ASSESSING MERCURY EXPOSURE RISK IN THE LAKE ZAPOTLÁN WATERSHED, MEXICO

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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ABSTRACT

Mercury is an environmental contaminant of global concern. The distribution of mercury in freshwater systems is poorly characterized in Mexico, despite widespread contamination from industrial and urban effluents. The land use, geology, and hydrology of the Lake Zapotlán basin, Mexico are conducive to the delivery of elevated mercury in water to the lake due to untreated wastewater discharge, deforestation, and local volcanic history. To assess a mercury exposure risk to fish consumers, the concentrations of total Hg (THg) in water inputs, surface waters, sediments, and the commercial catch of tilapia and carp were investigated. Results indicate that despite high particle-bound inputs of THg to the lake in runoff and wastewater, THg in sediments and surface waters were low. Dense *Typha latifolia* dominated wetlands are believed to retain THg inflow from water inputs. Concentrations of THg in tilapia and carp were low, suggesting low mercury bioavailability in this system.
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1. INTRODUCTION AND OBJECTIVES

1.1 MERCURY AS A GLOBAL CONTAMINANT

Mercury (Hg) is an environmental contaminant of global concern. The highly toxic organomercurial compound methylmercury (MeHg) has caused numerous human poisoning incidents worldwide, the most infamous in Minamata, Japan. During the 1950s near Minamata City, MeHg discharge from a local chemical plant bioaccumulated to extremely high levels in local seafood causing serious health consequences for more than 2000 people (Harada 1995). In Canada, the English-Wabigoon River system was heavily impacted by Hg inputs from a local chlor-alkali plant in the 1960s (Parks and Hamilton 1987). This resulted in extremely high Hg concentrations in locally consumed fish and in blood levels of aboriginal community residents (Wheatley et al. 1979; Parks and Hamilton 1987).

1.1.1 Mercury Biogeochemistry

Sources of Hg to aquatic systems can be from both point sources or atmospheric deposition. While some Hg is naturally occurring, it is believed that the majority of the Hg burden in the atmosphere is from anthropogenic sources (Benoit et al. 2003). In the gas phase, anthropogenic sources of atmospheric Hg include metal smelting, combustion of coal, municipal incineration of garbage, and chlor-alkali factories (Lindqvist et al. 1991). Natural Hg emissions from volcanic activity can also contribute to the atmospheric pool of Hg (Nriagu and Becker 2003). Anthropogenic point source discharges of Hg to water bodies can be from clinical and dental waste, paint production, fertilizer, gold mining, and various municipal and industrial effluents (Villanueva and Botella 1998). Natural Hg enrichment of soils and surface waters can result from localized geological sources that include volcanic emissions (Legittimo et al. 1986), hydrothermal activity (Craw et al. 2000), and underlying bedrock (Loukola-Ruskeeniemi et al. 2003).

Deposition from the large atmospheric pool of Hg to the earth’s surface is the major source of Hg in parts of the USA and Canada and elsewhere in the world (Lindqvist et al. 1991; Munthe et al. 2007). Deposition of Hg is sufficient to contaminate remote water bodies in the absence of any point source Hg discharge (Drevnick et al. 2007; Dittman and Driscoll 2009). The speciation of Hg emissions is primarily elemental Hg$^0$, ionic Hg(II), and particulate Hg (Russell Bullock 2000). Atmospheric Hg in its gaseous elemental state Hg$^0$ can travel long distances in the atmosphere until it undergoes oxidation to ionic Hg(II) species (Sakata and
Asakura 2007). Hg(II) has a greater affinity to bind to water and dust particles and fall out of the atmosphere onto terrestrial and aquatic ecosystems. Particulate Hg generally has a shorter atmospheric lifetime than other Hg species, and is deposited to the surface through dry deposition and scavenging by precipitation (Sakata and Asakura 2007).

While inorganic Hg is the primary input of Hg to most aquatic systems, it is MeHg that bioaccumulates in aquatic foodwebs and is the cause of health advisories (Benoit et al. 2003). Microbial-mediated production of MeHg is the dominant source of MeHg within aquatic ecosystems (Benoit et al. 2003). The variation of biogeochemical characteristics across spatial and temporal scales can have a strong influence on the amount of MeHg produced (Munthe et al. 2007). The production and bioaccumulation of MeHg in aquatic foodwebs is highly dependant on many biogeochemical factors including pH, sulfate chemistry, dissolved organic carbon, iron, bacterial activity, alkalinity, and primary productivity (Lang et al. 1993; Munthe et al. 2007).

Sulfate reduction by sulfate reducing bacteria (SRB) has been found to be the largest control on Hg methylation in aquatic systems (Benoit et al. 2003). However, recent research suggests several strains of iron-reducing bacteria are also capable of Hg methylation (Fleming et al. 2006; Kerin et al. 2006). Reductions in sulfur emissions in the USA have shown to decrease levels of Hg in fish in sulfate-limited waters despite no change in Hg deposition (Drevnick et al. 2007). SRB are obligate anaerobes, and given adequate labile carbon (electron donor) and sulfate (electron acceptor), can methylate bioavailable Hg(II) as a metabolic by-product (Compeau and Bartha 1985). In addition to lake sediments (Matilainen et al. 1999; Krabbenhoft et al. 1998a) and anoxic waters (Eckley et al. 2005), wetlands are sites of MeHg production in watersheds (St.Louis et al. 1996; Munthe et al. 2007). In tropical locations, the root zones of floating aquatic macrophyte vegetation have been identified as hotspots for MeHg production (Mauro et al. 1999; 2001). Macrophyte root zones are rich in labile carbon from root exudates and have been found to support Hg methylation by SRB (Guimarães et al. 1998).

One of the limiting factors on MeHg production is the uptake of Hg(II) into microbial cells. The uptake of Hg(II) by methylating bacteria can occur through the passive diffusion of neutral Hg species (Barkay et al. 1997) and through active bacterial uptake (Kelly et al. 2003). The binding of Hg with dissolved organic carbon (DOC) has been shown to influence MeHg production by limiting uptake into bacterial cells (Ravichandran 2004). In the Florida Everglades, USA, Gilmour et al. (1998) found that sulfate chemistry influenced Hg speciation, and thus MeHg production. MeHg production increased with increasing distance from
agricultural sulfate inputs, and was inversely related to sulfate reduction rates and porewater sulfide concentrations (Gilmour et al. 1998).

One proposed mechanism for Hg methylation within the bacterial cell is through the transfer of a methyl group by methylcobalamin via the acetyl-CoA synthase pathway (Benoit et al. 2003). However, it has been demonstrated that some SRB with this pathway are capable of Hg methylation while others are not, indicating that there may be a specific enzyme associated with this process that has yet to be discovered. The exact biochemical pathway(s) for SRB and other methylating strains of bacteria is still unclear (Benoit et al. 2003; Barkay and Wagner 2005; Fleming et al. 2006).

1.1.2 Mercury Bioaccumulation and Exposure Risk

MeHg is a potent neurotoxin that bioaccumulates in fish and presents a major health risk to fish consumers including humans (Munthe et al. 2007; Castoldi et al. 2008). The primary Hg exposure pathway to humans is through the consumption of fish (Dabeka et al. 2003; Hightower and Moore 2003; Health Canada 2007). MeHg primarily targets the central and peripheral nervous systems. While toxic effects are highly dependant on the magnitude and duration of exposure, common effects include paresthesia, malaise, blurred vision, constriction of the visual field, deafness, dysarthria, ataxia, and at high exposures coma and death (Clarkson 2002). Other toxicological effects of MeHg exposure may include certain cancers and endocrine system disruption (Harada 1995; ATSDR 1999). While low-level long term (chronic) exposure to MeHg in adults may not result in readily identified symptoms (Clarkson 2002), the fetus, infant, and young child are especially vulnerable. Symptoms of MeHg poisoning of children include cerebral palsy, deformity of limbs, and various other physical and mental developmental disabilities (Harada 1995). The risk of neurodevelopmental disabilities due to low-level chronic exposure of MeHg in children is still unclear due to many conflicting findings in epidemiological studies (Kwok et al. 2007). Recent research from the USA suggests that Latino children are at a higher risk of Hg exposure than other ethnic groups due to diet and potential exposure to traditional medicines containing elemental Hg (Carter et al. 2007).

1.1.3 Geographic Distribution of Current Mercury Research

 Much of the current framework for Hg biogeochemical cycling is based upon research from northern temperate regions in North America and Scandinavia. However, research from
subtropical and tropical areas such as the Florida Everglades, USA and the Amazon River Basin, South America have demonstrated a clear risk for Hg exposure in warmer regions (Fleming et al. 1995; Lebel et al. 1997). Very little research exists regarding the distribution of Hg in Mexico’s inland freshwater bodies (Pilgrim et al. 2000). Given that many of Mexico’s waterways are heavily polluted (Cortes et al. 2004), concern for Hg contamination is justified.

1.2 MERCURY IN MEXICO

While the monitoring of Hg-wet deposition is expanding across the USA and Canada, there is currently no ongoing monitoring of Hg deposition in Mexico. Additionally, the distribution of Hg is not well characterized across many of Mexico’s inland water bodies, despite widespread contamination of Hg and other contaminants from industrial and urban effluents (Soto-Galera et al. 1998; Avila-Pérez et al. 1999; Cortés et al. 2004; Avila-Pérez and Zarazúa-Ortega 2006). Avila-Pérez and Zarazúa-Ortega (2006) found alarmingly high THg concentrations (8.4-13.6 μg Hg/L) in a Mexican reservoir along the Lerma River. The Lerma River drains a large region of central Mexico, and receives minimally treated effluents from several municipalities and industrial areas. The widespread contamination of Mexico’s inland waterways with toxic effluents high in THg has the potential for substantial production and bioaccumulation of MeHg in aquatic biota. While some studies of Hg in fish from coastal regions of Mexico have exceeded World Health Organization (WHO) tissue consumption guidelines (Reimer and Reimer 2005; Ruelas et al. 2007), Hg in inland freshwater fisheries is poorly characterized. While there are few data regarding Hg in the freshwater fisheries of Mexico, conditions are favorable for a potentially large Hg exposure risk through the consumption of freshwater fish.

