THE APPLICATION OF MASS SPECTROMETRY IN ENVIRONMENTAL CHEMISTRY: INVESTIGATING BIOLOGICAL CYCLING OF ARSENIC, MERCURY AND GLYCINE BETAINE IN AQUATIC ECOSYSTEMS

by

Abdullah Mohammed Alowaifeer

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Ecology and Environmental Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2019

©COPYRIGHT

by

Abdullah Mohammed Alowaifeer

2019

All Rights Reserved

DEDICATION

To my parents and siblings. Without your prayers and support I would not be who I am today.

ACKNOWLEDGEMENTS

I would like to express my special appreciation to my advisor, Dr. Timothy R. McDermott, for his support and patience throughout my time in graduate school. Your guidance has been essential in my success as a student and will continue to benefit me throughout my career. I would also wish to express my gratitude to my co-advisor, Dr. Brian Bothner, you have been a tremendous mentor for me. Thank you for your encouragement and support. I would also like to thank my graduate committee for their continuous guidance and advice.

I would like to thank members of the McDermott lab, Bothner lab, Mass Spectrometry facility and the land resources and environmental sciences department for all their help. Finally, I am very grateful to the Saudi Food and Drug Authority for giving me the opportunity to pursue my PhD and for funding my research. Thank you for believing in me.

TABLE OF CONTENTS

1. INTRODUCTION 1

Arsenic in Aquatic Environments 1
Oxidation/Reduction 2
Methylation/Demethylation 3
Thioarsenicals 5
Arsenic Resistance and Toxicity... 6
Sediment Water Exchange 7
Arsenic in Phytoplankton.. 9
Arsenic in Zooplankton.. 13
Bioaccumulation and Biomagnification in Higher
Trophic Animals 15
Mercury in Aquatic Environments 20
Oxidation/Reduction 21
Methylation/Demethylation 22
Mercury Toxicity 23
Mercury Cycling 26
Mercury in Planktonic Organisms 29
Mercury in Aquatic Animals 32
Glycine Betaine in Aquatic Environments 39
Glycine Betaine Synthesis 40
Betaine Lipids 42
Fate of Glycine Betaine 44
Methylated Amines in Aquatic Environments... 45
Research Goals 48
References 55

2. METHOD AND METHOD DEVELOPMENT 75

Sample Preparation 75
Total Metal and Metalloids Extraction 75
Arsenic Species Extraction 75
Labeling Amines in Water with Dansly Chloride.................................... 76
Labeling Trimethylamine in Water with Ethylbromoacetate 77

TABLE OF CONTENTS – CONTINUED

Mass Spectrometer Analysis ... 77
Analysis of Total Metal and Metalloids by ICP-MS ... 77
Arsenic Species Analysis by IC- ICP-MS ... 78
Analysis of MMA and DMA by LC-MS ... 78
Analysis of TMA by LC-MS ... 79
Analysis of Glycine Betaine in Water by LC-MS ... 80
Analysis of Water-Soluble Methane by GC ... 80
References ... 84

3. BIOLOGICAL CYCLING OF ARSENIC AND MERCURY IN YELLOWSTONE LAKE ... 85

Contribution of Authors and Co-Authors ... 86
Manuscript Information Page ... 87
Abstract ... 87
Introduction ... 88
Materials and Methods ... 90
Sampling Location and Procedure ... 90
Analysis for Total Mercury and Arsenic ... 91
Analysis for Arsenic Species ... 91
Results ... 93
Total Mercury ... 93
Planktonic Arsenic ... 94
Arsenic in Cutthroat and Lake Trout ... 95
Discussion ... 97
References ... 108

4. EXAMING THE ROLE OF PHOTOAUTOTROPHS CONTRIBUTING TO GLYCINE BETAINE, METHYLATED AMINES AND METHANE IN OXIC RIVER WATERS ... 110

Contribution of Authors and Co-Authors ... 111
Manuscript Information Page ... 112
Abstract ... 112
Introduction ... 113

TABLE OF CONTENTS – CONTINUED

Materials and Methods 115
Sampling Location and Procedure 115
Labeling and Sample Preparation 117
Analysis for Methylated Amines and Glycine Betaine........ 118
Analysis for Water-Soluble Methane........ 118
Lake Water Microbial Activity 120
Results........ 121
Discussion 122
References........ 136

5. CONCLUDING REMARKS 138

6. REFERENCES CITED 142

LIST OF TABLES

Table Page

4.1 Physical and chemical measurments collected from the Madison River at different sampling dates in 2018 129

4.2 Physical and chemical measurments collected from the Gallatin River at different sampling dates in 2018 129

4.3 Physical and chemical measurments collected from the Yellowstone River at different sampling dates in 2018 130

LIST OF FIGURES

Figure Page

1.1 The two common inorganic arsenic species ... 50

1.2 Common methylated arsenic species ... 50

1.3 Common molecules with arsenic sugars ... 51

1.4 The three identified arsenolipid classes ... 51

1.5 Arsenobetaine proposed synthesis pathway ... 52

1.6 Glycine betaine formation pathways ... 52

1.7 The three identified betaine lipids found in numerous organisms ... 53

1.8 Identified pathways for the fate of betaine ... 53

1.9 The three known pathways for methylated amine degradation ... 54

2.1 Dansylation reaction scheme ... 81

2.2 TMA labeling reaction scheme ... 81

2.3 HPLC-ICP-MS chromatogram of arsenic species ... 82

2.4 LC-MS chromatogram of MMA and DMA ... 82

2.5 LC-MS chromatogram of TMA ... 83

2.6 LC-MS chromatogram of glycine betaine ... 83

3.1 General map of Yellowstone National Park with Yellowstone Lake shown ... 103

3.2 Planktonic total mercury ... 103

3.3 Total mercury concentration in lake trout and cutthroat trout as a function of fish organ and fish length groups ... 104

LIST OF FIGURES - CONTINUED

Figure Page

3.4 Total mercury concentration in lake trout and cutthroat trout as a function of fish organ and fish length 104

3.5 Planktonic total arsenic 105

3.6 Arsenic species profile in different planktonic size fractions 105

3.7 Total arsenic concentration in lake trout and cutthroat trout as a function of fish organ and fish length groups 106

3.8 Total arsenic concentration in lake trout and cutthroat trout as a function of fish organ and fish length 106

3.9 Relative proportion of identified arsenic species in different organs of length grouped (mm) cutthroat trout and lake trout 107

3.10 HPLC-ICP-MS chromatogram of arsenic species 107

4.1 General map of Yellowstone National Park with Yellowstone lake shown 126

4.2 General map of the state of Montana with major rivers as shown 126

4.3 Depth profiles of methane concentration, measured metabolites and environmental parameters in the upper water column of Yellowstone Lake 127

4.4 ^{13}C-Methane generation profiles of raw water samples spiked with various ^{13}C-labeled methylphosphonate (MPn) or methylamine (MeA) 127

4.5 Regression plots illustrating the potential relationships between methane and methylated amines 128

4.6 Regression plots illustrating potential relationships between methylated amines and glycine betaine 128

LIST OF FIGURES - CONTINUED

Figure Page

4.7 Temperature and chlorophyll a readings as a function of date and season 130

4.8 Total dissolved solids and water flow readings 131

4.9 Regression plots illustrating potential relationships between total suspended solids and water flow 131

4.10 Line graph showing water soluble methaneconcentrations at sampled rivers over time 132

4.11 Regression plots illustrating potential relationship found between measured metabolites in Madison river 132

4.12 Regression plots illustrating potential relationships between measured metabolites in the Madison river 133

4.13 Regression plots illustrating potential relationships between measured metabolites in Gallatin river 133

4.14 Regression plots illustrating potential relationships between between measured metabolites in the Gallatin river 134

4.15 Regression plots illustrating potential relationships between measured metabolites in the Yellowstone river 134

4.16 Regression plots illustrating potential relationships between measured metabolites in the Yellowstone river 135

4.17 Regression analysis illustrating potential relationship between Chlorophyll a and glycine betaine 135

ABSTRACT

Elemental cycling is a complex process that occurs abiotically and biotically. While abiotic cycling is well defined, biological cycling is more complex as it involves different microbes, animals and enzymes that govern its form and fate. In my project, I investigated the biological cycling of two of the most toxic elements known, arsenic and mercury. I examine their bioavailability, bioaccumulation and biomagnification in freshwater aquatic systems using Yellowstone lake as a study model. In addtion, the sources and sinks of glycine betaine, an important aquatic metabolite that contributes to the carbon and nitrogen cycle, is investigated in Yellowstone lake and three rivers located around the state of Montana. This research presented in this dissertation offers new insight on how arsenic and mercury cycle in aquatic systems and introduces a new hypothesis of the possible source of glycine betaine in freshwater ecosystem. Additionally, this project highlights a new and promising methodology to detect and quatify methylated amines in water samples.

CHAPTER ONE

INTRODUCTION

Arsenic in Aquatic Environments

Nicknamed as the king of poisons, arsenic was first discovered in 1200s by a German philosopher named Albertus Magnus. Arsenic is the 20th most abundant element in earth's crust, 14th in waterbodies and 12th in the human body. Arsenic is a metalloid with an atomic number of 33, an atomic weight of 74.921, and its electron configuration is [Ar] 3d10 4s2 4p3. Due to its half-filled P orbital, its oxidation state can change readily, and it can bond with multiple elements forming a variety of inorganic and organic compounds. This diversity in arsenic species gives it unique chemical and biological properties in the environment. Arsenic has 4 redox states (-3, 0, +3 and +5), depending on the redox potential and pH conditions. The most common redox forms are (+3) and (+5)[1], which occur when arsenic bonds with non-metals such as oxygen and sulfur, where arsenic donates the 3 electrons in the P orbital to form a +3 species or the two electrons in the 4S orbital plus the 3 electrons in the P orbital to form a +5 species.

<u>Oxidation and Reduction</u>

In water arsenic is present dominantly in the trivalent arsenite (As(III)) form or the pentavalent arsenate (As(V)) form. The ratio of As(III) to As(V) is dependent to some degree on the oxidizing condition (redox potential) of the water. The form of at which As(III) and As(V) is present is also pH dependent. Depending on environmental pH, As(III) can be H_3AsO_3, H_2AsO_3, $HAsO_3$ and AsO_3, and As(V) can be H_3AsO_4, H2AsO4, $HAsO_4$ and AsO_4 (Figure 1.1) [1]. The oxidation/reduction of arsenic can happen abiotically, however biological (i.e. microbial) transformations drive arsenic redox speciation in nature [2][3]. Prokaryotes have developed mechanisms to resist arsenic toxicity and some actually utilize it as an energy source. Chemolithotrophs have demonstrated the oxidation of As(III) to reduce oxygen, nitrate or chlorate[4]. Moreover, As(III) can be utilized as an electron donor for anoxygenic phototrophic growth[5]. These oxidation processes are controlled by two distinct enzymes: the AoxAB and ArxAB [4][5][6]. As for As(V) reduction, there are two mechanisms that have been described. The first one is believed to be a detoxifying mechanism to reduce As(V) to As(III) in order to discharge arsenic out of the cell. This process is mediated by ArsC reduced glutathione and thiol transfer protein glutaredoxin [7] [8]. The other mechanism is the dissimilatory arsenate reductases that occurs when As(V) is coupled with an electron donor with a lower redox potential [9]. This microbial As(V) respiration process happens under anaerobic conditions to provide energy to support bacterial growth. These microbes

can use a wide range of inorganic (e.g. sulfide) and organic electron donors such as acetate, lactate, formate and pyruvate [10].

Methylation/Demethylation

Arsenic methylation happens when a methyl group (-CH3) substitutes a hydroxyl group (-OH). This widespread biological process is important in arsenic transformation and metabolism in the environment [11]. The methylation pathway is a multistep process starting with As(V) reduction followed by a cycle of oxidation, methylation and reduction to form volatile and non-volatile species (Figure 1.2). The methyl group is transferred to As(III) from S-adenosylmethionine catalyzed by the S-adenosylmethionine methyltransferase enzyme with glutathione as electron donor[12]. Trivalent species are the products of ArsM catalysis [13], not pentavalent species as originally hypothesized by Challenger [14]. Methylation reactions are most often studied in aerobic culture conditions where the pentavalent species monomethylarsonic acid [MAs(V)] and dimethylarsinic acid [DMAs(V)] are observed. However, recent studies suggested that trivalent species methylarsenite [MAs(III)] and dimethylarsenite [DMAs(III)], the most toxic arsenicals known, are the products of the methylation process [12][15].

Presently, the ArsM-mediated As methylation pathway is the topic of important discussions, with two hypotheses currently being assessed [16]. The original reaction scheme proposed by Challenger [14] posits that the enzyme catalyzes a series of alternating oxidative methylations and reductions, using S-adenosylmethionine as the methyl donor to step-wise generate the pentavalent arsenicals MAs(V), DMAs(V) and

trimethylarsine oxide [TMAs(V)O]. Accordingly, the highly toxic trivalent species are intermediates, but are not primary reaction products.

Recently, Hayakawa et al. [17] offered a hypothesis suggesting the actual substrates of the methyltransferase are the glutathione (GSH) conjugates As(GS)3 and MAs(GS)2, and the products are the trivalent conjugates MAs(GS)2 and DMAs(GS) [16][13]. An important aspect of the Hayakawa hypothesis is that the conjugates are unstable, dissociating to the trivalent MAs(III) and DMAs(III), which can then spontaneously oxidize to their respective pentavalent counterparts upon exposure to oxygen. Marapakala et al. [18] showed that ArsM reaction rates are orders of magnitude greater with the As(III) glutathione conjugates as compared to the As(III) oxyanion. Subsequently, Ajees and Rosen [16] introduced an alternate reaction scheme that proposes a series of thiol exchange reactions that generate As(GS)3 as suggested by Hayakawa et al. [17] with the subsequent ArsM reaction intermediates either remaining associated with the enzyme and undergoing further methylation or may release from the enzyme. The latter would account for trivalent intermediates MAs(III) and DMAs(III). MAs(III) detoxification mechanisms involves ArsH, an organoarsenical oxidase capable of oxidizing trivalent methylated and aromatic arsenicals [19] and ArsI, which cleaves the carbon-arsenic bonds in methylated As(III) derivatives [20].

Thioarsenicals

Of the known naturally occurring arsenic containing compounds, perhaps the least understood are those that are thiolated, meaning bonded to an HS functional group. In natural environments, thioarsenicals are important components of arsenic biogeocycling in sulfide rich environment such as geothermal hot springs [21] or highly reducing subsurface groundwater environments[22]. Primarily, mono-, di-, tri- and tetrathioarsenicals are the compounds commonly found and studied. In addition to the requirement of sulfide, other environmental factors are extremely important in determining extent of formation, solubility, and stability of the thioarsenical compound. These include pH, redox conditions, and prevailing concentrations and ratios of As to SO4-2 and or H2S [23][24].

While microbial activity was emphasized above in discussing As(V) reduction and As(III) oxidation/methylation reactions and speciation, abiotic thiolation reactions appear to control arsenic thiolation [21][24]. Chemical equilibria reactions in (anoxic conditions) show that with increasing HS- : As ratios, the degree of thiolation tends to increase [24] [23] although there is some disagreement regarding whether the thioarsenical formed is trivalent [23] or pentavalent [24]. Abiotic H2S reduction of As(V) is highly pH dependent, reacting poorly around pH 7.0, even with H2S:As ratios of 100:1. Reaction rates increase markedly at low pH [13].

Arsenic Resistance and Toxicity

One of the arsenic resistance mechanisms known is through the extrusion of arsenic out of the cell via ArsB or Acr3 to prevent it from interaction with essential metabolic components Also, oxidation of As(III) to As(V) results in As(V), sorbing onto iron, manganese, aluminium (hydr)oxides and organic matter. In fact, sulfate reducers and iron oxidizers keep arsenic out of the cell by binding it to a metabolic product to from insoluble complexes. H2S and Fe(III)precipitates with As(V) and As(III) respectively, to form insoluble sulfide and iron complexes [25] [26].

There are no specific uptake transporters dedicated to arsenic, rather arsenic is adventitious and enters the cell through transporters for analogue molecules. As(V) is an analogue for phosphate, which is taken up by cells through phosphate transporters [27]. Once As(V) is in the cell, it replaces phosphate in biochemical reactions such as phosphorylation leading to the production of unstable arsenical by-products. As(V) reacts with adenosine 5-diphosphate ADP to form arsenate phosphate bond, however this bond is unstable due to arsenic having a relatively longer bond than phosphate. The unstable arsenoester bond spontaneously hydrolyses to ADP and As(V). This prevents ATP formation, thereby disrupting ATP synthesis, ATP-dependent transporters, glycolysis and signal transduction pathways [28].

As for As(III) , it has a structure similar to glycerol, therefore the major route of As(III) entry into the cell is passively through the glycerol transport system

(aquaglyceroporins) [29]. When inside the cell, As(III) has a strong affinity for sulfhydryl groups. Binding to free thiols can affect the structure and activity of various enzymes, receptors and transcription factors [30].

The degree of sulfur substitution is critical to the toxicity of thioarsenicals and is an example where a pentavalent arsenic species can be highly toxic. For example, DMAs(V) is of low relative toxicity, but thiolation to dimethylmonothioarsinic (DMMTAs(V)) greatly increases its toxicity, approaching that of As(III) and DMAs(III) [31], due to their efficient uptake and high production of reactive oxygen species. However, further sulfidication to form dimethyldithioarsinic (DMDTAs(V)) reduces toxicity significantly [32]. Consequently, one cannot generalize regarding the toxicity of thiolated arsenicals.

Sediment Water Exchange

Inorganic arsenic is present in the soil in As(III) or As(V) depending on the oxic condition of the sediment. Previous screening studies have shown that the concentration of As(V) is higher in the sediment of marine and freshwater except in deep aphotic anoxic soils. Moreover, the concentration of inorganic arsenic generally increases in correlation with sediment depth [33].

In marine systems most of the arsenic reacts with sulfur to form AsS, arsenopyrite (FeAsS) and orpiment (As2S3) which have a low solubility and mobility in the water

column. However, the sulfur concentration is lower in freshwater systems making arsenic more bioavailable then marine waters [34]. The most dominant form of As(III) in anoxic sediment is Arsenolite (As2O3), which is mobile and can diffuse into the overlying water column to be transported as As(III) [35]. In oxidized layers of the sediment, As(V) is associated with hydrous iron and manganese oxide. When the sediment redox shifts to moderately reducing conditions, iron and manganese oxide begin to dissolve releasing adsorbed As(V) into the water column [36]. Inorganic arsenic becomes mobilized from the sediment during transitions in redox potential. During redox shifts, arsenic dissolution from metal oxides and sulfides becomes favorable. Therefore, releasing arsenic into the water column, which is then mixes with the overlying water column by sediment resuspension. However, the concentration of total arsenic in the water layer above the sediment remains lower than concentration in the sediment.

Traces of MAs(V), DMAs(V) and TMAs(V)O have been observed in sediments from marine and freshwater systems [37]. Organic arsenicals are believed to be present as a result of sediment microbial activity. These biological synthesized compounds are mobile and can be transformed into the overlying water column by diffusion. Organic arsenicals are either present freely in the water column or broken down to inorganic arsenic by water microbes [33].

Arsenic in Phytoplankton (photosynthetic producer)

Phytoplankton is composed of photosynthetic microorganisms, both prokaryotes and eukaryote. Due to their carbon fixing ability, phytoplankton are most often viewed as the base of the aquatic food web in marine and freshwater systems. It's estimated that there are over a million phytoplankton species, with a size range of 1 μm diameter to 60 m long multicellular thalli [38]. Beginning at the base of the food chain, phytoplankton are likely a major source of arsenic for higher trophic level organisms and an important player in arsenic cycling.

In oxygenated waters with a pH of 7- 8 arsenic and phosphorus exist as analogues (H2AsO4- and H2PO4-), and arsenic is accumulated through cellular phosphate transporters as aforementioned. Levy et. al [39] showed that the increases in phosphate concentration in freshwater microalgae *Chlorella sp.* growth medium decreased arsenic cellular uptake, demonstrating competition between arsenate and phosphate for cellular uptake. Therefore, phosphate availability regulates As(V) uptake in phytoplankton. Both As(III) and As(V) have been documented in a diverse number of marine and freshwater phytoplankton. In addition, MAs(V), MAs(III), DMAs(V) and DMAs(III), believed to be a result of detoxification processes, have been documented too [40] [41] [39]. Studies on arsenic cycling in aquatic systems often mention phytoplankton as the first organisms involved in arsenic methylation.

One of the distinctive organic arsenicals present in phytoplankton are arsenic compounds containing riboses or as familiarly known “Arsenosugars”. Arsenosugar compounds are one of the predominant arsenicals found in freshwater phytoplankton as well as marine algae [41]. Although there are 20 identified biologically synthesized arsenosugars, dimethylarsinylriboside and trimethylarsenoribosides are the two most commonly found and often associated with four compounds, glycerol - ribose, phosphate - ribose, sulfonate - ribose, and sulfate – ribose (Figure 1.3) [42]. It was proposed by Edmonds et. al. [43] that the biosynthesis of arsenosugars is facilitated by S-adenosylmethionine contribution of two methyl groups to As(III) followed by the addition of a ribose group to DMAs(III),. However, recently published work by Xue et al.[42] demonstrated that arsM, a gene which encodes for S-adenosylmethionine methyltransferase, is only responsible for the methylation conversation to DMAs(III), but not the addition of the ribose molecule. The addition of the ribose group to DMAs(III) is facilitated by another enzyme encoded to arsS, a newly discovered gene adjacent to the arsM gene. Although the entire mechanism is yet to be determined, Xue et. al. (2018) [44] presented evidence that arsS is responsible for arsenic sugar synthesis in cyanobacterium *Synechocystis sp.* However, more research is needed to fully understand this mechanism and whether it happens in eukaryotes. Arsenosugars are mainly metabolized in animal bodies to DMAs(V), however there is no specific information regarding their toxicity [45]. Arsenosugars are stable compounds and have been documented to accumulate in low trophic organisms, in both marine and freshwater environments [46].

One of the most dominant organic arsenicals in aquatic systems is arsenobetaine. Arsenobetaine is poorly reactive and one of the least toxic forms of arsenic that was first discovered in 1977 [47]. It gained a lot of attention due to its low toxicity and predominance in aquatic life. Although the formation process is still unknown, the presence of arsenobetaine is suspected to be an end result of a long detoxification process. In addition, arsenobetaine is the arsenic analogue of glycine betaine, a nitrogen containing betaine compound used as osmolytes in aquatic animals. Osmolytes are ionic compounds that are soluble in cell environments used by organisms in response to stresses such as salinity. These osmolytes offer protection and stabilization of native protein structures within cells during temperature or salinity fluctuations [48]. That is why arsenobetaine concentrations in marine organisms are much higher than those in freshwater organisms [46]. Gailer et al. [49] found that the efficiency of arsenobetaine uptake from the water by the blue mussel *Mytilus edulis* dropped when glycine betaine was added to the water. In addition, Clowes et al. [50] suggested a relationship between salinity and arsenobetaine concentration by demonstrating the increase of arsenobetaine levels in blue mussel when exposed to high-salinity water, and a decrease when salinity was experimentally decreased. Although this phenomenom was only investigated in blue mussel and rays from the northern Adriatic seas [51], the results are consistent that arsenobetaine may be regulated by salinity.

It has always been hypothesized that the formation of arsenobetaine happens at the bottom of the food chain. However, many screening studies conducted documented the absence of arsenobetaine in marine [52][53][54][55][56] and freshwater habitat

[57][41][58][59]. In a recent study, Foster et. al. [60] screened 14 different brown, red and green marine macroalgae and found traces of arsenobetaine (0.01-1.2 mg/kg) in seven of them. This was the only study to date that documented arsenobetaione in marine phytoplankton. The absence of arsenobetaine in phytoplankton, especially in freshwater ecosystems, suggest that it could be formed at higher trophic levels.

Arsenic can form lipid-soluble dimethylarsinoyl species as well. It was hypothesized that these lipid compounds might have a functional role within the cell membrane by replacing phospholipids when phosphorus is scarce [61][62]. These newly identified lipid compounds have been classified into three groups: arsenohydrocarbons (AsHC), arseno fatty acids (AsFA) and arsenosugarphospholipids (AsPL) (Figure 1.4). These compounds have been documented in cyanobacteria and brown algae [61][63][64]. In one of the recent studies, Petursdottir et. al [63] cultured three strains of filamentous brown alga *Ectocarpus sp.* with As(V) and low phosphate conditions. The major arsenic compound found in the lipid fraction was AsHC (up to 80%), while the rest was in AsPL form, supporting the hypothesis that arsenic lipid formation is controlled by phosphate concentration. Little is known about arseniolipids as more research is still needed to determine the synthesis pathway, function and toxicity of these compounds.

Thioarsenicals have also been identified and reported in marine red, green and brown macroalgae [55][56][60]. These thioarsenic species were detected sporadically and in low quantities. It was suggested that they were a product of decaying parts of damaged macroalgae. However, there are no evidence of thioarsenicals synthesis in the aquatic system to date.

<u>Arsenic in Zooplankton</u>

Zooplankton are microscopic aquatic organisms with a size of >200 micron, that can be either an herbivorous by feeding on phytoplankton or carnivorous by consuming smaller zooplankton. Most zooplanktons are small crustacea belonging to the Arthropod phylum such as Copepods, Daphnia and Artemia, with some exceptions that belong to the Mollusca, Cnidaria and Ctenophore Phylum. Zooplankton are the second trophic level in the aquatic food chain and their interaction with arsenic has been studied extensively.

In marine systems herbivorous zooplankton have been shown to contain inorganic and methylated arsenic with the major compound begin arsenic sugars [54]. However, in carnivorous zooplankton the major arsenic compound is arsenobetaine [54][55][37]. Shibata et al. [54] investigated arsenic content in the sea of Japan and reported that herbivorous zooplankton *Calanus sp.* contained mainly arsenosugars with arsenobetaine as a minor component, and carnivorous *Themisto sp.* and *Sagitta sp.* contained arsenobetaine as the major arsenic compound. The authors proposed that the arsenic compounds in zooplankton reflect their feeding habit. Edmonds et al. [55] showed similar results when herbivorous copepod *Gladioferens imparipes* was cultured in artificial seawater and feed phytoplankton containing mainly arsenosugars. The herbivorous copepod did not contain arsenobeatine at all. As for freshwater systems, arsenobetaine is almost absent and the major arsenicals found are inorganic and methylated versions with traces of arsenic sugars [59][67][68]. Only one study reports arsenobetaine in freshwater zooplankton that was conducted in Grace Lake (Northwest Territories, Canada), although

zooplankton from neighboring Yellowknife and Kam Lakes showed no presence of arsenobetaine [57].

The presence of arsenobetaine in marine zooplankton but not freshwater organisms supports the theory that it functions as a cellular osmolyte in saline environments. In addition, the absence of arsenobetaine in phytoplankton and herbivorous zooplankton suggests, as claimed by many, that the formation of arsenobetaine mainly happens at the carnivore tophic level. The exact pathway and enzymes involved in the formation of arsenobetaine is still unknown. However, there are a couple of proposed pathways suggested by Kunito et al. [69] based on observational studies. The first proposed pathway is through the degradation of trimethylarsenoribosides to arsenocholine, then an oxidation step to form asrenobetaine. This pathway was proposed based on the finding of trimethylated sugars in abalone *Haliotis rubra* [70] and gastropods [71] in intestinal tissues. Trimethylarsenoribosides are found as a minor component in higher trophic organisms and some argue that it may be depleted due to rapid transformation [60], but trimethylarsenoribosides is not a major arsenical in phytoplankton [42]. The second proposed pathway is through the degradation of dimethylarsinylriboside [72]. This is a popular proposal and widely described, as dimethylarsinylriboside is very commonly found in phytoplankton [42]. Dimethylarsinylriboside degrades to form dimethylarsenoethanol, which either methylates to form arsenocholine or is oxidized to form Dimethylarsenoacetate. Moreover, the oxidation of arsenocholine and /or the methylation of dimethylarsenoacetate produces arsenobetaine (Figure 1.5). The presence of

dimethylarsenoethanol has been documented in phytoplankton [72], and both arsenocholine and dimethylarsenoacetate were detected in zooplankton from marine environments [55][37]. This pathway seems to be relatively plausible although more field work and culture experiments are needed to identify genes and enzymes involved.

Bioaccumulation and Biomagnification in Higher Trophic Animals

Bioaccumulation and biomagnification are two terms that are often confused. Bioaccumulation is the organism-specific uptake and retention of a substance from the surrounding environment at a rate that exceeds its elimination by that organism [73]; in other words the boundary of interest for determining whether bioaccumulation has occurred is a single organism and its immediate environment. If a compound's concentration increases in an organism through time, that compound is said to be bioaccumulated. Biomagnification, by contrast, is the process in which the concentration of a given chemical increases through a food web by trophic transfers [73]. In this case, the only way to determine whether biomagnification has occurred is to compare at least two organisms, each occupying different trophic levels.

Since the first determination of arsenic in fish and other aquatic organisms by Thiergardt and by Gautier and Clausman in 1900 [74], scientists have explored the possibility of arsenic accumulation in food chains. Arsenic enters the aquatic food chain through dietary and non-dietary routes such as uptake through gills and skin. In theory, arsenic enters the food chain at the base level (phytoplankton) and flows up the aquatic

food web, which can consist of 3 to 5 trophic levels. In marine organisms, arsenic has been reported to bioaccumulate in high concentrations [75][76][55][77][78]. Attar et al. [79] reported bioaccumulation of total arsenic in 13 finfish species and three crustacean species from the Arabian Gulf. In addition, Zhang et al. [80] investigated the bioaccumulation and trophic transfer of arsenic in two marine fish seabream *Acanthopagrus schlegeli* and *Terapon jarbua* artificially fed for 28 day. Their work suggests that arsenobetaine contributed to the accumulation of total arsenic in marine fish, although the feed contained a mixture of inorganic and organic arsenic. These findings support the hypothesis that arsenobetaine is formed within the carnivorous organism. In general, > 90% of arsenic found in marine fish (top predators) is arsenobetaine, arsenocholine and DMAs(V), with arsenobetaine being the major form [75][81][74][82][83]. Kirby et al. [76] screened the marine animals living in the mangrove swamps located on the coast of New South Wales, Australia and reported that 81% of arsenic was arsenobetaine, <1% arsenocholine and <2% was in DMAs(V) form. Arsenobetaine occurs across all trophic levels in the marine food web and is the dominant form of arsenic. Furthermore, arsenobetaine has been documented to bioaccumulates and increases with trophic level [75]. Amlund et al. [84] exposed Atlantic salmon *Salmo salar L.* and Atlantic cod *Gadus morhua L.* to dietary arsenobetaine to investigate the accumulation, distribution, and elimination of arsenic. The duration of the feeding experiment was three months, followed by a three-month depuration period. Both species showed differences in the accumulation and elimination of arsenobetaine. Arsenic concentrations in Atlantic salmon bioaccumulated significantly in muscle, liver, and

kidney, with an elimination half-life from muscle tissue estimated to be 37 days. On the other hand, total arsenic concentration in Atlantic cod showed a significant bioaccumulation in muscle tissue only, with elimination half-lives from muscle estimated at approximately 77 days. These differences observed within two fish species raised in the same environment with identical feed indicate that there are multiple factors involved in arsenic accumulation. Additionally, these findings can explain the differences in arsenic levels observed among marine fish, which is noticeable when examining data from in situ screening experiments conducted in marine systems.

Arsenic biomagnification has also been investigated in multiple studies. Goessler et al. [85] documented arsenic biomagnification in a three-trophic-level, marine system in the southwestern Australian coast. The primary producer *Hormosira banksii* seaweed had an arsenic concentration of 27mg/Kg dry weight and the herbivore *Austrocochlea constricta* gastropod had 74 mg/Kg dry weight, while the third trophic level *Morula marginalba* carnivores gastropod had 233 mg/Kg dry weight. Although arsenic has been reported to biomagnify in marine system in other studies too [80], several studies have reported inconsistent trends [76][86][87].

Arsenic speciation patterns in marine and freshwater systems are very different, although, a few studies have reported arsenobetaine being the predominate species in freshwater organisms [88][89]. For example, Slejkovec et al. [89] tested 9 different freshwater fish species belonging to 4 different families for arsenic speciation and found that the major fraction of extractable arsenic was arsenobetaine, which accounted for 92% in some *Salmonidae* species, followed by DMAs(V). Similar results were seen by

Soeroes et al. [90] when they analyzed catfish farmed in groundwater with elevated arsenic concentrations. However, others have reported only trace amounts of arsenobetaine [91] or even the absence of it in freshwater systems. Kaise et al. [67] sampled five different carnivorous fish species from the Hayakawa River in Japan to examine arsenic species. They found that most arsenic was in the inorganic form (93%) followed by methylated versions DMAs(V) and TMAs(V)O (7%). Furthermore, Lawrence et al. [92] analyzed marine and freshwater fish available throughout Canada to compare their arsenic species content. Marine fish including haddock, halibut, cod, herring and mackerel all had arsenobetaine as the major arsenical, while freshwater fish including pike, bass, carp, pickerel, whitefish, yellow perch, and striped perch contained no arsenobetaine nor arsenocholine.

Bioaccumulation of arsenic in freshwater aquatic organisms such phytoplankton and zooplankton that are consumed by predator fishes has been documented by several studies [93][94][95]. However, such trends are not seen in all higher trophic levels. Chen et al. [96] conducted an experiment to investigate arsenic bioaccumulation in freshwater medaka *Oryzias mekongensis* species. Fish were subjected to 100ug/L of As(V) in their water tank for 28 days to simulate chronic exposure. Surprisingly, arsenic bioaccumulation in exposed fish increased gradually during the initial 21 day exposure period and then decreased at day 28. The authors concluded that an acclimation effect occurred to regulate arsenic levels in the fish body. Suhendrayatna et al [59] investigated arsenic bioaccumulation and biomagnification patterns in an aquatic freshwater food chain cultured under experimental conditions. The three-step food chain was comprised

of *C. vulgaris* (phytoplankton), *N. denticulate* (zooplankton) and *Tilapia mossambica* (predator fish). The experimental results of the food chain model showed that total arsenic concentration decreased for each step up the food chain, Furthermore, the predominant species of arsenic found in all trophic levels was in the inorganic form with traces of MAs(V) and DMAs(V). As with marine systems, arsenic biomagnification trends vary significantly in freshwater. Burger et al. [77] measured arsenic in 11 freshwater fish species from the Savannah River, USA representing different trophic levels of the food chain. They found that arsenic concentrations in fish of lower trophic level were higher than in fish of higher trophic levels.

Arsenic bound lipids have also been documented in several fish species. Arsenic lipids have been reported in fish flesh from a local fish market in Aberdeen, UK. Amayo et al. [97] analyzed sardine (*Sardina pilchardus*), mackerel (*Scombers combrus*), red mullet (*Mullussur muletus*) and brill(*Scophthalmus rhombus*), and identified eight arsenolipids belonging to AsFA and AsHC class. Supported by their findings, they suggested that arseniolipids are accumulated through fish diet. AsFA and AsHC arsenolipids have been reported in commercial canned cod liver too [98]. Limited knowledge is available on arsenolipids synthesis and metabolism, as more research needs to be conducted.

To conclude, marine and freshwater systems are two completely different environments that should be studied accordingly, especially in terms of arsenic cycling. Arsenic in marine fish has been studied extensively, but little has been done on freshwater organisms. There is strong evidence that arsenic in marine organisms

bioaccumulates and that the majority of arsenic found is arsenobetaine followed by methylated versions. The low toxicity of arsenobetaine and its higher concentration in top trophic level fish indicate that it is produced as a result of a detoxification mechanism, although synthesis pathways are still unknown. In addition, arsenobetaine is an analog for the osmolite betaine aldehyde, which explains the differences seen in arsenobetaine levels between marine and freshwater systems. Moreover, biomagnification patterns are inconsistent in marine and freshwater fish as well, suggesting that there are other factors that govern arsenic trophic transfer. In freshwater organisms, arsenic species and bioaccumulation patterns are very variable. Such variability between studies might be due to spatial and seasonal variability in arsenic mobilization, diet, species differences, microbiome and contamination. Further, arsenic exists in several different organic and inorganic forms, thus understanding and predicting arsenic bioaccumulation is challenging.

Mercury in Aquatic Environments

Mercury is a metal with an atomic number of 80, an atomic weight of 200.59, and its electron configuration is [Xe] 4f14 5d10 6s2. Mercury is a rare earth metal and its concentration ranges from 21 ug/Kg (lower crust) to 56 (upper crust) ug/Kg [99]

Mercury has seven stable isotopes (196Hg, 198Hg, 199Hg, 200Hg, 201Hg, 202Hg, and 204Hg) with a relative mass difference of 4%. It is unknown when mercury was first discovered, but there is evidence that it dates to the times of the Pharaohs. The

ancient Greeks, Romans and Chinese have all used mercury for medicinal purposes. Nowadays, mercury is used in wiring devices, lighting, and dental restorations. Due to the 2 electrons present in the outer 6s orbital, mercury has 3 oxidation states (0, +1 and +2). Inorganic mercury (Hg2+) is the dominant form found in the environment followed by elemental mercury (Hg0), which is volatile.

Oxidation/Reduction

Reduction of Hg2+ happens in waters and soils, biotically and abiotically. Several microbes that have been isolated from contaminated sediment have shown resistance to mercury. Davila et al. [100] managed to isolate fifteen mercury resistant *Pseudomonas* strains from a gold mining site in Peru. As pure cultures, these strains demonstrated the ability to reduce of mercury in solution. Bacteria uses the mercuric reductase enzyme (MerA) to convert Hg2+ to volatile Hg0 [101].The exact mechanism for the microbial reduction process is still to be further investigated. In addition to microbes, some algae have shown to have the capability to reduce Hg2+ too [102]. It is believed that these biological processes are a part of an elimination mechanism that produces vapor Hg0, which diffuses out of the cell into the atmosphere. It has been reported that the reduction of Hg2+ can occur abiotically by the free radicals associated with fluvic [103] and humic acid [104].

Hg0 can be readily oxidized in the atmosphere abiotically to form Hg2+. This happens when it interacts with ozone in the presence of water. The oxidation process can

take place in oxygenated natural waters as well [105]. Biological oxidation of Hg0 can occur in waters and sediment. Smith et al. [106] was the first to document aerobic soil bacteria *Bacillus* and *Streptomyces* oxidation of Hg0. Only aerobic microbes have been shown to oxidize mercury as research on anaerobic microbes is still lacking. Further, it has been reported that mammals [107] and plants [108] can oxidize Hg0 by catalase and peroxidase.

Methylation/Demethylation

Natural biotic and abiotic processes can methylate inorganic mercury to form methylmercury (CH3 – Hg) and dimethylmercury gas (CH3 – Hg – CH3). Many anaerobic sediment microorganisms have demonstrated the ability to enzymatically catalyze CH3Hg [109]. Sulfate and iron reducers are the main microbes responsible for the active uptake of Hg2+, methylation in the cytoplasm and export of CH3Hg out of the cell [110]. It was hypothesized that the donation of a methyl group is from methyl-tetrahydrofolate via methylcobalamin [111]. Microbial methylation remains poorly understood, especially in terms of CH3HgCH3 generation although, Woods et. al [112] has proposed microbial generation of CH3HgCH3, but this process is yet to be demonstrated. It was suggested that sulfate reducing bacteria can produce CH3HgCH3 by reacting CH3Hg with sulfide to form dimethylmercuric sulfide, which degrades to CH3HgCH3 [113]. Humic, fluvic and carboxylic acids have been shown to contribute to

CH3Hg formation abiotically [114][115]. However, the yield of such reactions is low compared to microbial production.

CH3Hg can undergo oxidative demethylation by anaerobic microbes to produce CO2, CH4 and Hg2+ [116]. However, the mechanism of oxidative demethylation is currently unknown, although some suggest that CH3Hg is an analogue for methylamine degraded by methanogens [117]. Enzyme mediated demethylation, which splits the carbon mercury bond, can occur in aerobic and anaerobic conditions. In fact, MerB enzyme has been identified in several microbes that demonstrated in vitro mercury demthylation [118]. Also, MerB enzyme has displayed the breakdown of different forms of organic mercury compounds such as the fungicide methylmercury chloride and the disinfectant phenylmercuric acetate [119]. In light-exposed environments, abitotic degradation of CH3Hg can occur under a wavelength of 280-400 nm. Further, the photodegradation of CH3Hg in wetland produces Hg0 as a major product [120]. The rate of methylmecury photodegradation in the environment is yet to be estimated.

Mercury Toxicity

In prokaryotic cells, it is believed that the major route for mercury uptake is by diffusion of mercury complexes through the cell wall [121] although, active uptake has been proven in some microbes [110]. Anaerobic sulfate reducers *Desulfovibrio desulfuricans* ND132 and *Geobacter sulfurreducens* PCA study models both have an active mercury transport system composed of sulfhydryl and carboxyl functional groups.

However, these microorganisms are mercury methylators. The presence of an active transport pathways in other prokaryotes is not known. Also, it has been suggested that mercury can be transported into the cell accidentally through uptake systems of other essential metals that form complexes with sulfide and thiols [110]. Mercury's high affinity to thiolate ligands makes cysteines the most vulnerable target once in the cell.

Mercury reacts with sulfhydryl groups therefore, altering tertiary and quaternary structure of proteins and interfering with their function. There is evidence that mercury can interfere with DNA transcription and protein synthesis as well [122]. Microbes have the ability to extrude mercury out of the cell [123], especially CH3Hg but, the mechanisms remains unknown.

