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Edaphic and vegetative controls on mercury cycling in oligohaline wetlands

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EDAPHIC AND VEGETATIVE CONTROLS ON MERCURY CYCLING IN OLIGOHALINE
WETLANDS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by
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For Dad

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ABSTRACT

With the expansion of the human population and associated industries there is a concomitant increase in both resource utilization and the production of waste and deleterious by-products. Mercury is a naturally-occurring toxic metal with a complicated and unique biogeochemical cycle, and is often a contaminant of ecotoxicological concern in unindustrialized aquatic habitats. The research described herein was designed to elucidate multiple aspects regarding the behavior of mercury in oligohaline wetland habitats, particularly with respect to edaphic and vegetative effects, through a monitoring and characterization study as well as a series of manipulative experiments. The observational study, conducted in the wetlands surrounding Lake Maurepas, Louisiana, indicated that during the time of the study, little reduction of sulfate to sulfide was occurring, and thus soil methyl mercury concentrations were quite minimal. Further, the total mercury concentrations in local vegetation were found to be typical of uncontaminated environments in the United States. The first experimental study was a hydroponic greenhouse study that evaluated the capacity of several species of local wetland plants to function as phytoremediation agents for aqueous mercury contamination. Several of the species tested demonstrated the ability to reduce aquatic inorganic mercury concentration, but generally not to a greater extent than has been shown for other plant species. The second experimental study assessed vegetative response and partitioning of elevated surface water inorganic mercury into the soils and vegetation in a wetland mesocosm. The concentration of mercury in the surficial soil became elevated, but mostly occurred in biologically unavailable forms, while substantial mercury uptake by plants occurred only in belowground tissues. The final experimental study investigated the potential stimulation of soil methyl mercury concentration via elevated surface water nutrients in a benchtop soil core incubation study. No evidence of significantly increased soil methyl mercury concentration was found.

CHAPTER I

REVIEW OF MERCURY CYCLING IN WETLAND ENVIRONMENTS

Toxic metals represent some of the most persistent contaminants found in ecosystems as they cannot be degraded into less toxic components, but rather only immobilized in less available forms or physically removed (Carvalho et al. 1999). The cycling and fate of toxic metals released into wetlands has been a major area of research over the last several decades (see reviews in Kadlec and Knight 1996; Odum 2000). Although the exact conditions under which metals become unavailable are specific to each metal and their immediate environment, many are generally unavailable under a similar range of conditions (Bourg and Loch 1995), allowing for some general governing principles to be recognized. Wetland systems are typically viewed as effective natural filters for metals in aqueous solution given that these habitats generally display edaphic conditions under which many toxic metals become biologically unavailable, e.g., moderate pH, moderate to highly reduced redox status, and substantial organic matter content (Gambrell et al. 1991; Gambrell 1994; Bourg and Loch 1995; Simpson et al. 2002). Metals in aqueous solution frequently enter wetlands sorbed to particles suspended in incoming land runoff (Kadlec and Knight 1996). These suspended particles fall out of solution as water velocity is reduced (i.e., vegetation baffling), and are removed by sedimentation processes as they are buried and soil conditions render the metals unavailable (Delaune and Gambrell 1996; Kadlec and Knight 1996). Given the correct biogeochemical conditions, metals can also become incorporated into insoluble sulfide compounds rendering them unavailable (Gambrell 1994; Kaplan et al. 2002). However, this sequestration of metals is contingent on the primary factors that control metal solubility in wetlands, such as pH, soil redox potential, sulfide availability, and soil organic matter, remaining in specific ranges (Gambrell 1994; Sumner 2000). It is well

documented that the oxidation of previously flooded soils results in altered biogeochemical characteristics (e.g., pH and redox potential) that render metals more available (e.g., Gambrell 1994; Sumner 2000; Siedel 2002; McKee et al. 2004).

The biogeochemistry of mercury and methyl mercury continues to be an area of intense environmental research, largely due to the ubiquity of mercury in the environment and the highly toxic nature of methylated mercury (e.g., Breteler et al. 1981; Schwesig and Krebs 2003; Kongchum et al. 2006). Mercury is a naturally occurring metallic element that may become of great environmental concern due to its atmospheric transport (Schuster et al. 2002; Driscoll et al. 2007) and its varied chemical states, which range from the minimally to extremely toxic and bioavailable (Morel et al. 1998). Thus, understanding the biogeochemistry governing the shifts in chemical states of mercury is critical for proper management of systems that could be potentially subjected to mercury influx, either through direct point contamination or long-term nonpoint atmospheric deposition. The bioaccumulation of mercury in aquatic and wetland habitats has been a frequent subject of scientific inquiry because of tragic events such as the large-scale poisoning that occurred at Minimata Bay in Japan, where pollution-induced disease was first diagnosed in 1956 (Kudo et al. 1998). Excellent progress has been made in elucidating the assimilation efficiencies and bioavailability of various mercury compounds as they are transferred through trophic systems comprised of phytoplankton, invertebrates, and fish (Boudou and Ribeyre 1981; Lawson and Mason 1998; Tsui and Wang 2004). Also, numerous observational studies have indicated that a large number of diverse terrestrial and aquatic habitats contain biotic compartments with elevated mercury levels (e.g., Colaço et al. 2006; Driscoll et al. 2007; McIntyre and Beauchamp 2007).

Chemical Forms of Methyl Mercury

Mercury occurs as several chemical species depending on the characteristics of the local environment (Morel et al. 1998). Methyl mercury, which is actually a group of various mercury species including dimethyl mercury, methyl mercury chloride, methyl mercury hydroxide, etc., is the most infamous due to substantial toxicity and a tendency to bioaccumulate (Morel et al. 1998). Although the various methyl mercury species vary somewhat toxicologically, most analytical techniques determine total methyl mercury, and therefore regulatory limits are often set and enforced based on total methyl mercury concentration (Morel et al. 1998). The form of methyl mercury occurring in a particular area is influenced by salinity, with methyl mercury chloride predominating under saline conditions, whereas methyl mercury hydroxide often predominates under freshwater conditions (Morel et al. 1998). For the sake of brevity, methyl mercury hereafter will be used in the usual sense as representing total methyl mercury species. Methyl mercury is formed primarily by the activities of sulfate reducing bacteria in anaerobic environments through incidental side chain interactions of mercury with various metabolic pathways (Ekstrom et al. 2003). The sulfate reducing bacteria are a diverse collection of bacteria that share the metabolic strategy of using oxidized sulfur compounds, such as sulfate, as final electron acceptors in respiration while organic compounds or hydrogen are oxidized (Postgate 1984). Methyl mercury has a high affinity for lipids and therefore a strong tendency to bioaccumulate up food webs as animal tissues are consumed and lipid components are retained by the consumer (Lawson and Mason 1998; Morel et al. 1998). Methyl mercury concentrations are therefore often highest in top-level aquatic predators with relatively long life spans, including many sport fish consumed by humans (Storelli et al. 2007).