1.3 RESEARCH OBJECTIVES

An important freshwater ecosystem in central Mexico is Lake Zapotlán. The lake and surrounding wetlands support large and diverse populations of resident and migratory waterfowl. Additionally, the lake supports an important local fishing economy for tilapia and carp, and is used for recreational activities by local educational institutions. Land use, geology and hydrology of the Lake Zapotlán basin, Mexico are conducive to the delivery of elevated Hg in water to the lake due to insufficient treatment of wastewater, deforestation in the basin, and local volcanic history. Wastewater can have high Hg concentrations due to inputs from industrial,
commercial, and domestic liquid wastes (Balough and Liang 1995). Deforestation can result in the increased erosion of soils and delivery of soil Hg (both geologically and atmospherically derived) to the wetlands and lake (Garcia and Carignan 2000). There are currently no published data from the Lake Zapotlán watershed on the distribution of Hg in any terrestrial or aquatic compartment. The primary objective of this research was to assess the potential for an elevated risk of MeHg exposure to human and other consumers of fish from Lake Zapotlán. The specific objectives were to identify the main inputs of total Hg (THg) to Lake Zapotlán, and assess the distribution of THg in the waters and sediments of the lake and wetlands. Additionally, THg in the muscle tissue of tilapia and carp from the local fishery were investigated to assess MeHg bioaccumulation in this system and the risk to consumers. It is hypothesized that waters and sediments of the lake may be elevated in THg from local inputs, which may result in increased production and bioaccumulation of MeHg in aquatic biota.
2. RESEARCH SITE DESCRIPTION

2.1 CLIMATE

The Lake Zapotlán basin has a distinct wet season from June to September with average annual rainfall of 813 mm (Ortiz-Jiménez et al. 2005). The northwest region of the basin receives more rainfall compared with the east-southeast (Ortiz-Jiménez et al. 2005). The average annual air temperature is 19.6ºC with a variation of ±5.9 ºC with maximum temperatures in July and minimum temperatures in January (Ortiz-Jiménez et al. 2005).

2.2 WATERSHED PHYSIOGRAPHIC SETTING AND LANDUSE

Lake Zapotlán (19° 34'-19° 53’ N, 103° 24'-103° 39’ W) sits in an endorheic basin (45000 ha) at an elevation of 1497 m (Figure 2.1). It is the second largest inland freshwater lake in Jalisco State, Mexico and is internationally recognized as a RAMSAR wetland, primarily due to its important habitat for several species of migratory waterfowl. A detailed background on the hydrology, geomorphology, climatology, and human activities in the basin is described in Ortiz-Jiménez et al. (2005).

As reported by Ortiz-Jiménez et al. (2005) the lake has a surface area of 11 10^6 m^2, a mean depth of 1.66 m, and a mean volume of 19.6 10^6 m^3. The lake sits along the Mexican Transvolcanic Belt and is bordered to the west by the Colima volcanoes which reach an elevation of 4,258 m. About 1 million years ago Lake Zapotlán was part of a larger inland waterway, but tectonic activity has segregated this body into separate basins which include Lake Chapala, Lake Sayula-Zacualco, and Lake Zapotlán (de Anda et al. 1998). The basin is home to about 150,000 inhabitants. The majority of the population resides in Ciudad Guzmán (C. Guzmán), the second largest city in the state.

About 51% of the surface area of the basin is used for agriculture and pasture land, 43 % is forested or vegetated, 2% is urban, and 4% is lake and wetland (Ortiz-Jiménez et al. 2005). Property ownership in the basin is divided among private, federal and state, ‘ejidal’ (collective land divisions), and indigenous sectors (Para, personal communication 2008). The forested uplands are increasingly being converted from forest to agriculture and pasture land. While the forests of the Nevado de Colima National Park have protected status, they continue to face pressure from illegal logging, cattle encroachment, and wildfires (de Anda, personal communication 2007).
The vegetation in the forested regions of the Lake Zapotlán basin is largely dry tropical coniferous and deciduous forest (Ortiz-Jiménez et al. 2005). While 42% of the world’s tropical and subtropical forests are characterized as dry forest (Murphy and Lugo 1986), the hydrology and biogeochemical cycles in these ecosystems have not been extensively researched compared to tropical rain forests (Campo et al. 1998).

2.3 WATERSHED HYDROLOGY

There are little published empirical data on the hydrology of Lake Zapotlán. The relative distribution of water inputs and outputs to the lake through a combination of empirical data and modeling is described by Ortiz-Jiménez et al. (2005). Water inputs to the lake are dominated by surface runoff (53%) during the wet season. Other water inputs include precipitation (22%), groundwater (14%), and sewage (11%). There are currently no perennial streams entering Lake
Zapotlán (Ortiz-Jiménez et al. 2005). Wastewater streams from the municipalities of C. Guzmán and San Sebastián del Sur are the only year-round water inputs to the lake. As the lake has no surface outflows, water losses are dominated by extraction (37%), evapotranspiration (34%), and evaporation (29%). The lake has a mean residence time of 7 months (Ortiz-Jiménez et al. 2005).

The region is characterized as having a wet season from May to October, while November through April remains relatively dry with little to no precipitation (Anaya et al. 2007). During the dry season and in between storm events, hillslopes, riparian zones, and receiving water bodies are hydrologically disconnected. The onset of the rainy season can drive rapid transport of materials accumulated over the dry season in surface runoff (Austin et al. 2004). Ephemeral streams have flow following storm events but quickly dry up. Some of the effects of land-use and land-cover changes in the watershed include reduced soil water storage, increased runoff and erosion, increased sediment loading, and increased lake level fluctuations following storm events (Ortiz-Jiménez et al. 2005).

2.4 LAKE CHARACTERISTICS

Lake Zapotlán is characterized as subtropical, shallow (mean 1.7m), and alkaline (Ortiz-Jiménez et al. 2005; 2006). The lake is considered well mixed most of the year due to winds (Ortiz-Jiménez et al. 2007). While the lake was historically characterized as eutrophic (Ortiz-Jiménez et al. 2006; Greenberg et al. 2008), its current eutrophic status is uncertain due to reductions in untreated wastewater discharge. Historically elevated nutrient concentrations in the lake stemmed from poorly treated sewage effluent and urban and agricultural runoff (Ortiz-Jiménez et al. 2006).

The construction of several municipal roads has physically segmented the lake into three regions and caused additional complications and changes in morphometry, hydrodynamics, aeration, and water quality of the lake (Ortiz-Jiménez et al. 2005). These roads created increasingly stagnant zones, which combined with the highly eutrophic conditions of the lake to create optimal conditions for the rapid colonization of aquatic weeds such as Cattail (*Typha latifolia*) and Water Hyacinth (*Eichhornia crassipes*) (Ortiz-Jiménez et al. 2005; 2006). A program to control the excessive growth of aquatic vegetation was initiated in 1995 and has reduced the current extent of floating and rooted stands of *T. latifolia* and *E. crassipes* to 30% of total lake surface area ($3.3 \times 10^6 m^2$; Ortiz-Jiménez et al. 2005). While the reduction of wetland area through this program has been beneficial to recreational and commercial users of the lake,
the shredding of wetland vegetation may impact water quality and Hg biogeochemistry in Lake Zapotlán.

_E. crassipes_ readily sequesters Hg and other heavy metals in plant tissues (Muramoto and Oki 1983). Plant tissues subject to mechanical shredding will quickly decompose and release stored Hg to water and sediment (Greenfield et al. 2007). Additional implications for shredding include a temporary spike in nutrient and dissolved organic carbon concentrations that increase microbial activity and enhance biological oxygen demand. Greenfield et al. (2007) reported that waters went completely anoxic for several weeks in a relatively slow moving slough in the Sacramento River Delta, USA following mechanical shredding of _E. crassipes_. This could enhance Hg methylation by creating prime conditions for the growth and activity of methylating SRB through the delivery of fresh organic matter, formation of anoxic conditions, and release of Hg from the shredded hyacinth tissues.

### 2.5 LAKE ZAPOTLÁN FISHERY

African tilapia (_Oreochromis_ spp.) and the Asian common carp (_Cyprinus carpio_) were introduced to Mexico in the 1960s-80s as an inexpensive source of protein for rural communities (Fitzsimmons 2000; Tapia and Zambrano 2003). Mexico is currently one of the largest producers of tilapia in the western hemisphere (Fitzsimmons 2000) with production of 110,000 tonnes in 2004 (Fitzsimmons 2006). It is estimated that the tilapia fisheries in Michoacán and Guerrero states alone support nearly 44,000 inhabitants (Jiménez-Badillo 2006). Tilapia and carp produced in Mexico are mostly consumed domestically with minimal export to international markets.

Lake Zapotlán supports an important local fishing economy for tilapia and carp. Two fishing cooperatives formed in the 1980s currently operate in the basin and employ around 60 individuals. Tilapia and carp are well adapted to poor water conditions and thrive in turbid eutrophic environments such as those seen in Lake Zapotlán (Tapia & Zambrano 2003; Shelton and Popma 2006). Up to 2 tonnes of carp and tilapia are brought in daily with an annual catch upwards of 600 tonnes in 2007 (Para, personal communication 2008). Tilapia and carp make up 68% and 32% of the total catch respectively (Ortiz-Jiménez et al. 2005). The total catch was in decline until 2006, when restocking efforts dramatically increased catch volumes. Tilapia are currently stocked annually in Lake Zapotlán with fish from a hatchery located in the region. Carp sustain a naturally reproducing population within the lake and are not stocked annually.
Tilapia and carp fished from Lake Zapotlán are sold locally and regionally in Guadalajara, Michoacán, Toluca, and Puebla states. Other species such as largemouth bass (Micropterus salmoides) are fished recreationally but do not make up any of the commercial catch. Endemic fish species such as Goodea atripinnis and Poeciliopsis infans are considered endangered, likely impacted by poor water quality and competition from exotic species (Soto-Galera et al. 1998; Canonico et al. 2005; Ortiz-Jiménez et al. 2005; Zambrano et al. 2006).

Tilapia and carp are often considered low-mercury fish (USEPA 2003; Levenson and Axelrad 2006). Low Hg bioaccumulation in these species may result from diet and relatively low trophic status (Zhou and Wong 2000). There are very little Hg data for these species in subtropical and tropical freshwater lakes of Mexico. McCrory et al. (2006) reported high Hg (>0.3 μg Hg/g w.w.) in 27% of tilapia sampled from a Hg impacted site in Nicaragua, indicating the potential for tilapia to bioaccumulate Hg to advisory levels (>0.3-0.5 μg Hg/g w.w.). While the mean concentration of common carp in investigations by the US Environmental Protection Agency (140 ng/g w.w.; USEPA 2003) was below consumption advisory levels, some samples and locations exceeded health guidelines.

2.6 POTENTIAL SOURCES OF MERCURY TO LAKE ZAPOTLÁN

Possible local sources of Hg to Lake Zapotlán are from untreated wastewater inputs (Bodaly et al. 1998), transport of particle bound Hg due to erosion in upslope deforested areas (Garcia and Carignan 2000), and natural enrichment from the nearby Colima volcano (Nriagu and Becker 2003). Wet and dry atmospheric deposition of Hg to the lake surface and watershed are also certainly contributors.

2.6.1 Mercury in Wastewater

Untreated wastewater inputs may represent a large year-round flux of Hg to the lake. Despite an increase in wastewater treatment in recent years, sustained high nutrient and Escherichia coli concentrations indicate continued loading of untreated wastewater to Lake Zapotlán (Greenberg et al. 2008; Greenberg 2009). Wastewater represents an estimated 11% of total water inputs to Lake Zapotlán and is the only surface water inputs during the dry season (Ortiz-Jiménez et al. 2005). Three wastewater treatment plants (WWTPs) are located in the Lake Zapotlán basin. Equipment maintenance and malfunction result in the continued discharge of untreated urban wastewater to the lake (10,300 m³/day from 1982-2003; Ortiz-Jiménez et al.
Untreated wastewaters can have high concentrations of Hg and MeHg, largely associated with suspended solids (Balogh and Liang 1995; Mugan 1996).