The toxicity of mercury to organisms in higher trophic levels depends on the form, dose and the rate of exposure. For instance, most mercury complexes are stable and insoluble therefore unavailable and are poorly absorbed [124]. Inorganic mercury is mainly found bound to a chloride ion to from mercuric salt (Hg – Cl). High exposure to mercuric salt is usually associated with local gastrointestinal and renal symptoms. These symptoms include the damage of gut mucosa, liver damage and renal tubular necrosis. However, neurological symptoms are less evident because inorganic mercury does not cross the blood-brain barrier efficiently [125]. The mode of toxicity for inorganic mercury is through its adherent to sulfhydryl groups on metallothionein and glutathione leading to loss of function. On the other hand, CH3Hg is the most toxic and biologically available form of mercury [126][127][128]. Many studies have been conducted on the exposure effects of CH3Hg on the mammalian body. Although human health effects of

chronic exposure to concentrations of CH3Hg is still disputable, CH3Hg poisoning has been linked to pathological changes in the heart and blood vessel system, the reproductive system, and the immune system. Symptoms may include paresthesia, blurred vision, and malaise. At higher doses it may lead to deafness, speech difficulties, and constriction of the visual field [129] but the carcinogenicity of CH3Hg is still under discussion. When material containing CH3Hg is ingested it is separated by gastric acid. Then, it combines with cysteine among the amino acids in the duodenum and almost 100% of it is absorbed. It only takes an ingested dose of CH3Hg 30–40 hours to be completely distributed throughout the body [130]. Afterwards, it combines with the hemoglobin of red blood cells and accumulates in the central nervous system and causes disorders of the neurons [131]. In addition, it combines with glutathione to make CH3Hg - glutathione compound, which is distributed to different tissues and organs through the blood vessels. Consequently, it easily crosses the blood-brain barrier and accumulates in the myelin membrane, causing hydrolysis to its phosphatidylethanolamine lipids composition. In case of pregnancy, the CH3Hg -glutathione compound can cross the placenta and accumulate in the brain of fetuses causing birth defects [131]. The mammalian body metabolizes CH3Hg by converting it to bivalent inorganic mercury and it undergoes oxidation and reduction, during which it releases oxygen radicals that causes severe damage to cells by activating the chain of lipid peroxidation of the cell membrane [132]. The main excretion paths of CH3Hg are through the bile and feces, but some is also excreted in sweat, urine, hair, nails and breast milk [130]. The half-life period of CH3Hg, that is, the time in which the content of CH3Hg in the body is reduced to half

through excretion, is 70 days on average [133]. When a person is exposed to CH_3Hg, CH_3Hg - glutathione compound in the blood moves to the follicle in the hair growth stage and is accumulated there. It has been proven that the mercury concentration in hair is proportional to the blood concentration [133].

Mercury Cycling

Mercury in its Hg^0 form is emitted into the atmosphere by natural and anthropogenic sources. These natural sources include: degassing of earth's crust and volcanic emissions, while the anthropogenic sources include coal combustion, mining and waste incineration. Anthropogenic inputs have led to a high increase of mercury concentration in air and water. In its latest mercury assessment report, the United Nations (UN) estimates the global mercury emission from anthropogenic source to be 2,220 tons, which represents 30% of total global emissions. The larger portion of the emission is from natural processes, which also involve recycling of previously introduced anthropogenic mercury. Once in the air, elemental mercury has a lifetime of several months and can be widely transported and dispersed across the globe [134]. When conditions are suitable, elemental mercury oxidize to Hg^{2+} then, wet or dry deposition occurs over water and land.

Anthropogenic activity has led to the increase of deposition, increasing the concentration of mercury in water. Lamborg et al. [134] found that human activity tripled the concentration of mercury in surface waters and calculated a 150% increase of mercury concentration in thermocline waters compared to the pre-anthropogenic era. In

the water, Hg^{2+} forms complexes with Cl^-, Br^- and $-OH$ depending on the salinity. Total mercury concentration in the water can vary widely depending on location. Screening of uncontaminated British estuaries reveled a very low concentration of 19 ng/L total mercury [135], while uncontaminated Tasmanian estuaries had 350 ng/L [136]. Lakes and estuaries close to natural or artificial mercury input sources tend to have a higher mercury concentration. In the water, Hg^{2+} is readily reduced biotically and abiotically to elemental mercury consequently, supersaturating surface water with mercury gas [134]. Mercury vapor can diffuse in and out of the water creating an equilibrium. Moreover, elemental mercury can be oxidized back to Hg^{2+} in oxygenated waters. Siciliano et al. [137] presented evidence that there is a correlation between the decrease in dissolved elemental mercury and microbial oxidase activity in freshwater lakes, proving that microbes play an important role in mercury cycling in natural waters.

Inorganic mercury can make strong complexes with freely floating sulfydroxyl-containing organic compounds, amino acids, hydroxy carboxylic acid and humic acid, which then sediment at the bottom of the water body [138]. CH_3Hg can also be found in natural waters, usually associated with - Cl or – OH. In fact, the concentration of elemental mercury decreases with depth and the concentration of CH_3Hg increases with depth, suggesting that the major source of CH_3Hg is benthic microbial activity [139][140]. In the sediment, most of the mercury is found complexed with dissolved organic matter (DOM) and sulfhydroxyl [141][138]. The production of CH_3Hg dominates in anoxic sediments primarily by sulfate reducers, while in oxic soils the demethylation process dominants [142]. In addition, the methylation process is inhibited

when a high concentration of hydrogen sulfide is present. This is due to the formation of insoluble mercury sulfide complexes that are unavailable biologically. However, as sulfide concentration decrease, inorganic mercury becomes bioavailable and is readily accumulated by microbes thus, the rate of methylation becomes higher [143][144][145]. Degradation of organic mercury occurs in anoxic sediments as well but at a lower rate than oxic sediments. CH_3Hg is degraded by sulfate reducers and methanogens to produce Hg^{2+} and methane. Methane is then oxidized by anaerobic methanotrophs to form carbon dioxide (CO_2) [146]. The demethylation process has been documented to happen in the sediment producing Hg^{2+}, which becomes soluble and mobile in the overlying water column [147]. Furthermore, the demethylation process can happen abiotically, but at a slower rate than biological degradation [148].

To conclude, mercury geochemical cycle is complex and is closely linked to sulfur cycling. Microbes govern the mercury cycling by oxidation, reduction, methylation and demethylation processes, although the extent of this role is yet to be fully understood. The presence of sulfate in anoxic sediments favors sulfate reducers and makes Hg^{2+} bioavailable as Hg-S complexes are not formed. Sulfur concentration in freshwaters is less than marine waters thus, mercury is more bioavailable to freshwater organisms. The role of methanogens and methanotrophs in the demethylation process suggests that mercury and carbon cycling is linked as well.

Mercury in Planktonic Organisms

Mercury can be present in the water column in different forms Hg0 ,Hg(OH)2, HgClOH, HgCl2 and CH3HgCl depending on pH and chloride concentration, although inorganic mercury is the most common chemical species [149][150]. The overall proportional distribution of mercury species in freshwater (Hg2+ > Hg0 = CH3Hg) is different than seawater (Hg2+ > Hg0 > CH3Hg) [151]. These differences are attributed to the differences in biological and physical reactions. To perform their cellular function, phytoplankton take up needed nutrients including trace metals from their surrounding environment. The incorporation of mercury at the base of the aquatic food web occurs efficiently in both freshwater and marine systems [152][153] and is thought to be the major route of mercury accumulation in food webs. In fact, bacteria and phytoplankton at the bottom of the food web may accumulated 10 times more mercury from the water than other higher trophic organisms [154]. The accumulation of mercury by lower trophic organisms plays a major role in mercury cycling in natural waters by modifying the species, influencing the concentration and the transport of dead matter to the sediment.

Phytoplankton have been documented to produce Hg0, which diffuses out of the cell and back into the environment. This reaction happens rapidly and is thought to be an acclimation mechanism to avoid accumulating mercury in the cell [155][156]. Such processes have been observed accompanied by the formation of HgS by algae, which is believed to be another method of detoxifying mercury [156][157]. The exact mechanism and enzymes involved is unknown, but an increase of glutathione, which is a precursor for phytochelatin synthesis, was recorded during these processes [157][158][159].

Methylation by phytoplankton has also been suggested, however current evidence is lacking [160][157][157]. Phytoplankton can also activity regulate the concentration of mercury in the water column. Luengen et al. [161] found that CH3Hg concentration in the San Francisco Bay estuary decreases during algal blooms and increases when algae decay, due to CH3Hg remineralization and increase production from sediment. In addition, Heimburger et al. [162] concluded in their study that during bacteria remineralization of small phytoplankton, concentration of CH3Hg in open oceans increases. The remineralization process of dead organic matter can occur in the water column as decaying phytoplankton sink towards the bottom or in the sediment by benthic organisms [163]. However, the difference in remineralization rates between sediment and water column is yet to be determined and will definitely be location dependent. Not all phytoplankton biomass will undergo the remineralization process, as higher trophic level organisms will feed on algae thereby, transferring mercury to the consumer and up the food chain.

Cationic metals are generally found bound to inorganic anion (hydroxide, carbonate, chloride, etc.) and to organic ligands (humic and fulvic acid) resulting in metal complexes that are hydrophilic. Phytoplankton outer membranes are mainly constructed as a lipid bilayer, which is impermeable to charged and polar molecules. Therefore, cationic metal uptake is usually facilitated by specific transport proteins [164][165]. Then, metals bind to intracellular ligands to be metabolized or go through the detoxification process. Mercury can either be taken up by active transporters like other metals or through passive diffusion. When bound to chloride, mercury complexes HgCl2

and CH_3HgCl become neutral and lipophilic with an Octanol - Water Partition Coefficient (Kow) of 3.3 and 1.7 respectively. These chemical properties allow such complexes to penetrate cell lipid bilayers into the cytoplasm. In contrary, hydroxyl complexes Hg $(OH)_2$ and CH_3HgOH have Kow of 0.05 and 0.07 respectively and therefore, cannot passively diffuse through the outer membrane [166]. This was supported by experiments preformed on artificial membranes that demonstrated the ability of $HgCl_2$ to cross lipid bilayers [167][168]. Mason et al [166] showed a linear correlation between uptake rates and Kow values of mercury species in marine diatom *Thalassiosira weissflogii*. Diatom toxic growth inhibition was found to be related to the concentration of $HgCl_2$ and CH_3HgCl, but not the total mercury concentration in the water column. However, mercury uptake cannot be attributed to passive diffusion alone, as freshwater phytoplankton can accumulate mercury where salinity is low. Pickhardt et al. [154] found that dead diatoms contained less Hg^{2+} and CH_3Hg than the living cell. The concentrations of Hg^{2+} and CH_3Hg in the cytoplasm dropped from 15.5% to 0.2% and from 64% to 4% respectively, when diatoms were heat killed. These findings suggest that mercury uptake is metabolically controlled by specific transporters. Moye et al. [169] cultured two green algae species (*Selenastrum capricomutum* and *Cosmarium botrytis*), one blue-green algae (*Schizothrix calcicola*) and one diatom (*Thalassiosira weissflogii*) with CH_3HgCl to study their uptake kinetics. Species differences in uptake rates were noticed as both the blue-green algae and diatom accumulated levels 20 times lower than the other two species. In addition, 30 day-old *Cosmarium botrytis* cells accumulated more mercury than 3-day old cells. Moreover, experiments with uncouplers, gamma-radiation

and light deprivation have all shown to affect uptake rates. The authors concluded that the evidences points to an active transport mechanism for CH3HgCl. To date, facilitated transporters involved in mercury uptake in phytoplankton are not determined, although overwhelming evidence point to it.

There are several factors that can influence the rate at which phytoplankton uptake mercury from their surrounding environment. The relative abundance of different organisms, organism size, DOM, salinity, and pH all influence uptake rates. During algal blooms, the concentration of mercury in algae and higher trophic level planktons decreases due to a lower degree of trace metal partitioning [170][154][152]. Organism surface area to volume ratio can also influence the efficiency of mercury uptake by phytoplankton, as smaller organisms with high surface to volume ratio uptake more mercury than larger ones [171]. DOM tends to have reduced sulfur sites that bind mercury, therefore higher DOM is associated with lower uptake rates [169][172][173]. Although higher pH reduces cation uptake due to competition at transporter binding sites, Le Faucheur et al. [174] found that freshwater algae *C. reinhardtii* accumulated 40% more mercury when pH dropped from 6.5 to 5.5, suggesting that mercury uptake could be proton mediated.

Mercury in Aquatic Animals

During the last decades, the formation, bioaccumulation and biomagnification of mercury in aquatic systems has been studied extensively, due to its high toxicity and the

nutritional value that seafood provides. Photosynthetic primary producers are generally thought to be the entry point of mercury into the aquatic food web, from which mercury is available for higher trophic organisms [163]. The transfer of mercury from the phytoplankton level to the herbivorous grazers depend on the species of mercury, algal quality and density [175]. Mercury distribution within the algal cell is species dependent as well, as Hg2+ binds to cell membrane, while CH3Hg is incorporated in the cytoplasm [176]. During nutrient trophic transfer, CH3Hg is more efficiently accumulated by zooplanktons than Hg2+ [152][177]. It was proposed that this is due to the zooplankton gut lining and digestive strategy that only absorbs soluble material [176]. Mason et al. [166] fed copepods the marine diatom T. weissflogii preloaded with mercury and observed high CH3Hg levels (62% of total mercury) compared to Hg2+ (15%) in the zooplankton. This was attributed to the high proportion of CH3Hg in the diatom cytoplasm (63%) compared with Hg2+ (9%).

Based on multiple published studies, the concentration of CH3Hg in zooplankton is double the amount reported in phytoplankton [178][179][180]. The low levels of CH3Hg in natural water and the efficiency of CH3Hg trophic transfer to zooplankton, indicate that phytoplankton are important participants in CH3Hg biomagnification [181]. Phytoplankton quality is also an important factor in the biomagnification of mercury. Zooplankton fed algae containing high carbon to phosphorus (C:P) ratio, tend to accumulate more mercury than low C:P fed zooplankton [182]. Karimi et al. [182] cultured Daphnia fed with two different quality of *Ankistrodesmus falcatus* phytoplankton and found that Daphnia fed algae with C:P = 139 had 30% less CH3Hg

than Daphnia fed algae with C:P = 1320. Similar results were documented by others [183], and it was explained that low C:P (high quality food) led zooplankton to have higher growth rate and slightly lower ingestion rates. The density of phytoplankton is another important factor in studying mercury trophic transfer. It was shown that higher algal growth rates, led to lower accumulation of CH_3Hg per algal cell, therefore lower transfer up the food web [184][152][177]. Adams et al. [184] measured the concentration of mercury in biotic and abiotic particles during thermal stratification in nine lakes in the Adirondack Mountains of New York State and found that algal density was negatively correlated with total mercury. Furthermore, Chen et al. [185] compared mercury concentration with physical and chemical readings from across150 lakes in northeastern USA, and found negative correlation between mercury concentration in zooplankton and fish with pH, SO_4, human land use and zooplankton density. Therefore, density plays a major role in both phytoplankton and zooplankton mercury uptake. In fact, zooplankton density influence on mercury accumulation is more important than other properties such as pH and DOM [175].

Zooplankton, the second level of aquatic food webs, are the primary food source for many fish species. Pelagic fish can feed on phytoplankton, zooplankton, a mixture of both, or prey on other fish species. Depending on the ecosystem, the top predators in aquatic food web are large carnivorous fish, birds and/or mammals including human. Since CH_3Hg is more readily accumulated by zooplanktons [152] and its concentration increases by a factor of 2 [179], it can be predicted that higher trophic level organisms will biomagnify CH_3Hg efficiently. In fact, Bloom et al. [186] examined 229 samples

from seven freshwater and eight saltwater fish species for inorganic and organic mercury. They found that 95% of total mercury was in CH_3Hg form, and that no differences in the organic portion was observed between freshwater and marine fish. Similar results have been published indicating CH_3Hg as the major contributor to mercury trophic transfer [187][188][189]. When CH_3Hg is ingested, it penetrates the gut wall and is accumulated rapidly [190], while Hg^{2+} is poorly absorbed and remains in the gut wall for up to 36 days [191]. Boudou et al. [191] conducted feeding experiments on rainbow trout to evaluate the trophic transfer of CH_3Hg and Hg^{2+}. Each fish received an average of 830 ug/Kg of $HgCl_2$ or CH_3HgCl_2 in their fry diet for 30 days. They found that 84% of CH_3Hg was assimilated and accumulated in liver, muscle, kidneys and spleen, while only 23% of Hg^{2+} was absorbed through the intestine. However, the amount of CH_3Hg accumulated is species dependent and different animal species will have different absorption rates for CH_3Hg and Hg^{2+}. Fowler et al. [192] fed mussels (*Mytilus galloprovincialis*) and benthic shrimp (*Lysmata seticaudata*) on phytoplankton containing CH_3Hg and Hg^{2+} for 35 days. They found that mussels accumulated twice as much CH_3Hg as Hg^{2+}, while the benthic shrimp accumulated 10 times more CH_3Hg than Hg^{2+}. Accumulation factors are not only governed by the predator but even the type of prey can affect absorption rates of CH_3Hg. Suzuki et al. [193] fed Japanese amberjack fish with Japanese anchovy fish and Japanese horse mackerel, and found that the absorption percentage of CH_3Hg was 67% and 88% respectively.

Regardless of the factors affecting absorption rates of CH_3Hg and Hg^{2+}, it remains that top predators contain the highest concentration of CH_3Hg and total mercury

in most screening studies [186][194][195][196]. Furthermore, the major source of accumulating CH3Hg is the diet, as concentration of CH3Hg in the water column are generally low [150]. However, aquatic animals can also accumulate mercury from the water through their gills [197]. Canli et al. [197] found that Norway lobsters can accumulate both CH3Hg and Hg2+ through their gills. However, Hg2+ was predominantly found in the gills, while CH3Hg was evenly distributed among tissues, indicating efficient absorption capabilities of CH3Hg through the gills. Equally uptake rates of CH3Hg and Hg2+ through the gills, and different organ distribution profiles was also found by Laporte et al. [198] in shore crabs. Laporte et al. [198] estimated the uptake of both CH3Hg and Hg2+ through the gills to be 1% of the total mercury detected in the animal. The difference between diet and gill uptake might be due to the lower concentration of mercury in the water compared to the diet, and that the dominant species natural waters is Hg2+, which has a weak uptake rate.

The distribution of mercury in the animal's organs does not seem to follow a pattern, as data is variable depending on animal and location. However, the liver accumulates the highest amount of mercury compared to other organs in most studies, due to absorption rout and its detoxification function [199][200][201][202]. Bargagli et al. [203] analyzed different organs of three top predator living in Terra Nova Bay (Antarctica) for total mercury. They found that the Weddell seal accumulated 40X more mercury in the liver than the muscle, and that the order of mercury distribution in the organs from highest to lowest was as follows: liver > spleen > muscles. In addition, the order of mercury organ distribution in Adelie penguins was liver > kidneys > muscles,

while Cod fish had an order of kidneys > muscles > liver. Neff [204] reported similar mercury organ distribution patterns in marine animals and mammals living in the gulf of Mexico. He found that marine mammals had a mercury distribution order of liver > kidneys > muscles, while marine birds had the highest concentration of mercury in their livers followed by their kidneys then muscles.

The variability in spatial mercury screening studies confirm that location is a major factor in trophic transfer. The various physical and biological processes differ regionally, causing the report of inconsistent results. The United States Geological Survey (USGS) measured total mercury concentrations in 1,486 fish representing 16 different species from 86 sites in 21 different national parks in the Western United States and published a report with their results [205]. Total mercury results in fish muscles varied widely with the lowest concentration measured close to 10 ug/kg and the highest concentration 1,100 ug/kg. The high variability was reported to be among parks and across different locations at a specific park. The importance of local processes in uptake concentration and biomagnification patterns is highlighted when comparing reported data on a specific species caught from different locations. Swordfish *Xiphias gladius* from the Azores Islands has an average total mercury concentration range of 1.25 – 9.3 mg/kg [196], while swordfish from the West Mediterranean has a mercury concentration range of 1.7 – 2.35 mg/kg [194], and fish caught form the north Pacific had a mercury concentration of 2.14 mg/kg [186].

High mercury concentrations in top predator remains an issue of great significance for human consumers, especially since anthropogenic inputs are increasing

yearly, resulting in more mercury becoming bioavailable. Dietz et al. [206] estimates that 74% to 94% of the mercury found in predatory birds and mammals living in the Arctic is of anthropogenic sources. The Environmental Protection Agency (EPA) and The Food and Drug Authority (FDA) both advise to avoid seafood containing levels exceeding 0.46 mg/kg and to limit consumption to once a week if concentration is between 0.23 to 0.46 mg/kg. Moreover, for seafood containing total mercury concentration between 0.15 to 0.23 mg/kg it is advised to limit consumption to twice a week, while for seafood with mercury lower than 0.15 mg/kg, three weekly serving are advised [207]. However, these are general guidelines as exact limits depend on serving amount and subjects weight, and age. Since global seafood consumption is increasing yearly (9.6 kg per capita in the 1960 to 20 kg per capita in 2016) [208], regular screening studies are necessary to insure public consumers safety.

The topic of mercury in aquatic food chains has been studied extensively and almost every aquatic animal has been analyzed for mercury due to its high toxicity and governmental agencies awareness. However, more research is still need especially, in terms of lower trophic level organisms and their interaction with mercury. The methylation process and the organisms involved are still not fully understood. Therefore, microbial and phytoplankton mercury interaction is an interesting subject for further exploration.

Glycine Betaine in Aquatic Environments

Glycine betaine ($(CH_3)_3N^+CH_2COOH$) or trimethylglycine as it may be referred to, is an amino-acid derivative, intracellular organic osmolyte. Under abiotic stress conditions, prokaryotic and eukaryotic cells are known to accumulate glycine betaine as an adjustment process. Since water moves in and out of the cell by osmosis, periods of fluctuating physical parameters such as salinity and temperature influence cellular components and the rate of metabolic reactions. Consequently, affected cells biosynthesize and/or directly uptake glycine betaine rapidly. The specific mechanism by which glycine betaine offers protection is still unknown, although it is believed that it prevents denaturing of intracellular proteins [209]. In addition, glycine betaine can act as a methyl group donor to support several cellular functions such as protein synthesis and DNA methylation [210].

Microorganisms, phytoplankton, plants and aquatic animals are all known to accumulate glycine betaine during stress conditions [211][212][213][214]. Kageyama et al. [215] studied the effect of sodium chloride (NaCl) concentration on the growth and chemical composition of the diatom *Thalassiosira pseudonana*. Intracellular glycine betaine content increased when NaCl concentration increased by ×0.5 suggesting a salt tolerance mechanism. However, the protection effect is limited as diatoms failed to grow when NaCl exceeded ×0.5. Similarly, plants have evolved to tolerate changing salt levels by accumulating glycine betaine as well [216]. In addition to salinity, temperature, CO_2, drought, UV radiation and heavy metals have all been documented to influence glycine betaine cellular content [215]. Spielmeyer et al. [217] cultured three algal strains

Thalassiosira pseudonana, *Phaeodactylum tricornutum* and *Emiliania huxleyi* to study the influence of temperature and CO2 on glycine betaine accumulation. They found that as temperature increased the levels of glycine betaine increased as well, however glycine betaine concentration decreased with elevated levels of CO2. Besides high temperature, cold stress responses can also stimulate cellular glycine betaine build-up. The fish pathogen Vibrio *anguillarum* accumulates glycine betaine as a protectant against cold temperatures [218]. In addition, glycine betaine in teleosts muscles increases with depth to inhibit the effect of hydrostatic pressure [219]

Glycine Betaine Synthesis

Although glycine betaine synthesis pathways have been determined, it is believed that active uptake into the cell is more favorable as it is energetically cheaper [218]. There are two glycine betaine uptake systems that has been identified in some bacterial species, 1) ATP-binding cassette (ABC) type transporters and 2) the betaine-choline-carnitine transporter (BCCT) family [220]. However, such transporters are yet to be determined in other aquatic organisms. There are two known pathways for glycine betaine synthesis, either through the oxidation of choline or by the methylation of glycine. Microbes, phytoplankton and aquatic animals have demonstrated the biosynthesis of glycine betaine through a two-step choline oxidation pathway. Choline is a methylated nitrogen compound widely found in aquatic environments at a reported concentration of 45 nM in seawater [221]. It is an essential compound for the synthesis of

phosphatidylcholine and sphingomyelin, which are two major phospholipids important for cellular membranes. Choline is oxidized to form betaine aldehyde by the enzyme choline dehydrogenase, which is then further oxidized by betaine aldehyde dehydrogenase to produce glycine betaine (Figure 1.6 A) [215]. In large aquatic animals and mammals, choline oxidation occurs mainly in the liver and kidneys [222][223]

The other pathway involves a three-step methylation of glycine to form sarcosine as a first step followed by dimethylglycine to then produce glycine betaine (Figure 1.6 B). This pathway has only been identified in one diatom species and some bacterial species. Surprisingly, the enzyme involved in catalyzing S-adenosyl methionine addition was found to differ between microorganisms. Nyyssola et al. [224] were the first to identify the three-step glycine methylation pathway in extreme halophiles and they documented the involvement of two enzymes, glycine sarcosine methyltransferase (EsGSMT) and sarcosine dimethylglycine methyltransferase (EsSDMT). Later, Waditee et al. [225] identified two different enzymes associated with glycine methylation in cyanobacteria, which they referred to as glycine sarcosine methyltransferase (ApGSMT) and dimethylglycine methyltransferase (ApDMT). Further, Lai et al. [226][227] identified three catalytic enzymes involved in glycine betaine synthesis in the halophilic *methanoarchaeon*, glycine sarcosine methyltransferase enzyme (MpGSMT), glycine sarcosine dimethylglycine methytransfer enzyme (MpGSDMT), and arcosine dimethylglycine methytransfer enzyme (MpSDMT). In addition, Kageyama et al. [215] searched the genome sequence of the diatom *Thalassiosira pseudonana* and found that all steps can be catalyzed by one enzyme in this organism, which they called glycine

sarcosine dimethylglycine methytransfer enzyme (TpGSDMT). Regardless of the enzymes involved the pathway and the production of glycine betaine in similar. Further investigation is required to explore the presence of such pathway in higher trophic organisms.

Betaine Lipids

Betaine lipids are glycerolipids that have a betaine head group instead of phosphorous attached to a diacylglycerol backbone by an ether bond. The ether bond makes these compounds more chemically stable than most phospholipids, which contain a phosphodiester linkage instead [228]. Betaine lipids have been documented in bacteria, phytoplankton, protozoa, fungi and plants [229][230][231][232]. To date, there are three identified betaine lipids diacylgyceryl-N-trimethylhomoserine (DGTS), diacylglyceryl hydroxymethyl-N,N,N-trimethyl-beta-alanine (DGTA) and diacylglyceryl carboxyhydroxymethylcholine (DGCC) (Figure 1.7) [233][234]. The synthesis of betaine lipids is controlled by the concentration of phosphate as mostly no betaine lipids have been documented during optimal culture conditions. During phosphate starvation periods, phospholipid is degraded and betaine lipids are synthesized to relocate phosphate to necessary metabolic processes as an adaptive strategy [232][235][236]. They only exception was found in the green algae *Chlamydomonas reinhardtii*, which has been shown to lack phosphatidylcholine and alternatively produces DGTS regardless of the phosphorus concentration [237]. However, betaine lipids have a different fatty acid

profile than phospholipids with higher unsaturated groups [238]. In addition, the concentration of betaine lipids to phospholipids in phytoplankton is varies widely among different species [239]. Furthermore, low temperature has been documented to stimulate DGTS synthesis in the microalga *Nannochloropsis oceanica* as well, but the metabolic benefit is still unclear [240]. Therefore, it was suggested that betaine lipids serve a complex role that includes substituting for phospholipids [234].

DGTS is the most commonly found betaine lipid and the most abundant in bacteria and phytoplankton. Consequently, it has been studied extensively, and its biosynthetic pathway, precursors, genes and enzymes have been identified. DGTS is synthesized by a two-step pathway, which starts by the addition of a homo-Ser moiety to diacylglycerol to produce diacylglycerylhomoserine, followed by the addition of three methyl groups to finally form DGTS. In prokaryotes models *Rhodobacter sphaeroides* and *Sinorhizobium meliloti*, steps one and two are catalyzed by the enzymes BtaA and BtaB respectively, with S-Adenosyl methionine as a cofactor [241][242]. While in the eukaryotic green alga model *C. reinhardtii*, both steps are catalyzed by one enzyme CrBTA1, that contains domains similar to BtaA and BtaB [243].

Betaine lipids are an interesting field of study, as their protection role and cellular functions are not fully understood. In addition, most research is focused on DGTS, while little is known about DGTA and DGCC. The pathway, genes and enzymes involved in the production of DGTA and DGCC are unknown. In addition, DGTS synthesis pathway has only been documented in bacterial and phytoplankton models, while little is known regarding their existence and metabolism in larger aquatic animals and mammals.

Fate of Glycine Betaine

Glycine betaine can be used by many microbes as a carbon and energy source. There are three identified pathways for glycine betaine microbial degradation, which occur aerobically and anaerobically. Under oxic conditions, marine microbial model *Pseudomonas aeruginosa* can oxidize glycine betaine to form dimethylglycine and formaldehyde using the monooxygenases enzyme glycine betaine demethylase [244]. Dimethylglycine can undergo further oxidation conducted by a heterodimeric flavin-linked oxidoreductase enzyme to form sarcosine and formaldehyde. Finally, the demethylation of sarcosine is carried out by a heterotetrameric enzyme to form glycine and formaldehyde as end product (Figure 1.8 A). Microbes utilize this pathway to generate formaldehyde to use it as a carbon source.

The other two pathways happen strictly under anoxic conditions to either produce dimethylglycine or Trimethylamine (TMA). Mueller et al. [245] were the first to demonstrate that two bacterial strains belonging to the genus *Eubacterium limosum* can grow anaerobically on glycine betaine as an energy source to produce dimethylglycine, acetate and butyrate. Moreover, methanogens isolated from marine sediments have also shown the ability to use glycine betaine as a carbon source to produce dimethylglycine, methane and CO2 [246]. However, these microbes were unable to further degrade or grow on dimethylglycine. The formation of TMA is also possible through the reduction of glycine betaine [247]. This pathway requires an electron donor such as formate or H2 in the presence of glycine betaine, to produce TMA in association with acetate, which is a fermentation by-product. This reaction is catalyzed by the glycine-specific protein B (PB

betaine), which has been purified from the gram-negative anaerobic bacteria *Eubacterium acidaminophilum* (Figure 1.8 B) [248][249].

Methylated Amines in Aquatic Environments

Amines are organic compounds that are derivatives of ammonia, that have one or more hydrogen atoms replaced by an organic ligand. The sources of amine emissions into the atmosphere include animal husbandry, fossil fuel burning, industrial processes and aquatic organisms. There are four amine compounds that are abundant and of high biological relevance, they are TMA ((CH3)3NH2), trimethylamine oxide (TAMO) ((CH3)3ONH2), dimethylamine (DMA) ((CH3)2NH2) and monomethylamine (MMA) ((CH3) NH2) [250]. These methylated nitrogenous compounds are ubiquitous in the environment and are found in gaseous form at room temperature.

As aforementioned, TMA is a precursor of glycine betaine, which can be used by microbes as a carbon and nitrogen source [251]. Large et al.[252] were the first to demonstrate the ability of *Aminobacter aminovorans* to grow on TMA as a sole carbon source by converting it to TAMO and formaldehyde. It was suggested that the enzyme TMA monooxygenase (Tmm) is responsible for the oxidation process. For aerobic microbes to utilize TMA as a carbon or a nitrogen source, TMA is first oxidized to TAMO, which is then converted to DMA by the enzyme trimethylamine N-oxide demethylase. DMA is then transformed to MMA using the dimethylamine monooxygenase enzyme, and lastly MMA is converted to ammonia to be used by the

microbe. The last reaction is catalyzed by enzyme N-methylglutamate dehydrogenase, and formaldehyde is produced at each step of methylated amine conversion (Figure 1.9 A) [252][253]. Formaldehyde can be used as a carbon source by many microbes, therefore TAMO, DMA and MMA can also be used as a carbon source by forming formaldehyde or as a nitrogen source by breaking it down to ammonia. Moreover, formaldehyde can be oxidized abiotically to form CO2, which is released into the atmosphere by diffusion [253].

In anaerobic aquatic environments, TMA, DMA and MMA can be utilized as methylotrophic substrates. The use of methylated amine by methanogens was first documented in high salinity environments, where glycine betaine and its precursors are dominant metabolites [254][255]. The three-step methane formation pathway starts with the conversion of TMA to DMA by the corrinoid protein methyltransferase, which is then demethylated by the coenzyme M to produce methyl-CoM. Further, methyl-CoM can either be reduced to produce methane or oxidized to form CO2. This pathway can be repeated similarly to utilize DMA and MMA to produce MMA and ammonia respectively in association with methane (Figure 1.9 B) [256][257].

Recently, Wang et al. [287] discovered a new pathway for MMA metabolism during their investigation of methane supersaturation of oxic waters, a phenomenon that is often referred to as the “methane paradox”. A freshwater isolate *Acidovorax sp.* YL-MeA-13 was demonstrated to grown aerobically on glycine betaine or MMA as a sole nitrogen source with methane being generated as a by-product. The 5’ pyridoxal phosphate-dependent reaction catalyzed by aspartate aminotransferase uses MMA and a-

ketoglutarate as substrates to produce methane and glutamate (Figure 1.9 C). Finally, they suggested that decaying phytoplankton are a major source for environmental MMA, by the breakdown of algal betaine (glycine betaine ➡ TMA ➡ TMAO ➡ DMA ➡ MMA ➡ methane).

The fate of gaseous methylated amines emitted to the atmosphere include wet and dry deposition, oxidation or acid-base reactions. The oxidation of methylamines happens with OH and O3 to produce hydrogen cyanide, and nitrous oxide [258]. These reactions occur rapidly with an estimated amine lifetime of two hours with respect to OH oxidation, and 5–10 hours for O3 oxidation, which is a minor reaction in comparison to OH oxidation [259]. In the presence of inorganic acids, methylated amines can also undergo acid–base reactions to form salt products such as HCl, HNO3 and H2SO4 [260]. Further, reactions between organic acids and methylated amines have been predicted to form amides [261]. Data on global atmospheric emissions and concentration of methylated amines is limited, due to the challenges of measurement and sophistication of model designs. Yu et al [262] simulated a model to predicate the global atmospheric emissions, sinks and oxidation of methylated amines. They found that TMA global emission was the highest (196 Gg N yr-1) followed by MMA (96.2 Gg N yr-1) then DMA, which was estimated at 38.3 Gg N yr-1. Atmospheric concertation was region dependent, as high elevation areas had less methylated amines then low altitude locations, and polar regions had lower levels in comparison to other locations due to lack to emissions. In general, atmospheric TMA was higher by a factor of 8 than DMA, while MMA was 2.5 times higher than DMA. The average concentrations of TMA, DMA and

MMA was estimated to range between 1 – 50 ppt, 0.1 – 10 ppt and 0.2 – 20 ppt respectively. The lower levels of atmospheric DMA in comparison to MMA and TMA could be due to its relative higher boiling point. TMA and MMA have a boiling point of 3 and -6 °C respectively, while DMA's boiling point is 7 - 9 °C.

To conclude, betaine and methylated amines are important metabolites in aquatic systems, that can be utilized by many microorganisms as a carbon and nitrogen source. In addition, the fate of betaine and methylated amines are closely associated, with phytoplankton and microbes being crucial players in their metabolism. Since betaine is a major source for methylated amines and two of three possible identified pathways for MMA degradation involve the production of methane, it can be assumed that biological methane production is related to betaine degradation.

Research Goals

1- Acquire skills in experimental design, sampling planning, procedures and execution.

2- Gain knowledge and expertise in method development, sample preparation, extraction protocols and laboratory troubleshooting.

3- Develop analytical skills and techniques to best utilize mass spectrometry in environmental investigations.

4- Study how arsenic and mercury is transferred through aquatic food webs by examining their bioaccumulation and biomagnification patterns in freshwater organisms, that includes microbes, phytoplankton, zooplankton and top predators.

6- Investigate arsenic and mercury uptake and distribution in freshwater fish by the analysis of arsenic species and total mercury in the gills, liver and muscle.

7- Develop a chromatographic method to detect and quantify MMA, DMA, TMA and betaine in water.

8- Investigate the possible relation between betaine, methylated amines and biological methane production in freshwater systems.

9- Explore the relationship between physical parameters (pH, temperature, water flow), biological readings (chlorophyll and phycocyanin) and metabolites (betaine, TMA, DMA, MMA) in river systems.

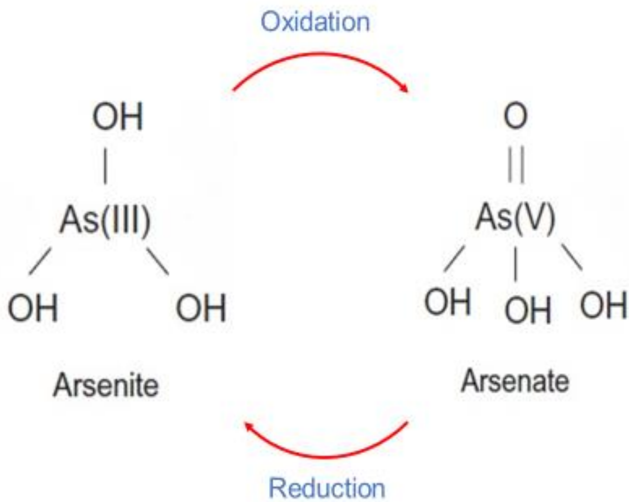


Figure 1.1 The two common inorganic arsenic species. The oxidation reduction reactions can occur biotically and abiotically.

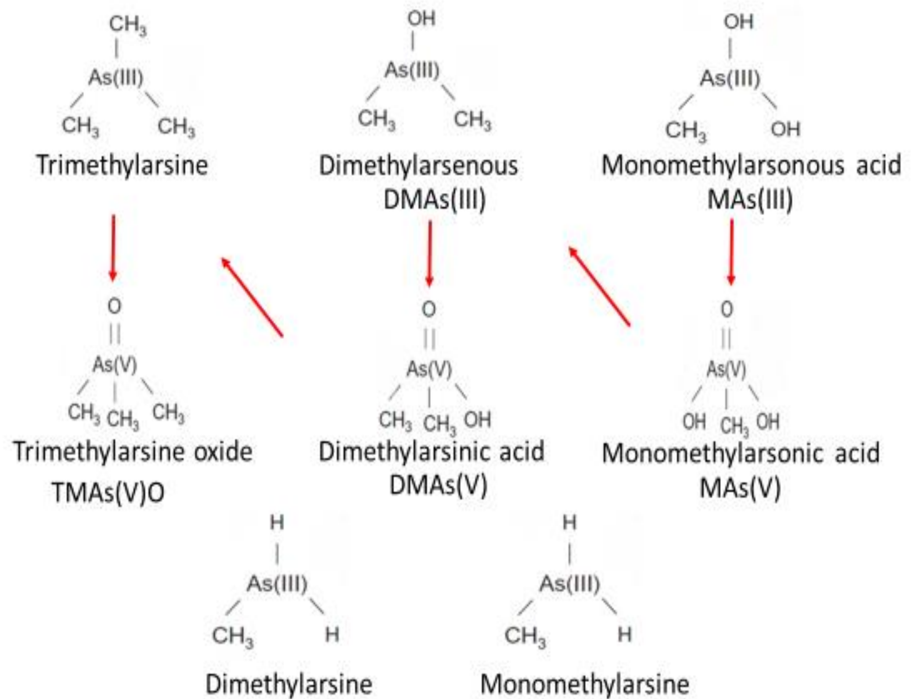


Figure 1.2 Common methylated arsenic species. Biological formation of these arsenicals depends on the microorganisms available and their metabolic capability to interact with arsenic.