Mercury in Aquatic Fauna

There is a greater public awareness of mercury contamination than many toxic metals due to public health warnings regarding excessive concentrations of mercury in some finfish and shellfish and the consequential potential dangers of consumption. These public concerns are heightened given public health statements encouraging the consumption of fish as a means to reduce coronary heart disease, with these cardiac benefits likely due to activity of omega-3 fatty acids (e.g., EPA and DHA) found primarily in seafood (Smith et al. 2009). Ingestion of contaminated fish tissue does represent the primary mechanism of mercury exposure by humans that are not associated with industrial activity utilizing mercury (Anderson et al. 2004; Storelli et al. 2007). Elevated levels of mercury in recreational fish have been reported since 1970 (Konrad, 1970). Johnsson et al. (2004) found a statistically significant increase in hair mercury levels of members of a Swedish population who reported one meal or more of freshwater fish per week compared with those who did not consume fish as frequently. Mercury concentrations are generally higher in larger, predatory fish because of the lipophilic nature of methyl mercury, which leads to biomagnification in aquatic food webs (Storelli et al. 2007). In contrast, invertebrates with shorter life spans, such as many shrimp species, have been shown to accumulate relatively little mercury as long as they are not held within a contaminated area. The United States FDA has maintained an advisory since 2001 against the consumption of shark, swordfish, king mackerel, and tilefish by women who are pregnant or who may become pregnant (Burger and Gochfel 2004). Research by Harris et al. (2007) indicates that faunal accumulation in many aquatic systems responds relatively quickly to either increases or decreases in mercury deposition, suggesting that the safety of fish for consumption could change substantially depending on changes to future environmental regulations that result in reduced mercury inputs.

Constraints on Vegetative Mercury Uptake

Many data gaps exist pertaining to the cycling of mercury through soil-water-plant systems, particularly in regard to vegetative factors controlling assimilation through food webs and biological sequestration. Because the inherent differences in the biochemistry and physiology of plant species result in differential paths and rates of mercury uptake, retention, and availability, considerable research is still needed to provide governing principles to determine plant-mediated mercury uptake and assimilation risk. The primary constraint of mercury accumulation by vegetation is the availability of mercury in the environment adjacent to the plant, encompassing precipitation and sorption phenomena (Evans 1989; Morel et al. 1998; Boening 2000), as well as ligand availability (Moreno et al. 2005). The ability of mercury to be absorbed across biological membranes is also dependent on the mercury species, with lipophilic HgCl_2 being the primary species accumulated under oxic conditions and the lipophilic CH_3HgCl being the primary species accumulated under anoxic conditions (Morel et al. 1998). Importantly, the capacity of a mercury species to be bioaccumulated is not dependent solely on the lipophilicity, which allows the metal to initially gain access to the cell, but the subsequent reactivity (Morel et al. 1998). For instance, $(\text{CH}_3)_2\text{Hg}$ and Hg^0 are both nonpolar species that can rapidly diffuse through biological membranes but, because of their lack of reactivity once inside the cell, they tend to diffuse out readily and accumulation does not occur (Cobbert and Goldsborough 2002). HgCl_2 and CH_3HgCl not only rapidly diffuse across biological membranes, but also react readily with intracellular components (e.g., proteins, nucleic acids), thus allowing them to be retained and bioaccumulate up food chains (Cobbert and Goldsborough 2002). It should be noted that CH_3HgCl has a greater tendency to bioaccumulate than HgCl_2 , likely a result of the Hg associated with HgCl_2 binding to those intracellular portions of

organisms that are excreted by predators (i.e., shell components) as opposed to CH_3HgCl , which binds with intracellular components that are retained (Morel et al. 1998).

Beyond the effects of the local environment and mercury speciation, the accumulation of available mercury by vegetation in terms of both rate and tissue localization varies substantially by species, likely due to species-specific biochemical or physiological processes (Boening 2000). Much of the research that has been conducted on mercury accumulation has focused on upland or salt marsh species, with relatively little data available for wetland species occurring in fresh water habitats (e.g., Breteler et al. 1981). The lack of mercury research on wetland vegetation represents a critical data gap for the management of natural and constructed fresh water wetlands that may become contaminated with mercury, as well as for the development of phytoremediation approaches appropriate to these habitat types. A more complete understanding of the role of vegetation in mercury cycling, and particularly interspecific variation of this vegetative cycling, could prove to be a key to determining risk in local environments. Further, the description of chelating compounds may provide basic information leading to the development of novel therapeutic techniques for the treatment of mercury poisoning.

Physiology of Vegetative Metal Uptake

Vegetative uptake of trace and toxic metals can occur through both the above- (foliar) and below- (root) ground portions of the plant (Prasad 1999). The importance of foliar uptake of toxic metals is likely to increase as the atmospheric deposition of toxic metals such as mercury continues (Prasad 1999). The uptake of toxic metals by roots has been the focus of vast ecotoxicological (McLaughlin et al. 1999) and phytoremediation-based (Clemens 2002; Weis and Weis 2004) research. The uptake of metals by plant roots generally occurs from the soil-water phase and thus the factors that control the solubility of metals can be regarded as

controlling their availability for plant uptake (Lasat 2002). Soil pH, redox status, and soil composition (organic matter and clay content) can be thought of as master variables controlling the concentration of metals in soil water and thereby the availability to local vegetation (Prasad 1999; Sparks 2003). Plants can directly affect local metal solubility, however, by altering the environment adjacent to the roots (e.g., rhizosphere acidification and/or local oxidation) and the release of metal-chelating organic compounds (Prasad 1999; Clemens et al. 2002). Members of the Poaceae family (i.e., the grasses) produce a specific class of non-protein amino acids referred to as phytosiderophores, which act to chelate Fe^{3+} and other metals, increasing the availability of these metals to the plant roots (Prasad 1999).

The physiologic uptake by plants of toxic metals that have moved into close proximity to the plant-root system is mediated by active and passive transport processes (Prasad 1999; Lambers and Colmer 2005). This is true of both metals that are essential at trace levels, but become toxic at higher concentrations (e.g., Cu, Zn, etc), as well as toxic metals with no known physiological function (e.g., Cd, Hg, etc). Although passive transport of metals into plant roots is documented, it plays a minor role compared with active transport in regard to metal uptake (Prasad 1999). Active transport of non-essential toxic metals can occur through the active transport sites of micronutrients in cases where the chemical form of the non-essential toxic metal is a close chemical mimic of an essential element. Generally this is a function of the ionic radius of the “unintended metal” being similar to that of the intended nutrient (Prasad 1999), such as Al^{3+} and Mg^{2+} (Kochian 1995). Examples of this include the active transport of arsenate through the high affinity phosphorous transport system (McLaughlin et al. 1999; Meharg and Hartley-Whitaker 2002) and the uptake of selenium due to its similarity to sulfur (Mayland et al. 1989; McLaughlin et al. 1999)

The accumulation of mercury into terrestrial crop plants is generally not considered to be a human health risk due to the minimal solubility of mercury in most agricultural soils (McLaughlin et al. 1999). However, Zheng et al. (2007) found total mercury levels that exceeded permissible levels for human consumption in multiple crop plants growing in fields close to a zinc smelting facility. Also, elevated levels of mercury have been found in the aquatic food plant *Ipomea aquatica* (water spinach) in Thailand (Göthberg and Greger 2006). Finally, a high correlation of elevated methyl mercury in human hair and in the rice constituting the base of the local diet was found in a Chinese village located near mercury polluted sediments (Feng et al. 2008).