Heavy metal (including Hg) inputs from industrial and municipal effluent discharge to natural water bodies in Mexico can originate from activities such as: food processing, leather processing, natural and synthetic textiles, paper production, rubber and polymer production, non-metallic mineral processing, wood and metal furniture fabrication, and equipment and machinery assembly (Avila-Pérez et al. 1999). Additional Hg inputs in municipal effluent can result from incomplete capture of dental and clinical waste (Adegbembo et al. 2002).

Municipal wastewater treatment works are highly effective (>90%) at the removal of Hg (Mugan 1996; Bodaly et al. 1998). The removal of Hg is primarily associated with the settling of solids during primary treatment and the conversion of dissolved organic material to flocculent material that is removed from the aqueous medium in the final steps of treatment (Goldstone et al. 1990). A small amount of Hg (4-6 ng Hg/m³) can be lost to evasion during the transport and collection of wastewater due to anoxic reducing conditions (Lindqvist et al. 1991; Mugan 1996). In a WWTP in St. Paul, USA, of the 248 g Hg/day entering into the plant, 95% is ultimately reemitted to the atmosphere through incineration of sludge, while only 4% is discharged to the Mississippi River via the effluent stream (Balogh and Liang 1995).
3. DISTRIBUTION OF TOTAL MERCURY IN WATERS, SEDIMENTS, AND VEGETATION IN LAKE ZAPOTLÁN, MEXICO

The objective of the research presented in this chapter is to assess the potential for elevated bioaccumulation of Hg in biota, and exposure risk to human consumers of fish from Lake Zapotlán. To accomplish this objective, a scoping study was undertaken to measure the distribution of THg in water (dissolved and particle-bound) and sediments of the wastewater discharges, seasonal surface runoff, wetlands, and the open lake on three sampling campaigns spanning a complete hydrological year over 2007-2008. An annual THg mass balance for Lake Zapotlán was developed using data from this study and estimates from the literature.

3.1 METHODS

3.1.1 Sample Acquisition – Water and Sediment

Sampling campaigns were conducted in October 2007 (late wet season), February 2008 (mid dry season), and in June/July 2008 (early-mid wet season). All sampling was taken using ultra-clean protocols. Technicians were gloved in the field with powder-free nitrile gloves using what is commonly referred to as the clean hands, dirty hands method. Water samples were acquired in sterile and clean 250 mL PETG bottles either as a grab sample or using an acid-washed Teflon® sampling line and peristaltic pump (Figure 3.1). Separate samples were taken for both filtered and unfiltered water samples. Samples for dissolved Hg analysis were filtered using an acid-cleaned Teflon® filter apparatus (Savillex Inc) and pre-muffled (500°C) glass-fiber filters (Whatman GFF 0.7μm). Water samples were acidified to 0.5% (by volume) using ultrapure trace metals grade hydrochloric acid.

Lake and wetland sediments were collected by boat and on foot using a small (6x6x6”) Eckman bucket sampler (Figure 3.2) and/or manual sediment coring by hand using clear acid-washed PVC tubing. The sample was returned to the surface, and the top 4 cm were subsampled (in duplicate) and transferred to a small leak-proof zip-closure bag using a sterile scoop. The bag was rolled to exclude air, double-bagged, labeled, and placed into a clean, dark cooler filled with ice until they were returned to the laboratory and frozen. Samples were kept frozen at -15°C and were transported on ice to the University of Toronto, Canada for analysis.
Figure 3.1 Sampling of wastewater stream using a Teflon® sampling line and peristaltic pump.

Figure 3.2 Collection of lake sediments using an Eckman bucket sampler.
An overview of sampling locations, the extent of the lake and wetlands, and locations of wastewater inputs to Lake Zapotlán is illustrated in Figure 3.3. Samples were taken from a total of nine lake locations, three untreated sewage outfall locations, and one location in the disconnected wetland (Oct 2007 and Feb 2008 only). The WWTP 1 canal was not sampled during the Oct 2007 sampling trip because its existence was unknown. The southern and northern wetlands receiving untreated sewage discharge were sampled intensively during July 2008.

Runoff water from four sampling locations along seasonal runoff streams were sampled following two storm events in July 2008. Atequizayán discharges to the southwest region of the lake into dense wetland vegetation. La Fortuna discharges to a small strip of seasonally flooded pasture in the northeast part of the lake. Los Guayabos originates to the southeast of C. Guzmán and is channeled into a concrete canal around the city. This canal collects urban runoff and eventually combines with the untreated effluents from WWTP 2 and is discharged to the south end of Lake Zapotlán.

For the first storm event the streams of Atequizayán, La Fortuna, and a downstream (includes urban runoff) location of Los Guayabos were sampled once. During the second storm event an upstream (minimal urban runoff) location of the Los Guayabos canal was sampled over a period of two hours. A total of six water samples were taken at 20 minute intervals to assess the variability in THg concentrations over the duration of this event. Samples of runoff water were collected as described for lake sampling. Rainwater was collected from the same storm events using a 0.25m diameter acid-washed Teflon® funnel that delivered rainwater directly into a 250 mL PETG bottle. For both storm events, two samples of rainwater were collected. Runoff/rainwater samples were acidified to 0.5% (by volume) using ultrapure hydrochloric acid.

3.1.2 Sample Acquisition - Vegetation Sampling

Vegetation was collected using the same general method as for sediments, except that the sample is manually transferred into the zip-closure bag. Sampling of *T. latifolia* was conducted in wetlands in proximity to wastewater outfalls. Relatively young and similar sized specimens of *T. latifolia* were selected for sampling. Foliage is a composite of three separate segments from the same individual. *T. latifolia* roots and rhizomes were collected through manual uprooting with the assistance of a 12” handsaw to loosen the plant from the sediments. *E. crassipes* was sampled at the north and south ends of the open water of the lake along the wetland fringe in
relative proximity to the wastewater outfalls. *E. crassipes* was collected from the San Sebastián del Sur wastewater canal in Oct 2007 and Feb 2008 but not during the sampling in June/July 2008. Increased water flow due to seasonal rains had removed *E. crassipes* from this location. *E. crassipes* was also sampled within the southern wetlands near the discharge of WWTP 2.

**Figure 3.3** Sampling locations for sediments, surface waters, wastewater treatment plants, runoff, and wastewater in the Lake Zapotlán Basin (Los Guayabos sampling above C. Guzmán not shown)
3.1.3 Ancillary Water Quality Measurements

Water temperature, dissolved oxygen, pH, and conductivity were measured at most sampling locations using a Hydrolab DS5X or a YSI 600QS water quality sonde. Measurements were taken at a standardized depth of 1m at all lake sites and at the surface in shallow wetland locations. Water samples for major ions and DOC were filtered using a Whatman GFF 0.7μm filter and frozen until analysis. Major ions were measured at the University of Toronto, Canada using a Dionex DX-500 Ion Chromatograph (Detection limit 0.04 mg/L, ongoing precision recovery 85-106%). DOC was measured at the University of Toronto using a Lachat IL 550 TOC/TN analyzer (Detection limit 0.6 mg C/L, ongoing precision recovery 97%) according to EPA Method 415.3: Determination of Total Organic Carbon and Specific uv Absorbance at 254 nm in Source Water and Drinking Water. The furnace temperature for sample combustion was set to 680°C.

3.1.4 Mercury Analysis

THg analysis in solid phase materials (sediment, soil, vegetation) was performed at the University of Toronto using a Milestone, Inc. DMA-80 Direct Mercury Analyzer following procedures outlined in USEPA Method 7473: Mercury in Solids and Solutions by Thermal Decomposition Amalgamation, and Atomic Absorption Spectrophotometry. Approximately 0.25-0.5 g of the sample was weighed into a nickel boat for analysis. Samples were analyzed as wet weight (w.w.) but final analytical concentrations are expressed as a standardized dry weight (d.w.) through the generation of a wet-to-dry weight conversion factor derived from oven-drying a subsample at 90°C for 24 hours. Prior to analysis, roots and foliage of T. latifolia were rinsed with 18MΩ purified water to remove sediment and dust (Millipore Milli-Q system). Average THg recovery of a lake sediment certified reference material to assess method accuracy was 94% (IAEA Sl-1, N=25) and the mean relative percent difference among triplicate measurements was 9% (N=67). The average blank measurement using empty nickel boats was 0.04 ng Hg (N=75). The detection limit calculated as three standard deviations of all blank measurements was 0.18 ng Hg.

Ultra-trace THg analysis in water was performed at the University of Toronto using a Tekran 2600 Automated Total Mercury System contained within a Class 100 Clean Room to minimize contamination of samples and equipment. THg determination in surface water
followed EPA Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS). This analysis utilized a Tekran model 2600 CVAFS Hg detector with automated sampler. Average recovery of a THg spike to assess method accuracy was 93% (N=11), relative percent difference among duplicate measurements was 13% (N=26), and the detection limit was 0.27 ng/L (N=39) calculated as three standard deviations of analysis of reagent water (18MΩ purified water).

3.1.5 Statistical Analysis

Statistical analysis using Microsoft Excel data analysis functions was conducted to assess spatial and temporal variability of results. The effects of seasonality and location on water and sediment THg concentrations were tested using ANOVA. If a statistical difference was found, the Tukey post-hoc test was utilized to determine which means differ from one another. Statistically significant differences were assumed at \( P \leq 0.05 \).

3.2 RESULTS

3.2.1 General Water Quality

Water quality parameters measured in situ in the open waters of the lake and disconnected wetland are presented in Table 3.1. Some parameters were not collected on all campaigns due to the use of slightly different instruments, instrument technical problems, or both. Average pH in lake surface water (8.7, range: 7.7-9, N=28) was basic and did not fluctuate greatly over the study period for most sampling locations. One low pH value of 7.7 was observed during the summer at the northernmost sampling site near the outfall of the San Sebastián del Sur wastewater. It is likely that storm runoff water from the northern catchments contributed to a localized drop in pH at the north end of the lake. Mean pH levels in the disconnected wetland surface waters were less than the lake for both Feb (wetland-8.0, N=1; lake-8.5, range: 8.0-8.9, N=9) and June/July (wetland-7.6, range: 7.4-8.3, N=9; lake-8.7, range: 7.7-9.1, N=9). Average lake temperature was coolest in February (19.1°C, range: 16.2-20.7, N=9) and warmest in October (26.2°C, range: 24.3-28.2, N=10). The disconnected wetland had slightly lower surface water temperature compared with the lake. The average depth of lake sampling stations was 2m (minimum and maximum depth of 0.3-4.5m respectively), with the deepest region found in the northeast.
Table 3.1 Various water quality parameters from the open water and wetland stations of Lake Zapotlán.