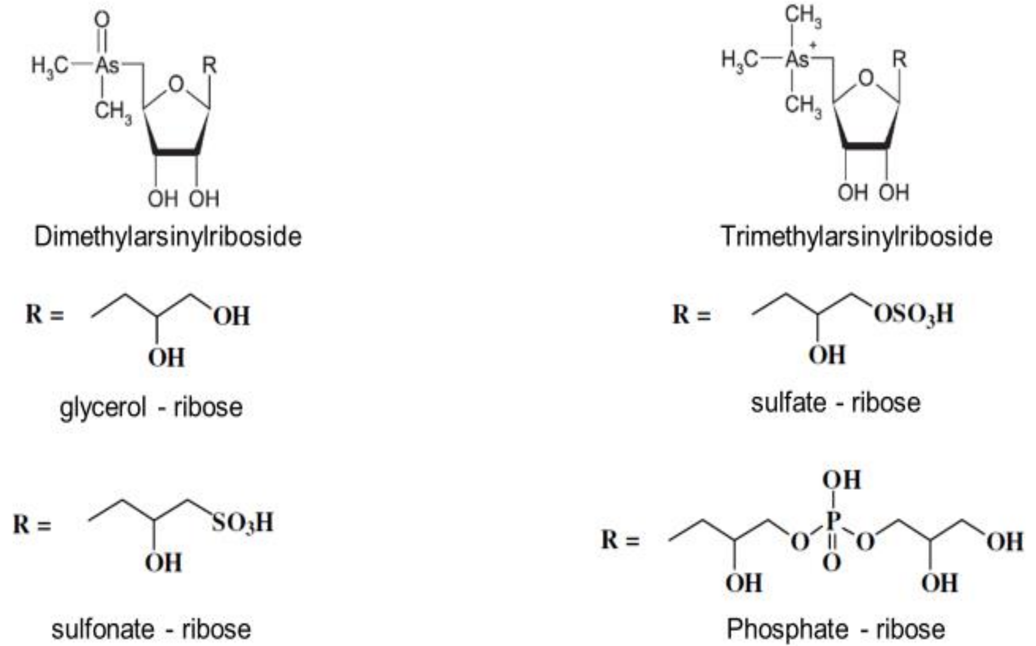


Figure 1.3 Common molecules with arsenic sugars

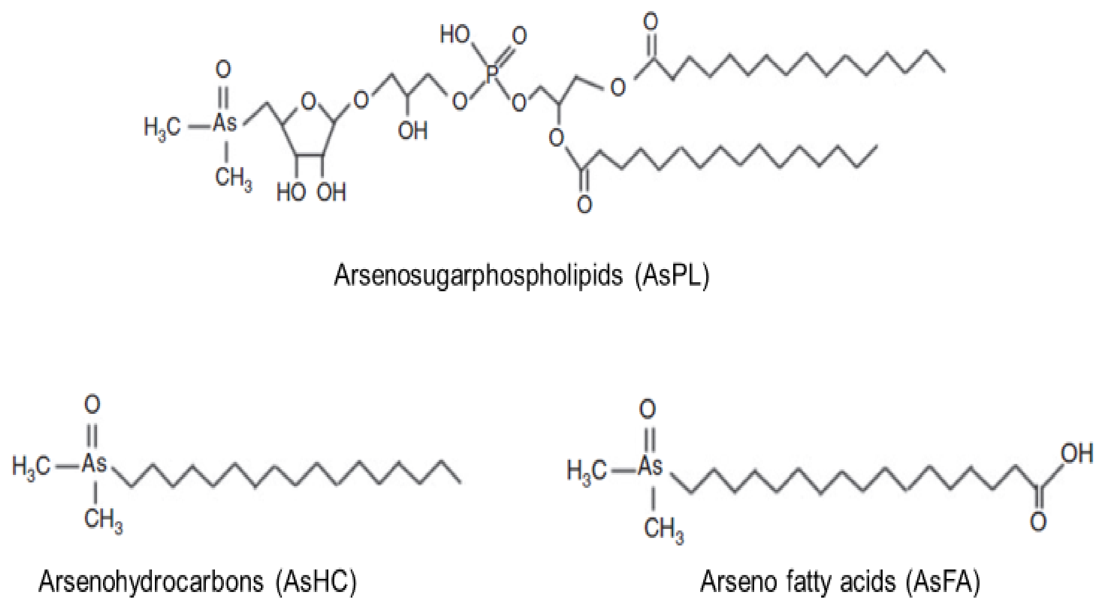


Figure 1.4 The three identified arsenolipid classes

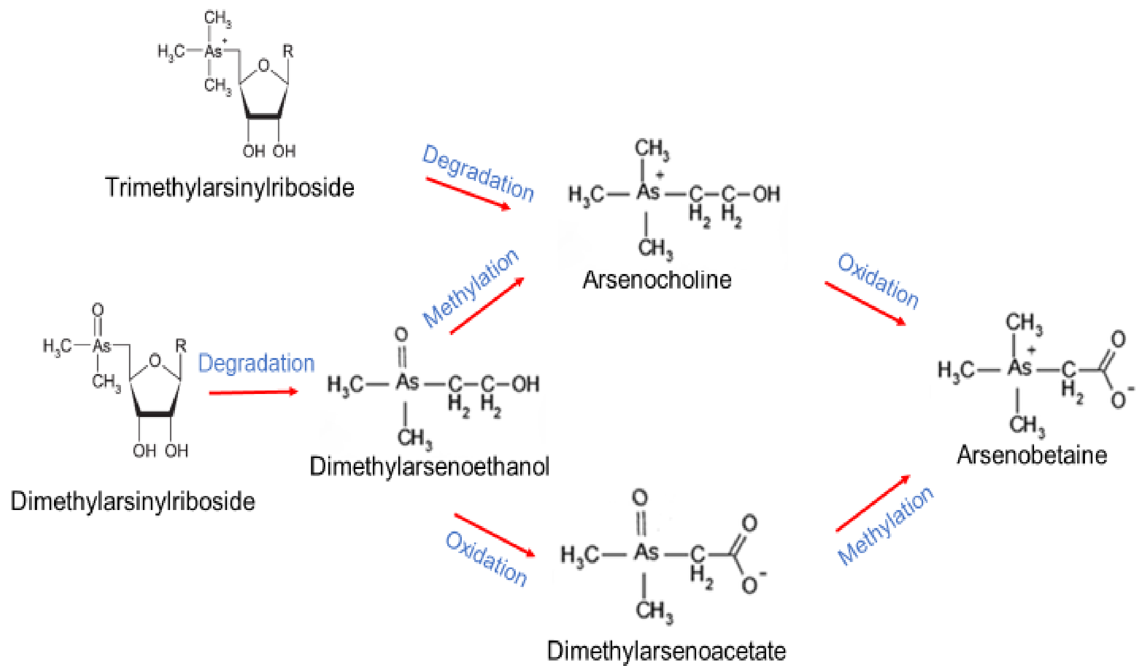
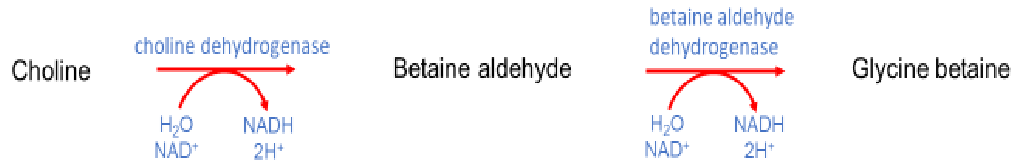


Figure 1.5 Arsenobetaine proposed synthesis pathways by Kunito et al. (59)



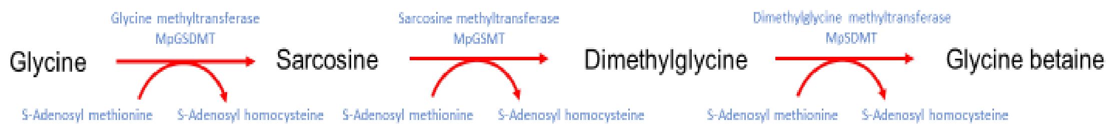
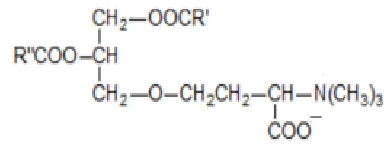
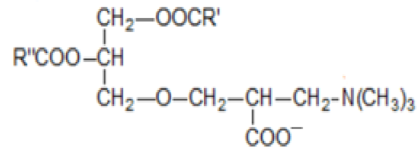


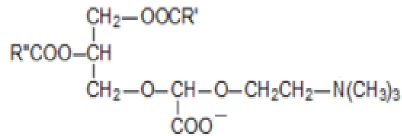
Figure 1.6 Glycine betaine formation pathways. A, Glycine betaine is formed by a two step pathway that involves the oxidation of choline and betaine aldehyde, which is facilitated by a dehydrogenase enzymes . B, The three step pathway include adding a methyl group at each step to finally produce glycine betaine



Diacylgyceryl-N-trimethylhomoserine (DGTS)



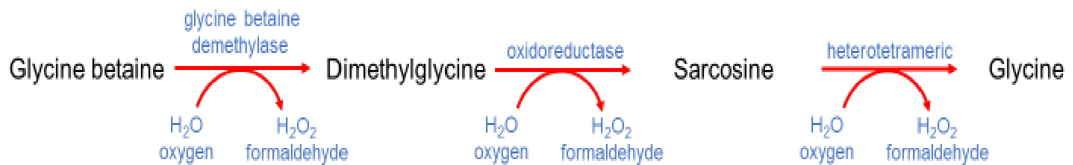
Diacylglyceryl hydroxymethyl-N,N,N-trimethyl-beta-alanine (DGTA)



Diacylglyceryl carboxyhydroxymethylcholine (DGCC)

Figure 1.7 The three identified betaine lipids found in numerous organisms

A. Pathway 1



B. Pathway 2

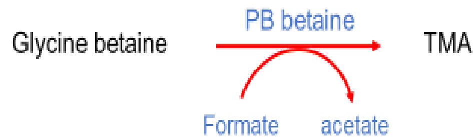
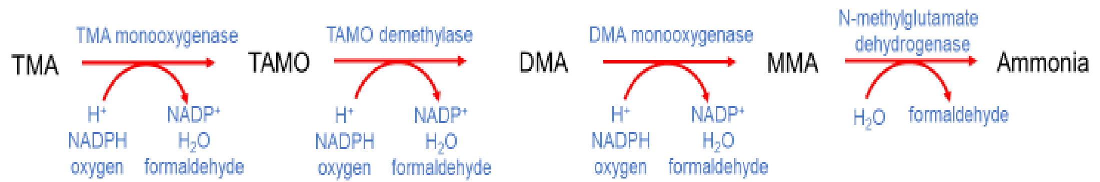
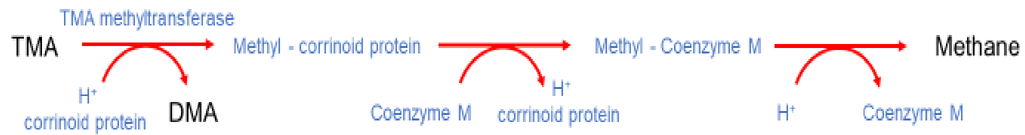


Figure 1.8 Identified pathways for the fate of betaine. A, This Pathway occurs aerobically to produce formaldehyde as a carbon source. B, anaerobically pathway to degrade betaine in the presence of formate to produce the carbon source acetate

A. Pathway 1



B. Pathway 2



C. Pathway 3

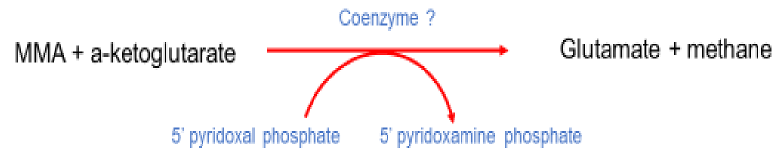


Figure 1.9 The three known pathways for methylated amine degradation. A, Aerobic TMA degradation to produce formaldehyde and ammonia, which can be used as a carbon and nitrogen source respectively. B, Three step pathway utilized by methanogens to break down TMA. This pathway is repeated for DMA and MMA to finally produce ammonia. C, the newly discovered pathway for aerobic methanogenesis, note missing coenzyme

References

[1] "Arsenic and arsenic compounds," *IARC Monogr. Eval. Carcinog. Risks to Humans*, 2011.

[2] J. F. Stolz and R. S. Oremland, "Bacterial respiration of arsenic and selenium," *FEMS Microbiol. Rev.*, 1999.

[3] R. E. Macur, J. T. Wheeler, T. R. McDermott, and W. P. Inskeep, "Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings," *Environ. Sci. Technol.*, 2001.

[4] R. S. Oremland, C. W. Saltikov, F. Wolfe-Simon, and J. F. Stolz, "Arsenic in the evolution of earth and extraterrestrial ecosystems," *Geomicrobiol. J.*, 2009.

[5] T. R. Kulp *et al.*, "Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California," *Science (80-.).*, 2008.

[6] G. L. Anderson, J. Williams, and R. Hille, "The purification and characterization of arsenite oxidase from Alcaligenes faecalis, a molybdenum-containing hydroxylase," *J. Biol. Chem.*, 1992.

[7] R. Mukhopadhyay, B. P. Rosen, L. T. Phung, and S. Silver, "Microbial arsenic: From geocycles to genes and enzymes," *FEMS Microbiology Reviews*. 2002.

[8] J. Messens and S. Silver, "Arsenate Reduction: Thiol Cascade Chemistry with Convergent Evolution," *Journal of Molecular Biology*. 2006.

[9] D. Ahmann, A. L. Roberts, L. R. Krumholz, and F. M. M. Morel, "Microbe grows by reducing arsenic [7]," *Nature*. 1994.

[10] J. F. Stolz, P. Basu, J. M. Santini, and R. S. Oremland, "Arsenic and Selenium in Microbial Metabolism," *Annu. Rev. Microbiol.*, 2006.

[11] R. Bentley and T. G. Chasteen, "Microbial Methylation of Metalloids: Arsenic, Antimony, and Bismuth," *Microbiol. Mol. Biol. Rev.*, 2002.

[12] J. Qin, B. P. Rosen, Y. Zhang, G. Wang, S. Franke, and C. Rensing, "Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase," *Proc. Natl. Acad. Sci.*, 2006.

[13] C. Fan, G. Liu, Y. Long, B. Rosen, and Y. Cai, "Thiolation in arsenic metabolism: a chemical perspective," *Metallomics*. 2018.

[14] F. Challenger, "Biological Methylation," *Chem. Rev*, vol. 36, pp. 315–361, 1945.

[15] J. Qin, C. R. Lehr, C. Yuan, X. C. Le, T. R. McDermott, and B. P. Rosen, "Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga," *Proc. Natl. Acad. Sci.*, 2009.

[16] A. A. Ajees and B. P. Rosen, “As(III) S-Adenosylmethionine Methyltransferases and Other Arsenic Binding Proteins,” *Geomicrobiol. J.*, 2015.

[17] T. Hayakawa, Y. Kobayashi, X. Cui, and S. Hirano, “A new metabolic pathway of arsenite: Arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19,” *Arch. Toxicol.*, 2005.

[18] K. Marapakala, J. Qin, and B. P. Rosen, “Identification of catalytic residues in the As(III) S-adenosylmethionine methyltransferase,” *Biochemistry*, 2012.

[19] J. Chen, H. Bhattacharjee, and B. P. Rosen, “ArsH is an organoarsenical oxidase that confers resistance to trivalent forms of the herbicide monosodium methylarsenate and the poultry growth promoter roxarsone,” *Mol. Microbiol.*, 2015.

[20] M. Yoshinaga and B. P. Rosen, “A C*As lyase for degradation of environmental organoarsenical herbicides and animal husbandry growth promoters,” *Proc. Natl. Acad. Sci.*, 2014.

[21] B. Planer-Friedrich, J. London, R. B. Mccleskey, D. K. Nordstrom, and D. Wallschläger, “Thioarsenates in geothermal waters of yellowstone National Park: Determination, preservation, and geochemical importance,” *Environ. Sci. Technol.*, 2007.

[22] V. K. Stucker, K. H. Williams, M. J. Robbins, and J. F. Ranville, “Arsenic geochemistry in a biostimulated aquifer: An aqueous speciation study,” *Environ. Toxicol. Chem.*, 2013.

[23] J. A. Smieja and R. T. Wilkin, “Preservation of sulfidic waters containing dissolved AS(III),” *J. Environ. Monit.*, 2003.

[24] S. Stauder, B. Raue, and F. Sacher, “Thioarsenates in sulfidic waters,” *Environ. Sci. Technol.*, 2005.

[25] K. A. Rittle, J. I. Drever, and P. J. S. Colberg, “Precipitation of arsenic during bacterial sulfate reduction,” *Geomicrobiol. J.*, 1995.

[26] D. B. Senn and H. F. Hemond, “Nitrate controls on iron and arsenic in an urban lake,” *Science (80-.).*, 2002.

[27] B. P. Rosen and Z. Liu, “Transport pathways for arsenic and selenium: A minireview,” *Environment International*. 2009.

[28] R. A. Goyer and T. W. Clarkson, “Toxic effects of metals.,” in *Casarett and Doull’s Toxicology: The Basic Science of Poisons.*, 2001.

[29] H. Bhattacharjee, R. Mukhopadhyay, S. Thiyagarajan, and B. P. Rosen, “Aquaglyceroporins: Ancient channels for metalloids,” *J. Biol.*, 2008.

[30] S. Li, Y. Chen, and B. P. Rosen, "Role of vicinal cysteine pairs in metalloid sensing by the ArsD As(III)-responsive repressor," *Mol. Microbiol.*, 2001.

[31] H. Naranmandura, N. Suzuki, K. Iwata, S. Hirano, and K. T. Suzuki, "Arsenic metabolism and thioarsenicals in hamsters and rats," *Chem. Res. Toxicol.*, 2007.

[32] T. Ochi, K. Kita, T. Suzuki, A. Rumpler, W. Goessler, and K. A. Francesconi, "Cytotoxic, genotoxic and cell-cycle disruptive effects of thio-dimethylarsinate in cultured human cells and the role of glutathione," *Toxicol. Appl. Pharmacol.*, 2008.

[33] K. J. Reimer and J. A. Thompson, "Arsenic speciation in marine interstitial water. The occurrence of organoarsenicals," *Biogeochemistry*, 1988.

[34] G. Tingzong, R. D. DeLaune, and W. H. Patrick, "The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium, and zinc in estuarine sediment," *Environ. Int.*, 1997.

[35] G. F. Riedel, J. G. Sanders, and R. W. Osman, "The effect of biological and physical disturbances on the transport of arsenic from contaminated estuarine sediments," *Estuar. Coast. Shelf Sci.*, 1987.

[36] P. H. Masscheleyn, R. D. Delaune, and W. H. Patrick, "Arsenic and Selenium Chemistry as Affected by Sediment Redox Potential and pH," *J. Environ. Qual.*, 1991.

[37] M. Takeuchi *et al.*, "Distribution and fate of biologically formed organoarsenicals in coastal marine sediment," *Appl. Organomet. Chem.*, 2005.

[38] M. Giordano, J. Beardall, and J. A. Raven, "CO_2 CONCENTRATING MECHANISMS IN ALGAE: Mechanisms, Environmental Modulation, and Evolution," *Annu. Rev. Plant Biol.*, 2005.

[39] J. L. Levy, J. L. Stauber, M. S. Adams, W. A. Maher, J. K. Kirby, and D. F. Jolley, "Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (Chlorella sp. and Monoraphidium arcuatum)," *Environ. Toxicol. Chem.*, 2005.

[40] L. A. Murray, A. Raab, I. L. Marr, and J. Feldmann, "Biotransformation of arsenate to arsenosugars by Chlorella vulgaris," *Appl. Organomet. Chem.*, 2003.

[41] S. MIYASHITA, S. FUJIWARA, M. TSUZUKI, and T. KAISE, "Rapid Biotransformation of Arsenate into Oxo-Arsenosugars by a Freshwater Unicellular Green Alga, *Chlamydomonas reinhardtii*," *Biosci. Biotechnol. Biochem.*, 2011.

[42] X. M. Xue *et al.*, "Arsenic Methyltransferase is Involved in Arsenosugar Biosynthesis by Providing DMA," *Environ. Sci. Technol.*, 2017.

[43] J. S. Edmonds and K. A. Francesconi, "Transformations of arsenic in the marine environment," *Experientia*. 1987.

[44] K. A. F. Xi-Mei Xue, Jun Ye, Georg Raber, Barry P. Rosen and and Y.-G. Z. Chan Xiong, Zhe Zhu, Christopher Rensing, "Identification of steps in the pathway of arsenosugar biosynthesis," *Environ. Sci. Technol.*, 2018.

[45] J. Feldmann and E. M. Krupp, "Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs?," *Anal. Bioanal. Chem.*, 2011.

[46] G. Caumette, I. Koch, and K. J. Reimer, "Arsenobetaine formation in plankton: A review of studies at the base of the aquatic food chain," *J. Environ. Monit.*, 2012.

[47] J. S. Edmonds, K. A. Francesconi, J. R. Cannon, C. L. Raston, B. W. Skelton, and A. H. White, "Isolation, crystal structure and synthesis of arsenobetaine, the arsenical constituent of the western rock lobster panulirus longipes cygnus George," *Tetrahedron Lett.*, 1977.

[48] M. B. Burg and J. D. Ferraris, "Intracellular organic osmolytes: Function and regulation," *Journal of Biological Chemistry*. 2008.

[49] J. Gailer, K. J. Lrgolic, K. A. Francesconi, and J. S. Edmondsxs, "Metabolism of arsenic compounds by the blue mussel mytilus edulis after accumulation from seawater spiked with arsenic compounds," *Appl. Organomet. Chem.*, 1995.

[50] L. A. Clowes and K. A. Francesconi, "Uptake and elimination of arsenobetaine by the mussel Mytilus edulis is related to salinity," *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.*, 2004.

[51] Z. Šlejkovec *et al.*, "Bioaccumulation of arsenic species in rays from the northern Adriatic Sea," *Int. J. Mol. Sci.*, 2014.

[52] P. Pengprecha, M. Wilson, A. Raab, and J. Feldmann, "Biodegradation of arsenosugars in marine sediment," *Appl. Organomet. Chem.*, 2005.

[53] S. Foster, D. Thomson, and W. Maher, "Uptake and metabolism of arsenate by anexic cultures of the microalgae Dunaliella tertiolecta and Phaeodactylum tricornutum," *Mar. Chem.*, 2008.

[54] Y. Shibata, M. Sekiguchi, A. Otsuki, and M. Morita, "Arsenic compounds in zoo- and phyto-plankton of marine origin," *Appl. Organomet. Chem.*, 1996.

[55] J. S. Edmonds, Y. Shibata, K. A. Francesconi, R. J. Rippingale, and M. Morita, "Arsenic Transformations in Short Marine Food Chains studied by HPLC-ICP MS," *Appl. Organomet. Chem.*, 1997.

[56] T. Kaise and S. Fukui, "The chemical form and acute toxicity of arsenic compounds in marine organisms," *Appl. Organomet. Chem.*, 1992.

[57] G. Caumette, I. Koch, E. Estrada, and K. J. Reimer, "Arsenic speciation in plankton organisms from contaminated lakes: Transformations at the base of the freshwater food chain," *Environ. Sci. Technol.*, 2011.

[58] S. Miyashita *et al.*, “Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS,” *Chemosphere*, 2009.

[59] Suhendrayatna, A. Ohki, T. Nakajima, and S. Maeda, “Studies on the accumulation and transformation of arsenic in freshwater organisms II. Accumulation and transformation of arsenic compounds by Tilapia mossambica,” *Chemosphere*, 2002.

[60] S. Foster and W. Maher, “Arsenobetaine and thio-arsenic species in marine macroalgae and herbivorous animals: Accumulated through trophic transfer or produced in situ?,” *J. Environ. Sci. (China)*, 2016.

[61] S. García-Salgado, G. Raber, R. Raml, C. Magnes, and K. A. Francesconi, “Arsenosugar phospholipids and arsenic hydrocarbons in two species of brown macroalgae,” *Environmental Chemistry*. 2012.

[62] B. A. S. Van Mooy *et al.*, “Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity,” *Nature*, 2009.

[63] A. H. Petursdottir, K. Fletcher, H. Gunnlaugsdóttir, E. Krupp, F. C. Kupper, and J. Feldmann, “Environmental effects on arsenosugars and arsenolipids in Ectocarpus (Phaeophyta),” *Environ. Chem.*, 2016.

[64] A. Raab, C. Newcombe, D. Pitton, R. Ebel, and J. Feldmann, “Comprehensive analysis of lipophilic arsenic species in a brown alga (Saccharina latissima),” *Anal. Chem.*, 2013.

[65] R. Tukai, W. A. Maher, I. J. McNaught, M. J. Ellwood, and M. Coleman, “Occurrence and chemical form of arsenic in marine macroalgae from the east coast of Australia,” *Mar. Freshw. Res.*, 2002.

[66] R. Tukai, W. A. Maher, I. J. McNaught, and M. J. Ellwood, “Measurement of arsenic species in marine macroalgae by microwave-assisted extraction and high performance liquid chromatography-inductively coupled plasma mass spectrometry,” *Anal. Chim. Acta*, 2002.

[67] T. Kaise *et al.*, “Biomethylation of Arsenic in an Arsenic-rich Freshwater Environment,” *Appl. Organomet. Chem.*, 1997.

[68] G. Caumette, I. Koch, M. Moriarty, and K. J. Reimer, “Arsenic distribution and speciation in Daphnia pulex,” *Sci. Total Environ.*, 2012.

[69] T. A. and S. T. Kunito .T, R. Kubota, J. Fujihara, *Reviews of Environmental Contamination and Toxicology*. Springer, New York, 2008.

[70] J. Kirby, W. Maher, and D. Spooner, “Arsenic occurrence and species in near-shore macroalgae-feeding marine animals,” *Environ. Sci. Technol.*, 2005.

[71] S. Khokiattiwong, N. Kornkanitnan, W. Goessler, S. Kokarnig, and K. A. Francesconi, “Arsenic compounds in tropical marine ecosystems: Similarities between mangrove forest and coral reef,” *Environ. Chem.*, 2009.

[72] E. Duncan, S. Foster, and W. Maher, “Uptake and metabolism of arsenate, methylarsonate and arsenobetaine by axenic cultures of the phytoplankton Dunaliella tertiolecta,” *Bot. Mar.*, 2010.

[73] M. Azizur Rahman, H. Hasegawa, and R. Peter Lim, “Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain,” *Environmental Research*. 2012.

[74] G. Lunde, “Occurrence and transformation of arsenic in the marine environment,” *Environ. Health Perspect.*, 1977.

[75] K. A. Francesconi and J. S. Edmonds, “Arsenic and Marine Organisms,” *Adv. Inorg. Chem.*, 1996.

[76] J. Kirby, W. Maher, A. Chariton, and F. Krikowa, “Arsenic concentrations and speciation in a temperate mangrove ecosystem, NSW, Australia,” in *Applied Organometallic Chemistry*, 2002.

[77] J. Burger *et al.*, “Metal levels in fish from the Savannah river: Potential hazards to fish and other receptors,” *Environ. Res.*, 2002.

[78] J. P. Meador, D. W. Ernest, and A. Kagley, “Bioaccumulation of arsenic in marine fish and invertebrates from Alaska and California,” *Arch. Environ. Contam. Toxicol.*, 2004.

[79] K. M. Attar, M. Zamil El-Faer, T. N. Rawdah, and B. S. Tawabini, “Levels of arsenic in fish from the Arabian Gulf,” *Mar. Pollut. Bull.*, 1992.

[80] W. Zhang, Z. Guo, Y. Zhou, L. Chen, and L. Zhang, “Comparative contribution of trophic transfer and biotransformation on arsenobetaine bioaccumulation in two marine fish,” *Aquat. Toxicol.*, 2016.

[81] P. J. Peshut, R. J. Morrison, and B. A. Brooks, “Arsenic speciation in marine fish and shellfish from American Samoa,” *Chemosphere*, 2008.

[82] S. Foster, W. Maher, E. Schmeisser, A. Taylor, F. Krikowa, and S. Apte, “Arsenic species in a rocky intertidal marine food chain in NSW, Australia, revisited,” *Environ. Chem.*, 2006.

[83] M. Morita and Y. Shibata, “Chemical form of arsenic in marine macroalgae,” *Applied Organometallic Chemistry*. 1990.

[84] H. Amlund, K. A. Francesconi, C. Bethune, A. K. Lundebye, and M. H. G. Berntssen, “Accumulation and elimination of dietary arsenobetaine in two species of fish, Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.),” *Environ. Toxicol. Chem.*, 2006.

[85] W. Goessler, W. Maher, K. J. Irgolic, D. Kuehnelt, C. Schlagenhaufen, and T. Kaise, “Arsenic compounds in a marine food chain,” *Fresenius. J. Anal. Chem.*, 1997.

[86] W. A. Maher, S. D. Foster, A. M. Taylor, F. Krikowa, E. G. Duncan, and A. A. Chariton, “Arsenic distribution and species in two Zostera capricorni seagrass ecosystems, New South Wales, Australia,” *Environ. Chem.*, 2011.

[87] K. A. Francesconi, W. Goessler, S. Panutrakul, and K. J. Irgolic, “A novel arsenic containing riboside (arsenosugar) in three species of gastropod,” *Sci. Total Environ.*, 1998.

[88] K. Shiomi, Y. Sugiyama, K. Shimakura, and Y. Nagashima, “Arsenobetaine as the major arsenic compound in the muscle of two species of freshwater fish,” *Appl. Organomet. Chem.*, 1995.

[89] Z. Šlejkovec, Z. Bajc, and D. Z. Doganoc, “Arsenic speciation patterns in freshwater fish,” *Talanta*, 2004.

[90] C. Soeroes *et al.*, “Arsenic speciation in farmed Hungarian freshwater fish,” *J. Agric. Food Chem.*, 2005.

[91] J. Zheng and H. Hintelmann, “Hyphenation of high performance liquid chromatography with sector field inductively coupled plasma mass spectrometry for the determination of ultra-trace level anionic and cationic arsenic compounds in freshwater fish,” *J. Anal. At. Spectrom.*, 2004.

[92] J. F. Lawrence, H. B. S. Conacher, P. Michalik, and G. Tam, “Identification of Arsenobetaine and Arsenocholine in Canadian Fish and Shellfish by High-Performance Liquid Chromatography with Atomic Absorption Detection and Confirmation by Fast Atom Bombardment Mass Spectrometry,” *J. Agric. Food Chem.*, 1986.

[93] S. Maeda, R. Inoue, T. Kozono, T. Tokuda, A. Ohki, and T. Takeshita, “Arsenic metabolism in a freshwater food chain,” *Chemosphere*, 1990.

[94] C. Y. Chen and C. L. Folt, “Bioaccumulation and diminution of arsenic and lead in a freshwater food web,” *Environ. Sci. Technol.*, 2000.

[95] R. P. Mason, J. M. Laporte, and S. Andres, “Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish,” *Arch. Environ. Contam. Toxicol.*, 2000.

[96] L. Chen, W. Zhang, Z. Guo, and L. Zhang, “Effects of acclimation on arsenic bioaccumulation and biotransformation in freshwater medaka Oryzias mekongensis after chronic arsenic exposure,” *Environ. Pollut.*, 2018.

[97] K. O. Amayo, A. Raab, E. M. Krupp, T. Marschall, M. Horsfall, and J. Feldmann, “Arsenolipids show different profiles in muscle tissues of four commercial fish species,” *J. Trace Elem. Med. Biol.*, 2014.

[98] U. Arroyo-Abad, J. Mattusch, T. Reemtsma, and C. Piechotta, “Arsenolipids in commercial canned cod liver: An occurrence and distribution study,” *Eur. J. Lipid Sci. Technol.*, 2014.

[99] K. H. Wedepohl, “The composition of the continental crust,” *Geochim. Cosmochim. Acta*, 1995.

[100] J. S. Dávila and J. H. Custodio, “Mercury Reduction by Bacteria Isolated from Informal Mining Zones,” *Adv. Mater. Res.*, 2009.

[101] T. Barkay, S. M. Miller, and A. O. Summers, “Bacterial mercury resistance from atoms to ecosystems,” *FEMS Microbiology Reviews*. 2003.

[102] D. BEN- BASSAT and A. M. MAYER, “Light- Induced Hg Volatilization and O2Evolution in Chlorella and the Effect of DCMU and Methylamine,” *Physiol. Plant.*, 1978.

[103] R. K. Skogerboe and S. A. Wilson, “Reduction of Ionic Species by Fulvic Acid,” *Anal. Chem.*, 1981.

[104] B. Allard and I. Arsenie, “Abiotic reduction of mercury by humic substances in aquatic system - an important process for the mercury cycle,” *Water, Air, Soil Pollut.*, 1991.

[105] M. Amyot, G. A. Gill, and F. M. M. Morel, “Production and loss of dissolved gaseous mercury in coastal seawater,” *Environ. Sci. Technol.*, 1997.

[106] T. Smith, K. Pitts, J. A. McGarvey, and A. O. Summers, “Bacterial oxidation of mercury metal vapor, Hg(0),” *Appl. Environ. Microbiol.*, 1998.

[107] M. Ogata and H. Aikoh, “Mechanism of metallic mercury oxidation in vitro by catalase and peroxidase,” *Biochem. Pharmacol.*, 1984.

[108] S. H. Du and S. C. Fang, “Catalase activity of C3 and C4 species and its relationship to mercury vapor uptake,” *Environ. Exp. Bot.*, 1983.

[109] J. W. Vonk and A. K. Sijpesteijn, “Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi,” *Antonie Van Leeuwenhoek*, 1973.

[110] J. K. Schaefer, S. S. Rocks, W. Zheng, L. Liang, B. Gu, and F. M. M. Morel, “Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria,” *Proc. Natl. Acad. Sci.*, 2011.

[111] M. Berman, T. Chase, and R. Bartha, “Carbon flow in mercury biomethylation by Desulfovibrio desulfuricans,” *Appl. Environ. Microbiol.*, 1990.

[112] J. M. Wood, F. S. Kennedy, and C. G. Rosen, “Synthesis of methyl-mercury compounds by extracts of a methanogenic sacterium [20],” *Nature*. 1968.

[113] F. Baldi, “Microbial Transformation of Mercury Species and Their Importance in the Biogeochemical Cycle of Mercury,” *Met. Ions Biol. Syst.*, 1997.

[114] J. H. Weber, "Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment," *Chemosphere*, 1993.

[115] R. Falter, "Experimental study on the unintentional abiotic methylation of inorganic mercury during analysis: Part 1: Localisation of the compounds effecting the abiotic mercury methylation," in *Chemosphere*, 1999.

[116] R. S. Oremland, C. W. Culbertson, and M. R. Winfrey, "Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation," *Appl. Environ. Microbiol.*, 1991.

[117] M. C. Marvin-Dipasquale and R. S. Oremland, "Bacterial methylmercury degradation in Florida everglades peat sediment," *Environ. Sci. Technol.*, 1998.

[118] J. K. Schaefer, J. Letowski, and T. Barkay, "Mer-mediated resistance and volatilization of Hg(II) under anaerobic conditions," *Geomicrobiol. J.*, 2002.

[119] J. L. Schottel, "The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of Escherichia coli," *J. Biol. Chem.*, 1978.

[120] I. Suda, M. Suda, and K. Hirayama, "Degradation of methyl and ethyl mercury by singlet oxygen generated from sea water exposed to sunlight or ultraviolet light," *Arch. Toxicol.*, 1993.

[121] J. M. Benoit, C. C. Gilmour, and R. P. Mason, "The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3)," *Environ. Sci. Technol.*, 2001.

[122] D. W. Gruenwedel and D. S. Lu, "Changes in the sedimentation characteristics of DNA due to methylmercuration," *Biochem. Biophys. Res. Commun.*, 1970.

[123] C. C. Gilmour *et al.*, "Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation," *Appl. Environ. Microbiol.*, 2011.

[124] R. A. Bernhoft, "Mercury toxicity and treatment: A review of the literature," *Journal of Environmental and Public Health*. 2012.

[125] T. Suzuki, T. I. Takemoto, S. Shishido, and K. Kani, "Mercury in human amniotic fluid," *Scand. J. Work. Environ. Heal.*, 1977.

[126] N. E. Selin, "Global Biogeochemical Cycling of Mercury: A Review," 2010.

[127] R. B. Jonas, C. C. Gilmour, D. L. Stoner, M. M. Weir, and J. H. Tuttle, "Comparison of methods to measure acute metal and organometal toxicity to natural aquatic microbial communities," *Appl. Environ. Microbiol.*, 1984.

[128] S. M. Ullrich, T. W. Tanton, and S. A. Abdrashitova, "Mercury in the aquatic environment: A review of factors affecting methylation," *Critical Reviews in Environmental Science and Technology*. 2001.

[129] ATSDR, “Toxicological profile for mercury: U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry,” *Toxicol. PROFILE Mercur.*, 1999.

[130] S. M. Silbernagel *et al.*, “Recognizing and preventing overexposure to methylmercury from fish and seafood consumption: Information for physicians,” *Journal of Toxicology*. 2011.

[131] Y. S. Hong, Y. M. Kim, and K. E. Lee, “Methylmercury exposure and health effects,” *J. Prev. Med. Public Heal.*, 2012.

[132] D. R. Abernethy, A. J. DeStefano, T. L. Cecil, K. Zaidi, and R. L. Williams, “Metal impurities in food and drugs,” *Pharmaceutical Research*. 2010.

[133] “Environmental Health Criteria 101: Methylmercury,” *Environ. Heal. Criteria*, 1990.

[134] C. H. Lamborg *et al.*, “A global ocean inventory of anthropogenic mercury based on water column measurements,” *Nature*, 2014.

[135] R. J. Law, M. J. Waldock, C. R. Allchin, R. E. Laslett, and K. J. Bailey, “Contaminants in seawater around England and Wales: Results from Monitoring surveys, 1990-1992,” *Mar. Pollut. Bull.*, 1994.

[136] R. Plaschke, G. Dal Pont, and E. C. V. Butler, “Mercury in waters of the Derwent Estuary - Sample treatment and analysis,” *Mar. Pollut. Bull.*, 1997.

[137] S. D. Siciliano, N. J. O'Driscoll, and D. R. S. Lean, “Microbial reduction and oxidation of mercury in freshwater lakes,” *Environ. Sci. Technol.*, 2002.

[138] and M. L. S. Cristie Dalland, Eva Schumacher, “The Adsorption of Organomercury Compounds from Seawater onto Sedimentary Phases,” *Org. Mar. Geochemistry*, vol. 305, pp. 369–381, 1986.

[139] R. P. Mason and W. F. Fitzgerald, “The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean,” *Deep. Res. Part I*, 1993.

[140] R. P. Mason, K. R. Rolfhus, and W. F. Fitzgerald, “Methylated and elemental mercury cycling in surface and deep ocean waters of the North Atlantic,” *Water, Air, Soil Pollut.*, 1995.

[141] S. Moore, J.W., Ramamoorthy, “Heavy Metals in Natural Waters. Applied Monitoring and Impact Assessment,” *Springer Verlag*, 1984.

[142] G. Compeau and R. Bartha, “Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions,” *Appl. Environ. Microbiol.*, 1984.

[143] M. Berman and R. Bartha, “Control of the Methylation process in a mercury-polluted aquatic sediment,” *Environ. Pollution. Ser. B, Chem. Phys.*, 1986.

[144] J. M. Benoit, R. P. Mason, and C. C. Gilmour, "Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and implications for availability to methylating bacteria," *Environ. Toxicol. Chem.*, 1999.

[145] H. Hintelmann, K. Keppel-Jones, and R. D. Evans, "Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability," *Environ. Toxicol. Chem.*, 2000.

[146] K. Pak and R. Bartha, "Products of mercury demethylation by sulfidogens and methanogens," *Bull. Environ. Contam. Toxicol.*, 1998.

[147] K. Nakamura, M. Sakamoto, H. Uchiyama, and O. Yagi, "Organomercurial-volatilizing bacteria in the mercury-polluted sediment of Minamata Bay, Japan," *Appl. Environ. Microbiol.*, 1990.

[148] J. M. Wood, "Biological processes involved in the cycling of elements between soil or sediments and the aqueous environment," *Hydrobiologia*, vol. 149, no. 1, pp. 31–42, 1987.

[149] M. Ravichandran, "Interactions between mercury and dissolved organic matter - A review," *Chemosphere*. 2004.

[150] Y. Wu and W. X. Wang, "Intracellular speciation and transformation of inorganic mercury in marine phytoplankton," *Aquat. Toxicol.*, 2014.

[151] K. Leopold, M. Foulkes, and P. Worsfold, "Methods for the determination and speciation of mercury in natural waters-A review," *Analytica Chimica Acta*. 2010.

[152] P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum, "Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web," *Sci. Total Environ.*, 2005.

[153] R. W. H. Carroll, J. Memmott, J. J. Warwick, C. H. Fritsen, J. C. J. Bonzongo, and K. Acharya, "Seasonal variation of mercury associated with different phytoplankton size fractions in Lahontan Reservoir, Nevada," *Water. Air. Soil Pollut.*, 2011.

[154] P. C. Pickhardt and N. S. Fisher, "Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies," *Environ. Sci. Technol.*, 2007.

[155] R. P. Mason, F. M. M. Morel, and H. F. Hemond, "The role of microorganisms in elemental mercury formation in natural waters," *Water, Air, Soil Pollut.*, 1995.

[156] D. Kelly, K. Budd, and D. D. Lefebvre, "Mercury analysis of acid- and alkaline-reduced biological samples: Identification of meta-cinnabar as the major biotransformed compound in algae," *Appl. Environ. Microbiol.*, 2006.

[157] D. J. A. Kelly, K. Budd, and D. D. Lefebvre, "Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae," *Arch. Microbiol.*, 2007.

[158] E. Morelli, R. Ferrara, B. Bellini, F. Dini, G. Di Giuseppe, and L. Fantozzi, "Changes in the non-protein thiol pool and production of dissolved gaseous mercury in the marine diatom Thalassiosira weissflogii under mercury exposure," *Sci. Total Environ.*, 2009.

[159] S. Devars, C. Avilés, C. Cervantes, and R. Moreno-Sánchez, "Mercury uptake and removal by euglena gracilis," *Arch. Microbiol.*, 2000.

[160] O. R. and C. J. Watras, "Microbial Mercury Methylation in Aquatic Environments: A Critical Review of Published Field and Laboratory Studies," *Environ. Sci. Technol*, vol. 53, pp. 4–19, 2019.

[161] A. C. Luengen and A. R. Flegal, "Role of phytoplankton in mercury cycling in the San Francisco Bay estuary," *Limnol. Oceanogr.*, 2009.

[162] L. E. Heimbürger *et al.*, "Methyl mercury distributions in relation to the presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, North-western Mediterranean)," *Geochim. Cosmochim. Acta*, 2010.

[163] C. J. Watras, R. C. Back, S. Halvorsen, R. J. M. Hudson, K. A. Morrison, and S. P. Wente, "Bioaccumulation of mercury in pelagic freshwater food webs," in *Science of the Total Environment*, 1998.

[164] K. Simkiss and M. G. Taylor, "Transport of metals across membranes," in *Metal speciation and bioavailability in aquatic systems*, 1995.

[165] M. Hanikenne, S. S. Merchant, and P. Hamel, "Transition Metal Nutrition: A Balance Between Deficiency and Toxicity," in *The Chlamydomonas Sourcebook 3-Vol set*, 2009.