Interactions of Metals with Nutrients and Elements

The interaction of metals and nutrients is complex, with both positive and negative effects on metal availability possible. For example, elevated levels of anionic nutrients may provide a large number of appropriate binding sites for metals (Gothberg et al. 2004), thus reducing the bioavailable fraction. Conversely, elevated levels of cationic nutrients can competitively inhibit the uptake of metals at root sites (Greger 1999; Gothberg et al. 2004). However, there are also a number of mechanisms by which elevated nutrients can increase metal uptake. For instance, increased nutrient levels, barring an associated stress inhibiting growth, increase growth rate and biomass, thus typically enhancing the uptake of available metals (Ekvall and Greger 2002; Gothberg et al. 2004). Toxic metals are believed to generally reduce leaf phosphorus levels, and as a result, photosynthetic rate (Krupa et al. 2002). However, increasing leaf phosphorus levels could be anticipated to reduce the impact of cadmium on photosynthetic rate (Krupa et al. 2002). Because, as described below, mercury is exceedingly efficient at substituting for manganese in chlorophyll molecules, an excess of manganese may prove to be

protective through competitive effects (Krupa et al. 2002). Selenium is believed to play a protective role in mercury intoxication in mammals (see review by Cuvin-Aralar and Furness 1991). Ekelund and Danilov (2001) found that elevated selenium reduced oxidative stress and restored photosynthetic function in the protist *Euglena gracilis*. It is possible that selenium could exert some protective influence on plants subjected to mercury contamination.

Organic Acids, Phytochelatins, and Metallothioneins

One mechanism by which plants tolerate elevated toxic metal levels is through the production of organic ligands that bind the metals, thereby preventing the metals from interacting with cellular components such as proteins, lipid membranes, and nucleic acids, thereby reducing the detriment to the cell (Sanità di Toppi et al. 2002). Three major classes of ligands that are produced for metal detoxification include organic acids, phytochelatins, and metallothioneins (Sanità di Toppi et al. 2002). Organic acids can be exuded from the plant root, rendering metals non toxic before they are absorbed by the plant or used to bind toxic metals after the absorption into the plant tissue (Matsumoto 2002). Organic acid toxic metal complexes are often located in plant cell vacuoles, suggesting that this is the area of long-term toxic metal sequestration (Matsumoto 2002). Generally, citrate is thought to confer the greatest protection against toxic metals, followed by malate and then oxalate (Matsumoto 2002). The production of peptide-based ligands for the detoxification of toxic metals in plant cells is a well documented aspect of vegetative tolerance to toxic metals (Zenk 1996; Cobbert and Goldsborough 2002). Generally, two large categories of these peptides are recognized: 1) phytochelatins, which are generated enzymatically from a glutathione or related substrate, without specific genetic coding, and, 2) metallothioneins, which are peptides produced directly from genetic coding (Zenk 1996; Cobbert and Goldsborough 2002). Although metallothioneins are known to occur in some plants,

phytochelatins appear to be essentially ubiquitous and vastly more important in the tolerance of heavy metals (Sanita di Toppi et al. 2004). Phytochelatins are generally based on a skeleton of various lengths of γ -GluCys di-peptides with a terminal Gly (Zenk 1996; Cobbert and Goldsborough 2002). The sulfur-rich nature of phytochelatins makes them ideal for binding toxic metal ions and the phytochelatin-metal compounds are frequently sequestered in the vacuole, thereby reducing the toxicity of the metal (Zenk 1996; Cobbert and Goldsborough 2002). Although phytochelatins are known to be widely occurring in plants, and even other organismal groups such as fungi and animals, variation exists in and among plant species in terms of the type and quantity of phytochelatin that can be produced (Zenk 1996; Cobbert and Goldsborough 2002; Sanita di Toppi et al. 2007). The production of phytochelatins has been demonstrated by several researchers to be associated with greater tolerance to various mercury compounds by *Arabidopsis* (Li et al. 2006), *Hydrilla verticillata* (Gupta et al. 1998), and *Vallisneria spiralis* (Gupta et al. 1998). Interestingly, Rellan-Alvarez (2006) found that maize (*Zea mays*) did not produce phytochelatins when exposed to 6 and 30 μ M concentrations of mercury, but that plant growth was severely inhibited.

Mercury Specific Plant Responses

Studies regarding the bioaccumulation and toxicity of mercury to plants have often focused on agricultural crops (e.g., Warman et al. 1995; Patra and Sharma 2000), although there have been some studies on wetland plants, typically as potential bioremediation agents (see De et al. 1985; Sridhar 1988; Sinha et al. 1996). As stated above, mercury uptake and toxicity is typically species specific, thereby limiting the approach of using model species to estimate these effects (Krishnan et al. 1988; Patra and Sharma 2000). Of particular interest is that *Schoenoplectus americanus*, a common wetland plant species in the Pass Manchac (Louisiana)

area, was found to bioaccumulate mercury at 11.2 times the sediment concentration (Gilbert 1990). Many wetland plant species tend to accumulate mercury from sediments in root tissues (Coquery and Welbourn 1994; Ribeyre and Boudou 1994); importantly many herbivores and omnivores that would likely feed on this material in the Lake Maurepas system either directly consume root material (e.g., nutria: Llewellyn and Shaffer 1993; Ford and Grace 1998), or consume detrital matter that may incorporate belowground tissues (e.g., crawfish: Alcorlo et al. 2004). Interestingly, for many plant species mercury concentrations in aboveground tissues are not correlated with sediment mercury concentrations, but rather with atmospheric mercury concentrations, indicating that atmospheric mercury is the primary source of mercury to these tissues and that there is typically little translocation of mercury from belowground plant tissues to aboveground tissues (Patra and Sharma 2000). Mercury is known to affect photosynthesis through a variety of mechanisms (Patra and Sharma 2000). Mercury has been found to increase the activity of chlorophyllase in rice more than either zinc or copper, thereby reducing photosynthetic integrity (Mysliwa-Kurdziel and Strzalka 2002). The central magnesium atom of chlorophyll structures can be substituted by other metals, including mercury, under low light levels (Patra and Sharma 2000). Mercury is generally believed to be more effective than other toxic metals in this substitution (Krupa et al. 2002). Several studies have shown that chlorophyll fluorescence decreases subsequent to mercury exposure, indicating impairment of photosystem II by this metal (Mysliwa-Kurdziel and Strzalka 2002). Bernier et al. (1992) found that the photosystem II portion of the photosynthetic cycle in *Hordeum vulgare* was disrupted by mercury addition as indicated by inhibition of oxygen production and chlorophyll fluorescence. Further study on the physiological effects of mercury on *Hordeum vulgare* by Bernier et al. (1992) indicates that mercury disruption of photosynthesis occurs at the electron donor portion of

photosystem II. De et al. (1985) examined the response of *Pistia stratiotes* to the addition of mercury and determined that chlorophyll, protein, and RNA content, as well as dry weight and protease/catalase content, were decreased, while peroxidase activity and amino acid concentrations increased, with increased mercury addition.