<table>
<thead>
<tr>
<th></th>
<th>Lake Temp (°C)</th>
<th>DO (mg/L)</th>
<th>pH</th>
<th>Cond. (μS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake (N=10)</td>
<td>26.2</td>
<td>8.3</td>
<td>8.9</td>
<td>684</td>
</tr>
<tr>
<td>Max</td>
<td>28.2</td>
<td>9.8</td>
<td>9.0</td>
<td>690</td>
</tr>
<tr>
<td>Min</td>
<td>24.3</td>
<td>6.3</td>
<td>8.7</td>
<td>679</td>
</tr>
<tr>
<td>Feb 2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake (N=9)</td>
<td>19.1</td>
<td>7.9</td>
<td>8.5</td>
<td>1048</td>
</tr>
<tr>
<td>Max</td>
<td>20.7</td>
<td>10.4</td>
<td>8.9</td>
<td>1533</td>
</tr>
<tr>
<td>Min</td>
<td>16.2</td>
<td>4.1</td>
<td>8.0</td>
<td>975</td>
</tr>
<tr>
<td>Disconnected Wetland (N=1)</td>
<td>18.1</td>
<td>5.4</td>
<td>8.0</td>
<td>1288</td>
</tr>
<tr>
<td>June/July 2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake (N=9)</td>
<td>24.2</td>
<td>N/A</td>
<td>8.7</td>
<td>944</td>
</tr>
<tr>
<td>Max</td>
<td>26.5</td>
<td>N/A</td>
<td>9.1</td>
<td>1923</td>
</tr>
<tr>
<td>Min</td>
<td>21.2</td>
<td>N/A</td>
<td>7.7</td>
<td>317</td>
</tr>
<tr>
<td>Disconnected Wetland (N=9)</td>
<td>Mean</td>
<td>N/A</td>
<td>7.6</td>
<td>1411</td>
</tr>
<tr>
<td>Max</td>
<td>21.6</td>
<td>N/A</td>
<td>7.7</td>
<td>1886</td>
</tr>
<tr>
<td>Min</td>
<td>20.1</td>
<td>N/A</td>
<td>7.4</td>
<td>1083</td>
</tr>
</tbody>
</table>

While there is some variability among sites, generally the surface waters are well oxygenated. Conductivity values were higher for the dry season (Feb) sampling trip than other sampling trips. This may be accounted for by changing wind patterns that stir up sediments into the water column combined with a reduction in lake volume during the dry period (Ortiz-Jiménez et al. 2007). Average conductivity was higher in the disconnected wetland (1349 μS/cm, range: 1083-1886, N=10) than in the lake (892 μS/cm, range: 317-1923, N=28), likely due to the wastewater discharge and limited water exchange with the lake.

Average DOC concentrations in lake and wetland surface waters was high (48.1 mg C/L; range 12.6-91.6, N=38). Average DOC concentrations in the lake were 50.0 mg C/L (range: 35.7-60.8 mg C/L, N=18) from October-June, but fell to 26.6 mg C/L (range: 14.4-46.0 mg C/L, N=5) in July. DOC concentrations in the lake are very high in comparison to other global water bodies. A review of over 7,000 lakes worldwide show that average DOC concentrations in surface waters are 7.6 mg C/L, with < 0.4% of lakes having concentrations greater than 40 mg C/L (Sobek et al. 2007).
3.2.2 Mercury Inputs to Lake Zapotlán

Total Mercury in Municipal Wastewater Discharges. Wastewater THg concentrations from the three discharges are illustrated in Figure 3.4. THg concentrations in untreated wastewater discharges to Lake Zapotlán from C. Guzmán (WWTP 1 and 2) averaged 484 ng/L (range: 134-942, N=9) across the study period (Figure 3.4). The average THg input from the San Sebastián del Sur wastewater outfall was much lower (34.5 ng/L, range: 17.5-64.2, N=4), reflecting the smaller size and decreased urbanization of this area compared with C. Guzmán. THg was dominantly in the particulate phase in the C. Guzmán (93%) and San Sebastián del Sur (86%) discharges. Lower THg measured during the Oct and June/July sampling periods are likely due to dilution during the wet season.

![Figure 3.4](image)

**Figure 3.4** Mean concentrations of THg in unfiltered waters in untreated wastewater inputs to Lake Zapotlán (± range of values). Oct: N=1 (Plant 2), 2 (San Sebastián del Sur); Feb: N=1(Plant 1), 2(Plant 2), 1(San Sebastián del Sur); June/July: N=2 (Plant 1), 3 (Plant 2), 2 (San Sebastián del Sur)

An initial assessment of the C. Guzmán WWTPs demonstrated high THg removal efficiencies for both particle-bound (97-98%) and dissolved THg (82%) with effluent THg concentrations <10.0 ng Hg/L (Figure 3.5). However, high THg in wastewater streams entering the lake confirm that there is currently significant bypass of wastewater from these facilities. While wastewater inputs to the lake do not exceed Mexican wastewater discharge guidelines (0.005 mg/L; SEMARNAT 1996) for the protection of aquatic life in rivers (no guideline exists for lakes), these concentrations are cause for concern considering that wastewater input estimates make up a considerable proportion of water inputs to the lake (11%; Ortiz-Jiménez et al. 2005).
Volume estimates of the annual discharge of untreated wastewater into Lake Zapotlán were determined from data provided by the Jalisco State Water Commission (CEAJ 2008). Estimates are based on the volume of water treated for drinking water, assuming a 30% loss, and then subtracting the estimated volume of treated wastewater:

\[
\text{Volume Untreated Wastewater Discharge} = \text{Volume of Treated Water} \times 0.7 - \text{Volume Treated}
\]

**Figure 3.5** C. Guzmán wastewater treatment plant influent and treated effluent particulate and dissolved phase THg, July 2008 (N=1 for each treatment).

In C. Guzmán treated drinking water volumes were estimated at 2,677 m$^3$/day based upon an average treatment volume of 280 L/person/day with a population of 95,619 in 2007. In 2007 the two WWTPs in C. Guzmán were treating an average of 147.3 L/sec (CEAJ 2008). Estimated untreated wastewater inputs from C. Guzmán are 70 L/sec. Since the WWTP in San Sebastián del Sur (population 5,000) was not in operation during this study, approximately 11 L/sec of wastewater flows directly to the lake from this community. It is estimated that approximately 6050 m$^3$/day of untreated wastewater enters Lake Zapotlán from C. Guzmán and 950 m$^3$/day from San Sebastián del Sur. Based on these values and the average concentrations of THg (C. Guzmán = 484 ng/L; San Sebastián del Sur = 34.5 ng/L) in wastewater, it is estimated that 1.08 kg Hg/yr is discharged to Lake Zapotlán in untreated wastewater. Approximately 90% of THg inputs are associated with particles.
Total Mercury in Rainfall and Runoff. During our sampling campaign, stream channels only had surface flow following several large storm events in July, with little to no flow after a 24-hour period following the storm event. Average THg in rainwater from the two storm events was 5.1 ng/L (range: 0.5-11.5 ng/L, N=4). This average is less than reported at an atmospheric monitoring station in central-eastern Mexico in Hidalgo State (10.7 ng/L, N=38; NAPD 2005) and is similar to THg in rainwater seen in the northeast USA (range: 4.0-6.0 ng/L; NADP 2007). The mean unfiltered THg concentration in four wet season runoff streams following two storm events was relatively high (130 ng/L, range: 39.0-253, N=9; Table 3.2). Dissolved THg averaged 22.6 ng/L (range: 4.7-43.0, N=9) indicating THg is highly associated to particles (83%).

Table 3.2 Mean THg in unfiltered and filtered waters from four separate surface water inflows to Lake Zapotlán following two storm events in July, 2008.

<table>
<thead>
<tr>
<th></th>
<th>Unfiltered THg (ng/L)</th>
<th>Dissolved THg (ng/L)</th>
<th>% Particulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>130</td>
<td>22.6</td>
<td>83</td>
</tr>
<tr>
<td>Maximum</td>
<td>253</td>
<td>43.0</td>
<td>-</td>
</tr>
<tr>
<td>Minimum</td>
<td>38.8</td>
<td>4.7</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

Estimated annual inputs of runoff and precipitation are 18.91 and 7.76 $10^6$ m$^3$, respectively, composing 53% and 22% of total water inputs, respectively (Ortiz-Jiménez et al. 2005). To estimate the annual flux of THg in runoff and precipitation to Lake Zapotlán the annual volumes were multiplied by the average THg concentrations measured in this study. It is estimated that 2.46 kg of Hg is discharged annually to the lake in seasonal runoff streams, predominantly associated with particles (83%). Estimated annual THg input from precipitation is 0.040 kg.

3.2.3 Total Mercury in Lake Zapotlán Water and Sediments

Lake Sediments. THg in lake sediments averaged 53.6 ng Hg/g d.w. (range: 14.6-136, N=34) (Figure 3.6). While the average lake sediment concentration increased slightly in the wet season (71.1 ng/g d.w., range: 64.3-92.2, N=5), there was no statistically significant difference based
upon a single factor ANOVA ($P = 0.26$). This slight increase in sediment concentration in July (wet season) could be due to the settling of particulate inputs from runoff streams.

Variability around the mean value is driven by proximity to wastewater outfalls. The mean THg concentration in sediment in the southern end of the lake closest to the WWTP2 outfall (99.8 ng/g d.w., range: 84.4-136 ng/g d.w., $N=4$) was double the mean concentration found in the open lake (48.8 ng/g d.w., range: 14.6-92.2 ng/g d.w., $N=31$). Sediment THg concentrations are below Environment Canada’s sediment quality guidelines of 170 ng/g with a probable effects level (PEL) of 486 ng/g (CCME 2002). The PEL is the concentration of chemical likely to cause an adverse effect to aquatic biota.

![Figure 3.6](image)

**Figure 3.6** Mean THg in Lake Zapotlán sediments (± range of values). $N=11$ (Oct), 9 (Feb and June), 5 (July)

**Surface Waters.** Concentrations of THg in unfiltered lake surface water averaged 2.4 and 2.2 ng/L in October 2007 and February 2008 (range: 0.9-4.6, $N=20$), respectively, but increased at the onset of the rains to 4.2 and 5.6 ng/L for June and July 2008 (range: 2.1-10.7, $N=14$), respectively (Figure 3.7). As found by Avila-Pérez et al. (2006), dilution driven by greater reservoir volume in October and February compared to June/July are one explanation for reduced surface water THg concentrations. Variability in lake surface water THg concentrations can also be explained by proximity to wastewater outfalls. The mean THg concentration at sampling stations closest to the WWTP 2 and San Sebastián del Sur wastewater outfalls was 6.6
ng Hg/L (range: 2.1-11.0, N=6) while other lake stations averaged 2.6 ng Hg/L (range: 0.9-5.0, N=27). Surface water concentrations in the main body of Lake Zapotlán were similar to remote lakes in the northeast USA (range: 0.5-5.0 ng/L; Dittman and Driscoll 2009). Lake concentrations are well below Environment Canada’s water quality guideline of 26.0 ng Hg/L in natural waters for the protection of aquatic life (CCME 2001).