[166] R. P. Mason, J. R. Reinfelder, and F. M. M. Morel, "Uptake, toxicity, and trophic transfer of mercury in a coastal diatom," *Environ. Sci. Technol.*, 1996.

[167] J. Gutknecht, "Inorganic mercury (Hg2+) transport through lipid bilayer membranes," *J. Membr. Biol.*, 1981.

[168] E. Bienvenue *et al.*, "Transport of mercury compounds across bimolecular lipid membranes: Effect of lipid composition, pH and chloride concentration," *Chem. Biol. Interact.*, 1984.

[169] H. A. Moye, C. J. Miles, E. J. Phlips, B. Sargent, and K. K. Merritt, "Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water," *Environ. Sci. Technol.*, 2002.

[170] C. Y. Chen and C. L. Folt, "High plankton densities reduce mercury biomagnification," *Environ. Sci. Technol.*, 2005.

[171] N. S. Fisher, P. Bjerregaard, and S. W. Fowler, "Interactions of marine plankton with transuranic elements. 1. Biokinetics of neptunium, plutonium, americium, and californium in phytoplankton," *Limnol. Oceanogr.*, 1983.

[172] P. R. Gorski, D. E. Armstrong, J. P. Hurley, and D. P. Krabbenhoft, "Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga," *Environ. Pollut.*, 2008.

[173] A. C. Luengen, N. S. Fisher, and B. A. Bergamaschi, "Dissolved organic matter reduces algal accumulation of methylmercury," *Environ. Toxicol. Chem.*, 2012.

[174]Verine Le Faucheur, Y. Tremblay, C. Fortin, and P. G. C. Campbell, "Acidification increases mercury uptake by a freshwater alga, Chlamydomonas reinhardtii," *Environ. Chem.*, 2011.

[175] S. Le Faucheur, P. G. C. Campbell, C. Fortin, and V. I. Slaveykova, "Interactions between mercury and phytoplankton: Speciation, bioavailability, and internal handling.," *Environ. Toxicol. Chem.*, 2014.

[176] J. R. Reinfelder and N. S. Fisher, "The assimilation of elements ingested by marine copepods," *Science (80-.).*, 1991.

[177] P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum, "Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs," *Proc. Natl. Acad. Sci.*, 2002.

[178] C. J. Watras and N. S. Bloom, "Mercury and methylmercury, in individual zooplankton: Implications for bioaccumulation," *Limnology and Oceanography*. 1992.

[179] C. R. Hammerschmidt and W. F. Fitzgerald, "Bioaccumulation and trophic transfer of methylmercury in Long Island Sound," *Arch. Environ. Contam. Toxicol.*, 2006.

[180] D. S. Becker and G. N. Bigham, "Distribution of mercury in the aquatic food web of Onondaga Lake, New York," in *Water, Air, and Soil Pollution*, 1995.

[181] M. T. K. Tsui and W. X. Wang, "Uptake and Elimination Routes of Inorganic Mercury and Methylmercury in Daphnia magna," *Environ. Sci. Technol.*, 2004.

[182] R. Karimi, C. Y. Chen, P. C. Pickhardt, N. S. Fisher, and C. L. Folt, "Stoichiometric controls of mercury dilution by growth.," *Proc. Natl. Acad. Sci.*, 2007.

[183] N. M. Lawson and R. P. Mason, "Accumulation of mercury in estuarine food chains," *Biogeochemistry*, 1998.

[184] R. M. Adams, M. R. Twiss, and C. T. Driscoll, "Patterns of mercury accumulation among seston in lakes of the Adirondack Mountains, New York," *Environ. Sci. Technol.*, 2009.

[185] C. Y. Chen, R. S. Stemberger, N. C. Kamman, B. M. Mayes, and C. L. Folt, "Patterns of Hg bioaccumulation and transfer in aquatic food webs across multi-lake studies in the northeast US," *Ecotoxicology*, 2005.

[186] N. S. Bloom, "On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue," *Can. J. Fish. Aquat. Sci.*, 1992.

[187] D. R. Young *et al.*, "Trophic structure and pollutant concentrations in marine ecosystems of southern California," *Calif. Coop. Ocean. Fish. Investig. Reports*, 1980.

[188] J. N. Morgan, M. R. Berry, and R. L. Graves, "EFFECTS OF COMMONLY USED COOKING PRACTICES ON TOTAL MERCURY CONCENTRATION IN FISH AND THEIR IMPACT ON EXPOSURE ASSESSMENTS," *J. Expo. Anal. Environ. Epidemiol.*, 1997.

[189] W. J. Sydeman and W. M. Jarman, "Trace metals in seabirds, Steller sea lion, and forage fish and zooplankton from central California," *Mar. Pollut. Bull.*, 1998.

[190] R. J. Pentreath, "The accumulation of organic mercury from sea water by the plaice, Pleuronectes platessa L," *J. Exp. Mar. Bio. Ecol.*, 1976.

[191] A. Boudou and F. Ribeyre, "Experimental study of trophic contamination of Salmogairdneri by two mercury compounds - HgCl2 and CH3HgCl - analysis at the organism and organ levels," *Water. Air. Soil Pollut.*, 1985.

[192] S. W. Fowler, M. Heyraud, and J. La Rosa, "Factors affecting methyl and inorganic mercury dynamics in mussels and shrimp," *Mar. Biol.*, 1978.

[193] M. H. Teruaki SUZUKI, "Experimental Investigation on the Biological Concentration of Mercury-II," *Nippon SUISAN GAKKAISHI*, vol. 41, no. 2, pp. 225–231, 1975.

[194] A. Pastor, F. Hernández, M. A. Peris, J. Beltrán, J. V. Sancho, and M. T. Castillo, "Levels of heavy metals in some marine organisms from the western Mediterranean area (Spain)," *Mar. Pollut. Bull.*, 1994.

[195] R. Dietz, F. Riget, and P. Johansen, "Lead, cadmium, mercury and selenium in Greenland marine animals," in *Science of the Total Environment*, 1996.

[196] L. R. Monteiro and H. D. Lopes, "Mercury content of swordfish, Xiphias gladius, in relation to length, weight, age, and sex," *Mar. Pollut. Bull.*, 1990.

[197] M. Canli and R. W. Furness, "Mercury and cadmium uptake from seawater and from food by the Norway lobster Nephrops norvegicus," *Environ. Toxicol. Chem.*, 1995.

[198] J. M. Laporte, J. P. Truchot, N. Mesmer-Dudons, and A. Boudou, "Bioaccumulation of inorganic and methylated mercury by the gills of the shore crab Carcinus maenas: Transepithelial fluxes and histochemical localization," *Mar. Ecol. Prog. Ser.*, 2002.

[199] D. W. Kuehl and R. Haebler, "Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (Tursiops truncatus) collected during an unusual mortality event in the Gulf of Mexico, 1990," *Arch. Environ. Contam. Toxicol.*, 1995.

[200] M. Nigro and C. Leonzio, "Intracellular storage of mercury and selenium in different marine vertebrates," *Mar. Ecol. Prog. Ser.*, 1996.

[201] M. Havelková, L. Dušek, D. Némethová, G. Poleszczuk, and Z. Svobodová, "Comparison of mercury distribution between liver and muscle - A biomonitoring of fish from lightly and heavily contaminated localities," *Sensors*, 2008.

[202] M. Yoon *et al.*, "Total and Methyl Mercury Concentrations in Antarctic Toothfish (Dissostichus mawsoni): Health Risk Assessment," *Bull. Environ. Contam. Toxicol.*, 2018.

[203] R. Bargagli, F. Monaci, J. C. Sanchez-Hernandez, and D. Cateni, "Biomagnification of mercury in an Antarctic marine coastal food web," *Mar. Ecol. Prog. Ser.*, 1998.

[204] Jerry M Neff, "Metal and Organic Chemicals Aassociated with Oil and Gas well produced water: Bioaccumulation, Fates, and Effects in the Marine Environment," *Rep. offshore Oper. Comm.*, p. 357, 1997.

[205] and C. M. F. P. Collin A. Eagles-Smith, James J. Willacker Jr., "Mercury in Fishes from 21 National Parks in the Western United States—Inter- and Intra-Park Variation in Concentrations and Ecological Risk," 2014.

[206] R. Dietz, P. M. Outridge, and K. A. Hobson, "Anthropogenic contributions to mercury levels in present-day Arctic animals-A review," *Science of the Total Environment*. 2009.

[207] "EPA-FDA Fish Advice." [Online]. Available: https://www.epa.gov/fish-tech/epa-fda-fish-advice-technical-information.

[208] FAO, *FAO Yearbook. Fisheries and Aquaculture Statistics*. 2016.

[209] L. Murdock *et al.*, "Analysis of strains lacking known osmolyte accumulation mechanisms reveals contributions of osmolytes and transporters to protection against abiotic stress," *Appl. Environ. Microbiol.*, 2014.

[210] R. Obeid, "The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway," *Nutrients*. 2013.

[211] J. Boch, B. Kempf, and E. Bremer, "Osmoregulation in Bacillus subtilis: Synthesis of the osmoprotectant glycine betaine from exogenously provided choline," *J. Bacteriol.*, 1994.

[212] N. Garcia, J. A. Lopez Elias, A. Miranda, M. Martinez Porchas, N. Huerta, and A. Garcia, "Effect of salinity on growth and chemical composition of the diatom Thalassiosira weissflogii at three culture phases," *Lat. Am. J. Aquat. Res.*, 2012.

[213] B. Rathinasabapathi *et al.*, "Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the first step of glycine betaine synthesis in plants: Prosthetic group characterization and cDNA cloning," *Proc. Natl. Acad. Sci.*, 1997.

[214] J. Dragolovich, "Dealing with salt stress in animal cells: The role and regulation of glycine betaine concentrations," *J. Exp. Zool.*, 1994.

[215] H. Kageyama, Y. Tanaka, and T. Takabe, "Biosynthetic pathways of glycinebetaine in Thalassiosira pseudonana; functional characterization of enzyme catalyzing three-step methylation of glycine," *Plant Physiol. Biochem.*, 2018.

[216] R. Waditee-Sirisattha, M. Singh, H. Kageyama, D. Sittipol, A. K. Rai, and T. Takabe, "Anabaena sp. PCC7120 transformed with glycine methylation genes from Aphanothece halophytica synthesized glycine betaine showing increased tolerance to salt," *Arch. Microbiol.*, 2012.

[217] A. Spielmeyer and G. Pohnert, "Influence of temperature and elevated carbon dioxide on the production of dimethylsulfoniopropionate and glycine betaine by marine phytoplankton," *Mar. Environ. Res.*, 2012.

[218] Y. Ma, Q. Wang, X. Gao, and Y. Zhang, "Biosynthesis and uptake of glycine betaine as cold-stress response to low temperature in fish pathogen Vibrio anguillarum," *J. Microbiol.*, 2017.

[219] P. H. Yancey, M. D. Rhea, K. M. Kemp, and D. M. Bailey, "Trimethylamine oxide, betaine and other osmolytes in deep-sea animals: depth trends and effects on enzymes under hydrostatic pressure.," *Cell. Mol. Biol. (Noisy-le-grand).*, 2004.

[220] C. Chen and G. A. Beattie, "Pseudomonas syringae BetT is a low-affinity choline transporter that is responsible for superior osmoprotection by choline over glycine betaine," *J. Bacteriol.*, 2008.

[221] M. A. Roulier, B. Palenik, and F. M. M. Morel, "A method for the measurement of choline and hydrogen peroxide in seawater," *Mar. Chem.*, 1990.

[222] P. C. B. Sizeland, S. T. Chambers, M. Lever, L. M. Bason, and R. A. Robson, "Organic osmolytes in human and other mammalian kidneys," *Kidney Int.*, 1993.

[223] J. R. Treberg, “The accumulation of methylamine counteracting solutes in elasmobranchs with differing levels of urea: a comparison of marine and freshwater species,” *J. Exp. Biol.*, 2006.

[224] A. Nyyssölä, J. Kerovuo, P. Kaukinen, N. Von Weymarn, and T. Reinikainen, “Extreme halophiles synthesize betaine from glycine by methylation,” *J. Biol. Chem.*, 2000.

[225] R. Waditee *et al.*, “Isolation and functional characterization of N-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism Aphanothece halophytica,” *J. Biol. Chem.*, 2003.

[226] M. C. Lai, C. C. Wang, M. J. Chuang, Y. C. Wu, and Y. C. Lee, “Effects of substrate and potassium on the betaine-synthesizing enzyme glycine sarcosine dimethylglycine N-methyltransferase from a halophilic methanoarchaeon Methanohalophilus portucalensis,” *Res. Microbiol.*, 2006.

[227] S. J. Lai and M. C. Lai, “Characterization and regulation of the osmolyte betaine synthesizing enzymes GSMT and SDMT from halophilic methanogen Methanohalophilus portucalensis,” *PLoS One*, 2011.

[228] W. R. Riekhof, S. Naik, H. Bertrand, C. Benning, and D. R. Voelker, “Phosphate starvation in fungi induces the replacement of phosphatidylcholine with the phosphorus-free betaine lipid diacylglyceryl-N,N,N-trimethylhomoserine,” *Eukaryot. Cell*, 2014.

[229] M. Kato, M. Sakai, K. Adachi, H. Ikemoto, and H. Sano, “Distribution of betaine lipids in marine algae,” *Phytochemistry*, 1996.

[230] K. Kunzler and W. Eichenberger, “Betaine lipids and zwitterionic phospholipids in plants and fungi,” *Phytochemistry*, 1997.

[231] A. Shemi, D. Schatz, H. F. Fredricks, B. A. S. Van Mooy, Z. Porat, and A. Vardi, “Phosphorus starvation induces membrane remodeling and recycling in Emiliania huxleyi,” *New Phytol.*, 2016.

[232] C. Benning, Z. H. Huang, and D. A. Gage, “Accumulation of a novel glycolipid and a betaine lipid in cells of rhodobacter sphaeroides grown under phosphate limitation,” *Arch. Biochem. Biophys.*, 1995.

[233] V. M. Dembitsky, “Betaine ether-linked glycerolipids: Chemistry and biology,” *Progress in Lipid Research*. 1996.

[234] J. P. Canãvate, I. Armada, J. L. Riós, and I. Hachero-Cruzado, “Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae,” *Phytochemistry*, 2016.

[235] O. Geiger, V. Röhrs, B. Weissenmayer, T. M. Finan, and J. E. Thomas-Oates, "The regulator gene phoB mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-N,N,N-trimethylhomoserine in Rhizobium (Sinorhizobium) meliloti," *Mol. Microbiol.*, 1999.

[236] I. Khozin-Goldberg and Z. Cohen, "The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte Monodus subterraneus," *Phytochemistry*, 2006.

[237] A. G. and W. E. Christian Giroud, "Lipids of Chlamydomonas reinhardtii. Analysis of Molecular Species and Intracellular Site(s) of Biosynthesis," *Plant Cell Physiol*, vol. 29, no. 4, pp. 587–595, 1988.

[238] L.-J. Dolch *et al.*, "A Palmitic Acid Elongase Affects Eicosapentaenoic Acid and Plastidial Monogalactosyldiacylglycerol Levels in Nannochloropsis," *Plant Physiol.*, 2017.

[239] J. P. Cañavate, I. Armada, and I. Hachero-Cruzado, "Interspecific variability in phosphorus-induced lipid remodelling among marine eukaryotic phytoplankton," *New Phytol.*, 2017.

[240] H. Murakami, T. Nobusawa, K. Hori, M. Shimojima, and H. Ohta, "Betaine Lipid Is Crucial for Adapting to Low Temperature and Phosphate Deficiency in Nannochloropsis," *Plant Physiol.*, 2018.

[241] I. M. López-Lara *et al.*, "Phosphorus-Free Membrane Lipids of *Sinorhizobium meliloti* Are Not Required for the Symbiosis with Alfalfa but Contribute to Increased Cell Yields Under Phosphorus-Limiting Conditions of Growth," *Mol. Plant-Microbe Interact.*, 2005.

[242] R. M. Klug and C. Benning, "Two enzymes of diacylglyceryl-O-4'-(N,N,N,-trimethyl)homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium Rhodobacter sphaeroides," *Proc. Natl. Acad. Sci.*, 2001.

[243] W. R. Riekhof, B. B. Sears, and C. Benning, "Annotation of genes involved in glycerolipid biosynthesis in Chlamydomonas reinhardtii: Discovery of the betaine lipid synthase BTA1Cr," *Eukaryot. Cell*, 2005.

[244] M. J. Wargo, "Homeostasis and catabolism of choline and glycine betaine: Lessons from Pseudomonas aeruginosa," *Applied and Environmental Microbiology*. 2013.

[245] E. Mueller, K. Fahlbusch, R. Walther, and G. Gottschalk, "Formation of N,N-dimethylglycine, acetic acid, and butyric acid from betaine by Eubacterium limosum," *Appl. Environ. Microbiol.*, 1981.

[246] A. J. Watkins, E. G. Rousse, R. J. Parkes, and H. Sass, "Glycine betaine as a direct substrate for methanogens (methanococcoides spp.)," *Appl. Environ. Microbiol.*, 2014.

[247] B. Möller, R. Oßmer, B. H. Howard, G. Gottschalk, and H. Hippe, "Sporomusa, a new genus of gram-negative anaerobic bacteria including Sporomusa sphaeroides spec. nov. and Sporomusa ovata spec. nov.," *Arch. Microbiol.*, 1984.

[248] K. Hormann and J. R. Andreesen, "Reductive cleavage of sarcosine and betaine by Eubacterium acidaminophilum via enzyme systems different from glycine reductase," *Arch. Microbiol.*, 1989.

[249] M. Meyer, K. Granderath, and J. R. Andreesen, "Purification and Characterization of Protein PBof Betaine Reductase and its Relationship to the Corresponding Proteins Glycine Reductase and Sarcosine Reductase from Eubacterium Acidaminophilum," *Eur. J. Biochem.*, 1995.

[250] X. Ge, A. S. Wexler, and S. L. Clegg, "Atmospheric amines - Part I. A review," *Atmospheric Environment*. 2011.

[251] E. L. Barrett and H. S. Kwan, "Bacterial Reduction of Trimethylamine Oxide," *Annu. Rev. Microbiol.*, 1985.

[252] C. M. Large PJ, Boulton CA, "The reduced nicotinamide-adenine dinuclotide phosphate- and oxygen-dependent N-oxygenation of trimethylamine by Pseudomonas aminovorans," *Biochem*, vol. 128, pp. 137–138, 1972.

[253] Y. Chen, N. A. Patel, A. Crombie, J. H. Scrivens, and J. C. Murrell, "Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase," *Proc. Natl. Acad. Sci.*, 2011.

[254] G. M. King, M. J. Klug, and D. R. Lovley, "Metabolism of Acetate, Methanol, and Methylated Amines in Intertidal Sediments of Lowes Cove, Maine.," *Appl. Environ. Microbiol.*, 1983.

[255] D. Giani, L. Giani, Y. Cohen, and W. E. Krumbein, "Methanogenesis in the hypersaline Solar Lake (Sinai)," *FEMS Microbiol. Lett.*, 1984.

[256] T. C. Tallant and J. A. Krzycki, "Methylthiol:coenzyme M methyltransferase from Methanosarcina barkeri, an enzyme of methanogenesis from dimethylsulfide and methylmercaptopropionate," *J. Bacteriol.*, 1997.

[257] D. J. Ferguson, N. Gorlatova, D. A. Grahame, and J. A. Krzycki, "Reconstitution of dimethylamine:coenzyme M methyl transfer with a discrete corrinoid protein and two methyltransferases purified from Methanosarcina barkeri," *J. Biol. Chem.*, 2000.

[258] G. W. Schade and P. J. Crutzen, "Emission of aliphatic amines from animal husbandry and their reactions: Potential source of N2O and HCN," *J. Atmos. Chem.*, 1995.

[259] D. Y. Lee and A. S. Wexler, "Atmospheric amines - Part III: Photochemistry and toxicity," *Atmospheric Environment*. 2013.

[260] S. M. Murphy *et al.*, "Secondary aerosol formation from atmospheric reactions of aliphatic amines," *Atmos. Chem. Phys.*, 2007.

[261] K. C. Barsanti and J. F. Pankow, "Thermodynamics of the formation of atmospheric organic particulate matter by accretion reactions-Part 3: Carboxylic and dicarboxylic acids," *Atmos. Environ.*, 2006.

[262] F. Yu and G. Luo, "Modeling of gaseous methylamines in the global atmosphere: Impacts of oxidation and aerosol uptake," *Atmos. Chem. Phys.*, 2014.

[287] Qian Wang, Abdullah Alowaifeer, Patricia Kerner, Narayanaganesh Balasubramanian, Angela Patterson, John E. Dore, Brian Bothner, and Timothy R. McDermott "Aerobic bacterial metabolism of methylamine or glycine betaine generates methane, contributing to methane oversaturation in oxic waters"

CHAPTER TWO

METHOD AND METHOD DEVELOPMENT

Sample preparation

Total Metal and Metalloids Extraction

Sample is weighed and placed into a 50ml falcon tube, and then 5 ml of nitric acid is added. Caps are placed over the tubes without sealing to allow for ventilation. Samples are then autoclaved to facilitate complete digestion (20 min sterilization, slow exhaust) as described by Lozano et al. [263]. Samples is then diluted to make a 50x dilution. At this point, samples are ready for analysis.

Arsenic Species Extraction

Sample were weighed and placed into a 50ml falcon tube, and then 5 ml of distilled water was added. The samples were digested using regular microwave at 100W for 15 minutes as described by Hirata et al. [264]. After cooling, samples were filtered through a 3-kDa-cutoff Amicon Ultrafilter and refrigerated at 4 ◦C until analysis. Due to the failure to separate Arsenobeatine and As(III), hydrogen peroxide was added to the sample making a finally concentration of 10%. Hydrogen peroxide oxidized As(III) to As(V). Thus, arsenobetaine concentration can be determined and the concentration of As(III) was quantified by subtracting the concentration of As(V) after the addition of hydrogen peroxide from the initial concentration of As(V).

Labeling Amines in Water with Dansly Chloride

Methylated amines are volatile and have a relatively small mass, which makes it challenging to detect and quantify. MMA and DMA and have an average mass of 31.057 and 45.08 Dalton (Da) respectively. The detection and quantification of amines has significantly improved with the introduction of dansylating labeling. Dansyl chloride [Dns-Cl, 1 -dimethylaminonaphthalene-5-Sulfonyl chloride] is a derivatizing agent for the detection of amino acids and phenols. This procedure was first suggested by Weber [265], to label free amino groups in proteins. Ever since, multiple studies have been published on Dns-Cl reaction rates and hydrolysis. Gros et. al. [266] found that at a pH of 9.5 the highest reactivity of Dns-Cl with amino acids can be achieved, while maintaining low hydrolysis. The increase in the pH of the solution, will lead to a shift of the unreactive protonated amino group into its reactive basic form, which will then allow for the reaction with Dns-Cl to occur (Figure 2.1).

The dansylation procedure was performed as described by Schut et. al [267]. A Hamilton gastight syringe was used to draw 50ul of water from the sample serum bottle. Water was then placed into a 250ul polypropylene analysis vial and pH was adjusted to ~9.5 with 2ul of 160mM sodium hydroxide. Dns-Cl prepared in acetonitrile (20 mg/ml) was added to the sample in a volume of 46ul. Sample is then Incubated for 30min at room temperature. After the incubation period, pH was adjusted to ~4 with 2ul of 10% formic acid. At this point sample is ready for analysis.

Labeling Trimethylamine in Water with Ethylbromoacetate

As with MMA and DMA, TMA is also volatile at room temperature and has a mass of 59.11 Da. Therefore, using a label agent can be beneficial to facilitate the analysis. Ethyl bromoacetate was used to label the compounds of interest prior to analysis by LCMS. The labelling procedure was performed as described by Johnson [268]. A Hamilton gastight syringe was used to draw 90ul of water from the sample serum bottle. Water was then placed into a 250ul polypropylene analysis vial then, 10ul of (20mg/ml acetonitrile) ethyl bromoacetate was added to the analysis vial. Samples are then incubated for 30min at room temperature (Figure 2.2). At this point sample is ready for analysis.

Mass Spectrometer Analysis

Analysis of Total Metal and Metalloids by ICP-MS

Standard solutions for total arsenic and mercury were freshly prepared on the day of analysis and an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) with an octopole collision cell reaction (Agilent Technologies, Waldbronn, Germany). The ICP operating conditions were 1500 W generator power, 1 L/ gas flow, 1 L/ nebulizer gas flow, and 1 mL/min sample uptake rate. The ion lenses of the ICP-MS were optimized to achieve the highest signal/background ratio for the m/z 75 for arsenic and m/z 202 for mercury with a reaction collusion cell (He, 3 mL min–1). The internal standards used with the analysis were lithium, gallium, scandium, rhodium, and iridium.

Arsenic Species Analysis by IC- ICP-MS

Analysis was performed on a high-performance liquid chromatography (HPLC) (Series 2000; Perkin- Elmer, Waltham, MA) equipped with a Jupiter® 5 µm C18 300 Å reverse-phase column (250 mm × 4.6 mm; Phenomenex, Torrance, CA) eluted isocratically with a mobile phase consisting of 3 mM malonic acid, 5 mM tetrabutylammonium hydroxide and 5% methanol (v/v), pH 5.6, with a flow rate of 1 ml min−1 at 25°C. The HPLC was coupled with an ICP- MS (ELAN DRC- e; Perkin-Elmer) to determine arsenic concentration (Figure 2.3).

Analysis of MMA and DMA by LC-MS

Methylamine hydrochloride and Dimethylamine hydrochloride were purchased from Sigma-Aldrich and the concentration was quantified based on a four-point calibration curve. Chromatography experiment done on an Agilent 6538 Q-TOF mass spectrometry, positive mode, equipped with a reversed-phase Agilent Zorbax Eclepse Plus C18 column (2.1x150mm). Solvent A was 0.1% formic acid in HPLC water, and solvent B was 0.1% formic acid in acetonitrile. The 15 min binary gradient elution profile was as follows: t) 1min, 0% B; t) 11 min, 55% B; t) 14 min, 100% B; t) 15 min, 0% B. The wavelength was 320nm, the flow rate was 600 µL/min, and the sample injection volume was 10 µL. Limit of detection was determined to be 20nM for MMA and 8nM for DMA (Figure 2.4).

Analysis of TMA by LC-MS

Trimethylamine hydrochloride was purchased from Sigma-Aldrich and the concentration was quantified based on a four-point calibration curve. Chromatography experiment done on an Agilent 6538 Q-TOF mass spectrometry, positive mode, equipped with a normal-phase Waters ACQUITY BEH HILIC 1.7uM column (2.1x100mm). Solvent A was 0.1% formic acid in HPLC water, and solvent B was 0.1% formic acid in acetonitrile. The 4.7 min binary gradient elution profile was as follows: t) 0min, 90% B; t)2min, 70% B; t) 3.6 min, 60% B; t) 4.7 min, 90%B. The flow rate was 400 µL/min, and the sample injection volume was 5 µL. Limit of detection was determined to be 10nM (Figure 2.5).

Analysis of Glycine Betaine in Water by LC-MS

For detection of glycine betaine, standard was purchased from Sigma-Aldrich and the concentration was quantified based on a four-point calibration curve. A Hamilton gastight syringe was used to draw 90ul of water from the sample serum bottle. Water was then placed into a 250ul polypropylene analysis vial. Then, 10ul of methanol was added giving a 10% concentration. Sample is then vortexed for 30s. At this point sample is ready for analysis. Chromatography experiment done on an Agilent 6538 Q-TOFmass spectrometry, positive mode, equipped with a normal-phase Waters ACQUITY BEH HILIC 1.7uM column (2.1x100mm). Solvent A was 0.1% formic acid in HPLC water, and solvent B was 0.1% formic acid in acetonitrile. The 4.7 min binary gradient elution profile was as follows: t) 0min, 90% B; t)2min, 70% B; t) 3.6 min, 60% B; t) 4.7 min,

90%B. The flow rate was 400 μL/min, and the sample injection volume was 10 μL. Limit of detection was determined to be 1nM (Figure 2.6).

Analysis of Water-Soluble Methane by GC

The 120 mL water samples collected in the serum vials was injected with 5mL ultra-high purity nitrogen (N2) to create a headspace. Gas from the headspace was analyzed by gas chromatography HP 5890 using flame ionization detection. High purity methane gas was purchased and diluted using air bags to generate a three point calibration curve and the original methane concentration in solution was calculated using Henry's Law and solubility equations [269].

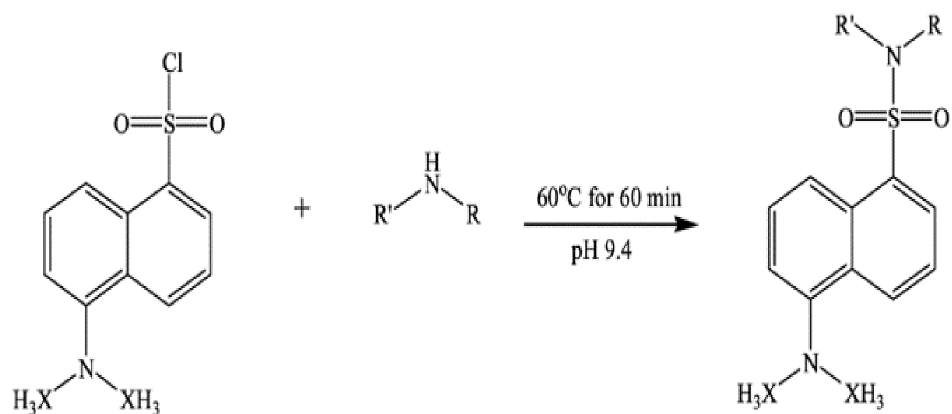


Figure 2.1 Dansylation reaction scheme. The use of dansly chloride to label MMA and DMA to facilitate detection and quantification as described by Guo et. al [254]

Figure 2.2 TMA labeling reaction scheme. The use of ethylbromoacetate to label TMA to facilitate detection and quantification as described by Johnson [255].

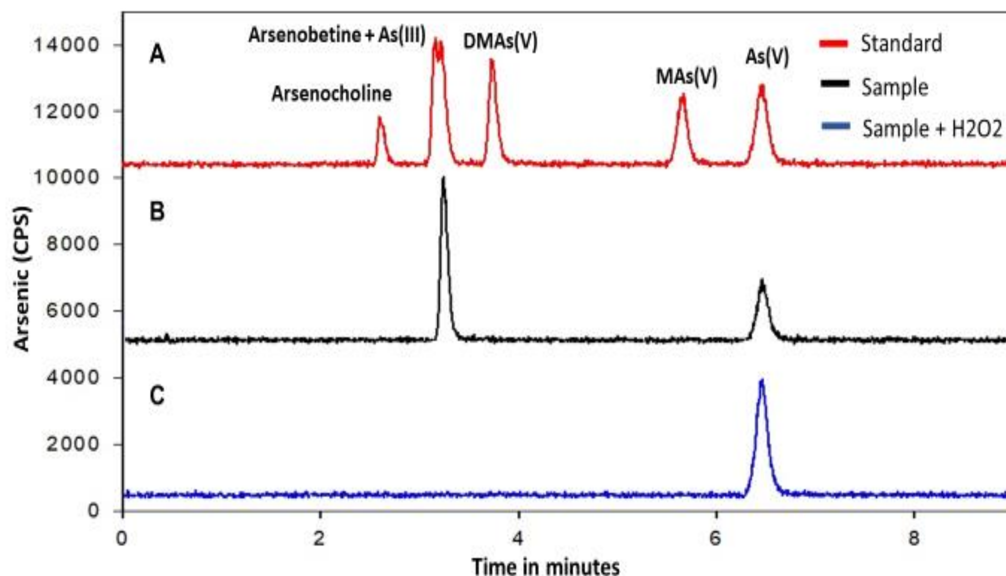


Figure 2.3 HPLC–ICP–MS chromatogram of arsenic species. A, standard run of available arsenic species for quantification. B, Sample run that contains two peaks. C, the addition of hydrogen peroxide to the sample for confirmation and quantification of As(III)

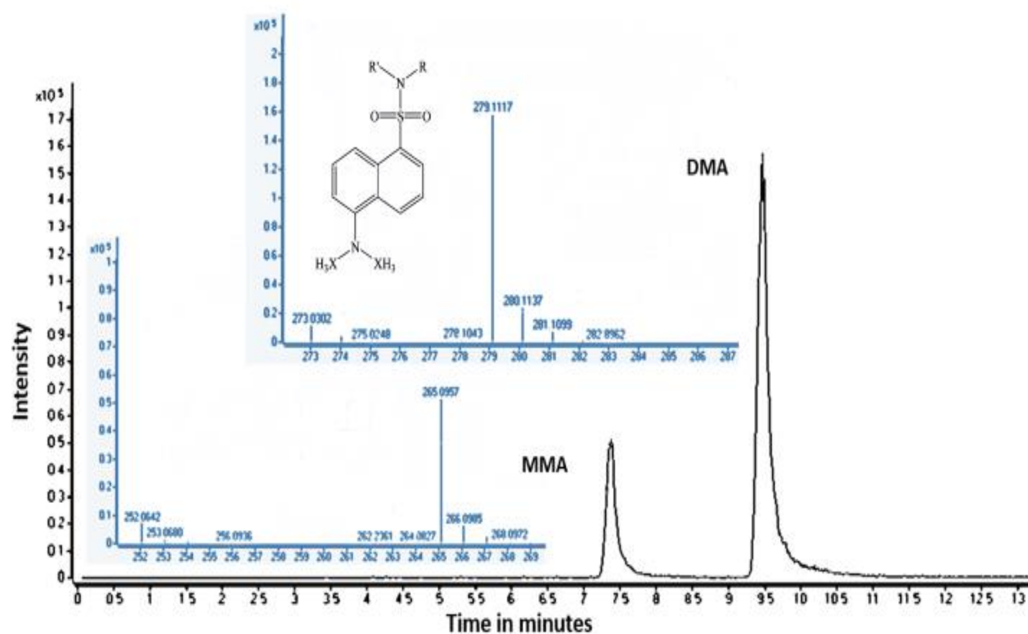


Figure 2.4 LC-MS chromatogram of MMA and DMA. Labeled MMA and DMA with dansly chloride run on C18 column. Retention time was 7.5 and 9.5 minutes and the m/z was 265.09 and 279.11 for MMA and DMA respectively.

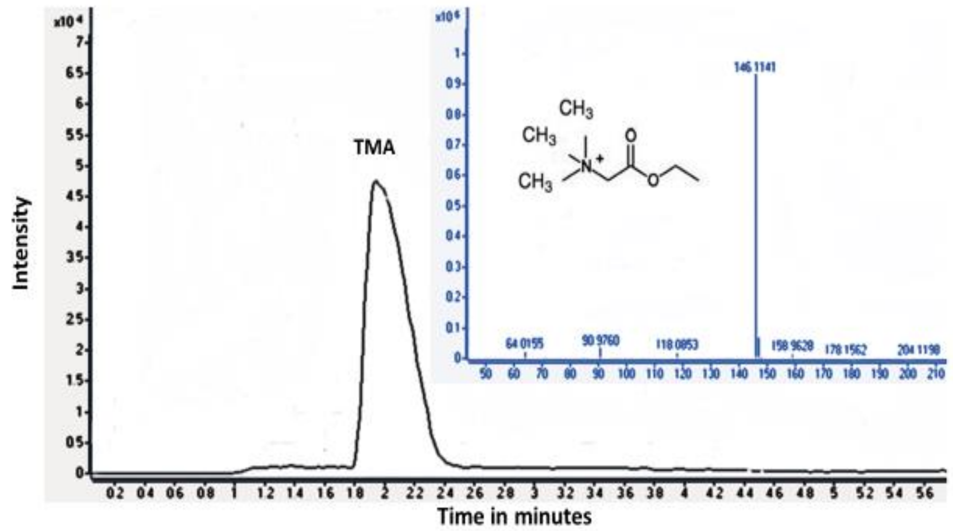


Figure 2.5 LC-MS chromatogram of TMA. Labeled TMA with ethylbromoacetate run on BEH HILIC. Retention time was 2 minutes and the m/z was 146.11.

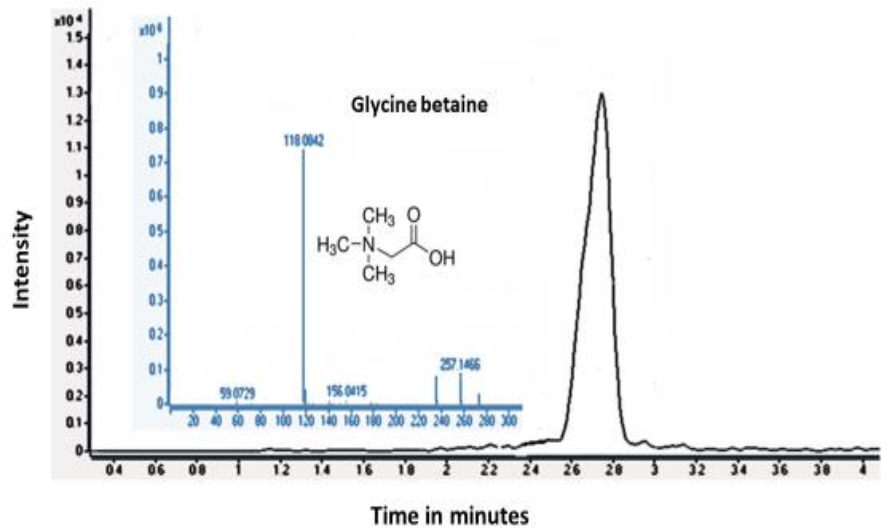


Figure 2.6 LC-MS chromatogram of glycine betaine. Run on BEH HILIC. Retention time was 2.6 minutes and the m/z was 118.08.

References

[263] R. O. Lozano-Rodríguez, E., Luguera, M., Lucena, J.J., Carpena-Ruiz, "Evaluation of two different acid digestion methods in closed systems of trace elements determination in plants.," *Quim. Anal*, vol. 14, no. 27–30, 1995.

[264] S. Hirata and H. Toshimitsu, "Determination of arsenic species and arsenosugars in marine samples by HPLC-ICP-MS," in *Applied Organometallic Chemistry*, 2007.

[265] W. G, "Polarization of the fluorescence of macromolecules: II. Fluorescent conjugates of ovalbumin and bovine serum albumin," *Biochem*, vol. 51, pp. 155–167, 1952.

[266] C. Gros and B. Labouesse, "Study of the Dansylation Reaction of Amino Acids, Peptides and Proteins," *Eur. J. Biochem.*, 1969.

[267] G. J. Schut *et al.*, " The catalytic mechanism of electron-bifurcating electron transfer flavoproteins (ETFs) involves an intermediary complex with NAD + ," *J. Biol. Chem.*, 2018.

[268] D. W. Johnson, "A flow injection electrospray ionization tandem mass spectrometric method for the simultaneous measurement of trimethylamine and trimethylamine N-oxide in urine," *J. Mass Spectrom.*, 2008.

[269] D. A. Wiesenburg and N. L. Guinasso, "Equilibrium Solubilities of Methane, Carbon Monoxide, and Hydrogen in Water and Sea Water," *J. Chem. Eng. Data*, 1979.

CHAPTER THREE

BIOLOGICAL CYCLING OF ARSENIC AND MERCURY IN YELLOWSTONE LAKE

Contribution of Authors and Co-Authors

Manuscript in Chapter Three

Author: Abdullah Alowaifeer

Contributions: Sampling, sample preparation, sample analysis, analyzed data, generated figures, wrote manuscript.

Co-Author: Masafumi Yoshinaga

Contributions: sample analysis.

Co-Author: Patricia E. Bigelow

Contributions: Provided fish samples.

Co-Author: Brian Bothner

Contributions: Provided insight and interpretation of results, edited manuscript.

Co-Author: Timothy R. McDermott

Contributions: Sampling, Provided insight and interpretation of results, edited manuscript.

Manuscript Information Page

Authors: Abdullah Alowaifeer, Masafumi Yoshinaga, Patricia E. Bigelow, Brian Bothner, Timothy R. McDermott

Status of Manuscript:
_ X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-review journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

BIOLOGICAL CYCLING OF ARSENIC AND MERCURY IN YELLOWSTONE LAKE

The following work is currently in progress to be submitted for publication

Abdullah Alowaifeer[1,2], Masafumi Yoshinaga[3], Patricia E. Bigelow[4], Brian Bothner[2], and Timothy R. McDermott[1]*

[1]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717

[2]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 Montana State University, Bozeman, MT 59717

[3]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL 33199

[4]US National Park Service, Center for Resources, Fisheries and Aquatic Sciences Program, Yellowstone National Park, WY 82190

*To whom correspondence should be addressed:
Timothy R. McDermott
Department of Land Resources and Environmental Sciences
Montana State University
Bozeman, MT 59717
E-mail: timmcder@montana.edu

Abstract

Arsenic and mercury are toxic elements that are widely present in aquatic environments. However, marine environments have captured most of the interest of researchers, while little research has been conducted in freshwater systems. In this study, I assessed the effect of the lake floor hydrothermal activity as a potential input of arsenic and mercury into the Yellowstone Lake food chain. Arsenic and mercury were examined

across trophic levels beginning with bacteria and archaea on up to the main fish species, Yellowstone cutthroat trout and its predator the lake trout. Arsenic and mercury concentrations were higher in plankton from waters near geothermal vents. Moreover, mercury concentration biomagnified with trophic levels, while arsenic behaved differently. Most arsenic was present in the inorganic form, followed by methylated versions with the absence of arsenobetaine. In both fish, mercury bioaccumulated in all organs tested, with greatest levels occurring in the liver followed by the muscles then the gills, and a relationship between mercury concentration and size was established. However, arsenic did not bioaccumulate nor biomagnify, and each organ had a different arsenic distribution profile. The liver was the organ with highest arsenic content in both species, which was dominated by methylated arsenicals, while fish muscle surprisingly had arsenobetaine as the major arsenicals. Further, the gills had inorganic arsenic as the main arsenic species, indicating that arsenic accumulation by freshwater organisms is complex and controlled by organism uptake and arsenical metabolism.