Summary

Although considerable research has been conducted concerning the environmental fate and risk of mercury in the environment, substantial data gaps remain, particularly regarding the role of vegetation either from an ecotoxicological standpoint or as a remediative tool. The general vegetation responses to mercury can be surmised from the literature, such as background levels of tissue mercury concentrations for uncontaminated environments and the tendency for mercury assimilated by belowground structures not to translocate to aboveground structures. However, mercury exhibits complex interactions with various environmental factors and thus addressing phytoaccumulation and phytoremediation questions requires particular attention to how these factors may vary from one environment to another. Similarly, the influence of the local environment on microbial processes involving mercury is of critical importance in understanding ecotoxicological risk, although this aspect of mercury research is much more frequently addressed. As scientific tools have evolved, however, it has become possible to more explicitly investigate factors believed to govern these microbial interactions. It is the intent of the research detailed in the following chapters to expand upon the current knowledge base of mercury cycling and fate in oligohaline wetland systems with special attention to the role of herbaceous vegetation. Specifically, in chapter 2, I investigate the concentrations of mercury in the soils and vegetation of the Lake Maurepas wetlands and how soil biogeochemistry may alter mercury dynamics in a field assessment. The intent of this research is to expand the

understanding of the roles played by soil and vegetation in mercury dynamics of oligohaline wetlands. In chapter 3, I examine the utility of several common oligohaline herbaceous species for mercury phytoremediation applications in a sand-culture study. Through this research the knowledge base for mercury phytoremediation will be expanded by investigating several species that have not been previously assessed. This is followed in chapter 4 by a study that elucidates the response of simulated oligohaline wetland systems to moderate inorganic mercury contamination using static aquaria and representative wetland soils. The intent of this study is to resolve current data gaps regarding the fate of mercury in wetland soils and vegetation exposed to an acute mercury contamination event. The final research chapter elucidates how altered nutrient loads and salinity levels could affect mercury methylation using a bench top soil core incubation study. This research effort is intended to provide a greater understanding of how mercury methylation dynamics in oligohaline soils could respond to eutrophication scenarios.

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CHAPTER II

TOTAL MERCURY, METHYL MERCURY, AND RELEVANT BIOGEOCHEMICAL SOIL CHARACTERISTICS IN AN OLIGOHALINE WETLAND

Introduction

The cycling of mercury in aquatic systems, particularly oligohaline wetlands, continues to be an area of intense research due to the extremely toxic and bioaccumulative nature of some mercury species favored in these environments (e.g., methyl mercury; Lacerda and Fitzgerald 2001). For many regions the introduction of mercury into aquatic environments is believed to occur via atmospheric deposition of mercury released by the combustion of fossil fuels; this is currently hypothesized for the northern Gulf of Mexico as well (National Science and Technology Council Committee 2004). Wetlands are often considered a source of methyl mercury to adjacent water bodies, with methyl mercury formation within these wetlands primarily mediated by sulfate-reducing bacteria (Compeau and Bartha 1985; Gilmour et al. 1992; St. Louis et al. 1994; Guentzel 2009). The biomagnification of methyl mercury through aquatic food webs is very efficient, with the majority of mercury (~85% or more) in edible fish tissue occurring as methyl mercury (Boudou and Ribeyre 1981; Lawson and Mason 1998). Extensive fish consumption advisories are currently in place for many water bodies worldwide and throughout Louisiana because of elevated mercury content (Bellanger et al. 2000). Due to the integral nature of wetlands in the cycling of methyl mercury, any efforts to reduce mercury levels in important sport fish species will require elucidation of current background mercury concentrations and a greater understanding of mercury dynamics in adjacent wetlands.

Multiple factors can potentially regulate the concentration of methyl mercury in aquatic soils and sediments, including the concentration of microbially-available mercury, labile organic matter, soil redox status, as well as the abundance and ratio of sulfate and sulfide (Lambertsson

and Nilsson 2006; Merritt and Amirbahman 2009). To be methylated, inorganic mercury must be available to appropriate (i.e., sulfate-reducing) bacteria and this availability is mediated by a number of soil and water constituents, such as bulk organic matter as well as dissolved organic matter and inorganic ligands (Compeau and Bartha 1985; Ravichandran 2004; Lambertsson and Nilsson 2006). The concentrations of sulfate and sulfide are related to mercury methylation both by affecting microbial sulfate reduction via sulfate acting as a limiting reactant and also through limiting inorganic mercury availability through sulfide precipitation (Compeau and Bartha 1985; Beniot et al. 1999; Benoit et al. 2003; Han et al. 2008). The collective effects of sulfate and sulfide governing microbial methylation of mercury result in the existence of a relatively narrow sulfate range generally considered optimal for methylation (Benoit et al. 2003; Hollweg et al. 2009). Soil redox potential, a consequence largely of the energetics of microbial metabolism, determines at what point sulfate reduction is thermodynamically favorable relative to other alternative electron acceptors (Reddy and Delaune 2008). Thus, soil redox potentials above those indicative of sulfate reduction should represent conditions that are unfavorable for mercury methylation (Delaune et al. 2004).

Investigations of mercury dynamics in vegetation have often focused either on aspects of phytoremediation or the role of vegetation in atmospheric mercury cycling. This dichotomy reflects the normal environmentally relevant scenarios in which mercury contamination is problematic: acute and substantial contamination of a habitat (e.g., industrial or mining activity) versus chronic introduction of mildly elevated mercury contamination through atmospheric deposition.

Breteler et al. (1981) investigated uptake of mercury by *Spartina alterniflora* in both experimentally contaminated plots as well as in marshes historically subjected to elevated aqueous mercury introduction from industrial activity and found higher levels of mercury in

roots than shoots under both control and elevated mercury conditions. Further, it appeared that recent exposure to mercury (experimentally contaminated plots) did not result in elevated mercury levels in *S. alterniflora* tissues whereas, elevated mercury concentrations were found in *S. alterniflora* taken from the marsh subjected to aqueous industrial effluents (Breteler et al. 1981). Canaario et al. (2007) examined mercury accumulation by *Sarcocornia fruticosa*, *Halimione portulacoides*, and *Spartina maritime* in several Portuguese salt marshes and also found a tendency for mercury concentrations to be higher in belowground material than in aboveground material. Interestingly, Canaario et al. (2007) determined that soil methyl mercury levels were much greater in vegetated than unvegetated soils, likely due to the enhancement of microbial activity by plant roots. Similarly, Windham-Myers et al. (2009) found that the removal of wetland macrophytes reduced soil methyl mercury concentrations, again likely through affecting the local microbial community.