![Graph showing THg concentrations in Lake Zapotlán from October 2007 to July 2008](image)

**Figure 3.7** Mean THg in unfiltered and filtered surface waters of Lake Zapotlán (± range of values). Significant differences for THg between dates are indicated with different letters; Tukey test ($\alpha = 0.05$). N=11 (Oct), 9 (Feb), 9 (June), 5 (July)

The percent of THg in the particulate phase was 33% and 23% in October and February, respectively, but increased to 70% and 78% in June and July, respectively (Figure 3.7). October and February total and particulate THg concentrations are statistically different from July based upon a single factor ANOVA and Tukey test ($\alpha = 0.05$). Dissolved phase THg did not differ among sampling dates ($P = 0.14$). The predominance of particulate phase THg is explained by the influx of particle-bound THg to the lake in natural runoff, and re-suspension of sediments from winds (Ortiz-Jiménez et al. 2007). Additionally, June and July were characterized by higher primary productivity (Ortiz-Jiménez et al. 2006) which may scavenge Hg and MeHg from the water column resulting in higher particulate bound Hg concentrations (Chen and Folt 2005).
3.2.4 Total Mercury in Impounded Wetland Water and Sediments

While sediment and water concentrations in the main body of the lake were below environmental quality guidelines, mean concentrations in the disconnected wetland frequently exceeded Environment Canada environmental quality levels. Mean THg in sediment and surface water in this wetland is shown in Figures 3.8 and 3.9, respectively. Average THg in sediment across the three sampling periods in the disconnected wetland (391 ng/g d.w., range: 84.0-860, N=9) was considerably higher than the open water lake stations (53.6 ng Hg/g d.w., range: 14.6-136, N=34). Similarly, average THg in unfiltered surface water (48.0 ng/L, range: 1.9-240, N=11) was higher than open water lake stations (3.3 ng/L, range: 0.9-10.7 ng/L, N=33).

![Figure 3.8](image) Mean THg in sediments in the disconnected wetland of Lake Zapotlán (± range of values), probably effects level according to Environment Canada is 486 ng Hg/g d.w. N= 1 (Oct), 1 (Feb), 9 (July)

The results from this survey reveals high spatial variability in THg concentrations in both water and sediment. Average unfiltered water and sediment THg concentrations within the dense *T. latifolia* wetlands closest to the wastewater outfall were higher (120 ng/L, range:13.7-240, N=5; 520 ng/g d.w., range: 381-863 ng/g d.w., N=4; respectively) than those in the open water stations (6.3 ng/L, range: 3.1-9.3 ng/L, N=4; 270 ng/g d.w., range: 84.0-490 ng/g d.w., N=4; respectively) (Figure 3.10). Decreasing particle-bound THg loads in surface waters along a gradient away from water inflows [116 ng/L (inflow), 2.3 ng/L (after wetland)] suggests that particle bound THg was being retained in the wetland, likely through settling. Additionally,
decreased dissolved THg away from water inflows [8.1 ng/L (inflow), 3.9 (after wetland) ng/L] suggests sorption to vegetation and particulate matter within the wetlands.

Figure 3.9 Mean THg (log scale) in unfiltered water in the disconnected wetland of Lake Zapotlán (± range of values), Environment Canada water quality guideline is 26 ng/L. N= 1 (Oct), 1 (Feb), 9 (July)

A similar reduction in THg was evident in areas of the southern wetlands receiving wastewater discharge. During a survey of this wetland in July 2008, THg in unfiltered surface water near wastewater inputs was 49.3 ng/L, and at the sampling location farthest from wastewater inputs and nearer the lake, inputs were 19.8 ng/L. Simultaneous sampling in the southern end of the lake revealed a lower THg concentration in unfiltered surface water (10.7 ng/L) compared to that near wastewater inputs (Figure 3.10). Surface water data suggest that there was a reduction in THg of approximately 38.6 ng/L as the wastewater moved through the wetlands. Sediment THg concentrations in the southern wetlands (average 68.8 ng/gdw, range: 47-101, N=8) and lake (91 ng/g d.w., N=1) were similar to each other.
3.2.5 Total Mercury in Floating and Rooted Macrophyte Vegetation

*E. crassipes.* There was very little coverage of *E. crassipes* over Lake Zapotlán during the sampling period due to management efforts to control this invasive species. Concentrations of THg in above water (blade and stalk) and below water (fine and woody root) plant mass in the lake and wastewater streams are presented in Table 3.3. Higher average THg concentrations in foliage and roots were found in the northern (25.9, 55.8 ng/g d.w., N=4, respectively) compared with that of the southern (11.4, 30.3 ng/g d.w., N=9, respectively) end of the lake (Table 3.3). The highest concentrations of THg were found in individuals growing within the San Sebastián del Sur wastewater canal and south canal discharge. The mean THg in root tissues of individuals growing in wastewater streams (130.1 ng/g d.w., range: 30.0-460 ng/g d.w., N=5) was higher than in individuals sampled from the lake.
Table 3.3 Mean THg in foliage and root tissues of *E. crassipes* (October 2007-July 2008).

<table>
<thead>
<tr>
<th></th>
<th>South Lake</th>
<th>North Lake</th>
<th>San Sebastián del Sur Wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foliage</td>
<td>Root</td>
<td>Foliage</td>
</tr>
<tr>
<td>Mean THg (ng Hg/gdw)</td>
<td>11.4</td>
<td>30.3</td>
<td>25.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>16.9</td>
<td>94.6</td>
<td>58.6</td>
</tr>
<tr>
<td>Minimum</td>
<td>8.8</td>
<td>5.6</td>
<td>7.0</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

*T. latifolia*. Root and foliage THg concentrations in *T. latifolia* from wetlands are shown in Table 3.4. As with sediment and water concentrations, vegetation THg concentrations were variable in the disconnected wetland. Neither root THg nor foliage THg was correlated with underlying sediment and water THg concentrations. A spatial pattern of THg in *T. latifolia* roots and foliage across Lake Zapotlán was evident. The averages of THg in foliage and roots follow a similar pattern to water and sediment THg: segregated wetland (32.8, 113 ng/g d.w., respectively) > south lake (11.0, 16.7 ng/g d.w., respectively) > north lake (4.4, 14.7 ng/g d.w., respectively) (Table 3.4).

Table 3.4 Mean THg in foliage and root tissues of *T. latifolia*, July 2008.

<table>
<thead>
<tr>
<th></th>
<th>South Wetland</th>
<th>Disconnected Wetland</th>
<th>North Wetland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foliage</td>
<td>Root</td>
<td>Foliage</td>
</tr>
<tr>
<td>Mean THg (ng/g d.w.)</td>
<td>11.0</td>
<td>16.7</td>
<td>32.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>20.7</td>
<td>34.9</td>
<td>179</td>
</tr>
<tr>
<td>Minimum</td>
<td>3.6</td>
<td>5.1</td>
<td>2.6</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

3.3 DISCUSSION

Wastewater and runoff inputs to Lake Zapotlán have high particle-bound THg concentrations that exceed Environment Canada water quality guidelines for the protection of aquatic life. Similarly, Avila-Pérez (2006) found that 89-92% of high Hg inputs to a Mexican reservoir from the contaminated Lerma River were associated with particles. While wastewater THg inputs are high, they are similar to those published elsewhere. Mugan (1996) found that
mean influent Hg concentrations to several treatment works in Wisconsin, USA ranged from 125-820 ng Hg/L, with individual measurements as high as 3000 ng Hg/L. The average concentration of THg in untreated sewage in Winnipeg, Canada was substantially lower than in this study (61 ng Hg/L; Bodaly et al. 1998).

It is estimated that 550,000 tonnes of sediments are discharged to the lake per year due to a combination of high slopes in the basin, erodible soils, deforestation, agriculture, and urban growth (Ortiz-Jiménez et al. 2005). The conversion of tropical dry forests to pasture and agricultural lands decreases soil cover, consequently diminishing infiltration rates and evapotranspiration, and increases runoff and erosion (Maass et al. 2005). As reviewed by Munthe et al. (2007), agriculturally impacted watersheds tend to export more Hg in the particulate phase in surface runoff compared to forested watersheds due to soils erosion; however this Hg may be less available for methylation and bioaccumulation in aquatic organisms.

The distribution of THg in water inputs, surface waters, sediments, and vegetation in Lake Zapotlán is illustrated in Figure 3.11. The highest concentrations of THg in sediments, waters, and vegetation are found within the wetlands of Zone A, followed by Zone A*, Zone B, and Zone C (Figure 3.11). The elevated concentrations found in Zone A are likely a reflection of untreated wastewater inputs, a dense T. latifolia-dominated wetland, disconnection from the lake due to a municipal road, and sediment retention due to low erosion and resuspension. Sediment concentrations in Zone A were in the range found in Avila-Pérez et al. (2006) in a mercury-impacted reservoir along the Lerma River (230-540 ng/g d.w.). The elevated concentrations of THg in waters and sediments of Zone B also reflect proximity to a major wastewater input. Unlike Zone A, the THg is not as concentrated in sediments due to increased rates of mass transport of particles into the main body of the lake.

Given that wastewater represents a significant water input to the lake, it is surprising that surface waters and lake sediments in Zone C do not reflect the high levels of THg inputs. Typha spp. are often cited for their ability to reduce heavy metals in contaminated effluents through the settling of particulate loads and bioconcentration of metals in plant tissues (Hadad et al. 2006; Maine et al. 2007; Sundberg and Hassan 2007). The wetland areas of Lake Zapotlán are acting as a buffer, effectively retaining both particle-bound and dissolved THg inflow from the wastewater and seasonal streams. Nutrient trends also indicate that the wetland fringe is improving water quality inputs to the lake. Lake surface waters had reduced average
<table>
<thead>
<tr>
<th>Water Input/Lake Zone</th>
<th>Particulate THg Water (ng/L)</th>
<th>Dissolved THg Water (ng/L)</th>
<th>THg Sediment (ng/g d.w.)</th>
<th>THg T. latifolia (ng/g d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Guzmán Wastewater</td>
<td>448 (±105, N=9)</td>
<td>35.5 (±5.7, N=9)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>San Sebastián del Sur Wastewater</td>
<td>29.8 (±9.7, N=4)</td>
<td>4.7 (±1.1, N=4)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Runoff (4 locations)</td>
<td>107 (±22.4, N=9)</td>
<td>22.6 (±4.8, N=9)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ZONE A (Within Typha)</td>
<td>108 (±44.7, N = 5)</td>
<td>8.1 (±0.7, N=9)</td>
<td>520 (±110, N=4)</td>
<td>Foliage: 32.8 (±16.3, N=11) Root: 113 (±57.7, N=11)</td>
</tr>
<tr>
<td>ZONE A* (Open Water)</td>
<td>2.3 (±0.8, N=4)</td>
<td>3.9 (±1.3, N=4)</td>
<td>270 (±82.7, N=4)</td>
<td>N/A</td>
</tr>
<tr>
<td>ZONE B</td>
<td>16 (±4.4, N=6)</td>
<td>4.6 (±0.9, N=6)</td>
<td>68 (±7.1, N=15)</td>
<td>Foliage: 11.0 (±2.2, N=11) Root: 16.7 (±4.1, N = 11)</td>
</tr>
<tr>
<td>ZONE C</td>
<td>1.3 (±0.2, N=26)</td>
<td>1.4 (±0.1, N=26)</td>
<td>48 (±2.5, N=25)</td>
<td>Foliage: 4.4 (±0.9, N=3) Root: 14.7 (±6.8, N=3)</td>
</tr>
</tbody>
</table>

**Figure 3.11** Summary figure of the spatial distribution of average THg in water, sediment, and *T. latifolia* in Lake Zapotlán, Mexico across all sampling periods (± standard error).

concentrations of phosphorus [below detect (0.04 mg/L), N=25] and sulfate (12 mg/L, N=29) compared with average wastewater inputs (phosphorus = 10 mg/L, N=12; sulfate = 39 mg/L, N=12). Constructed treatment wetlands in the Everglades Agricultural Area, USA (with
abundant *Typha* growth) also demonstrated reductions in both THg and phosphorus (Miles and Fink 1998). The wetland fringe of Lake Zapotlán appears to have an important function in improving water quality in Lake Zapotlán.