Introduction

Arsenic and mercury are found in the environment due to natural and anthropogenic sources [270] [271]. Arsenic is a as class 1 carcinogen according to the World Health Organization [1] , while mercury is a neurotoxin that can cause brain and liver damage [272]. However, arsenic toxicity is species-dependent as inorganic arsenic forms arsenate [As(V)] and arsenite [As(III)] are more toxic than pentavalent versions

monomethylarsonic acid [MAs(V)] and dimethylarsinic acid [DMAs(V)] [273], while arsenobetaine and arsenocholine, commonly found in aquatic systems, are essentially non-toxic [274]. In freshwater aquatic food webs, mercury has been extensively studied and is known to accumulate and biomagnify [275] [276] whereas, little has been done on arsenic freshwater biogeochemical-cycling [46].

Dissolved inorganic arsenic dominates freshwaters followed by MAs(V) and DMAs(V), which are planktonically driven [277]. Nonetheless, most arsenic species found in freshwater phytoplankton are As(V) and As(III), while arsenobetiane is absent [58][57]. Although it was hypothesized that the formation of arsenobetaine occurs at the bottom of the food chain, many freshwater and marine screening studies show other pathways are evident but are at present unknown and uncharacterized [53][46]. Arsenobetiane is an analogue for the osmolyte glycine betaine, and marine fish accumulate more arsenobetiane than their freshwater counterparts. Presumably this is due to differences in salinity. Fish can also methylate ingested inorganic arsenic to form MAs(V) and DMAs(V), which is believed to be a detoxification mechanism[278].

Yellowstone Lake is the home for several types of fish, with the lake trout and the cutthroat trout being the best known and studied. The lake trout is nonnative in Yellowstone Lake and preys on the cutthroat, and thus threatens the survival of native Yellowstone cutthroat trout and species that depend on it, such as grizzly bears, bald eagles, and river otters. In 1994, Yellowstone National Park (YNP) started a gillnetting and trap netting program to control the growing numbers of lake trout [279]. Anglers also contribute to lake trout management, as they were encouraged to fish for lake trout, and

are required to kill all lake trout caught. All of the fish caught by commercial gillnetting operation are killed and returned to the lake. Lake trout caught by sport fisherman can be kept, but YNP has established consumption limits because of the high mercury content. A previous study conducted by the United States Geological Survey (USGS) revealed the presence of arsenic and mercury in the lake, which was concluded to be arising from hydrothermal vents on the lake floor [280].

Here I examined the arsenic species and total mercury accumulation and magnification in a freshwater system. Yellowstone Lake aquatic food web is used as our study model. I examine the spatial effect on the accumulation of these metal(loid)s in lower trophic levels and two fish species cutthroat trout and lake trout. We note that the study conducted by the USGS was in a different part of the lake and did not examine lower planktonic size fractions nor the effect of fish length on arsenic and mercury bioaccumulation or biomagnification. In addition, this study focuses on arsenic species accumulation and its organ distribution patterns (liver, muscles and gills).

Material and methods

Sampling Location and Procedure

Sampling was conducted over a three-year period (2014, 2015 and 2016) during the summer months of June July and August. Two locations were sampled, the Inflated Plain region (44.320˚N, 110.212˚W) in the northern part of the lake, where high output hydrothermal vents are numerous; and at Promontory Point in the southern part of the lake (44.417˚N, 110.314˚W), where no vents are present (Figure 3.1). Lake water from a

depth of 10 meters was pumped to the boat deck via a high-volume peristaltic pump at each site. Water was passed through two zooplankton nets with nominal filtration sizes of 20 and 60 microns (um), with the resulting captured plankton biomass from each separately transferred to a Falcon tube. The filtrate was collected in a 10-liter Nalgene carboy and transferred to the laboratory where the water was filtered through Whatman® nylon membrane filters to collect 0.1, 0.8 and 3 um plankton based on a liter basis.

The YNP Fisheries Services (Dr. Pat Bigelow) provided all 48 cutthroat trout and 60 lake trout used in this study. Fish from each species was divided into three groups based on their length, which I used as a proxy for age. The length categories in millimeter (mm) were, small to mid-length <300 mm, mid-length 300-500 mm and large fish >500 mm. The length classification was based on fish spawning and maturity life stages [281]. Fish attaining a length of 300 mm are considered adult at which time their feeding habits change from that of fry and juvenile fish. Fish were cut open by inserting clean scissors right behind the pectoral fin to open the abdominal cavity to collect the liver. To extract the gills, the operculum bone was removed to expose the gills, which were cut off using decontaminated scissors. Then, skin is peeled off and fish muscle was cut off and homogenized using a regular blender. All gathered samples were stored in -80 °C freezer until analysis.

Analysis for total mercury and arsenic

Fish organs, 20 and 60 um zooplankton samples were weighed and separately placed into a 50ml Falcon tube, to which 5 ml of nitric acid was added. For smaller organism size fractions, one liter of lake water (from carboy) was filtered through the 3, 0.8 and 0.1 um filters (nanoplankton, microplankton, etc), and was also placed in a Falcon tube and 5 ml of nitric acid was added. Caps were placed over the tubes without sealing to allow for ventilation. Samples were then autoclaved to facilitate complete digestion (20 min sterilization, slow exhaust) [263], and then diluted 50 x.

Standard solutions for total arsenic and mercury were freshly prepared on the day of analysis. An Agilent 7500ce ICP-MS with an octopole collision cell reaction (Agilent Technologies, Waldbronn, Germany) was used for the analysis. The ICP-MS was optimized to achieve the highest signal/background ratio for the m/z 75 for arsenic and m/z 202 for mercury with a reaction collusion cell (He, 3 mL min–1). The internal standards used with the analysis were lithium, gallium, scandium, rhodium, and iridium.

Analysis for arsenic species

Samples were weighed and placed into a 50ml falcon tube, 5 ml of distilled water was added. The samples were digested using a regular microwave at 100W for 15 minutes [264]. After cooling, samples were filtered through a 3-kDa-cutoff Amicon Ultrafilter and the analysis was performed immediately (within 24 hr) to avoid long storage. Diffculties arose in separating arsenobetaine and As(III), and thus to differentiate

these arsenicals hydrogen peroxide was added to the sample making a final concentration of 10%. Hydrogen peroxide oxidizes As(III) to As(V), whereas it does not affect arsenobetaine. Thus, arsenobetaine can be determined by subtracting the concentration of As(V) after the addition of hydrogen peroxide from the initial concentration of arsenic. Arsenic species analysis was performed on a high-performance liquid chromatography (HPLC) (Series 2000; Perkin- Elmer, Waltham, MA) equipped with a Jupiter® 5 µm C18 300 Å reverse-phase column (250 mm × 4.6 mm; Phenomenex, Torrance, CA) eluted isocratically with a mobile phase consisting of 3 mM malonic acid, 5 mM tetrabutylammonium hydroxide and 5% methanol (v/v), pH 5.6, with a flow rate of 1 ml min−1 at 25°C. The HPLC was coupled with an ICP- MS (ELAN DRC- e; Perkin-Elmer) to determine arsenic concentration.

Results

Total Mercury

Total mercury concentration in water and plankton sizes 0.1, 0.8 and 3 um were normalized on a liter bases, as they were unmeasurable by mass, while the 20 and 60 um size fraction were calculated on a mass basis. Dissolved average (± SE) total mercury concentration in the Inflated Plain water (17 ng/L ± 8) was higher than water concentration from Promontory Point (4 ng/L ± 0.7). Similarly, total mercury in all planktonic size fractions was greater at Inflated Plain than Promontory Point, with the exception of the 0.1 um plankton, where concentrations in Inflated Plain (105 ng/L ± 30) and Promontory Point (119 ng/L ± 17) overlapped (Figure 3.2). Although the standard

error in the 0.8 and 3 um overlay in both sampling locations, the general trend is an increase in concentration with increasing size. Average concentration at the highest size fraction 20 and 60 um were 1119 ng/Kg and 1429 ng/Kg for Inflated Plain and 686 ng/Kg and 889 ng/Kg for Promontory Point respectively.

In both lake trout and cutthroat species, the concentration of mercury was higher in the liver followed by the muscles then the gills (Figure 3.3). Surprisingly, no differences were found in mercury content between species in the three analyzed organs. However, when comparing length groups, there was strong evidence that larger fish (>500 mm) accumulated more mercury than the other mid- length (300-500 mm) and small fish (<300 mm) (ANOVA, $P<0.05$). Similarly, significant differences ($P<0.05$) were observed for both the liver (Figure 3.3 B) and the gills (Figure 3.3 C) between length groups. Regression muscle mercury against fish length revealed an R^2 value of 0.45 in cutthroat and 0.53 for lake trout ($P<0.05$) (Figure 3.4 A). Similarly, a relationship was found for liver ($R^2 = 0.41$ and 0.53), and gills ($R^2 = 0.51$ and 0.52) for cutthroat and lake trout fish, respectively ($P<0.05$).

Planktonic Arsenic

Dissolved arsenic in samples from Inflated Plain was 15ug/L ± 1.4, while water from Promontory Point was found to contain 10ug/L ± 0.2. As with mercury, all trophic levels in the Inflated Plain had higher total arsenic than in Promontory Point (Figure 3.5). The largest difference between the two sampling locations was seen in the 20 and 60 um trophic level (microplankton), where the difference in arsenic concentration between lake

locations exceeded 10 folds and was statistically significant even though the Inflated Plain microplankton arsenic content was quite variable However, average total arsenic in Promontory Point was constant with increasing trophic position. (0.1um = 51ug/L ± 8, 0.8um = 45ug/L ± 12, 3um = 40ug/L ± 7, 20um = 32ug/L ± 3, 60um = 36ug/L ± 5).

Although the planktonic level was dominated by inorganic arsenic, the arsenic species profiles were different in both sampling sites (Figure 3.6 A). In Inflated Plain, 60-70% of the arsenic in all plankton trophic levels was found to be As(V), and MAs(V) was only found in the 0.8- 60 um size fraction, accounting for < 20% of the total arsenic. As(III) was also a minor component in all plankton trophic levels. On the other hand, Promontory Point plankton had higher organic arsenic content especially in the 3 and 20 □m size fractions, where more than 50% was methylated arsenic. Moreover, DMAs(V) was present in 0.1, 0.8 and 3 um sizes, but in less than 15% of the total arsenic content. As with Inflated Plain, arsenobetaine and arsenocholine were also not present (Figure 3.6 B).

Arsenic in Cutthroat Trout and Lake Trout

Total arsenic in both fish species was higher in the liver, followed by the gills and then the muscles. Over all, lake trout had higher arsenic content in all organs than cutthroat trout. In cutthroat muscle, no relation was found between arsenic and fish size (Figure 3.8 A), and the arsenic concentration was constant among all age-groups (p-value > 0.9). However, there was moderate evidence that the largest tested lake trout muscle had lower arsenic than the mid-length fish (p <0.1) but, no linear relationship was found

between concentration and length with an R^2 value of 0.003 (Figure 3.8 A). Similarly, the large lake trout gills had lower arsenic than mid-length fish ($p < 0.1$), while no difference was seen in cutthroat trout (Figure 3.7 C). In addition, no linear relationship was seen between arsenic gill content and fish length (Figure 3.8 C). However, small fish liver of both species contained higher arsenic than larger groups, and the calculated p-value for the ANOVA test was 0.05 and 0.09 for cutthroat and lake trout respectively (Figure 3.7 B). Consequently, linearity plots showed no relation between liver arsenic and fish length (Figure 3.8 B).

Arsenic species profile was different between the two fish species and even among organs. The muscle of small and mid-length cutthroat trout contained <30% As(V), <20% As(III), <31% MAs(V), <20% DMAs(V) and <25% arsenobetaine, but in large cutthroat As(III) and arsenobetaine were the major species representing 41% and 36% respectively. Arsenobetaine was the dominant arsenical in lake trout muscle, representing more than 55% of total arsenic in all length groups and all other arsenical were in minor levels (Figure 3.9 A). In cutthroat trout liver, methylated arsenicals were the major species found and inorganic arsenics were a relatively minor component. On the other hand, lake trout had arsenobetaine in addition to MAs(V) and DMAs(V) as the major arsenicals detected in their livers (Figure 3.9 B). Moreover, arsenocholine was only found in the liver of both species and as a minor arsenical. In contrast, inorganic arsenic was the major component in the gills of both species, exceeding 50% of total arsenic in cutthroat trout. Arsenobetaine and MAs(V) were also major arsenical in the gills representing more than 20% each of the total arsenic content (Figure 3.9 C). The arsenic species profile of the gills did no show significant differences as fish increase in length.

Finally, there were three unidentified peaks retained at 4.7, 5.1 and 8.3 minutes detected in the liver of both fish species that we were unable to identify due to the lack of standards (Figure 3.10). In addition, the 4.7 minutes peak was also detected in muscle of both fish species as well.

Discussion

The spatial variation in the concentration of dissolved mercury and arsenic between Inflated Plain and Promontory Point suggests that these elements can be used as hydrothermal-indicators and that the presence of hydrothermal vents is a natural input source for theses metal(loid)s. Such inputs affect the bioavailability and magnification rates as observed by the difference in concentration between sampling sites in all plankton size fraction (Figures 3.2 & 3.5). Other natural and anthropogenic inputs (i.e., and dry deposition) are not believed to differ, as the distance between the two sampling locations is less than 8 miles. The increase of total mercury concentration with increasing trophic level at both locations was as expected, which demonstrates the biomagnification characteristics of mercury in biological systems. Mercury levels in phytoplankton size classes (~11 and ~243 um) from the West Thumb region of Yellowstone Lake, another hydrothermal location at the lake, was reported by Chaffee et al [280] to be at 200 ug/Kg for both sizes. This is higher compared to 110 and 140 ug/Kg detected respectively in the 20 and 60 um size fractions from the Inflated Plain, which indicates higher bioavailability and inputs rates at the West Thumb. As with mercury, arsenic concentration in phytoplankton from the West Thumb was 10 fold higher than from the Inflated Plain

[280]. However, arsenic does not biomagnify in the lower trophic levels as mercury does (see Figure 3.5). This could be due to the several factors that govern arsenic uptake and metabolism.

Phytoplankton uptake of arsenic has been suggested to be controlled by the level of phosphate in the water [39] and the metabolic arsenical by products are organism-dependent. Organic arsenicals, which are believed to be an end-product to a detoxification pathway, are extruded out of the cell more efficiently than inorganic versions, thus affecting biomagnification patterns [39][40][41]. The ratio of dissolved As(V) to As(III) was 3/1 in both sampling sites, which is excepted as the dissolved oxygen concentration at the 10 meter sampling depth was 8.5 mg/L. However, the different distribution of arsenicals in lower trophic levels between the two locations, specifically the presence of DMAs(V) in Promontory Point only, suggest that organisms vary between sites (Figure 3.6). Kan et al. [282] found that microbial population structures in Yellowstone Lake appear influenced by geothermal inputs. Varying microbial communities could explain the different arsenical profiles between sampling sites, as different microbes deal with arsenic differently through oxidation, reduction, methylation and extrusion rates. These metabolic processes are governed by the microbial structure and expressed genes. The absence of arsenocholine and arsenobetaine in all phytoplankton size-fractions support the hypothesis that they are formed during the ingestion and metabolism of arsenic sugars in higher trophic animals, rather than at the base of the aquatic food web [46].

Our total mercury data in the muscle, liver and gills of both studied species display the classical bioaccumulation property mercury is known for. Larger fish

accumulated more mercury than smaller fish of the same species and a relatively strong correlations between length and concentration was demonstrated (see Figure 3.4). This claim was supported by the R^2 values and P-value <0.05 of the regression analysis. Despite the fact that cutthroat trout and lake trout differ in terms of their feeding habitats [281], they accumulated similar amounts of mercury, suggesting a minor trophic level effect. Fish lengths >500 mm accumulated the highest amount of mercury in both fish species in the liver followed by the muscles then the gills (Figure 3.4). This is expected due to the physiology and function of the hepatic portal system that transfers digested nutrients to the liver for anabolic and catabolic processes. Freshwater fish absorb their salt needs directly from the water through the gills, therefore the gills relatively low mercury content could be linked to the low mercury concentration in the water (ug/kg levels). In both fish species, mercury concentration in the muscle did not exceed 0.2 mg/kg and the food and drug authority (FDA) mercury action level is set at 1 mg/kg. It is important to note that permissible limits for mercury are determined based on age, weight and consumption portions.

Total arsenic was found to accumulate in the liver of both species in higher concentrations compared to the other two organs, due to aforementioned physiological reasons. However, unlike mercury, the gills accumulated more arsenic than the muscles, which could be due to the higher concentrations of dissolved arsenic compared to mercury levels in the water. The effect of trophic level and habitat on arsenic accumulation is clear when comparing its organ levels between the two fish species, with lake trout being higher in the trophic scale and having higher arsenic content as expected (Figure 3.7). However, the results of this study suggest that arsenic does not

bioaccumulate as observed with mercury. No relationship was found between fish length and arsenic concentration in all three analyzed organs (Figure 3.8). In fact, the concentration of arsenic in cutthroat trout muscle was similar in all fish length groups, while large lake trout had less arsenic content compared to mid-length fish. Similar results were observed in the gills of both fish too. On the other hand, the liver of small length fish had higher arsenic content, which decrease with bigger groups. Such observations are not unique and have been documented in freshwater systems by Chen et al. [94] and Suhendrayatna et al. [69]. It was suggested that freshwater fish can develop an acclimation effect as a defense mechanism to overcome arsenic toxicity. Although the exact process involved has never been suggested, observations in our study point to similar metabolic responses.

The different distribution of arsenicals among organs and fish species suggest that arsenic metabolism is complex and controlled by multiple factors. The accumulation of arsenobetaine in the muscles of large cutthroat and lake trout is surprising since both are freshwater fish (Figure 3.9). The relationship between arsenobetaine retention and salinity has been documented in many species but our results suggest other possible explanations. The high arsenobetaine levels in the muscles suggest other factors controlling arsenobetaine besides salinity. Since arsenobetaine is nontoxic, it could be an end product to a detoxification mechanism that protects both marine and freshwater fish against arsenic toxicity. The absence of arsenobetaine at all planktonic levels and the high concentration found in large fish clearly suggest that arsenobetaine biosynthesis is taking place in the fish body. In addition, arsenocholine was only detected in the liver (Figure 3.9) and the proposed pathway for arsenobetaine formation involves a one-step oxidation

of arsenocholine [69], which could possibly occur in the liver, since oxidation is a common metabolic function. Moreover, such hypotheses have been previously proposed based on similar findings [46].

The decrease in total arsenic in the liver is accompanied by a decrease in methylated arsenicals. The liver is the organ responsible for the methylation process and the reduction of the methylation rate seen with fish length could be a reason for higher arsenic accumulation in the muscles, which is seen in mid-length and large fish in both species. Lastly, the domination of inorganic arsenic in the gills supports the hypothesis that arsenic uptake from the water column through the gills is a major arsenic entry route. Freshwater fish acquire their salt needs from the water through the gills by action of the sodium and potassium ATPase pump, and chloride cotransporter enzyme. In fact, there are many factors that control the mitotic activity of cells in fish gills, like growth hormones and cortisol [283]. The synthesis of such hormones is age and species dependent, which can explain the higher inorganic content in the gills of cutthroat trout than lake trout (Figure 3.9).

To conclude, arsenic and mercury biologically cycle differently in freshwater systems. While mercury accumulated and biomagnified in a predictable manner, arsenic on the other hand behaved differently. This is due to the chemical properties of arsenic that in cables it to form many versions that involve multiple pathways, some of which are still unknown. In addition, higher trophic organisms can methylate and biodegrade arsenicals unlike mercury, where only the demethylation process has been confirmed. The high concentration of arsenobetaine in freshwater organisms opens the question to its relationship to glycine betaine and salinity. Therefore, the pathway involved in

arsenobetaine synthesis needs to be identified in order to fully understand what is believed to be a detoxification mechanism.

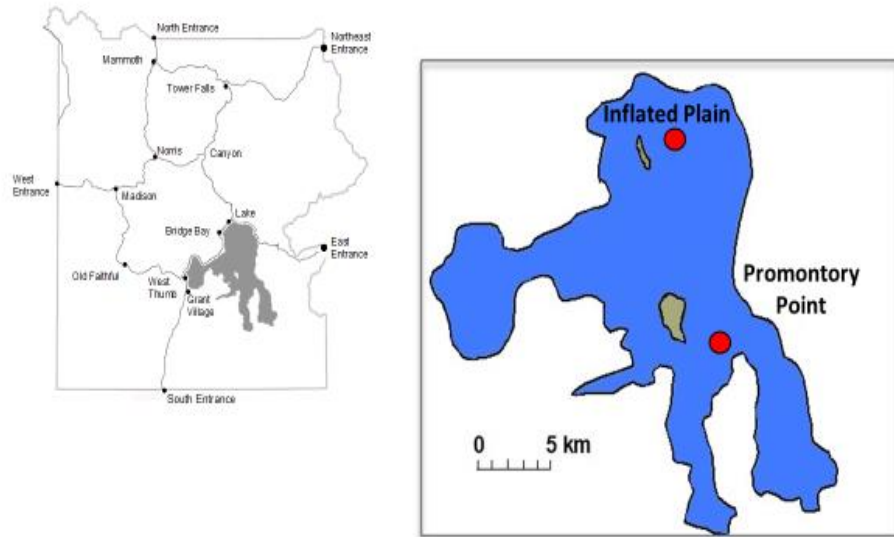


Figure 3.1 General map of Yellowstone National Park with Yellowstone lake shown. Red dot indicates the approximate sampling location near Inflated Plain and Promontory Point.

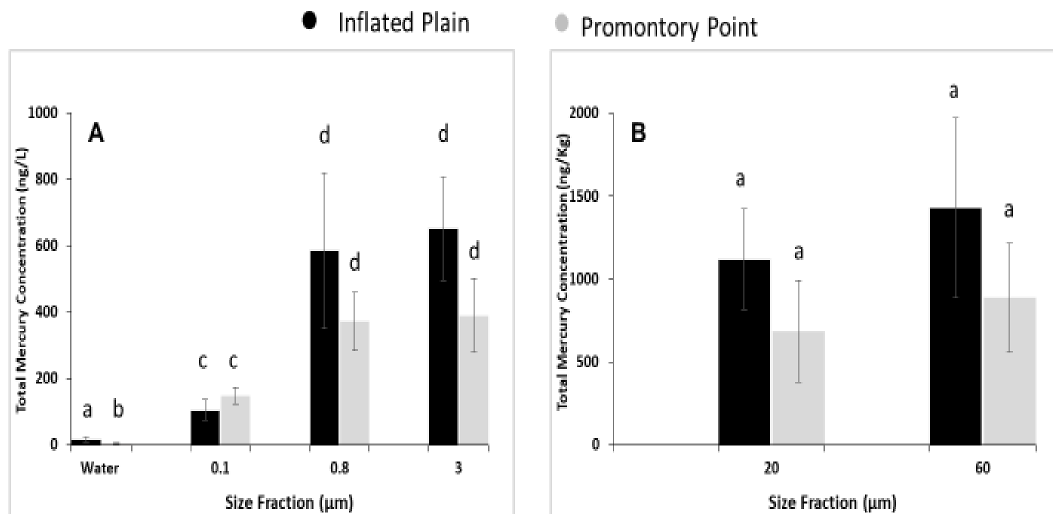


Figure 3.2 Planktonic total mercury. **A)** Bar graph showing concentration of total mercury in three plankton size fractions 0.1, 0.8 and 3 µm normalized on a liter basis. **B)** mercury concentration in 20 and 60 µm size plankton fractions normalized on a weight basis. Data bar having different letter designations are statistically different @ p-value <0.05

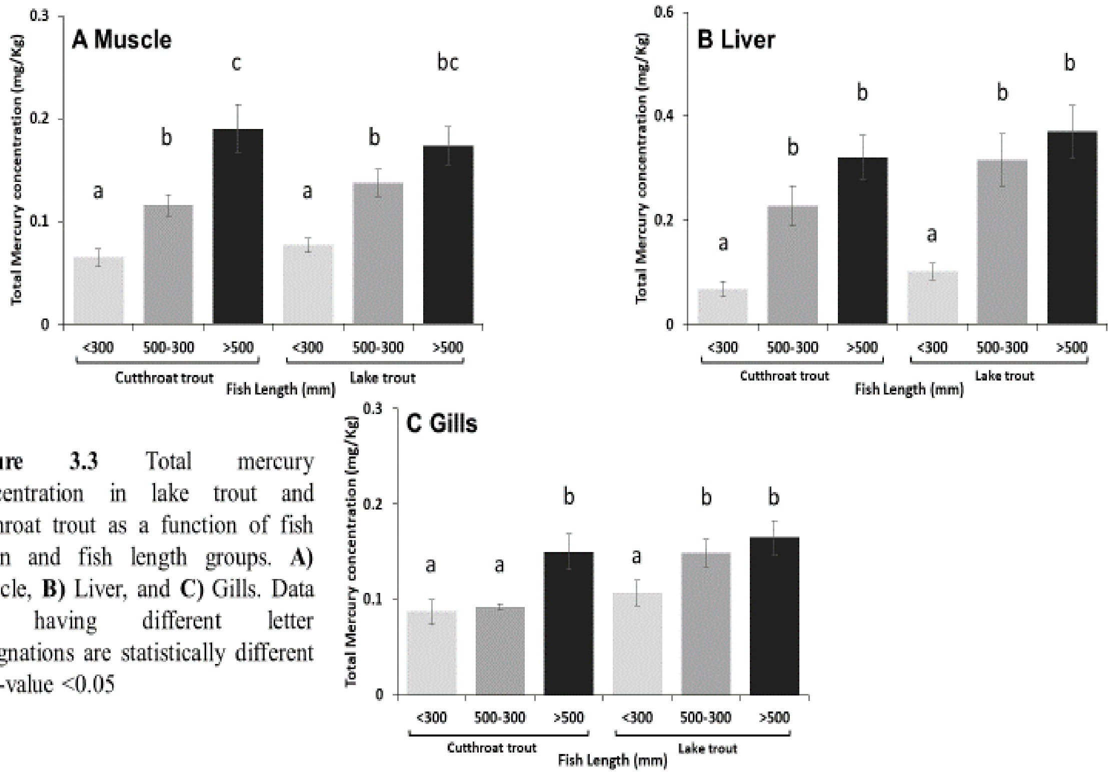


Figure 3.3 Total mercury concentration in lake trout and cutthroat trout as a function of fish organ and fish length groups. **A)** Muscle, **B)** Liver, and **C)** Gills. Data bar having different letter designations are statistically different @ p-value <0.05

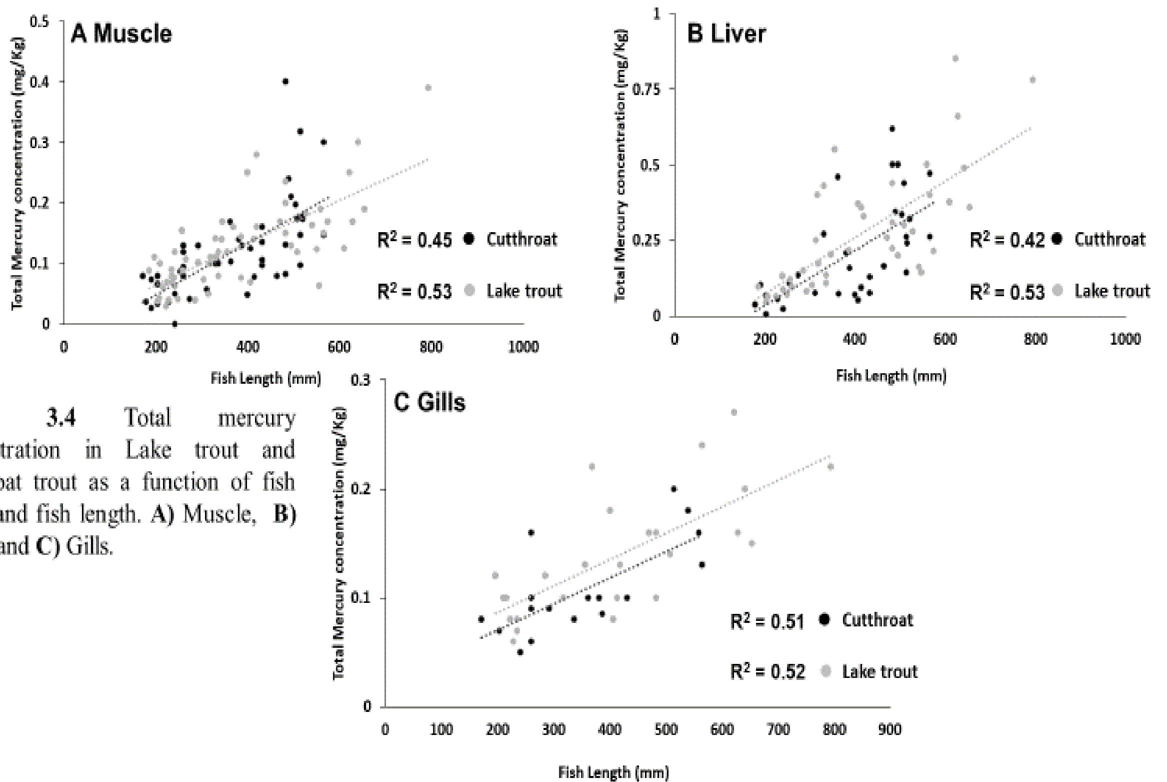


Figure 3.4 Total mercury concentration in Lake trout and Cutthroat trout as a function of fish organ and fish length. **A)** Muscle, **B)** Liver, and **C)** Gills.

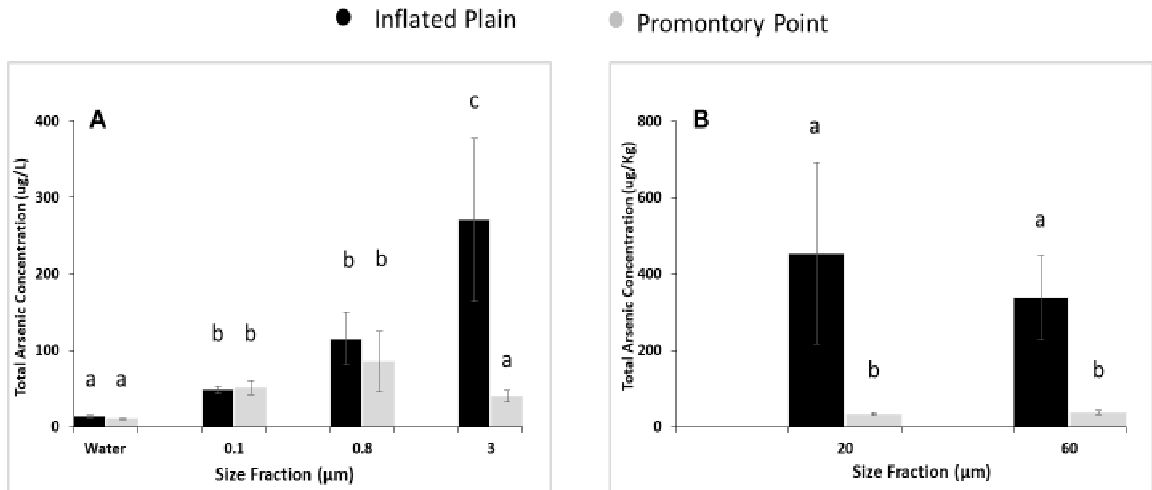


Figure 3.5 Planktonic total arsenic. **A)** Concentration of total arsenic in three plankton size fractions 0.1, 0.8 and 3 µm normalized on a liter basis. **B)** arsenic concentration in 20 and 60 µm plankton size fractions normalized on a weight basis. Data bar having different letter designations are statistically different @ p-value <0.05

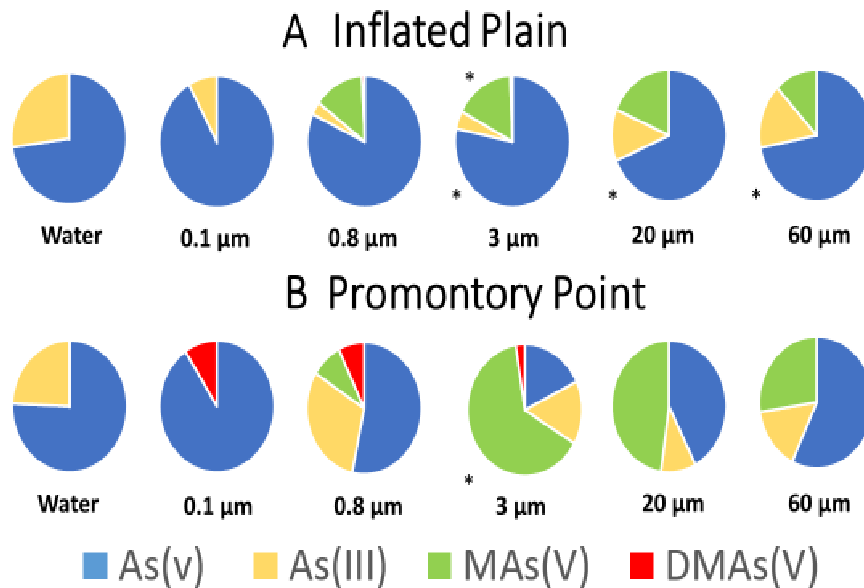


Figure 3.6 Arsenic species profiles in different planktonic size fractions. **A)** planktons from Inflated plain enriched with hydrothermal vents. **B)** Planktons from Promontory Point not known to be associated with lake floor hydrothermal vents. * Statistically significant differences in arsenical concentration between planktonic size fractions @ p-value <0.05

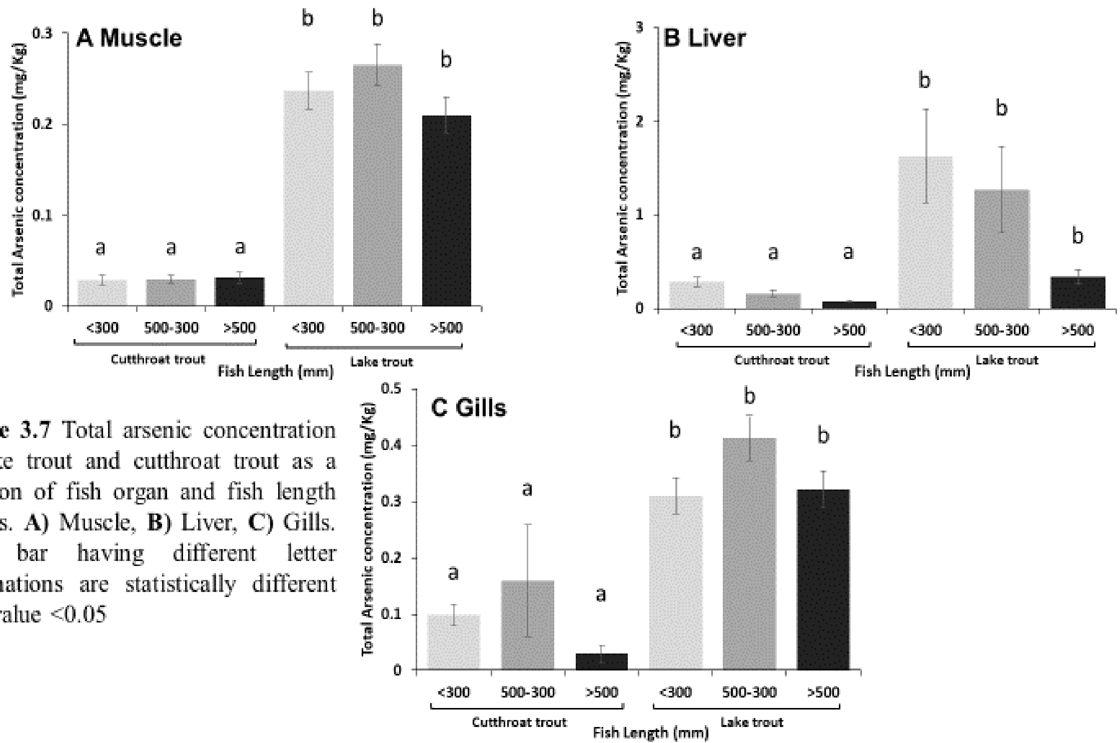


Figure 3.7 Total arsenic concentration in lake trout and cutthroat trout as a function of fish organ and fish length groups. **A)** Muscle, **B)** Liver, **C)** Gills. Data bar having different letter designations are statistically different @ p-value <0.05

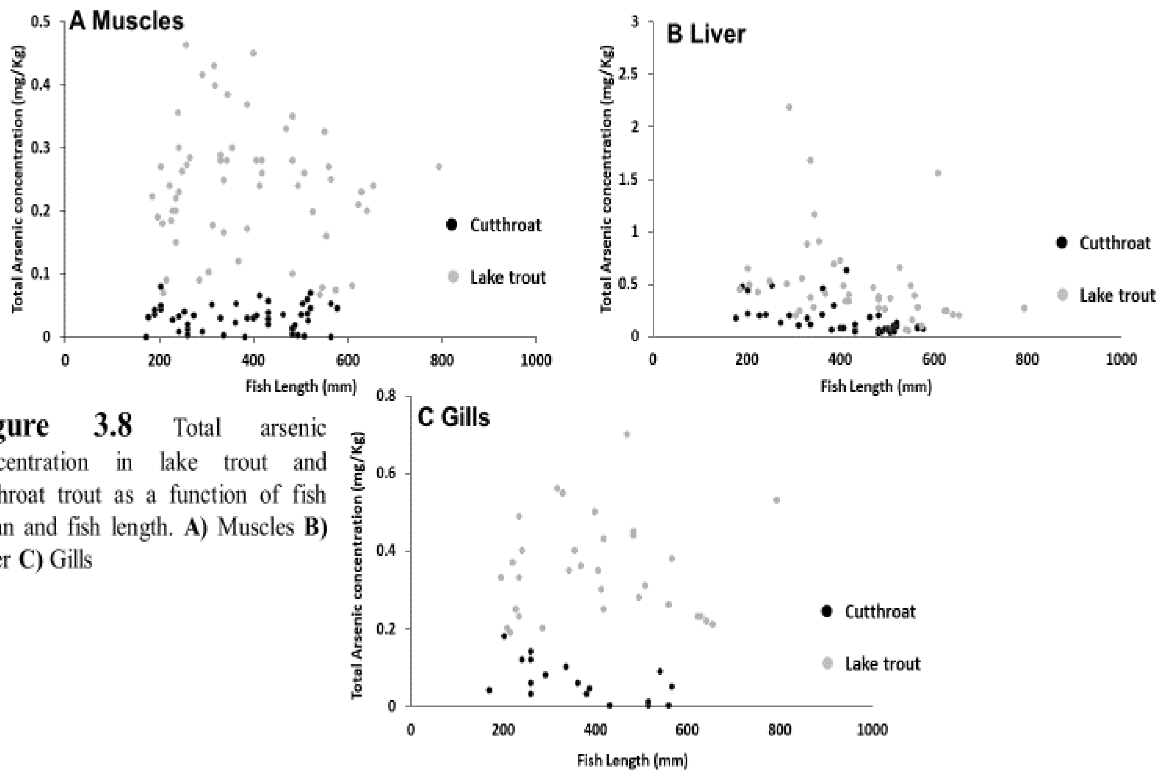


Figure 3.8 Total arsenic concentration in lake trout and cutthroat trout as a function of fish organ and fish length. **A)** Muscles **B)** Liver **C)** Gills

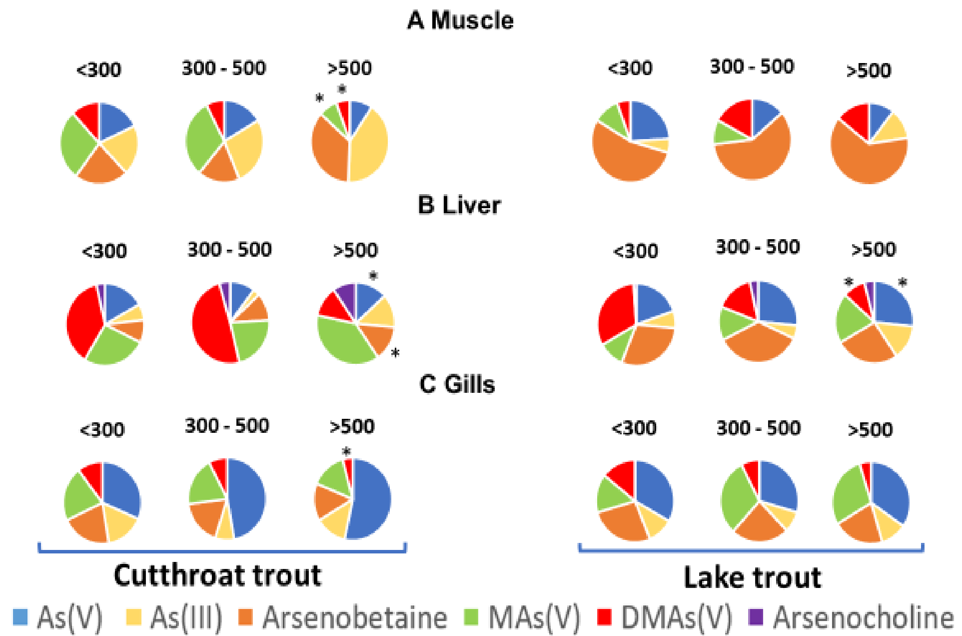


Figure 3.9 Relative proportion of identified arsenic species in different organs of length grouped (mm) Cutthroat trout and Lake trout. **A)** Muscle, **B)** Liver, **C)** Gills. * Statistically significant differences in arsenical concentration between fish size classes @ p-value <0.05

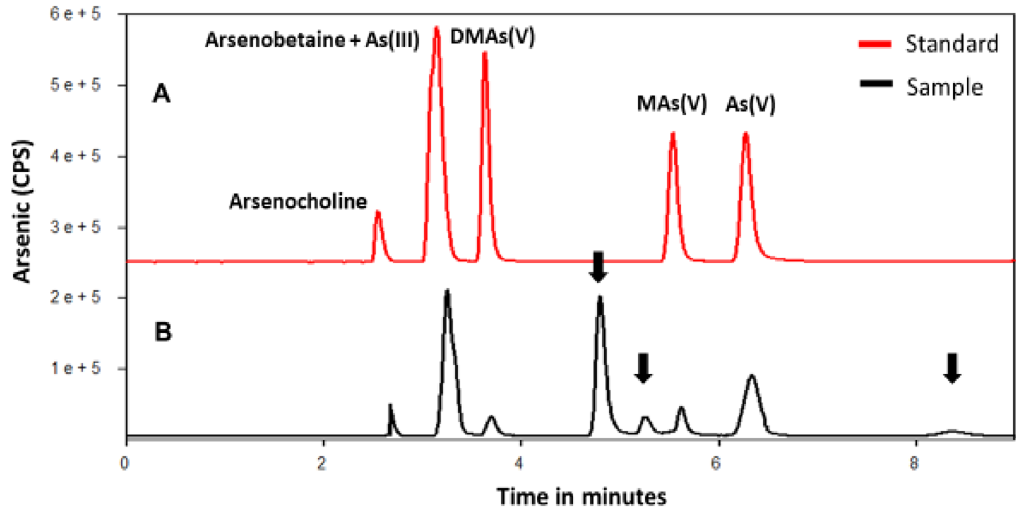


Figure 3.10 HPLC–ICP–MS chromatogram of arsenic species. A, standard run of available arsenic species for quantification. B, Lake trout liver sample illustrating three unidentified peaks

References

[1] "Arsenic and arsenic compounds," *IARC Monogr. Eval. Carcinog. Risks to Humans*, 2011.