Several thorough studies investigating total and methyl mercury concentrations of soils, sediments, and waters of the Lake Maurepas wetlands have recently been completed (Delaune et al. 2008; Yu et al. 2008; Delaune et al. 2009). However, no assessments of total mercury concentrations in tissues of macrophytes for this region are currently available and background concentrations of mercury for several of these species (*Saururus cernuus*, *Pontedaria cordata*, *Sagittaria lancifolia*) are currently unavailable in the peer-reviewed literature. Further, additional information relating methyl mercury concentrations to biogeochemical characteristics would be useful. Thus, the research detailed herein is intended to provide insight into mercury dynamics by addressing the following research objectives: 1) to elucidate the potential mediation of methyl mercury concentration in wetland soil by various biogeochemical factors, and 2) to determine the total mercury concentration in wetland soils and in the adjacent above- and below-

ground herbaceous wetland vegetation tissues, and to assess potential relationships among these compartments.

Materials and Methods

Experimental Design

A field study was implemented in the Lake Maurepas wetlands at six sites intended to be representative of common vegetative habitats, including Joyce Wildlife Management Area (N 30 24' 00.42", W 90 25' 43.86"), Amite River (N30 18.0' 17.9", W 90 36' 35.59"), Blind River (N 30 15' 8.73", W 90 39' 7.84"), Reserve Relief (N 30 08' 31.11", W 90 32' 41.30"), Tobe Canal (N 30 09' 32.34", W 90 28' 37.86"), and Turtle Cove (N 30 17' 35.51", W 90 25' 43.86") (see Fig. 1-1 for site map). A subset of these sites, Blind River, Reserve Relief, Tobe Canal, and Turtle Cove, occur along the southern portion of Lake Maurepas and represent a gradient of salinity and sulfate concentrations (Shaffer et al. 2003). The final two sites are located along the northern portion of Lake Maurepas and were chosen to provide further spatial and environmental information. Four, 1-m² permanent plots were established within each of these sites by marking opposite corners of plots with PVC pipes, yielding a total of 24 plots. All plots were essentially monospecific throughout the study with the dominant species at each site as follows: Amite River: *Saururus cernuus*, Blind River: *Pontederia cordata*; Joyce WMA: *Typha latifolia*; Reserve Canal: *Peltandra virginica*; Tobe Canal: *Sagittaria lancifolia*; Turtle Cove: *Schoenoplectus acutus*. Seasonally determined metrics in each plot included: pore-water pH, pore-water conductivity/salinity, pore-water nutrient concentration (NO₃-NO₂-N, NH₄-N, SO₄-S), soil organic matter, soil redox potential (1 and 10 cm depths), soil total Hg, soil methyl Hg, as well as above- and below-ground plant tissue Hg. Plots were sampled in Spring (March 2007), Summer (July 2007), Fall (November 2007), and Winter (February 2008) to investigate possible effects of season. A repeated measures randomized block design with 4 blocks (i.e.,

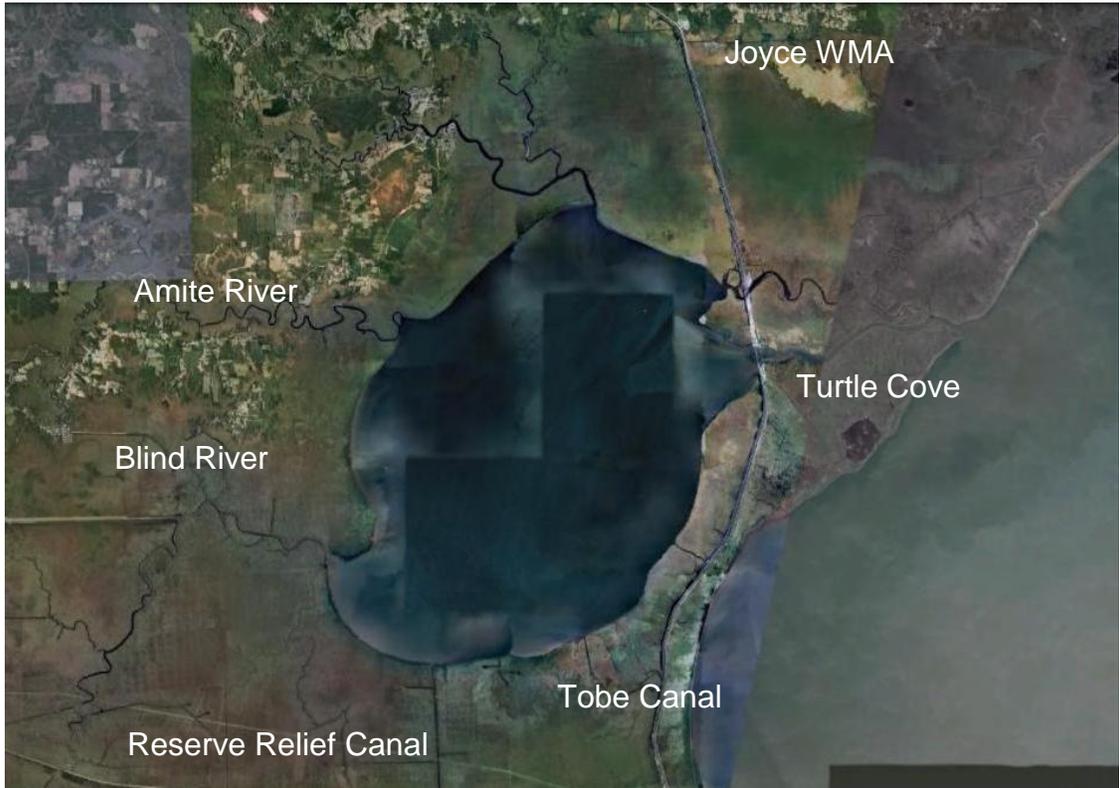


Fig. 2.1. Map of the field sites in the Lake Maurepas wetlands.

each plot treated as a block) yielded a 24 experimental plot experimental design. The statistical relationships were examined using the general linear model procedures of SAS 9.1 (SAS 2004).