### 3.4 MASS BALANCE OF TOTAL MERCURY

An approximate THg mass balance in Lake Zapotlán was assembled using the data collected in this study and various estimates from the literature. The inputs to Lake Zapotlán are wastewater, runoff, and wet deposition and the outputs are open water volatilization, wetland volatilization, water extraction, and fishing. Storage compartments include sediment, lake water, and wetland vegetation.

The components that are most certain and were calculated using data from this study and published studies from Lake Zapotlán are wastewater, runoff, water column storage, water extraction, sediment storage, and fish extraction. Vegetation storage is less certain due to estimated biomass values and high variability in root tissue THg concentrations from this study. Wet deposition is highly uncertain due to high variability in measured THg concentrations in precipitation. The components that are most uncertain and based entirely from estimates in the literature, are open water volatilization and wetland volatilization. The assumptions of this model are that THg inputs from dry deposition, groundwater inputs, and diffusive flux from sediments were negligible.

#### 3.4.1 Inputs

The estimated input of THg in runoff is 2.46 kg THg/yr (3.2.2). None of the runoff streams entering Lake Zapotlán are gauged, and flow volumes are based on water balance estimates in Ortiz-Jiménez et al. (2005). Additionally, the variability in runoff THg concentrations was high (range: 38.8-253 ng/L; 3.2.2) and sample size was low (N=9). For the mass balance we assume a ± 60% uncertainty based upon the variability in THg concentrations measured during this study. It is assumed that any error in the water volume is included in this uncertainty. For the mass balance the estimated input of THg in runoff is 0.98-3.9 kg THg/yr.

Wastewater contributes 1.08 kg/yr of THg loading (3.2.2). Similar to runoff, this estimate has uncertainty associated with large variability in THg concentrations (range: 130-940 ng/L) and unmeasured discharge volumes. While wastewater volume estimates based upon the provided data (CEAJ 2008) are somewhat certain, a ± 60% uncertainty is assumed for THg
inputs in wastewater due to the large variability in THg concentrations throughout the year. The estimated input of THg in wastewater is 0.43-1.8 kg THg/yr. Runoff accounts for a higher volume (18.92 $10^6$ m$^3$) of annual water inputs to the lake compared with wastewater (4.04 $10^6$ m$^3$) and therefore dominates Hg inputs (Ortiz-Jiménez et al. 2005). Approximately 90% of THg inputs in runoff and wastewater are associated with particles.

Wet deposition THg inputs (0.040 kg THg/yr) were very small in comparison to runoff and wastewater (Section 3.2.2). Due to high variability in THg measurements in precipitation (range: 0.5-11.0 ng/L) and a small sample size (N=4), an uncertainty of ± 90% is assumed for this estimate. For the mass balance the estimated input of THg in wet deposition is 0.004-0.076 kg THg/yr.

3.4.2 Outputs

Elemental Hg$^0$ volatilization from surface water can represent a considerable flux of dissolved THg to the atmosphere (Schroeder et al. 1992). The photoreduction of dissolved Hg$^{2+}$ and evasion from surface water is correlated with solar radiation, wind speed, and water temperature (Krabbenhoft et al. 1998b; Lindberg and Zhang 2000). While Hg evasion from tropical water bodies is not well characterized, the evasive loss of Hg from Lake Zapotlán could represent a substantial flux due to warm temperatures, high incoming radiation, frequent winds, and shallow waters. Additionally, high pH tends to increase the loss of volatile Hg$^0$ from lake waters (Winfrey and Rudd 1990). The annual evasion flux of THg was estimated using evasion rates from several studies over subtropical and tropical surface waters (8-28 $\mu$g Hg/m$^2$/yr; Lindberg and Zhang 2000; Lindberg et al. 2002; Muresan et al. 2007) and an approximate open water surface area of 7.7 $10^6$ m$^2$ (Ortiz-Jiménez et al. 2005). The estimated evasion rate from the open water areas of Lake Zapotlán is 0.060-0.220 kg THg/yr.

Mean annual Hg$^0$ flux over Typha was determined to be the largest THg flux from a constructed wetland in the Everglades (SFWMD 1999, Lindberg et al. 2005). Lindberg et al. (2002) demonstrated that the mean THg flux over Typha (175 $\mu$g Hg/m$^2$/yr) greatly exceeded emission rates from surface water alone (11 $\mu$g Hg/m$^2$/yr). The emission of Hg from Typha tissues occurs through sediment-Hg mobilization and subsequent transport within tissues via pressurized through-flow purging at sunrise and xylem transport during daytime transpiration (SFWMD 1999; Lindberg et al. 2005). The annual evasive flux of THg from wetlands was estimated using evasion rates from Typha in the Everglades and an estimated wetland surface
area of $3.3 \times 10^6$ m$^2$ (Ortiz-Jiménez et al. 2005). Lindberg et al. (2005) report a 60-70% uncertainty associated with their estimated evasion rates over *Typha* in the Florida Everglades due to spatial and temporal variability. The estimate of evasive flux over *Typha* in this mass balance assumes a similar uncertainty (± 70%). The estimated evasion rate from the vegetated areas of Lake Zapotlán is 0.17-0.98 kg THg/yr.

Water extraction THg output was estimated using a published extraction volume for 1983-2003 ($13.0 \times 10^6$ m$^3$/yr; Ortiz-Jiménez et al. 2005) and the average unfiltered surface water THg concentration in this study (3.3 ng/L; Section 3.2.5). The estimated output of THg through water extraction is (0.021-0.064 kg THg/yr). The range in water extraction outputs is based upon uncertainties in water balance calculations from Ortiz-Jiménez et al. (2005) and the fluctuation in surface water THg concentrations over the year (± 50% of annual mean).

Intensive fishing has been hypothesized to decrease fish THg concentrations in some lakes through reductions in THg and MeHg in the aquatic environment (Surette et al. 2006). The export of THg from Lake Zapotlán from commercial fishing activities was estimated using the harvest biomass in 2007 (600 tonnes), average THg concentrations in tilapia and carp (Ch. 4), and an estimated catch distribution of 68% tilapia and 32% carp (Ortiz-Jiménez et al. 2005). This output assumes a ±10% uncertainty due to the potential difference in the catch distribution during the study period compared with values published in Ortiz-Jiménez et al. (2005). The estimated output of THg in fishing is 0.0027-0.033 kg THg/yr. This value represents a very small output of THg from the lake.

### 3.4.3 Storage Compartments

THg storage in sediments was estimated to a 10 cm depth using a dry bulk density of 0.2-0.4 g/cm$^3$ (from estimates in Menounos 1997; Miles and Fink 1998; Ruiz et al. 2007), a lake area of $11 \times 10^6$ m$^2$, and an average lake THg sediment concentration of 53.6 ng/g d.w. (3.2.3). The estimated THg storage in sediments (0-10 cm) is 11-22 kg THg. The uncertainty associated with the range of sediment storage values reflects the range of estimated sediment bulk densities.

THg storage in lake water was estimated using a water volume of $19.61 \times 10^6$ m$^3$ (Ortiz-Jiménez et al. 2005) and the average THg concentration in unfiltered lake surface waters (3.3 ng/L; Section 3.2.3). Water volume (during 1995-2003) fluctuated between the dry and wet seasons from 10-30 $10^6$ m$^3$ (Ortiz-Jiménez et al. 2005). The estimated THg storage in lake water
is 0.033-0.098 kg THg. The uncertainty associated with lake water storage is due to the range of lake water volumes throughout the year.

*E. crassipes* readily sequesters Hg from polluted waters (Muramoto and Oki 1983; Lenka et al. 1990) and can bioconcentrate Hg several times greater than sediment Hg and several thousand times greater than water column Hg (Greenfield et al. 2007). The storage of THg in *E. crassipes* tissues was estimated using an approximate areal coverage of 2.2 $10^5$ m$^2$ (Ortiz-Jiménez et al. 2005), a biomass value of 334 g dw/m$^2$ (subtropical lake, Uruguay; Meerhoff et al. 2003), and the average THg concentration in roots and foliage (28.3 ng/g d.w., range 5.6-117 ng/g d.w., N=13; determined as part of this study). A ±50% uncertainty was assumed due to biomass estimates and high variability of THg concentrations in plant tissues. The estimated THg storage in *E. crassipes* is 0.001-0.003 kg THg, and is a very small THg sink in Lake Zapotlán.

While Hg has been shown to readily accumulate in *Typha* root tissues (de Souza et al. 1999, Sundberg and Hassan 2007), the origins of Hg in aboveground foliage is less certain. According to Fay and Gustin (2007) Hg in aboveground biomass of *T. latifolia* is primarily derived from the atmosphere and not underlying sediments and waters. Only belowground biomass is considered a THg sink in this mass balance. THg storage in the belowground biomass of *T. latifolia* was estimated using an approximate areal coverage of 3.3 $10^6$ m$^2$ (Ortiz-Jiménez 2005), a biomass value of 1500-2000 g dw/m$^2$ (Takashi et al. 2005; SFWMD 1999) and the average THg concentration in root tissue (63 ng/g d.w., range 0.3-774 ng/g d.w., N=19; determined as part of this study). A ±50% uncertainty was assumed due to biomass estimates and high variability of THg concentrations in root tissue. The estimated THg storage in the belowground biomass of *T. latifolia* is 0.21-0.63 kg THg.

### 3.4.4 Mass Balance Results

The results of the mass balance are illustrated in Figure 3.12. An inventory of annual THg fluxes shows 1.4-5.8 kg of inputs and 0.25-1.3 kg of outputs (Table 3.5). In a mass balance framework, accumulation is equal to the mass inputs minus the outputs, minus the change in storage, and accounting for any chemical reactions. Lake Zapotlán is thus a net sink for THg (0.1-5.5 kg THg/yr). The mass balance supports the hypothesis that a large THg sedimentation and sorption sink in the wetlands must account for the removal of particle-bound and dissolved THg inputs.
Figure 3.12 Mass balance of THg in Lake Zapotlán (fluxes in kg Hg/year). Methods used to calculate these estimates are described in the text.