[39] J. L. Levy, J. L. Stauber, M. S. Adams, W. A. Maher, J. K. Kirby, and D. F. Jolley, "Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (Chlorella sp. and Monoraphidium arcuatum)," *Environ. Toxicol. Chem.*, 2005.

[40] L. A. Murray, A. Raab, I. L. Marr, and J. Feldmann, "Biotransformation of arsenate to arsenosugars by Chlorella vulgaris," *Appl. Organomet. Chem.*, 2003.

[41] S. MIYASHITA, S. FUJIWARA, M. TSUZUKI, and T. KAISE, "Rapid Biotransformation of Arsenate into Oxo-Arsenosugars by a Freshwater Unicellular Green Alga, *Chlamydomonas reinhardtii*," *Biosci. Biotechnol. Biochem.*, 2011.

[46] G. Caumette, I. Koch, and K. J. Reimer, "Arsenobetaine formation in plankton: A review of studies at the base of the aquatic food chain," *J. Environ. Monit.*, 2012.

[53] S. Foster, D. Thomson, and W. Maher, "Uptake and metabolism of arsenate by anexic cultures of the microalgae Dunaliella tertiolecta and Phaeodactylum tricornutum," *Mar. Chem.*, 2008.

[57] G. Caumette, I. Koch, E. Estrada, and K. J. Reimer, "Arsenic speciation in plankton organisms from contaminated lakes: Transformations at the base of the freshwater food chain," *Environ. Sci. Technol.*, 2011.

[58] S. Miyashita *et al.*, "Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS," *Chemosphere*, 2009.

[69] T. A. and S. T. Kunito .T, R. Kubota, J. Fujihara, *Reviews of Environmental Contamination and Toxicology*. Springer, New York, 2008.

[263] R. O. Lozano-Rodríguez, E., Luguera, M., Lucena, J.J., Carpena-Ruiz, "Evaluation of two different acid digestion methods in closed systems of trace elements determination in plants.," *Quim. Anal*, vol. 14, no. 27–30, 1995.

[264] S. Hirata and H. Toshimitsu, "Determination of arsenic species and arsenosugars in marine samples by HPLC-ICP-MS," in *Applied Organometallic Chemistry*, 2007.

[270] K. Mandal, Badal and T. Suzuki, Kazuo, "Arsenic round the world: a review.," *Talanta*, 2002.

[271] F. M. M. Morel, A. M. L. Kraepiel, and M. Amyot, "THE CHEMICAL CYCLE AND BIOACCUMULATION OF MERCURY," *Annu. Rev. Ecol. Syst.*, 1998.

[272] D. Mergler *et al.*, "Methylmercury Exposure and Health Effects in Humans: A Worldwide Concern," *AMBIO A J. Hum. Environ.*, 2007.

[273] N. R. Akter KF, Owens G, Davey DE, "Arsenic Speciation and Toxicity in Biological Systems," *Rev Env. Contam Toxicol*, vol. 184, pp. 97–149, 2005.

[274] M. Kumaresan and P. Riyazuddin, "Overview of speciation chemistry of arsenic," *Current Science*. 2001.

[275] M. M. Chumchal *et al.*, "Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem," *Environ. Toxicol. Chem.*, 2011.

[276] M. G. Clayden, K. A. Kidd, B. Wyn, J. L. Kirk, D. C. G. Muir, and N. J. O'Driscoll, "Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes," *Environ. Sci. Technol.*, 2013.

[277] M. Azizur Rahman and H. Hasegawa, "Arsenic in freshwater systems: Influence of eutrophication on occurrence, distribution, speciation, and bioaccumulation," *Appl. Geochemistry*, 2012.

[278] S. Ciardullo, F. Aureli, A. Raggi, and F. Cubadda, "Arsenic speciation in freshwater fish: Focus on extraction and mass balance," *Talanta*, 2010.

[279] and S. T. O. Bigelow, P.E., T.M. Koel, D. Mahony, B. Ertel, B. Rowdon, "Protection of native Yellowstone cutthroat trout in Yellowstone Lake, Wyoming," Yellowstone National Park, Wyoming, USA., 2003.

[280] M. A. Chaffee *et al.*, "Applications of trace-element and stable-isotope geochemistry to wildlife issues, Yellowstone National Park and vicinity," *U.S. Geol. Surv. Prof. Pap.*, 2007.

[281] and J. M. S. Gresswell, R.E., C.S. Guy, M.J. Hansen, M.L. Jones, J.E. Marsden, P.J. Martinez, "Lake trout suppression in Yellowstone Lake: science review panel. Interim scientific assessment, 2014 performance year. A report to the superintendent," National Park Service, Yellowstone National Park, Wyoming, USA., 2015.

[282] J. Kan *et al.*, "Geochemistry and mixing drive the spatial distribution of free-living archaea and bacteria in yellowstone lake," *Front. Microbiol.*, 2016.

[283] S. D. McCormick, "Endocrine control of osmoregulation in teleost fish," *Am. Zool.*, 2001.

CHAPTER FOUR

EXAMINING THE ROLE OF PHOTOAUTOTROPHS CONTRIBUTING TO GLYCINE BETAINE, METHYLATED AMINES AND METHANE IN OXIC WATERS

Contribution of Authors and Co-Authors

Manuscript in Chapter Four

Author: Abdullah Alowaifeer

Contributions: Sampling, sample preparation, sample analysis, analyzed data, generated figures, wrote manuscript.

Co-Author: Qian Wang

Contributions: Sampling, sample preparation, sample analysis, analyzed data, generated figures.

Co-Author: Brian Bothner

Contributions: Provided insight and interpretation of results, edited manuscript.

Co-Author: Timothy R. McDermott

Contributions: Sampling, Provided insight and interpretation of results, edited manuscript.

Manuscript Information Page

Authors: Abdullah Alowaifeer, Qian Wang, Brian Bothner, Timothy R. McDermott

Status of Manuscript:
_ X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-review journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

EXAMINING THE ROLE OF PHOTOAUTOTROPHS CONTRIBUTING TO GLYCINE BETAINE, METHYLATED AMINES AND METHANE IN OXIC WATERS

The following work is currently in progress to be submitted for publication

Abdullah Alowaifeer[1,2], Qian Wang[1], Brian Bothner[2], and Timothy R. McDermott[1]*

[1]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717

[2]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 Montana State University, Bozeman, MT 59717

*To whom correspondence should be addressed:
Timothy R. McDermott
Department of Land Resources and Environmental Sciences
Montana State University
Bozeman, MT 59717
E-mail: timmcder@montana.edu

Abstract

Methane production has been thought to strictly occur under anaerobic conditions. However, newly emerging studies suggest that methanogenesis can happen aerobically and is not limited to carbon cycling. Several substrates have been shown to support methanogenesis, including methylated amines. Glycine betaine, a common precursor for methylated amines, is thought to be synthesized by aquatic organisms as a protectant osmolyte. Here I investigate the possible relationship between photoautotrophs and their contribution to glycine betaine synthesis in Yellowstone Lake and three oxic rivers in the

state of Montana. In addition, potential relationships between glycine betaine, methylated amines precursors and water-soluble methane was examined as well. This was achieved by water analysis for chlorophyll a, phycocyanin, glycine betaine, methylated amines, soluble methane and other physical parameters. TWO methane peaks were detected in Yellowstone Lake and ^{13}C-MMA was shown to be transformed to ^{13}C-methane, when spiked to lake water from the specific depth. Additionally, correlations were established between glycine betaine, methylated amine and methane in the lake water profile. The three subject rivers were found to differ in terms of biology and water chemistry. However, significant negative correlation between chlorophyll a and glycine betaine levels were found, from which I hypothesized that soluble glycine betaine in the river water is negatively correlated with apparent intact algae. Strong to moderate evidence of correlation between methylated amines in rivers suggest a metabolic relation among them. However, such correlations with glycine betaine were easier to demonstrate in the lake than the river.

Introduction

Glycine betaine ($(CH_3)_3N{+}CH_2COOH$), sometimes referred to as trimethylglycine, is an amino acid derivative and intracellular organic osmolyte accumulated under abiotic stress conditions by both prokaryotes and eukaryotes. Cells biosynthesize and/or directly uptake glycine betaine during fluctuating physical parameters such as increased salinity or temperature to prevent the denaturing of

intracellular proteins [209]. In addition, glycine betaine can act as a methyl group donor to support several cellular functions such as protein synthesis and DNA methylation [210]. Although uptake transporters are yet to be identified in phytoplankton, microbes can uptake glycine betaine through an ATP-binding cassette (ABC) type transporters or by the betaine-choline-carnitine transporter (BCCT) [220]. Moreover, phytoplankton synthesize glycine betaine either by the oxidation of choline [215] or through a three-step methylation of glycine [225]. In addition, phytoplankton can incorporate glycine betaine in their lipids to form betaine lipids, that are believed to be synthesized to functionally replace phospholipids during phosphate starvation periods [232][235][236].

In aquatic environments, glycine betaine can be utilized by methanogens as a carbon source to produce dimethylglycine, methane and CO_2 [246]. In addition, the anaerobic reduction of glycine betaine has been documented to form trimethylamine [TMA] and acetate [247]. TMA is a common precursor of glycine betaine, which can be used by microbes as a carbon and nitrogen source [251]. Trimethylamine oxide [TMAO], dimethylamine [DMA] and methylamine [MMA] are all possible products of TMA metabolism [252][253]. Under anaerobic conditions, TMA, DMA and MMA can be utilized as methylotrophic substrates for methanogens. The three-step methane formation pathway starts with the conversion of TMA to DMA by the corrinoid protein methyltransferase, which is then demethylated by the coenzyme M to produce methyl-CoM. Further, methyl-CoM can either be reduced to produce methane or oxidized to form CO_2. This pathway can be repeated similarly to utilize DMA and MMA to produce MMA and ammonia respectively in association with methane [256][257]. The aerobic

production of methane from glycine betaine and MMA has also been documented Wang et al. [287]. In addition, it was suggested that decaying phytoplankton are a major source for environmental MMA, being generated by the breakdown of algal betaine (glycine betaine ➡ TMA ➡ TMAO ➡ DMA ➡ MMA ➡ methane).

Although the aforementioned pathways were identified in different aquatic microbes, in my research I hypothesized that glycine betaine, TMA, DMA, MMA and methane are linked and biologically driven. Here I investigate the correlations between water-soluble methane, glycine betaine and its degradation metabolites in Yellowstone lake. Further, spatial and temporal relationships between these metabolites is examined in three rivers in the state of Montana in relation to chlorophyll a (algae proxy), phycocyanin (cyanobacteria proxy) and physical parameters (temperatures, total suspended solids [TDS], pH and water flow).

Material and Methods

Sampling Location and Procedure

Lake sampling was conducted on July 23, 2017 near Promontory point (44.4178N, 110.3148W) (Figure 4.1). A YSI EXO1 multiparameter sonde was used to measure temperature, dissolved O_2 and chlorophyll a in the water column at different depths. Water from these depths was collected in 120 mL serum vials, then immediately

sealed with grey chlorobutyl rubber stoppers and secured with aluminum crimps. Vials were injected immediately with 100µL of a saturated $HgCl_2$ solution and then stored at 4°C upon arrival to the laboratory prior to further analysis.

River sampling was conducted every two weeks in the summer of 2018 starting on the 11th of June till October 5th. Three locations were sampled Madison River (45.486827, 111.633973), Gallatin River (45.419213, 111.232977) and Yellowstone River (45.596810, 110.567113) (Figure 4.2). All three rivers are tributaries to the Missouri River. Sampling locations were selected based on accessibility and the United States Geological Survey (USGS) water parameter collection sites.

While standing on the edge of the water, A YSI EXO1 multiparameter sonde was submerged into the water to measure the river with respect to temperature, TDS, pH, dissolved O_2 and both chlorophyll a and phycocyanin fluorescence. Factory-based calibration was conducted in the field prior to sampling and the fluorescence sensor was calibrated in the laboratory with a rhodamine solution, using manufacturer recommended equations relating rhodamine fluorescence to chlorophyll a and phycocyanin fluorescence. To collect water samples, a 120 mL autoclaved glass serum vial was submerged into the water facing opposite direction of the current flow to facilitate the filling of the vial. Water vials were then immediately sealed with grey chlorobutyl rubber stoppers and secured with aluminum crimps. Vials were injected with 100µL of a saturated $HgCl_2$ solution and then stored at 4°C upon arrival to the laboratory prior to further analysis.

Labeling and Sample Preparation

The dansylation labeling procedure was performed as described by Schut et. al [267] to detect and quantity MMA and DMA. The glass serum vials were placed on ice prior to sample preparation. A Hamilton gastight syringe was used to draw 50ul of water from the sample serum bottle. Water was then placed into a 250ul polypropylene analysis vial and pH was adjusted to ~9.5 with 2ul of 160mM sodium hydroxide. Dansyl chloride [Dns-Cl, 1 -dimethylaminonaphthalene-5-Sulfonyl chloride] prepared in acetonitrile (20 mg/ml) was added to the sample in a volume of 46ul. Samples were then Incubated for 30min at room temperature. After the incubation period, the pH was adjusted to ~4 with 2ul of 10% formic acid. At this point, the sample is ready for analysis.

As for TMA, ethyl bromoacetate was used to label the compound prior to analysis.The labelling procedure was performed as described by Johnson [268]. A Hamilton gastight syringe was used to draw 90ul of water from the sample serum bottle. Water was then placed into a 250ul polypropylene analysis vial and 10ul of (20mg/ml acetonitrile) ethyl bromoacetate was added to the analysis vial. Samples were then incubated for 30min at room temperature. At this point, the sample is ready for analysis.

For the detection of glycine betaine, a Hamilton gastight syringe was used to draw 90ul of water from the sample serum bottle. Water was placed into a 250ul polypropylene analysis vial and 10ul of methanol was added giving a 10% methanol concentration. The sample was vortexed for 30s, after which it was ready for analysis.

Analysis of Methylated Amine and Glycine Betaine

Methylamine hydrochloride, dimethylamine hydrochloride, trimethylamine hydrochloride were purchased from Sigma-Aldrich and the concentration was quantified based on a four-point calibration curve. Chromatography experiment was done on an Agilent 6538 Q-TOF mass spectrometer. Limit of detection was determined to be 20nM for MMA, 8nM for DMA, 10nM for TMA and 1nM for glycine betaine.

Analysis of water-soluble methane

The 120 mL water samples collected in the serum vials were injected with 5mL ultra-high purity nitrogen (N2) to create a headspace. Gas from the headspace was analyzed by gas chromatography Hewlett Packard model 5890 gas chromatograph using flame ionization detection. High purity methane gas was purchased and diluted using air bags to generate a three-point calibration curve and the original methane concentration in solution was calculated using Henry's Law and solubility equations [269].

Lake water microbial activity

Lake water collected from where methane was detected, was used for ^{13}C-tracer experiments to determine which potential substrates might be contributing to aerobic methane production. Water was <80 um filtered at sampling location into sterile polyethylene carboy, which was kept cool and dark for transport back to the laboratory. Five ^{13}C-labeled compounds (99 atom%, Sigma-Aldrich) were tested as methanogenesis substrates: sodium bicarbonate, sodium formate, sodium acetate, methylphosphonic acid

(Mpn) and methylamine hydrochloride. Substrates were filter sterilized (0.2 um), then 1mL of stock solution (25mM) was transferred to autoclaved 70 mL serum vials with 50mL lake water. After, vials were sealed with grey chlorobutyl rubber stoppers and aluminum ring crimped. Incubations were carried out so as to mimic *in situ* conditions (128C; PAR = 70 mE m^{-2} s^{-1} via fluorescent lights; 16:8 h day/night cycle). At selected time points, 5 mL of filter-sterilized ambient air was injected into each vial, and then 5 mL of the pressurized headspace was analyzed for ^{13}C-labeled methane.

Results

The soluble methane profile of Yellowstone Lake water shows the presence of two peaks, one peak at 8m, and a broader peak located at 11.5m and 12m. Both peaks were located below the upper limit of the thermocline (Figure 4.3 A) and in >95 % oxygen saturated waters (Figure 4.3 B). Water from depths of interest were spiked with ^{13}C-labeled methanogen substrates show that 8m and 11.5m depth generate methane from Mpn, while 12m water generate methane using both MMA and Mpn (Figure 4.4). Concentration profiles of MMA, TMA and glycine betaine in the water column were quite similar, with MMA and glycine betaine being completely depleted at the 12m depth that coincided with the potential of the microbial community to transform MMA to methane (Figure 4.3 C). Regression analysis suggests there is a negative relationship between methane and these metabolites (Figure 4.5), and that the occurrence and concentration of these metabolites are correlated (Figure 4.6) and implies their metabolism is related.

The results of the physical data and water analysis collected from the three rivers is summarized in Tables 4.1 – 4.3. Each table contains recorded temperature, TDS, Chlorophyll a, Phycocyanin and pH collected at the sampling date as well as dissolved methane, MMA, DMA, TMA and glycine betaine reported in nM. The water flow data was obtained from the United States Geological Survey [USGS] national water information system website (https://waterdata.usgs.gov/nwis/rt). Oxygen reading were not reported as all river were saturated with oxygen (100% at the relevant temperature).

The water pH was slightly alkaline in all rivers but, fluctuated across sampling dates ranging between 8.2 - 8.4 in Madison river, 8.1 – 8.8 in the Gallatin and 7.7 – 9.0 in the Yellowstone. There was no relationship between water pH and temperature, TDS and water flow. Additionally, pH did not correlate to methane, glycine betaine or methylated amines concentrations.

Water temperatures peaked in mid-summer between July 16th and August 3rd at locations, and the lowest were recorded at the end of the sampling period. Comparing the temperature between the three rivers, Madison River had the highest average temperature followed by Yellowstone then Gallatin River. Broadly speaking, rivers with warmer water temperatures were associated with higher levers of chlorophyll a and phycocyanin (Figure 4.7 A & B), although no linear relationship was established between temperature, chlorophyll a and phycocyanin when examined as a function of all sampling dates. Phycocyanin levels were much lower than chlorophyll a as concentrations in Gallatin and Yellowstone Rivers did not exceed 0.09 and 0.1 ug/L respectively. In the Madison river, phycocyanin concentrations were also relatively lower than chlorophyll a levels,

including during the month of August, when concentrations were the highest and reached 0.65 ug/L, (Table 4.1).

Water flows decreased as the season progressed to autumn. The Yellowstone River had the highest water flows but the lowest TDS values (Figure 4.8). TDS was negatively correlated with water flow in all rivers; Madison river, $R^2 = 0.68$, $p<0.05$; Gallatin river, $R^2 = 0.92$, $p<0.05$; and Yellowstone river, $R^2 = 0.94$, $p<0.05$ (Figure 4.9).

Dissolved methane levels were highest in the Madison River followed by Yellowstone then Gallatin River (Figure 4.10). There was no apparent relationship between methane and temperature, chlorophyll a or phycocyanin. MMA, DMA, TMA and glycine betaine concentrations fluctuated, but with higher concentrations at later sampling points. Comparatively, glycine betaine levels were the lowest at all the time points, ranging between 0.6-5.7 nM in Madison river, 2.8-7.2 nM in Gallatin and 2.2-10.7 nM in Yellowstone river. TMA was the second lowest metabolite, followed by MMA then DMA, which had the highest concentration in all sampled rivers (Tables 4.1 - 4.3), ranging between 62 -936 nM in Madison river, 53 - 967 nM in Gallatin and 26 -1046 nM in Yellowstone river.

Regression analysis shows a moderately significance correlation between MMA and DMA ($R^2 = 0.67$, $p<0.05$) in the Madison river (Figure 4.11). However, no other relationship between metabolites was established in Madison River, as $R^2 = <0.2$ were considered weak. In the Gallatin River, a strong linear correlation was also observed between MMA and DMA ($R^2 = 0.94$, $p<0.05$), with both increasing in a similar pattern. However, MMA and TMA were not correlated ($R^2 = 0.32$, $p=0.15$) as were DMA and

TMA ($R^2 = 0.26$, p=0.19) (Figure 4.13). Glycine betaine was moderately correlated with MMA and DMA, ($R^2 = 0.33$, $p<0.05$) and ($R^2 = 0.53$, $p<0.05$) respectively, and glycine betaine and TMA were not correlated (Figure 4.14).

MMA and DMA were also strongly positively correlated in Yellowstone river as well. ($R^2= 0.85$, $p<0.05$). Correlations between MMA and TMA were weaker, but statistically significant ($R^2= 0.55$, $p<0.05$), while DMA and TMA concentrations were found to be strongly corelated ($R^2= 0.76$, $p<0.05$) (Figure 4.15). On the other hand, glycine betaine was strongly correlated with DMA ($R^2= 0.71$, $p<0.05$), and moderately correlated with MMA ($R^2= 0.41$, p=0.26) nor with TMA ($R^2= 0.51$, $p<0.26$) (Figure 4.16). Unlike lake water, correlation between methane and any of the four analyzed metabolites was not established. Finally, glycine betaine concentrations measured in all river were found to negatively correlate with chlorophyll a levels (Figure 4.17)

Discussion

The presence of methane in oxygen saturated waters in Yellowstone Lake and the microbial utilization of ^{13}C-MMA to produce ^{13}C-methane at 12m, support aerobic methane production using MMA as a substrate. This is further supported by the depletion of MMA where the methane peak was located and the negative correlation between measured MMA and methane. Strong correlations made between MMA, TMA and glycine betaine suggest a catabolic relation and that glycine betaine could be the source of methylated amines in the lake. Such observations support the TMA ➡TAMO ➡DMA

➡MMA degrading pathway. Demonstrating such correlation was challenging in rivers due to the dynamic complexity of such systems compared to a well-structured lake environment. In addition, relation between metabolites will depend on the extant biology, which will determine the relative activities of different known degradation pathways. However, MMA and DMA were found to always strongly correlate and thus could be metabolically related.

Recorded physical parameters and chemical analyses in the river study were not related to water pH, despite the basic properties of methylated amines. Fluctuating pH values throughout the study and differences between rivers suggest other biotic and abiotic factors are at play. The decrease in flow rate as the seasons progressed was expected with the seasonal reduction in snow melt at higher elevations. The strong evidence of an inverse correlation between water flow and TDS values are not unique and have been observed at other locations [284]. Higher flow rates lead to minimal interaction between river surface waters and the rocks and sediments on the river floor, resulting in a dilution effect on TDS levels [283].

A higher algal bloom was observed in Madison and Yellowstone Rivers between July 16th and August 3th, when water temperatures were highest. The Gallatin River had a lower average temperature, which was associated with lower chlorophyll a levels (Figure 4.7). Such observations support the fact that temperature is an important factor that drives the photosynthetic rate. Optimal temperature for freshwater algae ranges between 20 -30 °C [285]. Specifically, chlorophytes and diatoms, two common freshwater algal species, have an optimum growth temperature of (25.7 ± 0.1 °C) and

(24.0 ± 0.4 °C) respectively [286]. On the other hand, the optimal growth temperature for cyanobacteria is (30.6 ± 2.3 °C) [286], which is higher than observed temperatures in Gallatin and Yellowstone Rivers, where phycocyanin levels were relatively low. In the Madison River, phycocyanin levels increased slightly to 0.65 ug/L when temperatures reached 20°C, but temperatures did not exceed that mark, limiting cyanobacteria growth in comparison to algae. Consequently, no relationship was found between phycocyanin levels and the analyzed metabolites at each river.

The differences in dissolved methane between rivers suggests that methane sources and sinks vary between rivers. This may reflect different abundances of methanogens and methanotrophs, although this was not examined. Rivers with relatively higher mean temperatures had higher soluble methane concentrations, suggesting that temperature is a supporter of methane synthesis. The absence of correlation between methane and measured metabolites, points towards a complex metabolic degradation system that is yet to be fully understood. Presumably, the presence and concentrations of these metabolites are governed by the extant microbial community [246].

Higher DMA concentrations in comparison to MMA and TMA can be explained by higher water to atmospheric diffusion rates of MMA and TMA, as they have a boiling point of 3 and -6 °C, respectively, while DMA's boiling point is 7 - 9 °C. Such observations correspond with methylated amines modeling studies that conclude higher atmospheric concentrations of MMA and TMA than DMA [262]. Furthermore, glycine betaine degradation cannot account for all the methylated amines due to differences in concentrations. Thus, other sources (i.e., organic nitrogen compounds degradation) could

be of significance in freshwater rivers. In addition, low levels of glycine betaine are attributed to low salinity of freshwater systems, as glycine betaine is used by aquatic organisms as a protective osmolyte.

The negative linear correlation between chlorophyll a and glycine betaine, suggests that algae are an important source of glycine betaine in sampled rivers (Figure 4.17). Based on observations of this study, I hypothesize that as summer months are over and water temperatures decrease, algae die-off begins, resulting in degradation of their biomass and releasing glycine betaine. Glycine betaine is further degraded to its derivatives at high rates, but cannot account for all soluble methylated amines. Therefore, further investigation of other possible sources for glycine betaine and its metabolic products is needed. In addition, the lack of water microbial community profiles made it difficult to explain the differences in correlation significance between metabolites, which I attribute to varying microbial activity. The observed differences in temperature, phycocyanin and methane levels support the suggestion that the biology of these rivers is different.

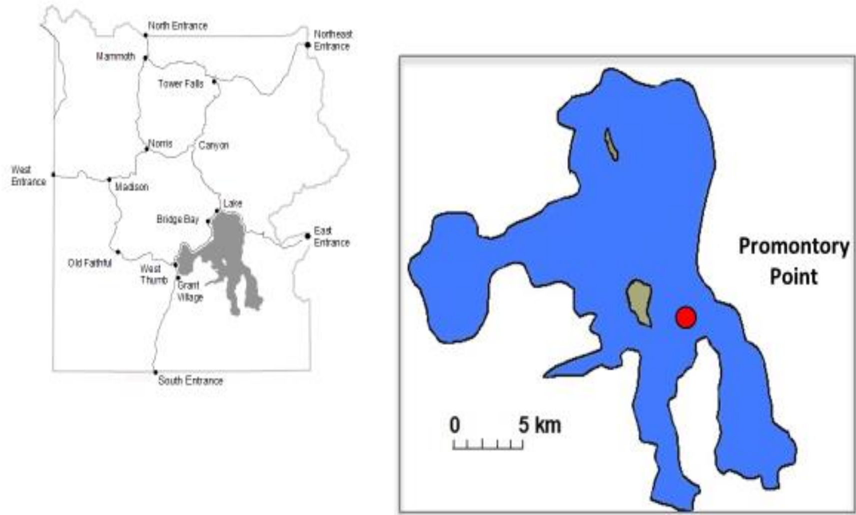


Figure 4.1 General map of Yellowstone National Park with Yellowstone lake shown. Red dot indicates the approximate sampling location near Promontory Point.

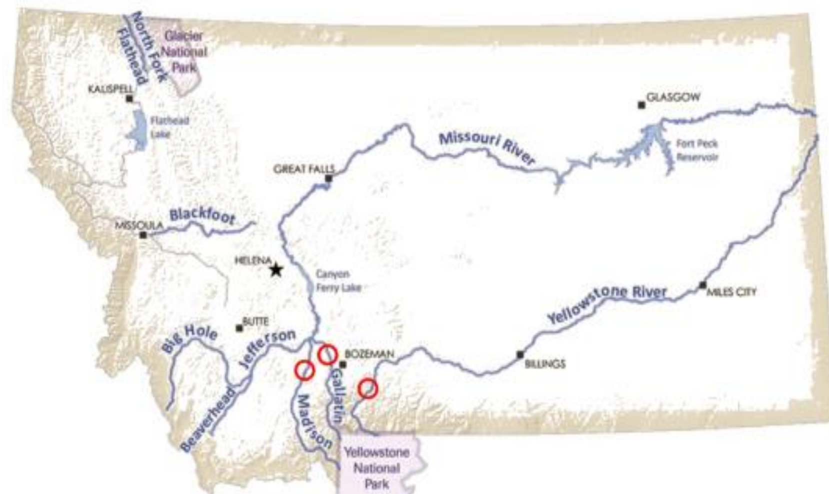


Figure 4.2 General map of the state of Montana with major rivers as shown. Red circles indicates the approximate sampling location at Madison river, Gallatin river and Yellowstone river.

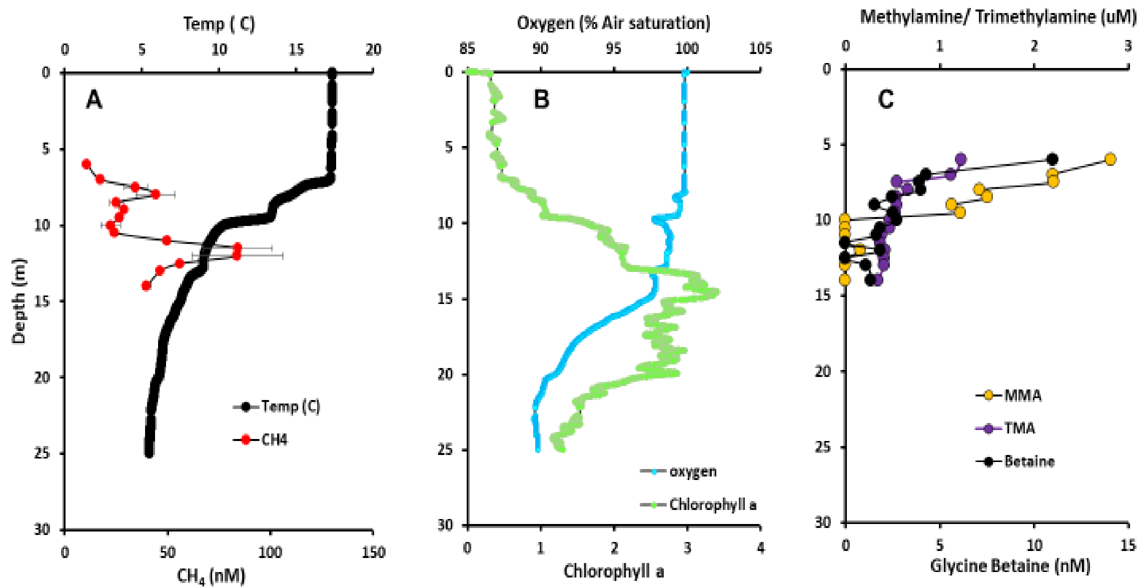


Figure 4.3 Depth profiles of methane concentration, measured metabolites and environmental parameters in the upper water column of Yellowstone Lake. **A)** Methane and temperature readings. **B)** Oxygen and chlorophyll a. **C)** Concentrations of MMA, TMA and glycine betaine.

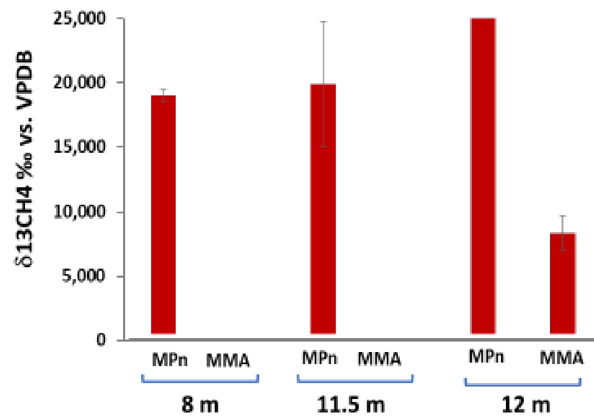


Figure 4.4 ^{13}C-Methane generation profiles of raw water samples spiked with ^{13}C-labeled methylphosphonate (MPn) or methylamine (MeA).

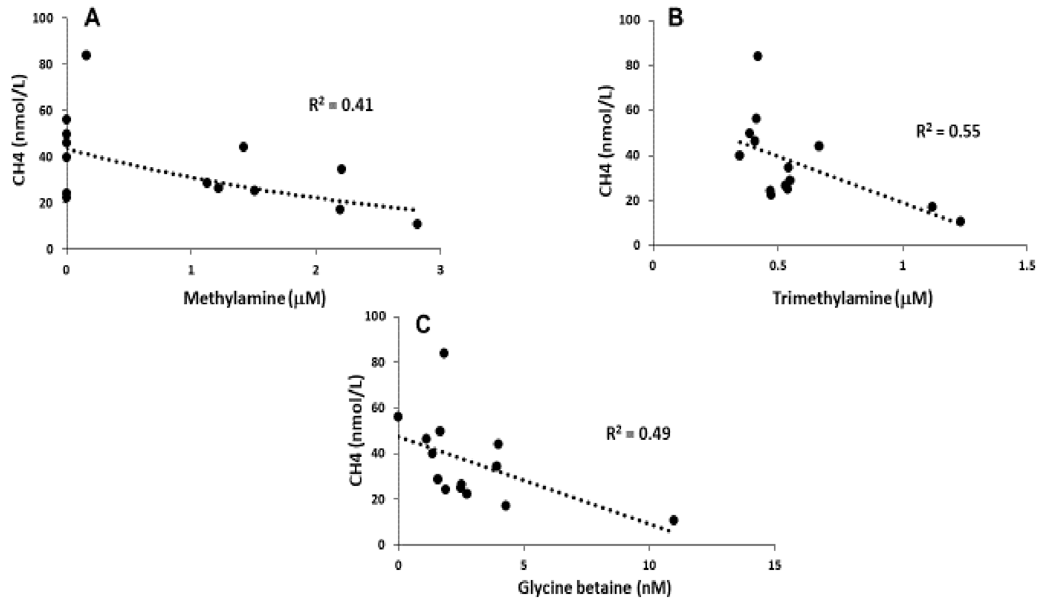


Figure 4.5 Regression plots illustrating the potential relationships between methane and methylated amines. A) CH_4 versus MMA, B) CH_4 versus TMA, and C) CH_4 versus glycine betaine.

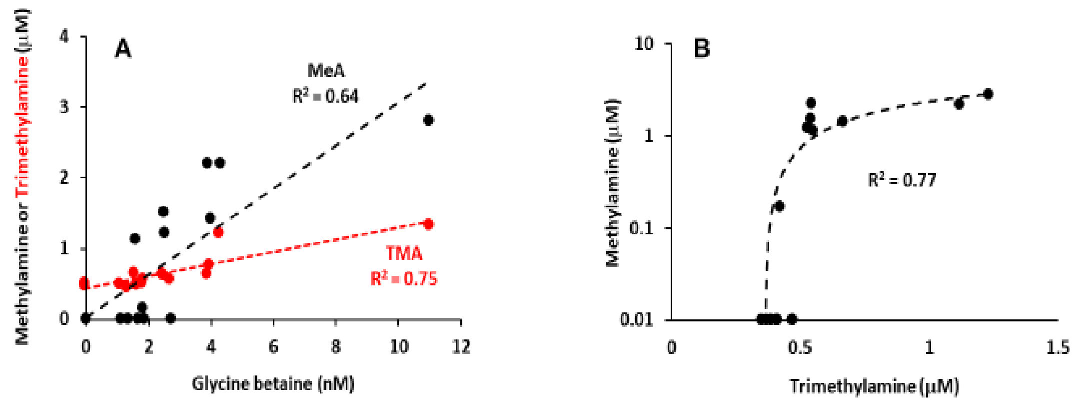


Figure 4.6 Regression plots illustrating potential relationships between methylated amines and glycine betaine. **A)** MMA and TMA versus glycine betaine, and **B)** MMA versus TMA.

Julian Date	Temperature (°C)	TDS (mg/L)	Chlorophyll a (ug/L)	Phycocyanin (ug/L)	pH	Water flow (ft³/sec)	Methane (nM)	MMA (nM)	DMA (nM)	TMA (nM)	Glycine Betaine (nM)
162	14	97	1.5	0.2	8.2	3700	-1.3	3.7	213.8	93.8	4.3
184	17.5	112	1.4	0.05	8.3	2590	6.5	85.2	499.5	72.1	2.3
197	21.4	127	2.4	0.1	8.4	2120	8.4	92.9	382.6	75.9	1.2
215	20.8	130	3.1	0.61	8.7	1870	4.2	59.6	166.4	46.7	1.1
229	19.6	132	2.2	0.65	8.6	1530	17.2	13.4	62.4	22.9	5.4
246	16.2	125	1.7	0.07	8.3	1510	16.8	70.3	721.8	36.7	2.5
260	14	124	1.1	0.06	8.3	1500	9.4	164.7	936.1	37.5	3.6
278	9.4	111	3.1	0.1	8.4	1780	-1.3	68.8	238.9	10.4	1.7

Table 4.1 Physical and chemical measurements collected from the Madison river at different sampling dates in 2018.

Julian Date	Temperature (°C)	TDS (mg/L)	Chlorophyll a (ug/L)	Phycocyanin (ug/L)	pH	Water flow (ft³/sec)	Methane (nM)	MMA (nM)	DMA (nM)	TMA (nM)	Glycine Betaine (nM)
162	5	63	0.8	0.07	8.1	3300	-3.6	0.4	53.4	14.2	3.4
184	10.1	90	0.7	0.06	8.4	1800	-4.4	41.7	170.3	4.6	4.3
197	14.2	120	0.3	0.03	8.6	1110	-4.7	44.7	195.7	3.4	3.6
215	13.2	134	0.4	0.09	8.8	740	-4.3	34.6	171.3	0.9	3.9
229	13.4	143	0.2	0.03	8.8	576	-4.5	34.2	376.2	7.6	10
246	9.9	135	0.2	0.04	8.5	517	-4.7	163.8	967.1	44.9	7.9
260	9	135	0.2	0.05	8.6	474	-3.9	38.6	153.7	40.9	4.7
278	6.5	118	0.5	0.04	8.8	603	-4.1	135.4	855.2	18.7	7.7

Table 4.2 Physical and chemical measurements collected from the Gallatin river at different sampling dates in 2018.

Julian Date	Temperature (°C)	TDS (mg/L)	Chlorophyll a (ug/L)	Phycocyanin (ug/L)	pH	Water flow (ft^3/sec)	Methane (nM)	MMA (nM)	DMA (nM)	TMA (nM)	Glycine Betaine (nM)
162	9.5	40	1.2	0.07	7.7	18400	3.8	35.4	26.4	9.8	4.3
184	14.3	54	0.5	0.1	7.9	10500	2.3	13.1	50.3	11.5	4.3
197	18.2	69	0.9	0.1	8.7	7780	3.7	47.4	156.4	10.8	6.7
215	17.9	82	1.5	0.1	8.7	5030	5.6	84.4	98.5	11.9	2.2
229	18.4	94	0.4	0.03	8.6	3060	4.8	20.1	61.1	12.8	6.8
246	15.2	96	0.5	0.04	8.8	2420	3.2	128.4	940.1	23.3	10.8
260	14.2	103	0.4	0.04	9	1970	2.2	194.5	1046.1	24.6	9.4
278	10	97	0.6	0.05	8.6	2030	1.6	109.8	475.4	8.587	7.6

Table 4.3 Physical and chemical measurements collected from the Yellowstone river at different sampling dates in 2018.