Data Collection

Soil redox potential was determined at 1 and 10 cm depths using three bright, Pt soil redox electrodes per depth and a calomel reference electrode as described in Patrick et al. (1996). Where available, soil pore-waters (composite sample 15 cm in depth) were collected using acid-washed soil sippers (see McKee et al. 1988, for a description of the technique). Immediately after collection of samples, one 3-ml aliquot of pore-water was expunged into an equal volume of antioxidant buffer (SAOB reagent, ThermoOrion Corporation) and analyzed for total dissolved sulfides using an Orion ion-selective electrode (Orion Research Inc.) within 24 hours after returning to the lab. A second aliquot for nutrient determination was expunged into a sample bottle and immediately placed on ice for transport back to the laboratory. Nutrient analysis was accomplished following EPA methods: 350.1 (ammonia), 353.2 (nitrate-nitrite), 375.4 (sulfate) and 1620 (total phosphorus) as described in APHA (2005). A third aliquot was expunged into a sample bottle for onsite determination of pore-water pH, conductivity, and salinity using hand held meters and approved EPA Methods (APHA 2005). Soil cores were collected to a depth of 15 cm using a 7.62-cm diameter thin-wall aluminum soil corer and placed into clean polyethylene bags. These soil cores were processed for the determination of total and methyl mercury following the methods outlined in Bloom (1989) for total and methyl mercury. An additional soil core was collected from each plot and placed into a preweighed, polyethylene bag for the determination of organic matter upon returning to the lab (Soil Testing and Plant Analysis Council 2000).

Plant cover was assessed through visual estimation (Barbour et al. 1999) in permanent plots. Samples of both above- and below-ground biomass for total mercury determination were

collected into clean polyethylene bags and, upon returning to the lab, rinsed with deionized water and blotted dry with Kimwipes. Thereafter, wet tissue samples were homogenized with stainless steel cutting tools, digested for a minimum of 12 hours in trace metal grade sulfuric and nitric acid at 100° C, oxidized with bromine chloride for a minimum of 12 hours, and then analyzed for total mercury concentration (see appendix to EPA method 1631 for details; APHA 2005).

Results

Total and Methyl Mercury Characterization

Significant effects were detected in total mercury concentrations in Lake Maurepas wetland soils in regard to season (Fig. 2.2 panel A; $F=2.80$, $P=0.0479$), site (Fig. 2.2 panel A; $F=3.57$, $P=0.0071$) and the interaction thereof (Fig. 2.2 panel A; $F=3.04$, $P=0.0012$). However, all values reported for total soil mercury fall within a range that would be considered background soil levels for the United States (Eisner 2006) and also within the range reported for the Lake Maurepas wetlands by Yu et al. (2008). Thus, these statistically significant effects, which likely result from microscale environmental differences, do not appear to represent concern as a contaminant. No significant differences in soil methyl mercury concentrations were detected in regard to season, site, or the interaction thereof (Fig. 2.2 panel C). Importantly, soil methyl mercury levels in this study were well within the normal range for an uncontaminated wetland, and actually occurred towards the lower end of the expected range. Significant effects were detected in total mercury concentrations in aboveground plant tissue in regard to the interaction of season and site (Fig. 2.2 panel C; $F= 4.51$, $P= 0.0170$), but not the main effect of season or

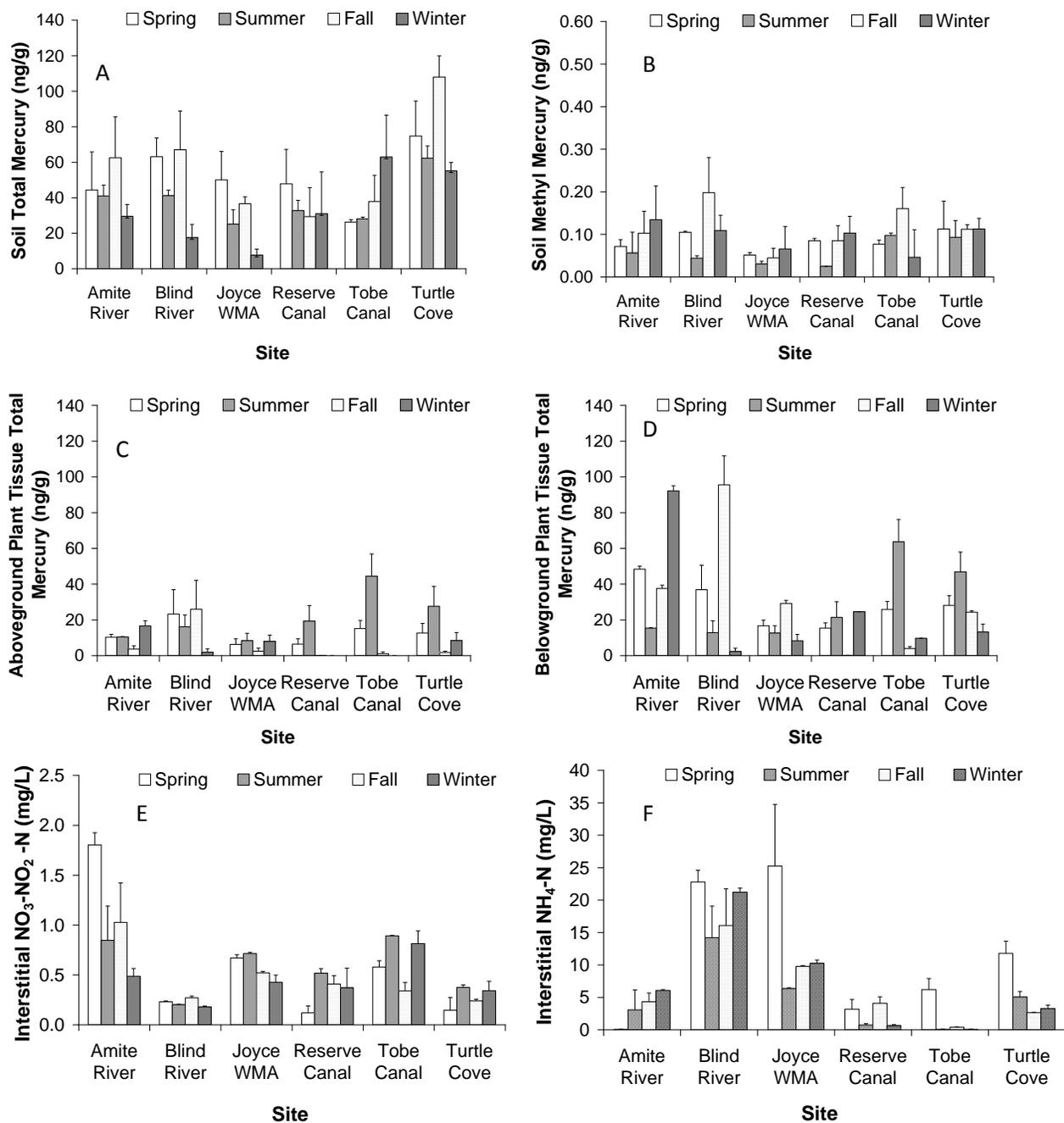


Fig. 2.2. Effect of site and season on soil total mercury (panel A), soil methyl mercury (panel B), aboveground plant tissue total mercury (panel C), belowground plant tissue total mercury (panel D), interstitial nitrate-nitrite (panel E), interstitial ammonium (panel F). All values are mean \pm 1 s.e..

site. Similarly for belowground plant tissue, no significant effect was detected among sites, but there was a significant effect of season (Fig. 2.2 panel D; $F=3.09$, $P=0.0051$) and a significant interaction of site and season was detected (Fig. 2.2 panel D; $F=4.67$, $P=0.0002$). As with total soil mercury concentration, all total below- and above-ground tissue mercury concentrations fell within a range considered background for wetland plant species in uncontaminated areas, suggesting that no undue environmental concern is warranted. The statistically significant differences in belowground plant tissue concentrations may reflect local scale phenomenon not captured by the experimental design.