Without invoking a large wetland sedimentation and sorption sink, THg in lake sediments and surface waters are expected to be higher than indicated by field surveys. Using the range of estimated sediment bulk density values (0.2-0.4 g/cm³) and the higher end of net sediment accumulation rates seen in nearby Lake Chapala (4 mm/yr; Fernex et al. 2001), THg concentrations in surface sediments would be estimated to be 68-522 ng/g d.w.

Table 3.5 THg mass balance results (fluxes in kg THg/yr, volumes from Ortiz-Jiménez et al. 2005 except for wastewater)

<table>
<thead>
<tr>
<th></th>
<th>Volume ($10^6 m^3$)</th>
<th>THg (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INPUTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runoff</td>
<td>18.91</td>
<td>0.98-3.9</td>
</tr>
<tr>
<td>Wastewater</td>
<td>2.56</td>
<td>0.43-1.8</td>
</tr>
<tr>
<td>Precipitation</td>
<td>7.76</td>
<td>0.0040-0.076</td>
</tr>
<tr>
<td><strong>OUTPUTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volatilization</td>
<td>-</td>
<td>0.23-1.2</td>
</tr>
<tr>
<td>Water Extraction</td>
<td>13.00</td>
<td>0.021-0.064</td>
</tr>
<tr>
<td>Fishing</td>
<td>-</td>
<td>0.0027-0.0033</td>
</tr>
<tr>
<td><strong>STORAGE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sediment</td>
<td>-</td>
<td>11-22</td>
</tr>
<tr>
<td>Vegetation</td>
<td>-</td>
<td>0.21-0.63</td>
</tr>
<tr>
<td>Water</td>
<td>19.612</td>
<td>0.033-0.098</td>
</tr>
</tbody>
</table>
This assumes that 90% of the THg inputs associated with particles are equally deposited throughout the lake. Additionally, lake surface water THg concentrations would be estimated to be higher (6-23 ng/L) than indicated by field surveys, assuming that the dissolved portion (10%) of THg inputs mixes equally throughout the lake volume (19.61 10^6 m^3; Ortiz-Jiménez et al. 2005). The results of this mass balance support the conclusion that wetlands are effectively buffering approximately 78-86% of THg inputs to the lake. This is similar to the THg removal efficiency (70%; Miles and Fink 1998) in the Everglades Agricultural Area, USA.

3.4 CONCLUSIONS

Both untreated wastewater and ephemeral streams represent substantial inputs of particulate bound THg to the lake-wetland complex of Lake Zapotlán. The distribution of THg in waters and sediments indicate that THg inputs are largely sequestered in the wetlands receiving these discharges. The disconnected wetland has the highest average concentrations of THg in sediments and water, which exceed current Environment Canada environmental quality guidelines for the protection of aquatic life. Elevated THg in this wetland is a consequence of a large THg load from wastewater inputs, dense wetlands that promote particle settling and input water interaction with plants and sediments, and a municipal road that restricts water exchange with the lake and protects the wetland sediments from erosion and suspension. It is likely that intensive surveys of other areas in the wetland fringe would reveal elevated THg concentrations in sediments due to interactions with natural runoff inputs.

Despite the high loading of THg from anthropogenic and natural sources, concentrations of THg in sediment and water in the majority of the open water lake sampling locations are low and are below Environment Canada environmental quality guidelines for the protection of aquatic life. The settling of particle-bound THg matter in the wetlands is likely the largest sink of THg inputs in Lake Zapotlán and the main reason for the minimal propagation of THg away from the wastewater discharges and ephemeral stream channels. Mass balance calculations indicate that wetlands are retaining an estimated 78-86% of THg inputs. The results of this study highlight the importance of the wetland fringe in improving the quality of water inputs to Lake Zapotlán.
4. CONCENTRATIONS OF MERCURY IN THE COMMERCIAL CATCH OF TILAPIA AND CARP IN LAKE ZAPOTLÁN, MEXICO

4.1 INTRODUCTION

High concentrations of THg entering Lake Zapotlán in untreated municipal wastewater discharges (mean = 484 ng Hg/L; Ch.3) and wet season runoff (mean = 130 ng Hg/L; Ch. 3) pose a potential for elevated bioaccumulation of THg in fish and an elevated fish consumption risk for humans and piscivorous birds. Wastewater and surface runoff compose nearly two-thirds of water inputs to the lake according to water balance model estimates in Ortiz-Jiménez et al. (2005). The THg load is primarily associated with particulate matter and is sequestered in extensive wetland sediments and vegetation along the lake margins. Despite reductions in THg loading to Lake Zapotlán, wetlands are frequently net sources of MeHg (St. Louis et al. 2006).

Research in the subtropical Everglades, USA has revealed a well-established link between enhanced MeHg production in subtropical wetlands and nutrient inputs from agricultural activities (Gilmour et al. 1998). These wetlands could promote extensive MeHg production and bioaccumulation in biota due to reducing anoxic conditions and high sediment THg. However, the large association of THg inputs with particles combined with elevated DOC concentrations in the lake may inhibit substantial bioaccumulation in aquatic biota (Dittman and Driscoll 2009, Munthe et al. 2007). To address this uncertainty, and assess whether or not a MeHg exposure risk to fish consumers exists, the concentration of THg in the commercial catch of tilapia and carp was investigated over three sampling campaigns in 2007-2008.

4.2 METHODS

4.2.1 Sampling

Fish specimens were obtained with the assistance of two fishing cooperatives that operate on Lake Zapotlán. As the main objective was to assess if there was a MeHg exposure risk to human consumers, samples of tilapia and carp were taken from the daily catch on three separate occasions; October 2007, February 2008, and July 2008 (Figure 4.1). The tilapia species Oreochromis aureus (Blue tilapia) is commercially stocked to the lake and represents the majority of the catch. A small number of Oreochromis niloticus (Nile tilapia) were also sampled from the catch, but it is unclear whether this species is currently stocked. Carp are currently not commercially stocked to the lake.
Commercial fishing is undertaken over the entire lake and wetlands. Fish are caught by monofilament net and returned live to the shore by the fishers. We sampled the daily catch as whole fish before gutting, and immediately placed the samples in a cooler on ice for same-day processing. At a laboratory at the University of Guadalajara in C. Guzmán, individual weight and length were recorded (Figure 4.1). Scales and otoliths were removed for aging. Using an ultra-clean stainless steel knife and clean techniques, a subsample of muscle was dissected from the predorsal area just above the lateral line. Fish muscle samples were double-bagged and frozen at -15°C until analysis. Samples were transported frozen to the University of Toronto, Canada and remained frozen until analysis.

![Figure 4.1 The two species investigated in this study, tilapia and common carp.](image)

**4.2.2 Mercury Analyses**

Fish THg analysis was performed at the University of Toronto, Canada, using the same methodology as for THg analysis in sediment and plant material (Section 3.1.4). National Research Council (NRC) certified reference materials DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas) were used to construct a calibration curve. Individual fish samples were thawed and a subsample no larger than 0.5 g was removed for analysis using a stainless steel
scalpel. Fish THg concentrations are reported on a wet-weight (ww) basis. Recoveries of certified reference materials to assess method accuracy was 98% (DORM-2, N = 13) and 93% (IAEA-407, N = 11). The mean relative percent difference among triplicate measurements was 10% (N = 33). Average blank measurements using empty nickel boats were 0.04 ng Hg, (N = 75). The detection limit defined as three standard deviations of all blank measurements was 0.18 ng Hg.

A subset of tilapia (N = 30) and carp (N = 21) were aged by the Ontario Ministry of Natural Resources in Dryden, Ontario. Tilapia otoliths were aged via the crack and burn preparation method outlined in Christensen (1964). Carp scales were aged via annulus counting according to Casselman (1983).

4.2.3 Risk Characterization

To evaluate human risk for tilapia and carp tissue consumption from Lake Zapotlán, methods from a Health Canada (2007) Hg fish consumption risk assessment were utilized. As reviewed by Health Canada (2007), provisional tolerable daily intakes (pTDI) for MeHg are 0.47 µg Hg/kg bodyweight/day for the general population, and 0.20 µg Hg/kg bodyweight/day for the protection of pregnant women and young children (<12 years). To evaluate the amount of tilapia and carp that would need to be consumed to exceed current Health Canada (2007) and World Health Organization (WHO 2003) guidelines the following equation was used:

\[
\text{Maximum Fish Muscle Intake (g/day)} = \frac{\text{pTDI (µg/kg bw/day) \times average body weight (kg)}}{[\text{MeHg concentration in fish muscle (µg/g w.w.)}]}
\]

Since MeHg was not measured in fish tissue, all fish THg was considered MeHg. As reviewed by Health Canada (2007) actual MeHg content in fish can vary (30-95 %), however risk assessors continue to make the conservative assumption of 100% MeHg. Average Canadian bodyweight (bw) and serving portion sizes were used (adults: bw 60 kg, portion size 150g; children: age 5-11 bw 26.4 kg, portion size 125g; children age 1-4: bw 14.4 kg, portion size 75g; Health Canada 2007).
4.2.4 Statistical Analysis

Statistical analysis of results using Microsoft Excel data analysis functions was conducted to assess spatial and temporal variability. The effects of seasonality and location on fish THg concentrations were tested using ANOVA. If a statistical difference was found, the Tukey post-hoc test was utilized to determine which means differ. Statistically significant differences were assumed at $P \leq 0.05$.

4.3 RESULTS

4.3.1 Fish Total Mercury from Lake

Results from the three sampling campaigns reveal low mean THg concentrations in both tilapia (3.6 ng/g w.w., range: 2.5-4.0, N=81) and carp (7.6 ng/g w.w., range: 5.2-11.6, N=41) (Table 4.1). While concentrations did not change markedly during the study period, some small statistically significant seasonal patterns are evident. For both tilapia and carp, average THg concentrations were the lowest during the dry season sampling (2.5, 5.2 ng/g w.w., respectively) and highest in the wet season (4.0, 11.6 ng/g w.w., respectively) (Table 4.1).