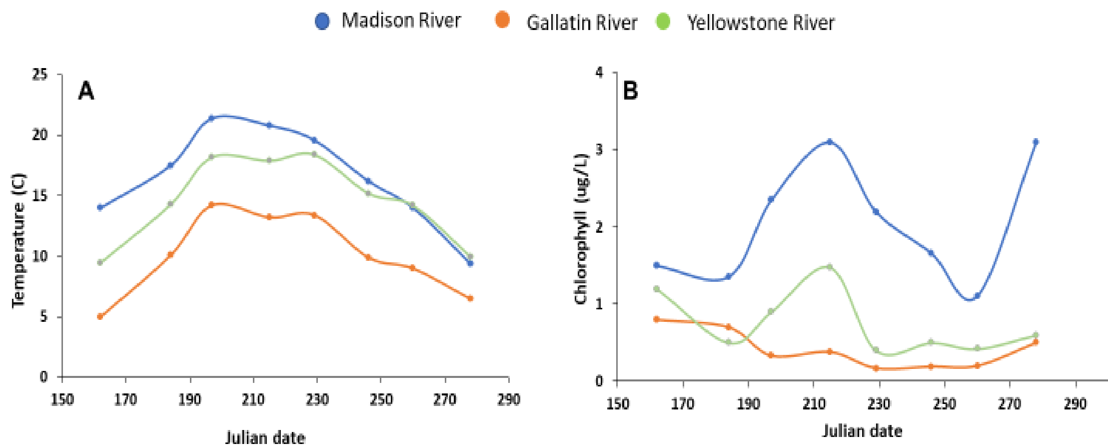


Figure 4.7 Temperature and chlorophyll a readings as a function of date and season. **A)** Temperature, and **B)** Chlorophyll a in sampled rivers.

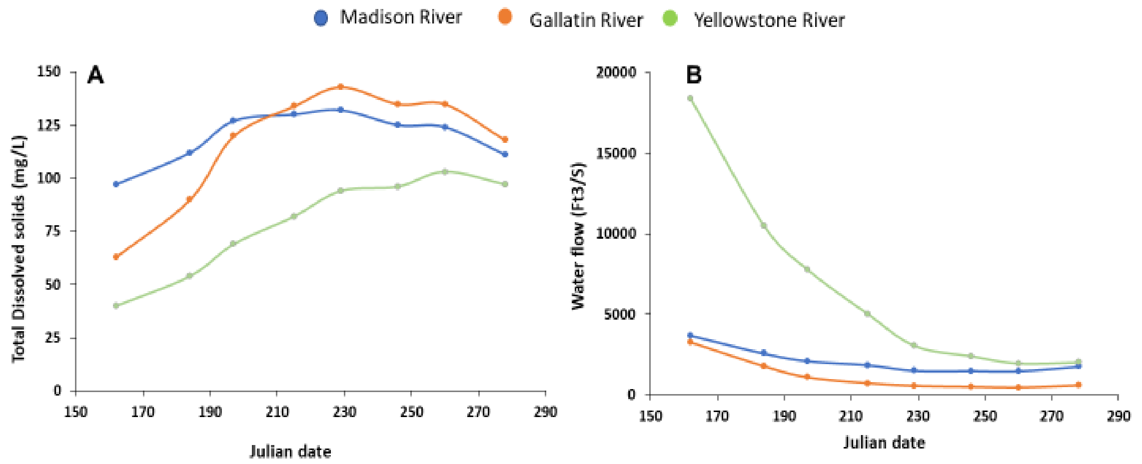


Figure 4.8 Total dissolved solids and water flow readings. **A)** Total dissolved solids, and **B)** water flow at sampled rivers.

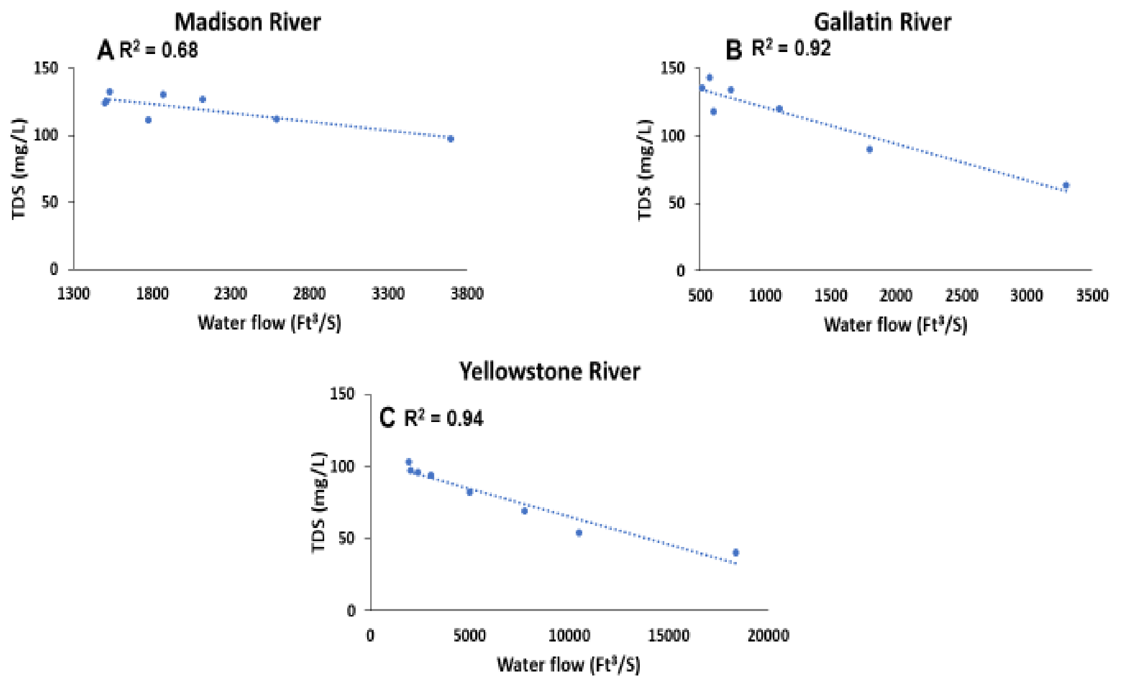


Figure 4.9 Regression plots illustrating potential relationships between total suspended solids and water flow in **A)** Madison river, **B)** Gallatin river, and **C)** Yellowstone river.

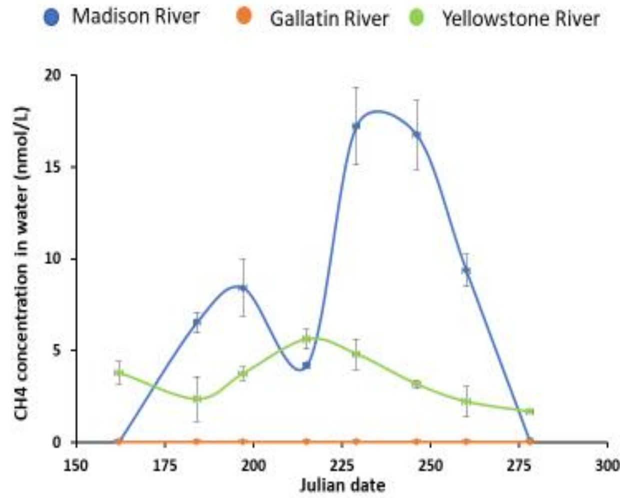


Figure 4.10 Line graph showing water soluble methane concentrations at sampled rivers over time.

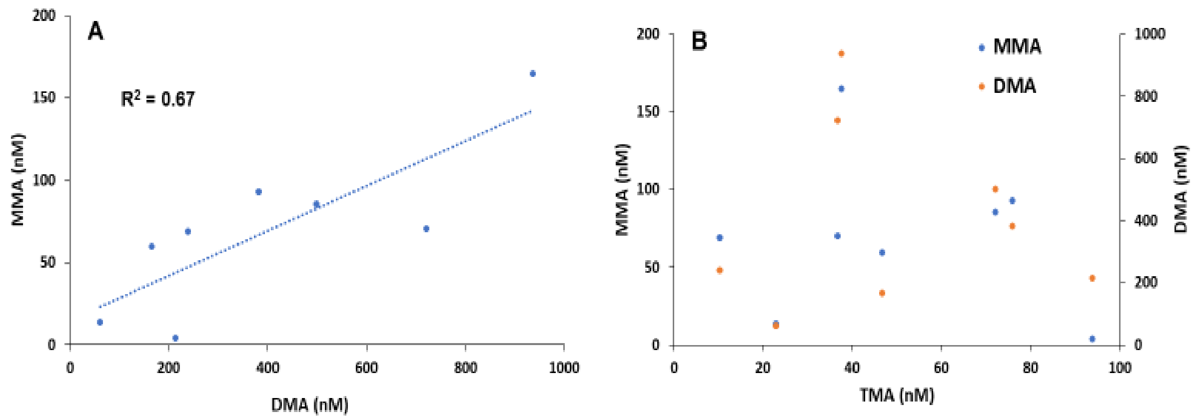


Figure 4.11 Regression plots illustrating potential relationship found between measured metabolites in Madison river. **A)** MMA in relation to DMA, **B)** MMA and DMA in relation to TMA.

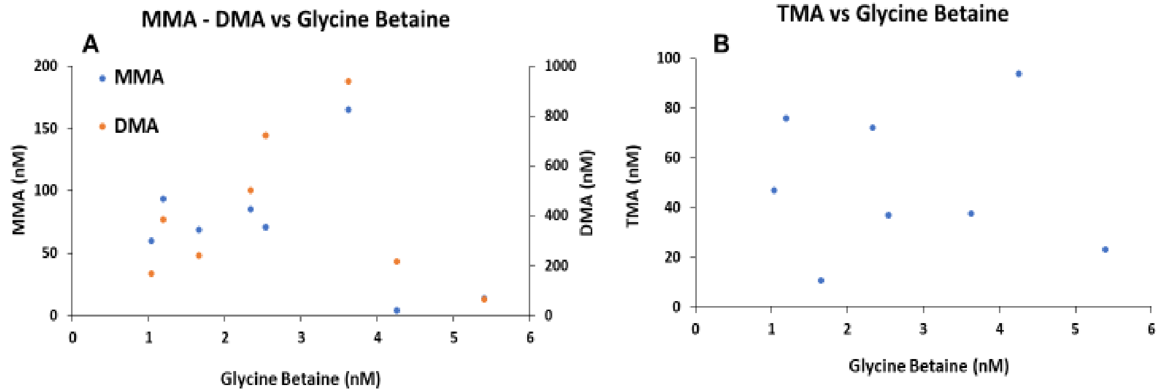


Figure 4.12 Regression plots illustrating potential relationships between measured metabolites in the Madison river. **A)** MMA and DMA in relation to glycine betaine, and **B)** TMA in relation to glycine betaine.

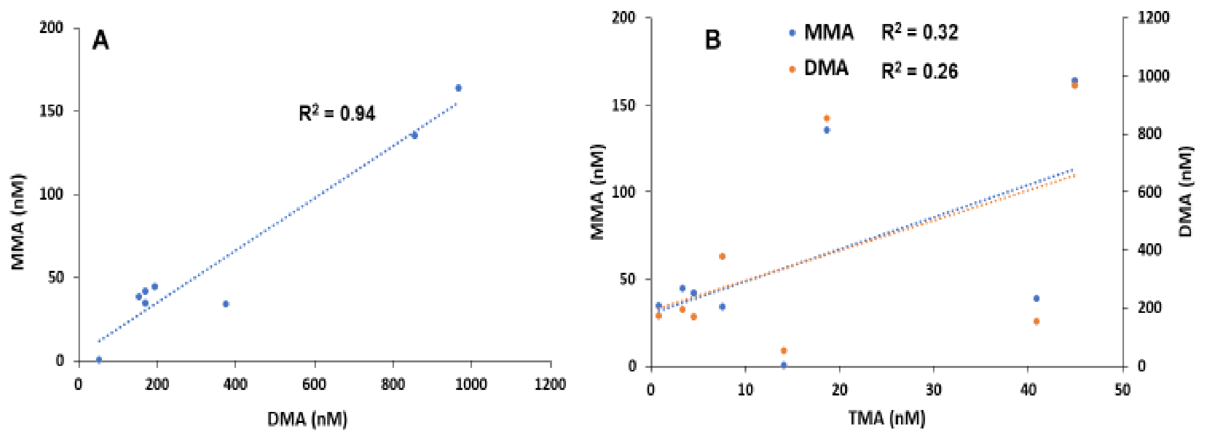


Figure 4.13 Regression plots illustrating potential relationships between measured metabolites in Gallatin river. **A)** MMA in relation to DMA, and **B)** MMA and DMA in relation to TMA.

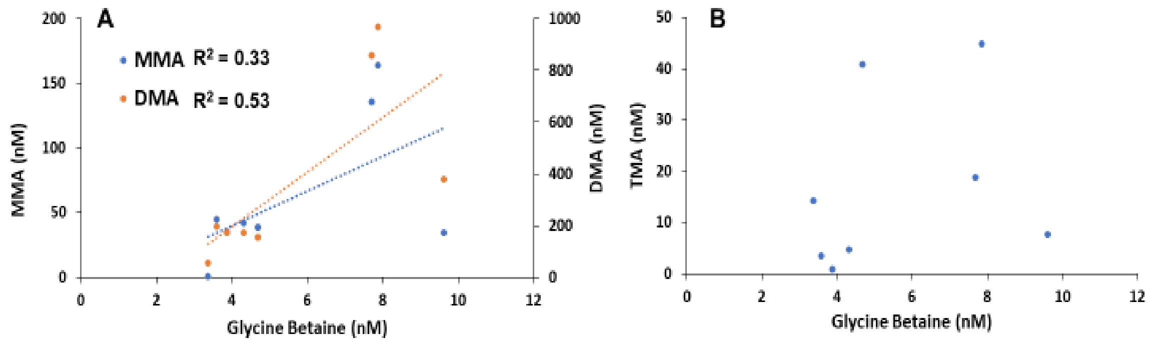


Figure 4.14 Regression plots illustrating potential relationships between between measured metabolites in the Gallatin river. **A)** MMA and DMA in relation to glycine betaine, and B) TMA in relation to glycine betaine.

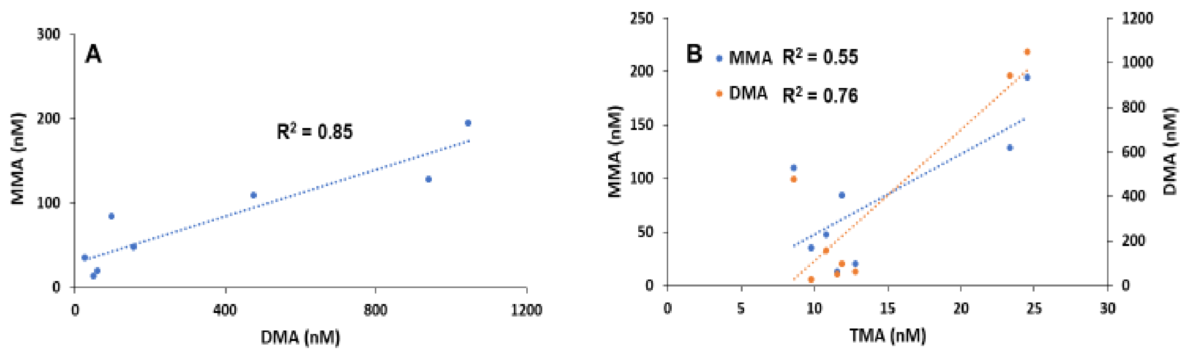


Figure 4.15 Regression plots illustrating potential relationships between measured metabolites in the Yellowstone river. **A)** MMA in relation to DMA, and **B)** MMA and DMA in relation to TMA.

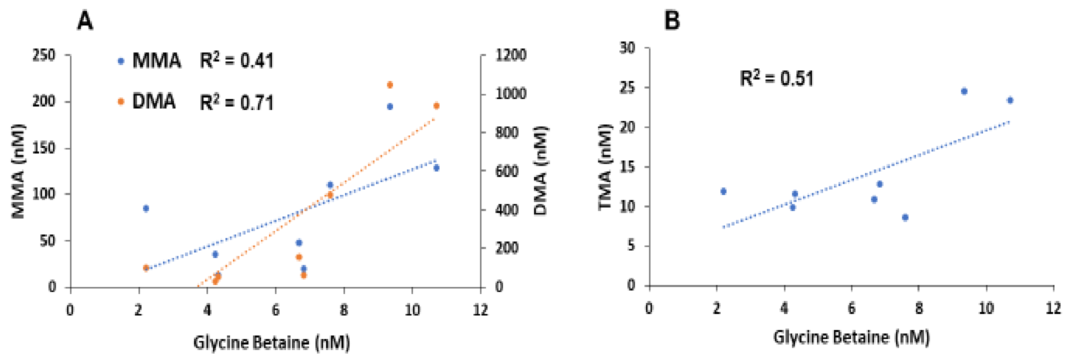


Figure 4.16 Regression plots illustrating potential relationships between measured metabolites in the Yellowstone river. **A)** MMA and DMA in relation to glycine betaine, and **B)** Linear relationship between TMA in relation to glycine betaine.

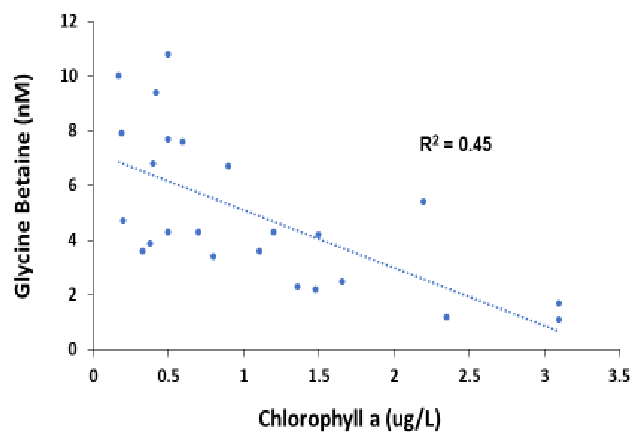


Figure 4.17 Regression analysis illustrating potential relationship between Chlorophyll a and glycine betaine. (R^2 = 0.45, P. <0.05).

References

[209] L. Murdock *et al.*, “Analysis of strains lacking known osmolyte accumulation mechanisms reveals contributions of osmolytes and transporters to protection against abiotic stress,” *Appl. Environ. Microbiol.*, 2014.

[210] R. Obeid, “The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway,” *Nutrients*. 2013.

[215] H. Kageyama, Y. Tanaka, and T. Takabe, “Biosynthetic pathways of glycinebetaine in Thalassiosira pseudonana; functional characterization of enzyme catalyzing three-step methylation of glycine,” *Plant Physiol. Biochem.*, 2018.

[220] C. Chen and G. A. Beattie, “Pseudomonas syringae BetT is a low-affinity choline transporter that is responsible for superior osmoprotection by choline over glycine betaine,” *J. Bacteriol.*, 2008.

[225] R. Waditee *et al.*, “Isolation and functional characterization of N-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism Aphanothece halophytica,” *J. Biol. Chem.*, 2003.

[232] C. Benning, Z. H. Huang, and D. A. Gage, “Accumulation of a novel glycolipid and a betaine lipid in cells of rhodobacter sphaeroides grown under phosphate limitation,” *Arch. Biochem. Biophys.*, 1995.

[235] O. Geiger, V. Röhrs, B. Weissenmayer, T. M. Finan, and J. E. Thomas-Oates, “The regulator gene phoB mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-N,N,N-trimethylhomoserine in Rhizobium (Sinorhizobium) meliloti,” *Mol. Microbiol.*, 1999.

[236] I. Khozin-Goldberg and Z. Cohen, “The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte Monodus subterraneus,” *Phytochemistry*, 2006.

[246] A. J. Watkins, E. G. Rousse, R. J. Parkes, and H. Sass, “Glycine betaine as a direct substrate for methanogens (methanococcoides spp.),” *Appl. Environ. Microbiol.*, 2014.

[247] B. Möller, R. Oßmer, B. H. Howard, G. Gottschalk, and H. Hippe, “Sporomusa, a new genus of gram-negative anaerobic bacteria including Sporomusa sphaeroides spec. nov. and Sporomusa ovata spec. nov.,” *Arch. Microbiol.*, 1984.

[251] E. L. Barrett and H. S. Kwan, “Bacterial Reduction of Trimethylamine Oxide,” *Annu. Rev. Microbiol.*, 1985.

[252] C. M. Large PJ, Boulton CA, “The reduced nicotinamide-adenine dinuclotide phosphate- and oxygen-dependent N-oxygenation of trimethylamine by Pseudomonas aminovorans,” *Biochem*, vol. 128, pp. 137–138, 1972.

[253] Y. Chen, N. A. Patel, A. Crombie, J. H. Scrivens, and J. C. Murrell, “Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase,” *Proc. Natl. Acad. Sci.*, 2011.

[256] T. C. Tallant and J. A. Krzycki, “Methylthiol:coenzyme M methyltransferase from Methanosarcina barkeri, an enzyme of methanogenesis from dimethylsulfide and methylmercaptopropionate,” *J. Bacteriol.*, 1997.

[257] D. J. Ferguson, N. Gorlatova, D. A. Grahame, and J. A. Krzycki, “Reconstitution of dimethylamine:coenzyme M methyl transfer with a discrete corrinoid protein and two methyltransferases purified from Methanosarcina barkeri,” *J. Biol. Chem.*, 2000.

[262] F. Yu and G. Luo, “Modeling of gaseous methylamines in the global atmosphere: Impacts of oxidation and aerosol uptake,” *Atmos. Chem. Phys.*, 2014.

[267] G. J. Schut *et al.*, “ The catalytic mechanism of electron-bifurcating electron transfer flavoproteins (ETFs) involves an intermediary complex with NAD + ,” *J. Biol. Chem.*, 2018.

[268] D. W. Johnson, “A flow injection electrospray ionization tandem mass spectrometric method for the simultaneous measurement of trimethylamine and trimethylamine N-oxide in urine,” *J. Mass Spectrom.*, 2008.

[269] D. A. Wiesenburg and N. L. Guinasso, “Equilibrium Solubilities of Methane, Carbon Monoxide, and Hydrogen in Water and Sea Water,” *J. Chem. Eng. Data*, 1979.

[283] S. D. McCormick, “Endocrine control of osmoregulation in teleost fish,” *Am. Zool.*, 2001.

[284] V. N. Sinyukovich, “Relationships between Water Flow and Dissolved Solids Discharge in the Major Tributaries of Lake Baikal,” *Water Resour.*, vol. 30, no. 2, pp. 186–190, 2003.

[286] J. O. Nalley, D. R. O’Donnell, and E. Litchman, “Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria,” *Algal Res.*, 2018.

[287] Qian Wang, Abdullah Alowaifeer, Patricia Kerner, Narayanaganesh Balasubramanian, Angela Patterson, John E. Dore, Brian Bothner, and Timothy R. McDermott “Aerobic bacterial metabolism of methylamine or glycine betaine generates methane, contributing to methane oversaturation in oxic waters”

CHAPTER FIVE

CONCLUDING REMARKS

Mass spectrometry is a very useful tool in the scientific research field if used in the correct manner. In my thesis work I present multiple conclusions derived by combining chemistry, laboratory techniques, and selection of the correct instrument. The absence of standards, instrument of choice or accessibility issues required working around such difficulties to achieve my goals. For instance, methylated amines gas standards were expensive to purchase and are commonly detected on GC with a flame ionized detector equipped with a packed column, which our laboratory lacked. These difficulties were overcome by using labeling techniques to conserve methylated amines in the liquid phase, therefore analyzing labeled amines with liquid chromatography, which was available in our laboratory with the possibility to be customized with different mobile phases and columns. Compounds of interests can be analyzed by many instruments and using different techniques but working with available tools and accessible funds is the challenge. Rising technical issues during analysis such as leaks, poor separation or high background were other obstacles that had to be dealt with. For instance, during the analysis of arsenic species arsenobetaine and arsenite were not chromatographically separating. Knowing that arsenite can be oxidized to arsenate, we were able to use hydrogen peroxide and quantify arsenite by calculating the amount of oxidized material. This approach is an example of using chemistry knowledge to troubleshoot analytical problems. Resolution and sensitivity are other crucial elements in

assessing the instrument of choice and setting limits of detection. Such specifications are improved with each new instrument generation, which makes it impractical to get my hands on the latest technology for my research. However, resolution and sensitivity can be improved to lower detection limits by many approaches. Such approaches include column equilibration, manipulating flow rate and selecting the correct column specification.

My interest in arsenic and mercury grew from their occurrence in the environment, especially in aquatic systems. Sea food is important diet source due to its high omega 3 levels in addition to its rich protein content. Sea products are consumed regularly by many and even on a daily basis by populations living near coastal areas. Therefore, the safety of such products is an important governmental responsibility to ensure consumer general health. Governmental agencies have set consumption permissible limits for arsenic and mercury based on total elemental concentrations. For mercury, the speciation analysis is both expensive and of great risk to technicians. Plus, both inorganic and methyl forms are extremely toxic, although methylmercury is more toxic than inorganic mercury. So, it was argued that differentiating is not advisable as permissible limits can be set based on a total concentration.

On the other hand, arsenic can be found in many versions, which have a wide range of toxic effects. Thus, setting limits based on a total concertation is not practical or potentially misleading. My results show that correlation can not be made by the amount of total arsenic, the toxic inorganic forms, or the non-toxic form arsenobetaine. Such arsenicals varied between species, trophic levels as well as environments, when

comparing my freshwater results to reported marine fish concentrations. In addition, the toxicity of many arsenicals remains unknown. The process at which arsenic sugars are formed, broken down or their toxic effects are yet to be fully understood. Similarly, thiol arsenicals are not studied very well. High arsenobetaine levels in freshwater fish, which is thought to be related to salinity osmolyte glycine betaine, suggest a complex detoxification mechanism that is yet to be understood. Discovering the underlying biosynthetic pathways and genetics, can give insight on to how aquatic animals can deal with arsenic toxicity. From which, new ideas can be generated to protect humans being exposed to high levels of toxic arsenicals. Therefore, I think more investment in arsenic related research is needed and more importantly, permissible levels for human aquatic food consumption need to be revised. Arsenic admissible levels should be set on a compound bases rather than a total value. Such goals can be achieved sufficiently, as arsenic speciation cost is decreasing, and mass spectrometry instruments are improving.

In my river research I was able to show a correlation between glycine betaine and algae (chlorophyll a) in Madison river, however such correlations were not obvious and need model generations. In my opinion, the relatively cold-water temperatures limited algal growth, which made it difficult to correlate with already low glycine betaine levels in freshwaters. Since glycine betaine has been shown to be related with salinity, I think studying its correlation with algal production in marine systems with higher average temperatures will yield better conclusions. Glycine betaine is a very interesting molecule, as it contains a nitrogen and a carbon atom, both which are essential elements in biology. Carbon and nitrogen have very complex biotic cycles and both atoms interact in glycine

betaine and its precursors. In addition, glycine betaine can be used by many microbes as a nitrogen and/or a carbon source. Moreover, glycine betaine can be broken-down to produce methane or ammonia, which are direct and indirect greenhouse gases. All the above emphasizes the importance of such molecule in biology, which has not gotten much scientific attention and its direct role in regard to cellular protection is yet to be fully understood.

REFERENCES CITED

[1] "Arsenic and arsenic compounds," *IARC Monogr. Eval. Carcinog. Risks to Humans*, 2011.

[2] J. F. Stolz and R. S. Oremland, "Bacterial respiration of arsenic and selenium," *FEMS Microbiol. Rev.*, 1999.

[3] R. E. Macur, J. T. Wheeler, T. R. McDermott, and W. P. Inskeep, "Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings," *Environ. Sci. Technol.*, 2001.

[4] R. S. Oremland, C. W. Saltikov, F. Wolfe-Simon, and J. F. Stolz, "Arsenic in the evolution of earth and extraterrestrial ecosystems," *Geomicrobiol. J.*, 2009.

[5] T. R. Kulp *et al.*, "Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California," *Science (80-.).*, 2008.

[6] G. L. Anderson, J. Williams, and R. Hille, "The purification and characterization of arsenite oxidase from Alcaligenes faecalis, a molybdenum-containing hydroxylase," *J. Biol. Chem.*, 1992.

[7] R. Mukhopadhyay, B. P. Rosen, L. T. Phung, and S. Silver, "Microbial arsenic: From geocycles to genes and enzymes," *FEMS Microbiology Reviews*. 2002.

[8] J. Messens and S. Silver, "Arsenate Reduction: Thiol Cascade Chemistry with Convergent Evolution," *Journal of Molecular Biology*. 2006.

[9] D. Ahmann, A. L. Roberts, L. R. Krumholz, and F. M. M. Morel, "Microbe grows by reducing arsenic [7]," *Nature*. 1994.

[10] J. F. Stolz, P. Basu, J. M. Santini, and R. S. Oremland, "Arsenic and Selenium in Microbial Metabolism," *Annu. Rev. Microbiol.*, 2006.

[11] R. Bentley and T. G. Chasteen, "Microbial Methylation of Metalloids: Arsenic, Antimony, and Bismuth," *Microbiol. Mol. Biol. Rev.*, 2002.

[12] J. Qin, B. P. Rosen, Y. Zhang, G. Wang, S. Franke, and C. Rensing, "Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase," *Proc. Natl. Acad. Sci.*, 2006.

[13] C. Fan, G. Liu, Y. Long, B. Rosen, and Y. Cai, "Thiolation in arsenic metabolism: a chemical perspective," *Metallomics*. 2018.

[14] F. Challenger, "Biological Methylation," *Chem. Rev*, vol. 36, pp. 315–361, 1945.

[15] J. Qin, C. R. Lehr, C. Yuan, X. C. Le, T. R. McDermott, and B. P. Rosen, “Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga,” *Proc. Natl. Acad. Sci.*, 2009.

[16] A. A. Ajees and B. P. Rosen, “As(III) S-Adenosylmethionine Methyltransferases and Other Arsenic Binding Proteins,” *Geomicrobiol. J.*, 2015.

[17] T. Hayakawa, Y. Kobayashi, X. Cui, and S. Hirano, “A new metabolic pathway of arsenite: Arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19,” *Arch. Toxicol.*, 2005.

[18] K. Marapakala, J. Qin, and B. P. Rosen, “Identification of catalytic residues in the As(III) S-adenosylmethionine methyltransferase,” *Biochemistry*, 2012.

[19] J. Chen, H. Bhattacharjee, and B. P. Rosen, “ArsH is an organoarsenical oxidase that confers resistance to trivalent forms of the herbicide monosodium methylarsenate and the poultry growth promoter roxarsone,” *Mol. Microbiol.*, 2015.

[20] M. Yoshinaga and B. P. Rosen, “A C*As lyase for degradation of environmental organoarsenical herbicides and animal husbandry growth promoters,” *Proc. Natl. Acad. Sci.*, 2014.

[21] B. Planer-Friedrich, J. London, R. B. Mccleskey, D. K. Nordstrom, and D. Wallschläger, “Thioarsenates in geothermal waters of yellowstone National Park: Determination, preservation, and geochemical importance,” *Environ. Sci. Technol.*, 2007.

[22] V. K. Stucker, K. H. Williams, M. J. Robbins, and J. F. Ranville, “Arsenic geochemistry in a biostimulated aquifer: An aqueous speciation study,” *Environ. Toxicol. Chem.*, 2013.

[23] J. A. Smieja and R. T. Wilkin, “Preservation of sulfidic waters containing dissolved AS(III),” *J. Environ. Monit.*, 2003.

[24] S. Stauder, B. Raue, and F. Sacher, “Thioarsenates in sulfidic waters,” *Environ. Sci. Technol.*, 2005.

[25] K. A. Rittle, J. I. Drever, and P. J. S. Colberg, “Precipitation of arsenic during bacterial sulfate reduction,” *Geomicrobiol. J.*, 1995.

[26] D. B. Senn and H. F. Hemond, “Nitrate controls on iron and arsenic in an urban lake,” *Science (80-.).*, 2002.

[27] B. P. Rosen and Z. Liu, “Transport pathways for arsenic and selenium: A minireview,” *Environment International*. 2009.

[28] R. A. Goyer and T. W. Clarkson, “Toxic effects of metals.,” in *Casarett and Doull’s Toxicology: The Basic Science of Poisons.*, 2001.

[29] H. Bhattacharjee, R. Mukhopadhyay, S. Thiyagarajan, and B. P. Rosen, “Aquaglyceroporins: Ancient channels for metalloids,” *J. Biol.*, 2008.

[30] S. Li, Y. Chen, and B. P. Rosen, “Role of vicinal cysteine pairs in metalloid sensing by the ArsD As(III)-responsive repressor,” *Mol. Microbiol.*, 2001.

[31] H. Naranmandura, N. Suzuki, K. Iwata, S. Hirano, and K. T. Suzuki, “Arsenic metabolism and thioarsenicals in hamsters and rats,” *Chem. Res. Toxicol.*, 2007.

[32] T. Ochi, K. Kita, T. Suzuki, A. Rumpler, W. Goessler, and K. A. Francesconi, “Cytotoxic, genotoxic and cell-cycle disruptive effects of thio-dimethylarsinate in cultured human cells and the role of glutathione,” *Toxicol. Appl. Pharmacol.*, 2008.

[33] K. J. Reimer and J. A. Thompson, “Arsenic speciation in marine interstitial water. The occurrence of organoarsenicals,” *Biogeochemistry*, 1988.

[34] G. Tingzong, R. D. DeLaune, and W. H. Patrick, “The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium, and zinc in estuarine sediment,” *Environ. Int.*, 1997.

[35] G. F. Riedel, J. G. Sanders, and R. W. Osman, “The effect of biological and physical disturbances on the transport of arsenic from contaminated estuarine sediments,” *Estuar. Coast. Shelf Sci.*, 1987.

[36] P. H. Masscheleyn, R. D. Delaune, and W. H. Patrick, “Arsenic and Selenium Chemistry as Affected by Sediment Redox Potential and pH,” *J. Environ. Qual.*, 1991.

[37] M. Takeuchi *et al.*, “Distribution and fate of biologically formed organoarsenicals in coastal marine sediment,” *Appl. Organomet. Chem.*, 2005.

[38] M. Giordano, J. Beardall, and J. A. Raven, “CO_2 CONCENTRATING MECHANISMS IN ALGAE: Mechanisms, Environmental Modulation, and Evolution,” *Annu. Rev. Plant Biol.*, 2005.

[39] J. L. Levy, J. L. Stauber, M. S. Adams, W. A. Maher, J. K. Kirby, and D. F. Jolley, “Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (Chlorella sp. and Monoraphidium arcuatum),” *Environ. Toxicol. Chem.*, 2005.

[40] L. A. Murray, A. Raab, I. L. Marr, and J. Feldmann, “Biotransformation of arsenate to arsenosugars by Chlorella vulgaris,” *Appl. Organomet. Chem.*, 2003.

[41] S. MIYASHITA, S. FUJIWARA, M. TSUZUKI, and T. KAISE, “Rapid Biotransformation of Arsenate into Oxo-Arsenosugars by a Freshwater Unicellular Green Alga, *Chlamydomonas reinhardtii*,” *Biosci. Biotechnol. Biochem.*, 2011.

[42] X. M. Xue *et al.*, “Arsenic Methyltransferase is Involved in Arsenosugar Biosynthesis by Providing DMA,” *Environ. Sci. Technol.*, 2017.

[43] J. S. Edmonds and K. A. Francesconi, “Transformations of arsenic in the marine environment,” *Experientia*. 1987.

[44] K. A. F. Xi-Mei Xue, Jun Ye, Georg Raber, Barry P. Rosen and and Y.-G. Z. Chan Xiong, Zhe Zhu, Christopher Rensing, “Identification of steps in the pathway of arsenosugar biosynthesis,” *Environ. Sci. Technol.*, 2018.

[45] J. Feldmann and E. M. Krupp, “Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs?,” *Anal. Bioanal. Chem.*, 2011.

[46] G. Caumette, I. Koch, and K. J. Reimer, “Arsenobetaine formation in plankton: A review of studies at the base of the aquatic food chain,” *J. Environ. Monit.*, 2012.

[47] J. S. Edmonds, K. A. Francesconi, J. R. Cannon, C. L. Raston, B. W. Skelton, and A. H. White, “Isolation, crystal structure and synthesis of arsenobetaine, the arsenical constituent of the western rock lobster panulirus longipes cygnus George,” *Tetrahedron Lett.*, 1977.

[48] M. B. Burg and J. D. Ferraris, “Intracellular organic osmolytes: Function and regulation,” *Journal of Biological Chemistry*. 2008.

[49] J. Gailer, K. J. Lrgolic, K. A. Francesconi, and J. S. Edmondsxs, “Metabolism of arsenic compounds by the blue mussel mytilus edulis after accumulation from seawater spiked with arsenic compounds,” *Appl. Organomet. Chem.*, 1995.

[50] L. A. Clowes and K. A. Francesconi, "Uptake and elimination of arsenobetaine by the mussel Mytilus edulis is related to salinity," *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.*, 2004.

[51] Z. Šlejkovec *et al.*, "Bioaccumulation of arsenic species in rays from the northern Adriatic Sea," *Int. J. Mol. Sci.*, 2014.

[52] P. Pengprecha, M. Wilson, A. Raab, and J. Feldmann, "Biodegradation of arsenosugars in marine sediment," *Appl. Organomet. Chem.*, 2005.

[53] S. Foster, D. Thomson, and W. Maher, "Uptake and metabolism of arsenate by anexic cultures of the microalgae Dunaliella tertiolecta and Phaeodactylum tricornutum," *Mar. Chem.*, 2008.

[54] Y. Shibata, M. Sekiguchi, A. Otsuki, and M. Morita, "Arsenic compounds in zoo- and phyto-plankton of marine origin," *Appl. Organomet. Chem.*, 1996.

[55] J. S. Edmonds, Y. Shibata, K. A. Francesconi, R. J. Rippingale, and M. Morita, "Arsenic Transformations in Short Marine Food Chains studied by HPLC-ICP MS," *Appl. Organomet. Chem.*, 1997.

[56] T. Kaise and S. Fukui, "The chemical form and acute toxicity of arsenic compounds in marine organisms," *Appl. Organomet. Chem.*, 1992.

[57] G. Caumette, I. Koch, E. Estrada, and K. J. Reimer, "Arsenic speciation in plankton organisms from contaminated lakes: Transformations at the base of the freshwater food chain," *Environ. Sci. Technol.*, 2011.

[58] S. Miyashita *et al.*, "Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS," *Chemosphere*, 2009.

[59] Suhendrayatna, A. Ohki, T. Nakajima, and S. Maeda, "Studies on the accumulation and transformation of arsenic in freshwater organisms II. Accumulation and transformation of arsenic compounds by Tilapia mossambica," *Chemosphere*, 2002.

[60] S. Foster and W. Maher, "Arsenobetaine and thio-arsenic species in marine macroalgae and herbivorous animals: Accumulated through trophic transfer or produced in situ?," *J. Environ. Sci. (China)*, 2016.

[61] S. García-Salgado, G. Raber, R. Raml, C. Magnes, and K. A. Francesconi, "Arsenosugar phospholipids and arsenic hydrocarbons in two species of brown macroalgae," *Environmental Chemistry*. 2012.

[62] B. A. S. Van Mooy *et al.*, "Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity," *Nature*, 2009.

[63] A. H. Petursdottir, K. Fletcher, H. Gunnlaugsdóttir, E. Krupp, F. C. Kupper, and J. Feldmann, "Environmental effects on arsenosugars and arsenolipids in Ectocarpus (Phaeophyta)," *Environ. Chem.*, 2016.

[64] A. Raab, C. Newcombe, D. Pitton, R. Ebel, and J. Feldmann, "Comprehensive analysis of lipophilic arsenic species in a brown alga (Saccharina latissima)," *Anal. Chem.*, 2013.

[65] R. Tukai, W. A. Maher, I. J. McNaught, M. J. Ellwood, and M. Coleman, "Occurrence and chemical form of arsenic in marine macroalgae from the east coast of Australia," *Mar. Freshw. Res.*, 2002.

[66] R. Tukai, W. A. Maher, I. J. McNaught, and M. J. Ellwood, "Measurement of arsenic species in marine macroalgae by microwave-assisted extraction and high performance liquid chromatography-inductively coupled plasma mass spectrometry," *Anal. Chim. Acta*, 2002.

[67] T. Kaise *et al.*, "Biomethylation of Arsenic in an Arsenic-rich Freshwater Environment," *Appl. Organomet. Chem.*, 1997.

[68] G. Caumette, I. Koch, M. Moriarty, and K. J. Reimer, "Arsenic distribution and speciation in Daphnia pulex," *Sci. Total Environ.*, 2012.

[69] T. A. and S. T. Kunito .T, R. Kubota, J. Fujihara, *Reviews of Environmental Contamination and Toxicology*. Springer, New York, 2008.

[70] J. Kirby, W. Maher, and D. Spooner, "Arsenic occurrence and species in near-shore macroalgae-feeding marine animals," *Environ. Sci. Technol.*, 2005.

[71] S. Khokiattiwong, N. Kornkanitnan, W. Goessler, S. Kokarnig, and K. A. Francesconi, "Arsenic compounds in tropical marine ecosystems: Similarities between mangrove forest and coral reef," *Environ. Chem.*, 2009.

[72] E. Duncan, S. Foster, and W. Maher, “Uptake and metabolism of arsenate, methylarsonate and arsenobetaine by axenic cultures of the phytoplankton Dunaliella tertiolecta,” *Bot. Mar.*, 2010.

[73] M. Azizur Rahman, H. Hasegawa, and R. Peter Lim, “Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain,” *Environmental Research*. 2012.

[74] G. Lunde, “Occurrence and transformation of arsenic in the marine environment,” *Environ. Health Perspect.*, 1977.

[75] K. A. Francesconi and J. S. Edmonds, “Arsenic and Marine Organisms,” *Adv. Inorg. Chem.*, 1996.

[76] J. Kirby, W. Maher, A. Chariton, and F. Krikowa, “Arsenic concentrations and speciation in a temperate mangrove ecosystem, NSW, Australia,” in *Applied Organometallic Chemistry*, 2002.

[77] J. Burger *et al.*, “Metal levels in fish from the Savannah river: Potential hazards to fish and other receptors,” *Environ. Res.*, 2002.

[78] J. P. Meador, D. W. Ernest, and A. Kagley, “Bioaccumulation of arsenic in marine fish and invertebrates from Alaska and California,” *Arch. Environ. Contam. Toxicol.*, 2004.