Pore-Water Nutrients

The pore-water nitrate-nitrite-N concentrations were significantly higher at the Amite River site than at other sites (Fig. 2.2 panel E; $F=22.16$, $P<0.0001$), particularly in the spring sampling, which resulted in a significant interaction (Fig. 2.2 panel E; $F=4.58$, $P<0.0001$). The pore-water ammonium-N concentrations were significantly higher at the Blind River site than at other sites (Fig. 2.2 panel F; $F=29.27$, $P<0.001$) and were significantly higher in spring than in other sampling periods (Fig. 2.2 panel F; $F=8.10$, $P<0.001$). A significant interaction of site and season was detected (Fig. 2.2 panel F; $F=1.98$, $P=0.034$), likely resulting from the Joyce, Tobe Canal, and Turtle Cove sites having greater pore-water ammonium-N in the spring than in other seasons, whereas the Blind River sites contained elevated pore-water concentrations for the entire study duration.

General Pore-Water Characteristics

The more saline-influenced sites, Tobe Canal and Turtle Cove, had significantly greater pore-water sulfate than the other sites (Fig. 2.3 panel A; $F=82.00$, $P<0.001$). A significant interaction of season and site was detected for pore-water salinity (Fig. 2.3 panel B; $F=4.97$, $P=0.003$), likely a result of the Amite River and Reserve Canal sites having similar salinity levels

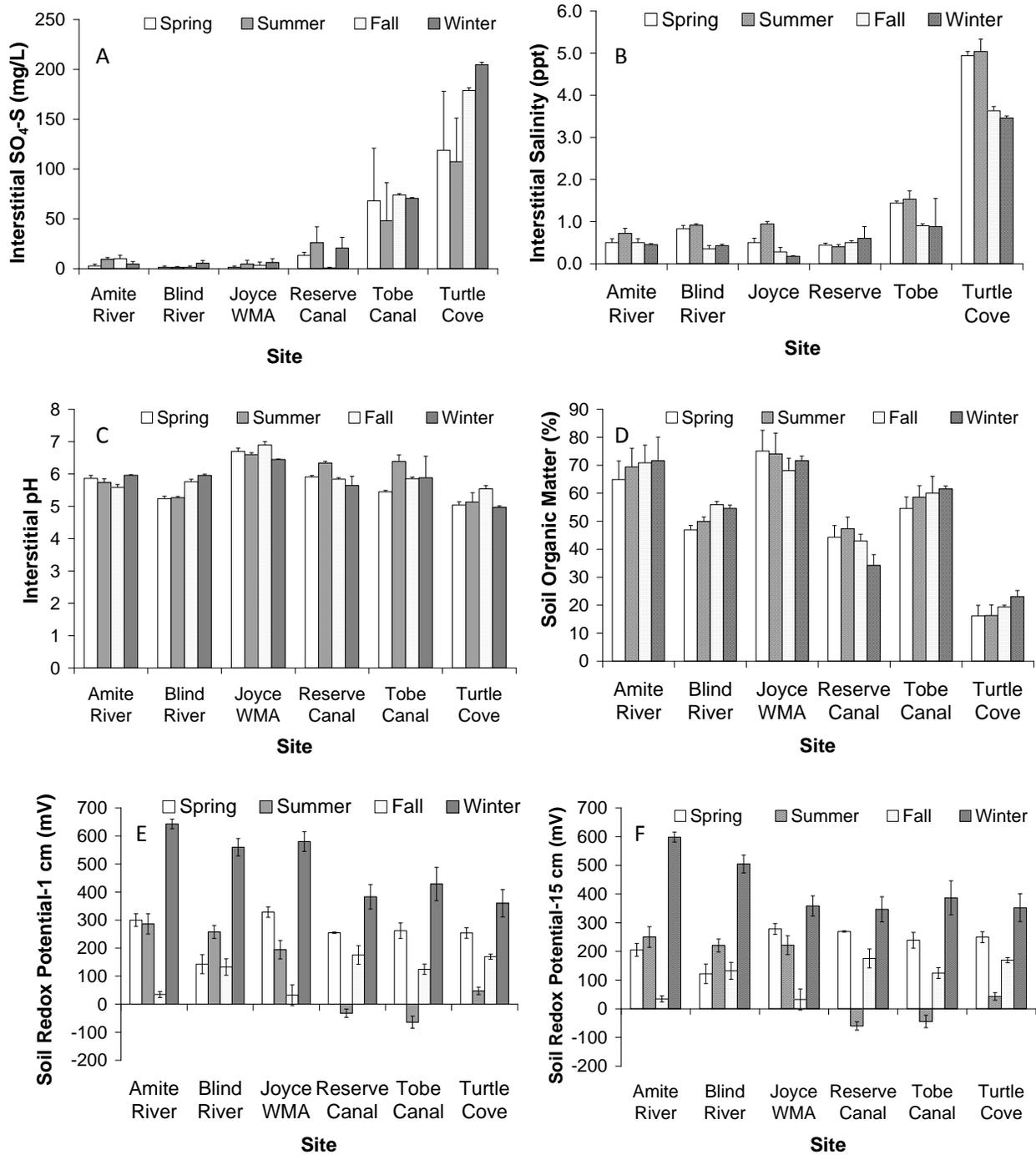


Fig. 2.3. Effect of site and season on interstitial sulfate (panel A), interstitial salinity (panel B), interstitial pH (panel C), soil organic matter (panel D), soil redox potential at 1 cm (panel E), and soil redox potential at 15 cm (panel F). All values are mean \pm 1 s.e..

throughout the study duration, whereas other sites had lower pore-water salinity levels in fall and winter. Similarly, a significant effect of site was also discerned in regard to pore-water salinity, which likely results from Tobe Canal and Turtle Cove being more saline than other sites (Fig. 2.3 panel B; $F=73.02$, $P<0.001$). No significant effect of season or interaction of season and site was found for pH. However, Turtle Cove pore-water was significantly more acidic than other the sites (Fig. 2.3 panel C; $F=5.17$, $P=0.003$).