4.3.2 Fish Total Mercury from Disconnected Wetland

In addition to the majority of the fish caught in the open lake, tilapia and carp were also taken from the disconnected wetland in Feb 2008 (for geographical reference see Figure 3.3). Average THg in tilapia (4.9 ng/g w.w., N=5) and carp (8.4 ng/g w.w., N=4) in the disconnected wetland were 95% and 62% higher than fish from the lake (2.5 ng/g w.w., N=24; 5.2 ng/g w.w. N=19), respectively (Table 4.1). Wetland carp were not significantly different from lake carp ($P = 0.10$). However, wetland tilapia were different from lake tilapia ($P = 0.001$) based upon a two-sample t-test ($\alpha = 0.05$). No fish were obtained from this wetland during the October 2007 and July 2008 sampling trips. Despite the small sample size this pattern suggests an increase in Hg bioaccumulation in tilapia and carp in the disconnected wetland. This zone has elevated THg in water and sediment compared to the open water areas of the lake (Ch. 3).
Table 4.1 Mean concentrations of THg in muscle tissue of tilapia and carp from lake and disconnected wetland, 2007-2008. For each species, significant differences between dates are indicated with different letters; Tukey test ($\alpha = 0.05$). *indicates a statistical difference between lake and wetland sample sets using a t-test ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Sampling Period</th>
<th>Species/Location</th>
<th>Mean [THg] (ng Hg/g w.w.)</th>
<th>Range</th>
<th>Sample Size (N)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2007</td>
<td>Tilapia/Lake</td>
<td>4.0 a</td>
<td>1.6-13.2</td>
<td>33</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Carp/Lake</td>
<td>8.0 ab</td>
<td>2.6-14.6</td>
<td>10</td>
<td>1.2</td>
</tr>
<tr>
<td>February 2008</td>
<td>Tilapia/Lake</td>
<td>2.5* b</td>
<td>1.7-5.1</td>
<td>24</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Tilapia/Wetland</td>
<td>4.9*</td>
<td>2.7-8.8</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Carp/Lake</td>
<td>5.2 a</td>
<td>2.6-17.1</td>
<td>19</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Carp/Wetland</td>
<td>8.4</td>
<td>3.7-14.7</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>July 2008</td>
<td>Tilapia/Lake</td>
<td>4.0 a</td>
<td>1.6-10.8</td>
<td>24</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Carp/Lake</td>
<td>11.6 b</td>
<td>2.9-28.8</td>
<td>12</td>
<td>2.3</td>
</tr>
</tbody>
</table>

4.3.3 Age and Length Trends

Tilapia ages ranged from 2-4 years and carp ranged from 2-6 years (Figure 4.2). The median age for both species was 3 years. Carp show a clear increase in length with age, with estimated growth rates of 5 cm/year length between 2-6 years (length = 4.82 (Age) + 19.23, $R^2=0.94$; Figure 4.2). Tilapia length is not different between 2-4 year old fish (Figure 4.2), indicating relatively slow growth rates beyond 2 years of age for this species in Lake Zapotlán. Similar to our findings, *O. aureus* growth rates in a Mexican reservoir were found to decrease beyond 2 years of age, with the greatest growth in the first year (Gómez-Márquez 1998; Jiménez-Badillo 2006). No studies on carp growth rates in Mexico are currently available.

THg concentrations in both tilapia and carp did not increase with total length (Figure 4.3). There is an improved relationship between average tilapia age and THg content ($R^2=0.79$; Figure 4.4), although average THg concentration only increased by 2 ng/g w.w. between 2 and 4 years of age. There was no THg – age relationship for carp ($R^2=0.02$; Figure 4.4). These generally weak relationships between Hg concentrations and length/age are uncommon in previous fish research. However, Taylor et al. (2005) found similarly reduced Hg/length ratios in *Oreochromis* spp. in Hg impacted waterways in Tanzania.
Figure 4.2. Comparison of mean length with age for tilapia and carp.
Figure 4.3 Mercury-total length comparisons for tilapia (N=86) and carp (N=45).
Figure 4.4 Mean THg-age relationship tilapia and carp (± range of values).
4.4 DISCUSSION

The results from this study demonstrate low THg concentrations in the muscle tissue of tilapia and carp from Lake Zapotlán. Zhou and Wong (2000) found that tilapia and common carp bioaccumulate less Hg than other species (black bass, silver carp, grass carp, bighead carp), primarily due to diet. Tilapia are generally herbivorous but are known to consume a wide variety of organisms including plankton, succulent green leaves, benthic organisms, aquatic invertebrates, larval fish, detritus, and decomposing organic matter (Shelton and Popma 2006). A study of the feeding habits of tilapia in a Mexican reservoir indicated that detritus and vascular plant tissues were the primary food source (Jiménez and Nepita 2000). Common carp root through benthic detritus to feed on invertebrates and insect larvae (Zhou and Wong 2000; Tapia and Zambrano 2003).

Ortiz-Jiménez and de Anda (2007) found that herbivorous zooplankton in Lake Zapotlán were nearly absent, possibly due to predation by *Chaoborus* larvae. Since zooplankton can be important species in the trophic transfer of MeHg in aquatic food webs (Pickhardt et al. 2005), the absence of these species in Lake Zapotlán may reduce bioaccumulation of MeHg in tilapia and carp. Zooplankton is likely to have higher THg concentrations compared to other components of the tilapia and carp diet (plant tissue, detritus). The absence of zooplankton species may result in a diet that is lower in THg than with more abundant zooplankton.

Studies in Hg-impacted waterways from gold mining in Tanzania revealed similarly low concentrations of THg in *Oreochromis* spp., despite high ambient Hg concentrations (Ikingura and Akagi 1996; van Straaten 2000; Taylor et al. 2005). Ikingura and Akagi (1996) hypothesize that Hg from mining activities is strongly bound to particulate matter and is not bioavailable. Hg data from Lake Zapotlán indicate that on average 90% of THg inputs in wastewater and runoff are associated with particulate matter (Ch. 3). Additionally, the resuspension of sediments and particulate matter in Lake Zapotlán due to constant winds and shallow waters may scavenge THg and MeHg from the water column and reduce bioavailability (Rudd and Turner 1983).

Despite high loading of THg to the waters and sediments of Lake Zapotlán, the biogeochemical conditions of surface waters appear to be unfavorable to MeHg production and bioaccumulation. Fish THg concentrations are inversely correlated to pH, alkalinity, and chlorophyll (Winfrey and Rudd 1990; Lange et al. 1993). Lange et al. (1993) found that largemouth bass had the highest THg in aquatic environments with pH less than 7, alkalinity less than 20 mg/l, and chlorophyll less than 10 µg/L. Additionally, nutrient rich systems tend to
decrease MeHg bioaccumulation in biota due to dilution in the phytoplankton and zooplankton biomass (Pickhardt 2002; Chen and Folt 2005). Lake Zapotlán represents an unfavorable environment for MeHg uptake in biota due to high pH (8-9), high alkalinity (192-300 mg/l as CaCO3; Ortiz-Jiménez et al. 2005; CIATEJ 2007), and high chlorophyll during the spring and summer (>88 μg/L; Ortiz-Jiménez et al 2006, 2007).

High average DOC concentrations (Section 3.2.1) in lake and wetland surface waters are likely to further reduce Hg uptake in fish. Hg complexation with organic matter at high DOC concentrations has been shown to limit Hg availability for methylation and uptake (Driscoll et al. 1995; Barkay et al. 1997; Choi et al. 1998). This process can be amplified at neutral or basic pH waters due to an absence of H+ ions which compete with Hg for binding sites on DOC (Barkay et al. 1997). Driscoll et al. (1995) found that THg concentrations in fish may actually decrease when water DOC concentrations exceed 8 mg C/L.

4.4.1 Consumption Risk

Fish THg concentrations in Lake Zapotlán were low even for unpolluted systems and are unlikely to represent a risk to consumers. Risk estimates reveal that individuals who consume tilapia and carp from Lake Zapotlán are unlikely to exceed safety guidelines. The amount of tilapia and carp needed to be consumed on a daily basis to exceed WHO guidelines is outlined in Table 4.2. Based on these calculations, it is unlikely that any member of the community consuming these fish will exceed their pTDI for MeHg. Average adults would have to consume 51 portions of tilapia and 22 portions of carp per day to exceed their pTDI. Even though these levels are lower for pregnant women and children, it is unlikely that individuals will exceed their limits. There is some uncertainty associated with average serving sizes and weights for each age class, as Canadian Hg risk assessment statistics were utilized.

Table 4.2 Maximum daily consumption limits for tilapia and carp from Lake Zapotlán, Mexico based upon current Health Canada and World Health Organization consumption guidelines.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean THg Concentration (ng/g w.w.)</th>
<th>Maximum Daily Consumption Limit (portions/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult Adult Child Child</td>
<td>Adult Child (child bearing) (5-11 years) (1-4 years)</td>
</tr>
<tr>
<td>Tilapia</td>
<td>3.7 51 22 11</td>
<td>10</td>
</tr>
<tr>
<td>Carp</td>
<td>8.4 22 9 5 5</td>
<td>5</td>
</tr>
</tbody>
</table>
4.5 CONCLUSIONS

Despite high loading of THg to Lake Zapotlán, concentrations of THg in tilapia and carp used for subsistence consumption or bound for local markets were extremely low. Results indicated that this aquatic system did not promote significant bioaccumulation and/or production of MeHg. Low fish THg concentrations are possibly due to decreased MeHg bioavailability through binding by particles and DOC. Additionally, high pH in this system may promote low rates of sulfate reduction and hence Hg methylation. The low trophic status and diet of tilapia and carp may also decrease Hg uptake. Risk assessment results indicate that there is currently a very low risk to Hg exposure through the consumption of tilapia and carp from any part of Lake Zapotlán.
5. SUMMARY AND CONCLUSIONS

The primary objective of this research was to assess the potential for an elevated risk of MeHg exposure to human and other consumers of fish from Lake Zapotlán. Based on the evidence gathered from this study, it appears that the risk of MeHg exposure from tilapia and carp consumption is very low to negligible. The concentrations of THg in tilapia and carp bound for local markets are extremely low relative to other ecosystems where the aquatic food web is more complex, and commercial fish are piscivorous and longer-lived. While there are few studies regarding Hg in subtropical freshwater fisheries in Mexico, the results are concurrent with other published trends. Our findings support low bioaccumulation of MeHg in aquatic systems with high pH, high alkalinity, high DOC, and nutrient rich conditions. Additionally, these results support previous studies that identify tilapia and carp as low Hg fish.

Water inputs to Lake Zapotlán from untreated municipal wastewater and wet season runoff were high in particle-bound THg. Despite high inputs to the lake, THg in sediments and surface waters of open water sampling locations were very low and did not exceed current Environment Canada environmental quality guidelines for the protection of aquatic life. Data for treated versus untreated wastewater discharges from treatment plants indicate that continuous operation of the water treatment facilities at full capacity would reduce the Hg from municipal discharges to low levels, virtually mitigating this source.

A mass balance of THg in Lake Zapotlán reveals that the lake is a net sink for THg inputs. Sedimentation and sorption of THg in wetlands is the most likely explanation for low THg concentrations in the open water areas of the lake. Elevated THg in sediments and waters in the southern and disconnected wetlands are likely a reflection of untreated wastewater inputs and dense *T. latifolia* dominated wetlands. The municipal road which disconnects the southeastern wetlands from the lake likely reduces sediment erosion and resuspension, effectively containing THg inputs to this region.

The results of this study highlight the importance of the wetland fringe in improving the quality of water inputs to Lake Zapotlán. *T. latifolia* wetlands surrounding the lake face increasing pressure from agricultural activities. Future management decisions regarding the removal of these wetlands should consider their important role in water quality improvements through sediment and THg retention. Loss of the wetland area, or a reconnection between the disconnected wetland and the open water by a removal of the roadway would potentially result in a much larger transport of THg to the open water system.
REFERENCES