[79] K. M. Attar, M. Zamil El-Faer, T. N. Rawdah, and B. S. Tawabini, “Levels of arsenic in fish from the Arabian Gulf,” *Mar. Pollut. Bull.*, 1992.

[80] W. Zhang, Z. Guo, Y. Zhou, L. Chen, and L. Zhang, “Comparative contribution of trophic transfer and biotransformation on arsenobetaine bioaccumulation in two marine fish,” *Aquat. Toxicol.*, 2016.

[81] P. J. Peshut, R. J. Morrison, and B. A. Brooks, “Arsenic speciation in marine fish and shellfish from American Samoa,” *Chemosphere*, 2008.

[82] S. Foster, W. Maher, E. Schmeisser, A. Taylor, F. Krikowa, and S. Apte, “Arsenic species in a rocky intertidal marine food chain in NSW, Australia, revisited,” *Environ. Chem.*, 2006.

[83] M. Morita and Y. Shibata, “Chemical form of arsenic in marine macroalgae,” *Applied Organometallic Chemistry*. 1990.

[84] H. Amlund, K. A. Francesconi, C. Bethune, A. K. Lundebye, and M. H. G. Berntssen, "Accumulation and elimination of dietary arsenobetaine in two species of fish, Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.)," *Environ. Toxicol. Chem.*, 2006.

[85] W. Goessler, W. Maher, K. J. Irgolic, D. Kuehnelt, C. Schlagenhaufen, and T. Kaise, "Arsenic compounds in a marine food chain," *Fresenius. J. Anal. Chem.*, 1997.

[86] W. A. Maher, S. D. Foster, A. M. Taylor, F. Krikowa, E. G. Duncan, and A. A. Chariton, "Arsenic distribution and species in two Zostera capricorni seagrass ecosystems, New South Wales, Australia," *Environ. Chem.*, 2011.

[87] K. A. Francesconi, W. Goessler, S. Panutrakul, and K. J. Irgolic, "A novel arsenic containing riboside (arsenosugar) in three species of gastropod," *Sci. Total Environ.*, 1998.

[88] K. Shiomi, Y. Sugiyama, K. Shimakura, and Y. Nagashima, "Arsenobetaine as the major arsenic compound in the muscle of two species of freshwater fish," *Appl. Organomet. Chem.*, 1995.

[89] Z. Šlejkovec, Z. Bajc, and D. Z. Doganoc, "Arsenic speciation patterns in freshwater fish," *Talanta*, 2004.

[90] C. Soeroes *et al.*, "Arsenic speciation in farmed Hungarian freshwater fish," *J. Agric. Food Chem.*, 2005.

[91] J. Zheng and H. Hintelmann, "Hyphenation of high performance liquid chromatography with sector field inductively coupled plasma mass spectrometry for the determination of ultra-trace level anionic and cationic arsenic compounds in freshwater fish," *J. Anal. At. Spectrom.*, 2004.

[92] J. F. Lawrence, H. B. S. Conacher, P. Michalik, and G. Tam, "Identification of Arsenobetaine and Arsenocholine in Canadian Fish and Shellfish by High-Performance Liquid Chromatography with Atomic Absorption Detection and Confirmation by Fast Atom Bombardment Mass Spectrometry," *J. Agric. Food Chem.*, 1986.

[93] S. Maeda, R. Inoue, T. Kozono, T. Tokuda, A. Ohki, and T. Takeshita, "Arsenic metabolism in a freshwater food chain," *Chemosphere*, 1990.

[94] C. Y. Chen and C. L. Folt, “Bioaccumulation and diminution of arsenic and lead in a freshwater food web,” *Environ. Sci. Technol.*, 2000.

[95] R. P. Mason, J. M. Laporte, and S. Andres, “Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish,” *Arch. Environ. Contam. Toxicol.*, 2000.

[96] L. Chen, W. Zhang, Z. Guo, and L. Zhang, “Effects of acclimation on arsenic bioaccumulation and biotransformation in freshwater medaka Oryzias mekongensis after chronic arsenic exposure,” *Environ. Pollut.*, 2018.

[97] K. O. Amayo, A. Raab, E. M. Krupp, T. Marschall, M. Horsfall, and J. Feldmann, “Arsenolipids show different profiles in muscle tissues of four commercial fish species,” *J. Trace Elem. Med. Biol.*, 2014.

[98] U. Arroyo-Abad, J. Mattusch, T. Reemtsma, and C. Piechotta, “Arsenolipids in commercial canned cod liver: An occurrence and distribution study,” *Eur. J. Lipid Sci. Technol.*, 2014.

[99] K. H. Wedepohl, “The composition of the continental crust,” *Geochim. Cosmochim. Acta*, 1995.

[100] J. S. Dávila and J. H. Custodio, “Mercury Reduction by Bacteria Isolated from Informal Mining Zones,” *Adv. Mater. Res.*, 2009.

[101] T. Barkay, S. M. Miller, and A. O. Summers, “Bacterial mercury resistance from atoms to ecosystems,” *FEMS Microbiology Reviews*. 2003.

[102] D. BEN- BASSAT and A. M. MAYER, “Light- Induced Hg Volatilization and O2Evolution in Chlorella and the Effect of DCMU and Methylamine,” *Physiol. Plant.*, 1978.

[103] R. K. Skogerboe and S. A. Wilson, “Reduction of Ionic Species by Fulvic Acid,” *Anal. Chem.*, 1981.

[104] B. Allard and I. Arsenie, “Abiotic reduction of mercury by humic substances in aquatic system - an important process for the mercury cycle,” *Water, Air, Soil Pollut.*, 1991.

[105] M. Amyot, G. A. Gill, and F. M. M. Morel, “Production and loss of dissolved gaseous mercury in coastal seawater,” *Environ. Sci. Technol.*, 1997.

[106] T. Smith, K. Pitts, J. A. McGarvey, and A. O. Summers, “Bacterial oxidation of mercury metal vapor, Hg(0),” *Appl. Environ. Microbiol.*, 1998.

[107] M. Ogata and H. Aikoh, “Mechanism of metallic mercury oxidation in vitro by catalase and peroxidase,” *Biochem. Pharmacol.*, 1984.

[108] S. H. Du and S. C. Fang, “Catalase activity of C3 and C4 species and its relationship to mercury vapor uptake,” *Environ. Exp. Bot.*, 1983.

[109] J. W. Vonk and A. K. Sijpesteijn, “Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi,” *Antonie Van Leeuwenhoek*, 1973.

[110] J. K. Schaefer, S. S. Rocks, W. Zheng, L. Liang, B. Gu, and F. M. M. Morel, “Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria,” *Proc. Natl. Acad. Sci.*, 2011.

[111] M. Berman, T. Chase, and R. Bartha, “Carbon flow in mercury biomethylation by Desulfovibrio desulfuricans,” *Appl. Environ. Microbiol.*, 1990.

[112] J. M. Wood, F. S. Kennedy, and C. G. Rosen, “Synthesis of methyl-mercury compounds by extracts of a methanogenic sacterium [20],” *Nature*. 1968.

[113] F. Baldi, “Microbial Transformation of Mercury Species and Their Importance in the Biogeochemical Cycle of Mercury,” *Met. Ions Biol. Syst.*, 1997.

[114] J. H. Weber, “Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment,” *Chemosphere*, 1993.

[115] R. Falter, “Experimental study on the unintentional abiotic methylation of inorganic mercury during analysis: Part 1: Localisation of the compounds effecting the abiotic mercury methylation,” in *Chemosphere*, 1999.

[116] R. S. Oremland, C. W. Culbertson, and M. R. Winfrey, “Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation,” *Appl. Environ. Microbiol.*, 1991.

[117] M. C. Marvin-Dipasquale and R. S. Oremland, “Bacterial methylmercury degradation in Florida everglades peat sediment,” *Environ. Sci. Technol.*, 1998.

[118] J. K. Schaefer, J. Letowski, and T. Barkay, “Mer-mediated resistance and volatilization of Hg(II) under anaerobic conditions,” *Geomicrobiol. J.*, 2002.

[119] J. L. Schottel, “The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of Escherichia coli,” *J. Biol. Chem.*, 1978.

[120] I. Suda, M. Suda, and K. Hirayama, “Degradation of methyl and ethyl mercury by singlet oxygen generated from sea water exposed to sunlight or ultraviolet light,” *Arch. Toxicol.*, 1993.

[121] J. M. Benoit, C. C. Gilmour, and R. P. Mason, “The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3),” *Environ. Sci. Technol.*, 2001.

[122] D. W. Gruenwedel and D. S. Lu, “Changes in the sedimentation characteristics of DNA due to methylmercuration,” *Biochem. Biophys. Res. Commun.*, 1970.

[123] C. C. Gilmour *et al.*, “Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation,” *Appl. Environ. Microbiol.*, 2011.

[124] R. A. Bernhoft, “Mercury toxicity and treatment: A review of the literature,” *Journal of Environmental and Public Health*. 2012.

[125] T. Suzuki, T. I. Takemoto, S. Shishido, and K. Kani, “Mercury in human amniotic fluid,” *Scand. J. Work. Environ. Heal.*, 1977.

[126] N. E. Selin, “Global Biogeochemical Cycling of Mercury: A Review,” 2010.

[127] R. B. Jonas, C. C. Gilmour, D. L. Stoner, M. M. Weir, and J. H. Tuttle, “Comparison of methods to measure acute metal and organometal toxicity to natural aquatic microbial communities,” *Appl. Environ. Microbiol.*, 1984.

[128] S. M. Ullrich, T. W. Tanton, and S. A. Abdrashitova, “Mercury in the aquatic environment: A review of factors affecting methylation,” *Critical Reviews in Environmental Science and Technology*. 2001.

[129] ATSDR, “Toxicological profile for mercury: U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry,” *Toxicol. PROFILE Mercur.*, 1999.

[130] S. M. Silbernagel *et al.*, “Recognizing and preventing overexposure to methylmercury from fish and seafood consumption: Information for physicians,” *Journal of Toxicology*. 2011.

[131] Y. S. Hong, Y. M. Kim, and K. E. Lee, “Methylmercury exposure and health effects,” *J. Prev. Med. Public Heal.*, 2012.

[132] D. R. Abernethy, A. J. DeStefano, T. L. Cecil, K. Zaidi, and R. L. Williams, "Metal impurities in food and drugs," *Pharmaceutical Research*. 2010.

[133] "Environmental Health Criteria 101: Methylmercury," *Environ. Heal. Criteria*, 1990.

[134] C. H. Lamborg *et al.*, "A global ocean inventory of anthropogenic mercury based on water column measurements," *Nature*, 2014.

[135] R. J. Law, M. J. Waldock, C. R. Allchin, R. E. Laslett, and K. J. Bailey, "Contaminants in seawater around England and Wales: Results from Monitoring surveys, 1990-1992," *Mar. Pollut. Bull.*, 1994.

[136] R. Plaschke, G. Dal Pont, and E. C. V. Butler, "Mercury in waters of the Derwent Estuary - Sample treatment and analysis," *Mar. Pollut. Bull.*, 1997.

[137] S. D. Siciliano, N. J. O'Driscoll, and D. R. S. Lean, "Microbial reduction and oxidation of mercury in freshwater lakes," *Environ. Sci. Technol.*, 2002.

[138] and M. L. S. Cristie Dalland, Eva Schumacher, "The Adsorption of Organomercury Compounds from Seawater onto Sedimentary Phases," *Org. Mar. Geochemistry*, vol. 305, pp. 369–381, 1986.

[139] R. P. Mason and W. F. Fitzgerald, "The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean," *Deep. Res. Part I*, 1993.

[140] R. P. Mason, K. R. Rolfhus, and W. F. Fitzgerald, "Methylated and elemental mercury cycling in surface and deep ocean waters of the North Atlantic," *Water, Air, Soil Pollut.*, 1995.

[141] S. Moore, J.W., Ramamoorthy, "Heavy Metals in Natural Waters. Applied Monitoring and Impact Assessment," *Springer Verlag*, 1984.

[142] G. Compeau and R. Bartha, "Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions," *Appl. Environ. Microbiol.*, 1984.

[143] M. Berman and R. Bartha, "Control of the Methylation process in a mercury-polluted aquatic sediment," *Environ. Pollution. Ser. B, Chem. Phys.*, 1986.

[144] J. M. Benoit, R. P. Mason, and C. C. Gilmour, "Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and implications for availability to methylating bacteria," *Environ. Toxicol. Chem.*, 1999.

[145] H. Hintelmann, K. Keppel-Jones, and R. D. Evans, “Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability,” *Environ. Toxicol. Chem.*, 2000.

[146] K. Pak and R. Bartha, “Products of mercury demethylation by sulfidogens and methanogens,” *Bull. Environ. Contam. Toxicol.*, 1998.

[147] K. Nakamura, M. Sakamoto, H. Uchiyama, and O. Yagi, “Organomercurial-volatilizing bacteria in the mercury-polluted sediment of Minamata Bay, Japan,” *Appl. Environ. Microbiol.*, 1990.

[148] J. M. Wood, “Biological processes involved in the cycling of elements between soil or sediments and the aqueous environment,” *Hydrobiologia*, vol. 149, no. 1, pp. 31–42, 1987.

[149] M. Ravichandran, “Interactions between mercury and dissolved organic matter - A review,” *Chemosphere*. 2004.

[150] Y. Wu and W. X. Wang, “Intracellular speciation and transformation of inorganic mercury in marine phytoplankton,” *Aquat. Toxicol.*, 2014.

[151] K. Leopold, M. Foulkes, and P. Worsfold, “Methods for the determination and speciation of mercury in natural waters-A review,” *Analytica Chimica Acta*. 2010.

[152] P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum, “Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web,” *Sci. Total Environ.*, 2005.

[153] R. W. H. Carroll, J. Memmott, J. J. Warwick, C. H. Fritsen, J. C. J. Bonzongo, and K. Acharya, “Seasonal variation of mercury associated with different phytoplankton size fractions in Lahontan Reservoir, Nevada,” *Water. Air. Soil Pollut.*, 2011.

[154] P. C. Pickhardt and N. S. Fisher, “Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies,” *Environ. Sci. Technol.*, 2007.

[155] R. P. Mason, F. M. M. Morel, and H. F. Hemond, “The role of microorganisms in elemental mercury formation in natural waters,” *Water, Air, Soil Pollut.*, 1995.

[156] D. Kelly, K. Budd, and D. D. Lefebvre, “Mercury analysis of acid- and alkaline-reduced biological samples: Identification of meta-cinnabar as the major biotransformed compound in algae,” *Appl. Environ. Microbiol.*, 2006.

[157] D. J. A. Kelly, K. Budd, and D. D. Lefebvre, "Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae," *Arch. Microbiol.*, 2007.

[158] E. Morelli, R. Ferrara, B. Bellini, F. Dini, G. Di Giuseppe, and L. Fantozzi, "Changes in the non-protein thiol pool and production of dissolved gaseous mercury in the marine diatom Thalassiosira weissflogii under mercury exposure," *Sci. Total Environ.*, 2009.

[159] S. Devars, C. Avilés, C. Cervantes, and R. Moreno-Sánchez, "Mercury uptake and removal by euglena gracilis," *Arch. Microbiol.*, 2000.

[160] O. R. and C. J. Watras, "Microbial Mercury Methylation in Aquatic Environments: A Critical Review of Published Field and Laboratory Studies," *Environ. Sci. Technol*, vol. 53, pp. 4–19, 2019.

[161] A. C. Luengen and A. R. Flegal, "Role of phytoplankton in mercury cycling in the San Francisco Bay estuary," *Limnol. Oceanogr.*, 2009.

[162] L. E. Heimbürger *et al.*, "Methyl mercury distributions in relation to the presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, North-western Mediterranean)," *Geochim. Cosmochim. Acta*, 2010.

[163] C. J. Watras, R. C. Back, S. Halvorsen, R. J. M. Hudson, K. A. Morrison, and S. P. Wente, "Bioaccumulation of mercury in pelagic freshwater food webs," in *Science of the Total Environment*, 1998.

[164] K. Simkiss and M. G. Taylor, "Transport of metals across membranes," in *Metal speciation and bioavailability in aquatic systems*, 1995.

[165] M. Hanikenne, S. S. Merchant, and P. Hamel, "Transition Metal Nutrition: A Balance Between Deficiency and Toxicity," in *The Chlamydomonas Sourcebook 3-Vol set*, 2009.

[166] R. P. Mason, J. R. Reinfelder, and F. M. M. Morel, "Uptake, toxicity, and trophic transfer of mercury in a coastal diatom," *Environ. Sci. Technol.*, 1996.

[167] J. Gutknecht, "Inorganic mercury (Hg2+) transport through lipid bilayer membranes," *J. Membr. Biol.*, 1981.

[168] E. Bienvenue *et al.*, "Transport of mercury compounds across bimolecular lipid membranes: Effect of lipid composition, pH and chloride concentration," *Chem. Biol. Interact.*, 1984.

[169] H. A. Moye, C. J. Miles, E. J. Phlips, B. Sargent, and K. K. Merritt, “Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water,” *Environ. Sci. Technol.*, 2002.

[170] C. Y. Chen and C. L. Folt, “High plankton densities reduce mercury biomagnification,” *Environ. Sci. Technol.*, 2005.

[171] N. S. Fisher, P. Bjerregaard, and S. W. Fowler, “Interactions of marine plankton with transuranic elements. 1. Biokinetics of neptunium, plutonium, americium, and californium in phytoplankton,” *Limnol. Oceanogr.*, 1983.

[172] P. R. Gorski, D. E. Armstrong, J. P. Hurley, and D. P. Krabbenhoft, “Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga,” *Environ. Pollut.*, 2008.

[173] A. C. Luengen, N. S. Fisher, and B. A. Bergamaschi, “Dissolved organic matter reduces algal accumulation of methylmercury,” *Environ. Toxicol. Chem.*, 2012.

[174]??verine Le Faucheur, Y. Tremblay, C. Fortin, and P. G. C. Campbell, “Acidification increases mercury uptake by a freshwater alga, Chlamydomonas reinhardtii,” *Environ. Chem.*, 2011.

[175] S. Le Faucheur, P. G. C. Campbell, C. Fortin, and V. I. Slaveykova, “Interactions between mercury and phytoplankton: Speciation, bioavailability, and internal handling.,” *Environ. Toxicol. Chem.*, 2014.

[176] J. R. Reinfelder and N. S. Fisher, “The assimilation of elements ingested by marine copepods,” *Science (80-.).*, 1991.

[177] P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum, “Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs,” *Proc. Natl. Acad. Sci.*, 2002.

[178] C. J. Watras and N. S. Bloom, “Mercury and methylmercury, in individual zooplankton: Implications for bioaccumulation,” *Limnology and Oceanography*. 1992.

[179] C. R. Hammerschmidt and W. F. Fitzgerald, “Bioaccumulation and trophic transfer of methylmercury in Long Island Sound,” *Arch. Environ. Contam. Toxicol.*, 2006.

[180] D. S. Becker and G. N. Bigham, “Distribution of mercury in the aquatic food web of Onondaga Lake, New York,” in *Water, Air, and Soil Pollution*, 1995.

[181] M. T. K. Tsui and W. X. Wang, "Uptake and Elimination Routes of Inorganic Mercury and Methylmercury in Daphnia magna," *Environ. Sci. Technol.*, 2004.

[182] R. Karimi, C. Y. Chen, P. C. Pickhardt, N. S. Fisher, and C. L. Folt, "Stoichiometric controls of mercury dilution by growth.," *Proc. Natl. Acad. Sci.*, 2007.

[183] N. M. Lawson and R. P. Mason, "Accumulation of mercury in estuarine food chains," *Biogeochemistry*, 1998.

[184] R. M. Adams, M. R. Twiss, and C. T. Driscoll, "Patterns of mercury accumulation among seston in lakes of the Adirondack Mountains, New York," *Environ. Sci. Technol.*, 2009.

[185] C. Y. Chen, R. S. Stemberger, N. C. Kamman, B. M. Mayes, and C. L. Folt, "Patterns of Hg bioaccumulation and transfer in aquatic food webs across multi-lake studies in the northeast US," *Ecotoxicology*, 2005.

[186] N. S. Bloom, "On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue," *Can. J. Fish. Aquat. Sci.*, 1992.

[187] D. R. Young *et al.*, "Trophic structure and pollutant concentrations in marine ecosystems of southern California," *Calif. Coop. Ocean. Fish. Investig. Reports*, 1980.

[188] J. N. Morgan, M. R. Berry, and R. L. Graves, "EFFECTS OF COMMONLY USED COOKING PRACTICES ON TOTAL MERCURY CONCENTRATION IN FISH AND THEIR IMPACT ON EXPOSURE ASSESSMENTS," *J. Expo. Anal. Environ. Epidemiol.*, 1997.

[189] W. J. Sydeman and W. M. Jarman, "Trace metals in seabirds, Steller sea lion, and forage fish and zooplankton from central California," *Mar. Pollut. Bull.*, 1998.

[190] R. J. Pentreath, "The accumulation of organic mercury from sea water by the plaice, Pleuronectes platessa L," *J. Exp. Mar. Bio. Ecol.*, 1976.

[191] A. Boudou and F. Ribeyre, "Experimental study of trophic contamination of Salmogairdneri by two mercury compounds - HgCl2 and CH3HgCl - analysis at the organism and organ levels," *Water. Air. Soil Pollut.*, 1985.

[192] S. W. Fowler, M. Heyraud, and J. La Rosa, "Factors affecting methyl and inorganic mercury dynamics in mussels and shrimp," *Mar. Biol.*, 1978.

[193] M. H. Teruaki SUZUKI, “Experimental Investigation on the Biological Concentration of Mercury-II,” *Nippon SUISAN GAKKAISHI*, vol. 41, no. 2, pp. 225–231, 1975.

[194] A. Pastor, F. Hernández, M. A. Peris, J. Beltrán, J. V. Sancho, and M. T. Castillo, “Levels of heavy metals in some marine organisms from the western Mediterranean area (Spain),” *Mar. Pollut. Bull.*, 1994.

[195] R. Dietz, F. Riget, and P. Johansen, “Lead, cadmium, mercury and selenium in Greenland marine animals,” in *Science of the Total Environment*, 1996.

[196] L. R. Monteiro and H. D. Lopes, “Mercury content of swordfish, Xiphias gladius, in relation to length, weight, age, and sex,” *Mar. Pollut. Bull.*, 1990.

[197] M. Canli and R. W. Furness, “Mercury and cadmium uptake from seawater and from food by the Norway lobster Nephrops norvegicus,” *Environ. Toxicol. Chem.*, 1995.

[198] J. M. Laporte, J. P. Truchot, N. Mesmer-Dudons, and A. Boudou, “Bioaccumulation of inorganic and methylated mercury by the gills of the shore crab Carcinus maenas: Transepithelial fluxes and histochemical localization,” *Mar. Ecol. Prog. Ser.*, 2002.

[199] D. W. Kuehl and R. Haebler, “Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (Tursiops truncatus) collected during an unusual mortality event in the Gulf of Mexico, 1990,” *Arch. Environ. Contam. Toxicol.*, 1995.

[200] M. Nigro and C. Leonzio, “Intracellular storage of mercury and selenium in different marine vertebrates,” *Mar. Ecol. Prog. Ser.*, 1996.

[201] M. Havelková, L. Dušek, D. Némethová, G. Poleszczuk, and Z. Svobodová, “Comparison of mercury distribution between liver and muscle - A biomonitoring of fish from lightly and heavily contaminated localities,” *Sensors*, 2008.

[202] M. Yoon *et al.*, “Total and Methyl Mercury Concentrations in Antarctic Toothfish (Dissostichus mawsoni): Health Risk Assessment,” *Bull. Environ. Contam. Toxicol.*, 2018.

[203] R. Bargagli, F. Monaci, J. C. Sanchez-Hernandez, and D. Cateni, “Biomagnification of mercury in an Antarctic marine coastal food web,” *Mar. Ecol. Prog. Ser.*, 1998.

[204] Jerry M Neff, “Metal and Organic Chemicals Aassociated with Oil and Gas well produced water: Bioaccumulation, Fates, and Effects in the Marine Environment,” *Rep. offshore Oper. Comm.*, p. 357, 1997.

[205] and C. M. F. P. Collin A. Eagles-Smith, James J. Willacker Jr., “Mercury in Fishes from 21 National Parks in the Western United States—Inter- and Intra-Park Variation in Concentrations and Ecological Risk,” 2014.

[206] R. Dietz, P. M. Outridge, and K. A. Hobson, “Anthropogenic contributions to mercury levels in present-day Arctic animals-A review,” *Science of the Total Environment*. 2009.

[207] “EPA-FDA Fish Advice.” [Online]. Available: https://www.epa.gov/fish-tech/epa-fda-fish-advice-technical-information.

[208] FAO, *FAO Yearbook. Fisheries and Aquaculture Statistics*. 2016.

[209] L. Murdock *et al.*, “Analysis of strains lacking known osmolyte accumulation mechanisms reveals contributions of osmolytes and transporters to protection against abiotic stress,” *Appl. Environ. Microbiol.*, 2014.

[210] R. Obeid, “The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway,” *Nutrients*. 2013.

[211] J. Boch, B. Kempf, and E. Bremer, “Osmoregulation in Bacillus subtilis: Synthesis of the osmoprotectant glycine betaine from exogenously provided choline,” *J. Bacteriol.*, 1994.

[212] N. Garcia, J. A. Lopez Elias, A. Miranda, M. Martinez Porchas, N. Huerta, and A. Garcia, “Effect of salinity on growth and chemical composition of the diatom Thalassiosira weissflogii at three culture phases,” *Lat. Am. J. Aquat. Res.*, 2012.

[213] B. Rathinasabapathi *et al.*, “Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the first step of glycine betaine synthesis in plants: Prosthetic group characterization and cDNA cloning,” *Proc. Natl. Acad. Sci.*, 1997.

[214] J. Dragolovich, “Dealing with salt stress in animal cells: The role and regulation of glycine betaine concentrations,” *J. Exp. Zool.*, 1994.

[215] H. Kageyama, Y. Tanaka, and T. Takabe, “Biosynthetic pathways of glycinebetaine in Thalassiosira pseudonana; functional characterization of enzyme catalyzing three-step methylation of glycine,” *Plant Physiol. Biochem.*, 2018.

[216] R. Waditee-Sirisattha, M. Singh, H. Kageyama, D. Sittipol, A. K. Rai, and T. Takabe, "Anabaena sp. PCC7120 transformed with glycine methylation genes from Aphanothece halophytica synthesized glycine betaine showing increased tolerance to salt," *Arch. Microbiol.*, 2012.

[217] A. Spielmeyer and G. Pohnert, "Influence of temperature and elevated carbon dioxide on the production of dimethylsulfoniopropionate and glycine betaine by marine phytoplankton," *Mar. Environ. Res.*, 2012.

[218] Y. Ma, Q. Wang, X. Gao, and Y. Zhang, "Biosynthesis and uptake of glycine betaine as cold-stress response to low temperature in fish pathogen Vibrio anguillarum," *J. Microbiol.*, 2017.

[219] P. H. Yancey, M. D. Rhea, K. M. Kemp, and D. M. Bailey, "Trimethylamine oxide, betaine and other osmolytes in deep-sea animals: depth trends and effects on enzymes under hydrostatic pressure.," *Cell. Mol. Biol. (Noisy-le-grand).*, 2004.

[220] C. Chen and G. A. Beattie, "Pseudomonas syringae BetT is a low-affinity choline transporter that is responsible for superior osmoprotection by choline over glycine betaine," *J. Bacteriol.*, 2008.

[221] M. A. Roulier, B. Palenik, and F. M. M. Morel, "A method for the measurement of choline and hydrogen peroxide in seawater," *Mar. Chem.*, 1990.

[222] P. C. B. Sizeland, S. T. Chambers, M. Lever, L. M. Bason, and R. A. Robson, "Organic osmolytes in human and other mammalian kidneys," *Kidney Int.*, 1993.

[223] J. R. Treberg, "The accumulation of methylamine counteracting solutes in elasmobranchs with differing levels of urea: a comparison of marine and freshwater species," *J. Exp. Biol.*, 2006.

[224] A. Nyyssölä, J. Kerovuo, P. Kaukinen, N. Von Weymarn, and T. Reinikainen, "Extreme halophiles synthesize betaine from glycine by methylation," *J. Biol. Chem.*, 2000.

[225] R. Waditee *et al.*, "Isolation and functional characterization of N-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism Aphanothece halophytica," *J. Biol. Chem.*, 2003.

[226] M. C. Lai, C. C. Wang, M. J. Chuang, Y. C. Wu, and Y. C. Lee, "Effects of substrate and potassium on the betaine-synthesizing enzyme glycine sarcosine dimethylglycine N-methyltransferase from a halophilic methanoarchaeon Methanohalophilus portucalensis," *Res. Microbiol.*, 2006.

[227] S. J. Lai and M. C. Lai, "Characterization and regulation of the osmolyte betaine synthesizing enzymes GSMT and SDMT from halophilic methanogen Methanohalophilus portucalensis," *PLoS One*, 2011.

[228] W. R. Riekhof, S. Naik, H. Bertrand, C. Benning, and D. R. Voelker, "Phosphate starvation in fungi induces the replacement of phosphatidylcholine with the phosphorus-free betaine lipid diacylglyceryl-N,N,N-trimethylhomoserine," *Eukaryot. Cell*, 2014.

[229] M. Kato, M. Sakai, K. Adachi, H. Ikemoto, and H. Sano, "Distribution of betaine lipids in marine algae," *Phytochemistry*, 1996.

[230] K. Kunzler and W. Eichenberger, "Betaine lipids and zwitterionic phospholipids in plants and fungi," *Phytochemistry*, 1997.

[231] A. Shemi, D. Schatz, H. F. Fredricks, B. A. S. Van Mooy, Z. Porat, and A. Vardi, "Phosphorus starvation induces membrane remodeling and recycling in Emiliania huxleyi," *New Phytol.*, 2016.

[232] C. Benning, Z. H. Huang, and D. A. Gage, "Accumulation of a novel glycolipid and a betaine lipid in cells of rhodobacter sphaeroides grown under phosphate limitation," *Arch. Biochem. Biophys.*, 1995.

[233] V. M. Dembitsky, "Betaine ether-linked glycerolipids: Chemistry and biology," *Progress in Lipid Research*. 1996.

[234] J. P. Canãvate, I. Armada, J. L. Riós, and I. Hachero-Cruzado, "Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae," *Phytochemistry*, 2016.

[235] O. Geiger, V. Röhrs, B. Weissenmayer, T. M. Finan, and J. E. Thomas-Oates, "The regulator gene phoB mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-N,N,N-trimethylhomoserine in Rhizobium (Sinorhizobium) meliloti," *Mol. Microbiol.*, 1999.

[236] I. Khozin-Goldberg and Z. Cohen, “The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte Monodus subterraneus,” *Phytochemistry*, 2006.

[237] A. G. and W. E. Christian Giroud, “Lipids of Chlamydomonas reinhardtii. Analysis of Molecular Species and Intracellular Site(s) of Biosynthesis,” *Plant Cell Physiol*, vol. 29, no. 4, pp. 587–595, 1988.

[238] L.-J. Dolch *et al.*, “A Palmitic Acid Elongase Affects Eicosapentaenoic Acid and Plastidial Monogalactosyldiacylglycerol Levels in Nannochloropsis,” *Plant Physiol.*, 2017.

[239] J. P. Cañavate, I. Armada, and I. Hachero-Cruzado, “Interspecific variability in phosphorus-induced lipid remodelling among marine eukaryotic phytoplankton,” *New Phytol.*, 2017.

[240] H. Murakami, T. Nobusawa, K. Hori, M. Shimojima, and H. Ohta, “Betaine Lipid Is Crucial for Adapting to Low Temperature and Phosphate Deficiency in Nannochloropsis,” *Plant Physiol.*, 2018.

[241] I. M. López-Lara *et al.*, “Phosphorus-Free Membrane Lipids of *Sinorhizobium meliloti* Are Not Required for the Symbiosis with Alfalfa but Contribute to Increased Cell Yields Under Phosphorus-Limiting Conditions of Growth,” *Mol. Plant-Microbe Interact.*, 2005.

[242] R. M. Klug and C. Benning, “Two enzymes of diacylglyceryl-O-4’-(N,N,N,-trimethyl)homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium Rhodobacter sphaeroides,” *Proc. Natl. Acad. Sci.*, 2001.

[243] W. R. Riekhof, B. B. Sears, and C. Benning, “Annotation of genes involved in glycerolipid biosynthesis in Chlamydomonas reinhardtii: Discovery of the betaine lipid synthase BTA1Cr,” *Eukaryot. Cell*, 2005.

[244] M. J. Wargo, “Homeostasis and catabolism of choline and glycine betaine: Lessons from Pseudomonas aeruginosa,” *Applied and Environmental Microbiology*. 2013.

[245] E. Mueller, K. Fahlbusch, R. Walther, and G. Gottschalk, “Formation of N,N-dimethylglycine, acetic acid, and butyric acid from betaine by Eubacterium limosum,” *Appl. Environ. Microbiol.*, 1981.

[246] A. J. Watkins, E. G. Rousse, R. J. Parkes, and H. Sass, “Glycine betaine as a direct substrate for methanogens (methanococcoides spp.),” *Appl. Environ. Microbiol.*, 2014.

[247] B. Möller, R. Oßmer, B. H. Howard, G. Gottschalk, and H. Hippe, “Sporomusa, a new genus of gram-negative anaerobic bacteria including Sporomusa sphaeroides spec. nov. and Sporomusa ovata spec. nov.,” *Arch. Microbiol.*, 1984.

[248] K. Hormann and J. R. Andreesen, “Reductive cleavage of sarcosine and betaine by Eubacterium acidaminophilum via enzyme systems different from glycine reductase,” *Arch. Microbiol.*, 1989.

[249] M. Meyer, K. Granderath, and J. R. Andreesen, “Purification and Characterization of Protein PBof Betaine Reductase and its Relationship to the Corresponding Proteins Glycine Reductase and Sarcosine Reductase from Eubacterium Acidaminophilum,” *Eur. J. Biochem.*, 1995.

[250] X. Ge, A. S. Wexler, and S. L. Clegg, “Atmospheric amines - Part I. A review,” *Atmospheric Environment*. 2011.

[251] E. L. Barrett and H. S. Kwan, “Bacterial Reduction of Trimethylamine Oxide,” *Annu. Rev. Microbiol.*, 1985.

[252] C. M. Large PJ, Boulton CA, “The reduced nicotinamide-adenine dinuclotide phosphate- and oxygen-dependent N-oxygenation of trimethylamine by Pseudomonas aminovorans,” *Biochem*, vol. 128, pp. 137–138, 1972.

[253] Y. Chen, N. A. Patel, A. Crombie, J. H. Scrivens, and J. C. Murrell, “Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase,” *Proc. Natl. Acad. Sci.*, 2011.

[254] G. M. King, M. J. Klug, and D. R. Lovley, “Metabolism of Acetate, Methanol, and Methylated Amines in Intertidal Sediments of Lowes Cove, Maine.,” *Appl. Environ. Microbiol.*, 1983.

[255] D. Giani, L. Giani, Y. Cohen, and W. E. Krumbein, “Methanogenesis in the hypersaline Solar Lake (Sinai),” *FEMS Microbiol. Lett.*, 1984.

[256] T. C. Tallant and J. A. Krzycki, “Methylthiol:coenzyme M methyltransferase from Methanosarcina barkeri, an enzyme of methanogenesis from dimethylsulfide and methylmercaptopropionate,” *J. Bacteriol.*, 1997.

[257] D. J. Ferguson, N. Gorlatova, D. A. Grahame, and J. A. Krzycki, “Reconstitution of dimethylamine:coenzyme M methyl transfer with a discrete corrinoid protein and two methyltransferases purified from Methanosarcina barkeri,” *J. Biol. Chem.*, 2000.

[258] G. W. Schade and P. J. Crutzen, “Emission of aliphatic amines from animal husbandry and their reactions: Potential source of N2O and HCN,” *J. Atmos. Chem.*, 1995.

[259] D. Y. Lee and A. S. Wexler, “Atmospheric amines - Part III: Photochemistry and toxicity,” *Atmospheric Environment*. 2013.

[260] S. M. Murphy *et al.*, “Secondary aerosol formation from atmospheric reactions of aliphatic amines,” *Atmos. Chem. Phys.*, 2007.

[261] K. C. Barsanti and J. F. Pankow, “Thermodynamics of the formation of atmospheric organic particulate matter by accretion reactions-Part 3: Carboxylic and dicarboxylic acids,” *Atmos. Environ.*, 2006.

[262] F. Yu and G. Luo, “Modeling of gaseous methylamines in the global atmosphere: Impacts of oxidation and aerosol uptake,” *Atmos. Chem. Phys.*, 2014.

[263] R. O. Lozano-Rodríguez, E., Luguera, M., Lucena, J.J., Carpena-Ruiz, “Evaluation of two different acid digestion methods in closed systems of trace elements determination in plants.,” *Quim. Anal*, vol. 14, no. 27–30, 1995.

[264] S. Hirata and H. Toshimitsu, “Determination of arsenic species and arsenosugars in marine samples by HPLC-ICP-MS,” in *Applied Organometallic Chemistry*, 2007.

[265] W. G, “Polarization of the fluorescence of macromolecules: II. Fluorescent conjugates of ovalbumin and bovine serum albumin,” *Biochem*, vol. 51, pp. 155–167, 1952.

[266] C. Gros and B. Labouesse, “Study of the Dansylation Reaction of Amino Acids, Peptides and Proteins,” *Eur. J. Biochem.*, 1969.

[267] G. J. Schut *et al.*, “ The catalytic mechanism of electron-bifurcating electron transfer flavoproteins (ETFs) involves an intermediary complex with NAD + ,” *J. Biol. Chem.*, 2018.

[268] D. W. Johnson, "A flow injection electrospray ionization tandem mass spectrometric method for the simultaneous measurement of trimethylamine and trimethylamine N-oxide in urine," *J. Mass Spectrom.*, 2008.

[269] D. A. Wiesenburg and N. L. Guinasso, "Equilibrium Solubilities of Methane, Carbon Monoxide, and Hydrogen in Water and Sea Water," *J. Chem. Eng. Data*, 1979.

[270] K. Mandal, Badal and T. Suzuki, Kazuo, "Arsenic round the world: a review.," *Talanta*, 2002.

[271] F. M. M. Morel, A. M. L. Kraepiel, and M. Amyot, "THE CHEMICAL CYCLE AND BIOACCUMULATION OF MERCURY," *Annu. Rev. Ecol. Syst.*, 1998.

[272] D. Mergler *et al.*, "Methylmercury Exposure and Health Effects in Humans: A Worldwide Concern," *AMBIO A J. Hum. Environ.*, 2007.

[273] N. R. Akter KF, Owens G, Davey DE, "Arsenic Speciation and Toxicity in Biological Systems," *Rev Env. Contam Toxicol*, vol. 184, pp. 97–149, 2005.

[274] M. Kumaresan and P. Riyazuddin, "Overview of speciation chemistry of arsenic," *Current Science*. 2001.

[275] M. M. Chumchal *et al.*, "Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem," *Environ. Toxicol. Chem.*, 2011.

[276] M. G. Clayden, K. A. Kidd, B. Wyn, J. L. Kirk, D. C. G. Muir, and N. J. O'Driscoll, "Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes," *Environ. Sci. Technol.*, 2013.

[277] M. Azizur Rahman and H. Hasegawa, "Arsenic in freshwater systems: Influence of eutrophication on occurrence, distribution, speciation, and bioaccumulation," *Appl. Geochemistry*, 2012.

[278] S. Ciardullo, F. Aureli, A. Raggi, and F. Cubadda, "Arsenic speciation in freshwater fish: Focus on extraction and mass balance," *Talanta*, 2010.

[279] and S. T. O. Bigelow, P.E., T.M. Koel, D. Mahony, B. Ertel, B. Rowdon, "Protection of native Yellowstone cutthroat trout in Yellowstone Lake, Wyoming," Yellowstone National Park, Wyoming, USA., 2003.

[280] M. A. Chaffee *et al.*, “Applications of trace-element and stable-isotope geochemistry to wildlife issues, Yellowstone National Park and vicinity,” *U.S. Geol. Surv. Prof. Pap.*, 2007.

[281] and J. M. S. Gresswell, R.E., C.S. Guy, M.J. Hansen, M.L. Jones, J.E. Marsden, P.J. Martinez, “Lake trout suppression in Yellowstone Lake: science review panel. Interim scientific assessment, 2014 performance year. A report to the superintendent,” National Park Service, Yellowstone National Park, Wyoming, USA., 2015.

[282] J. Kan *et al.*, “Geochemistry and mixing drive the spatial distribution of free-living archaea and bacteria in yellowstone lake,” *Front. Microbiol.*, 2016.

[283] S. D. McCormick, “Endocrine control of osmoregulation in teleost fish,” *Am. Zool.*, 2001.

[284] V. N. Sinyukovich, “Relationships between Water Flow and Dissolved Solids Discharge in the Major Tributaries of Lake Baikal,” *Water Resour.*, vol. 30, no. 2, pp. 186–190, 2003.

[285] C. U. Ugwu, H. Aoyagi, and H. Uchiyama, “Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of Chlorella sorokiniana,” *Photosynthetica*, 2007.

[286] J. O. Nalley, D. R. O’Donnell, and E. Litchman, “Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria,” *Algal Res.*, 2018.

[287] Qian Wang, Abdullah Alowaifeer, Patricia Kerner, Narayanaganesh Balasubramanian, Angela Patterson, John E. Dore, Brian Bothner, and Timothy R. McDermott “Aerobic bacterial metabolism of methylamine or glycine betaine generates methane, contributing to methane oversaturation in oxic waters”