General Soil Characteristics

No significant effect of season or interaction of season and site was found for soil organic matter. However, Turtle Cove had significantly less organic matter than other the sites (Fig. 2.3 panel D; $F=52.01$, $P<0.001$). A significant interaction of season and site was discerned for both surface (Fig. 2.3 panel E; $F=13.28$, $P<0.001$) and deep (Fig. 2.3 panel F; $F=34.12$, $P<0.001$) soil redox potential, with Reserve Canal, Tobe Canal, and Turtle Cove being more reduced in summer than the other field sites. A significant effect of season was also detected, for both surface (Fig. 2.3 panel E; $F=59.95$, $P<0.001$) and deep (Fig. 2.3 panel F; $F=111.16$, $P<0.001$) soil redox potential, with soils being much less reduced in winter than the other seasons.

As previously mentioned, water levels in the Lake Maurepas system were below average during the sampling year and sampling months. A United States Geological Survey water level gage located on the Amite River near the town of Maurepas reflects these trends in annual and monthly water levels in the Lake Maurepas system (Fig. 2.4, Table 2.1).

Discussion

This assessment of mercury levels in the Lake Maurepas wetlands generally indicates that, within all examined compartments, the total mercury concentrations are within a range considered to be representative of an uncontaminated wetland and that the wetland is safe for local users (Eisler 2006). The concentrations of total mercury reported in this study are within

Table 2.1. Annual average stream gauge height (m) at Amite River near Maurepas, Louisiana from USGS water level gage 07380215

Year	Average Gage Height (m)
2003	0.554
2004	0.560
2005	0.603
2006	0.467
2007	0.538
2008	0.580

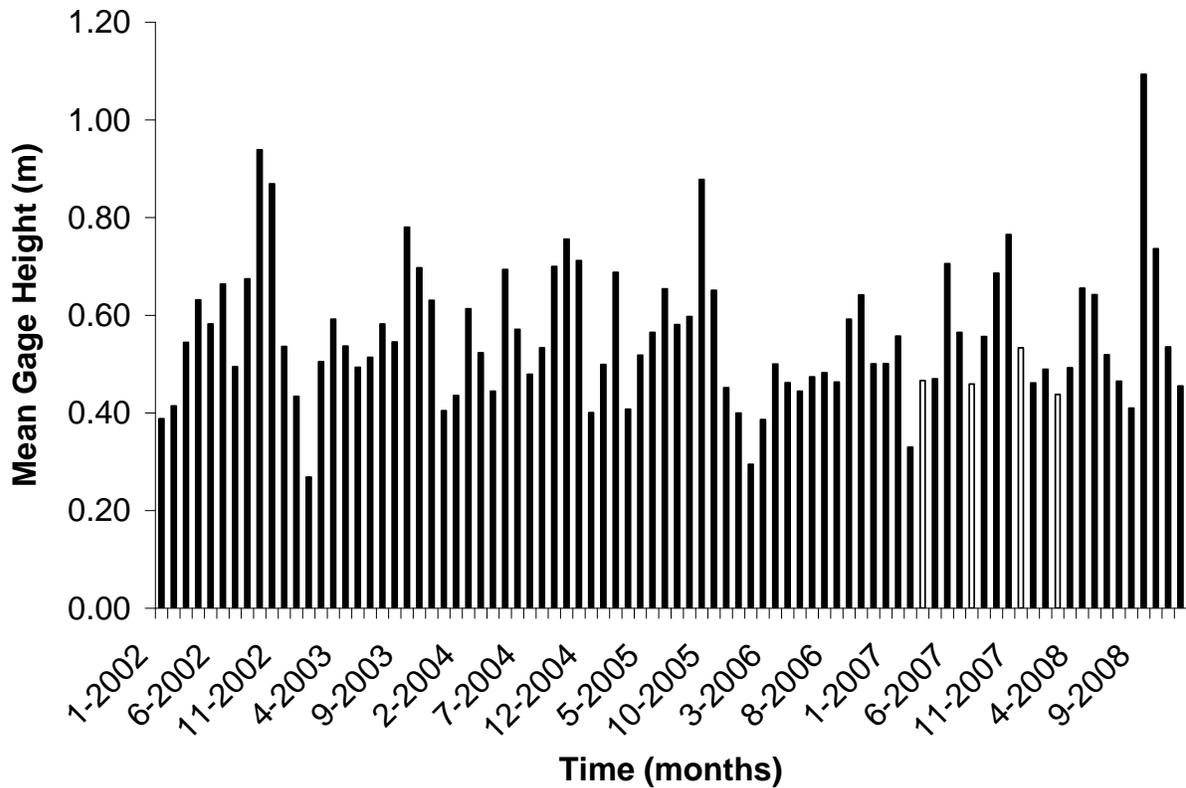


Fig. 2.4. Monthly average stream gauge height (m) at Amite River near Maurepas, Louisiana from USGS water level gage 07380215.

the range reported for Louisiana sediments and soils presented by other researchers, such as O'Rourke et al. (2001) 70-120 ng/g, Dupre et al. (1999) below detection to 250 ng/g, Kongchum et al. (2006) 78 -240 ng/g, Delaune et al. (2008) 10.6-177 ng/g, and Yu et al. (2008) 8.7-288.9 ng/g. These levels of mercury likely reflect the lack of elevated atmospheric deposition of mercury and the absence of major industrial sources of mercury in the Lake Maurepas area. Methyl mercury levels were determined in surficial soils, which are frequently the major site of mercury methylation in aquatic systems (Korthals and Winfrey 1987), and were also found to be within a range typical of an uncontaminated wetland (Eisler 2006). Interestingly, soil methyl mercury concentrations determined in this study are lower than those reported by Yu et al. (2008), which may reflect differences in hydrology prior to sampling. Water levels were low during this study, with wetland soils moist, but not saturated during each sampling event, whereas Yu et al. (2008) reported all soils either saturated or flooded. Similarly, Hall et al. (2008), investigated surface and pore-water total and methyl mercury in several Louisiana wetlands, including the Blind River area, and found higher levels of methyl mercury in freshwater wetlands compared to adjacent surface waters, suggesting that the wetlands may function as a net source of methyl mercury to these open water bodies. Results of this study, as well as Yu et al. (2008) and Hall et al. (2008), provide an interesting contrast to other oligohaline wetland systems where mercury dynamics have been studied, such as the Florida Everglades. Controlling factors of the methyl mercury concentrations in soils and biota of the Florida Everglades include sulfate and mercury loading (Gilmour and Krabbenhoft 2001). Reported soil total mercury concentrations in the Florida Everglades vary (100 ng/g: Drake et al. 1996; 157 ng/g: Kannan et al. 1998; 209 ng/g: Arfstrom et al. 2000), but are generally similar to, or slightly above, soil total mercury concentrations reported herein for the Lake Maurepas wetlands.

However, reported methyl mercury concentrations in Everglades soils (5 ng/g; Drake et al. 1996) are generally elevated compared to those found for the Lake Maurepas wetlands. Discussion of these findings highlights the need for multiple year investigations as well as the need for a thorough understanding of the local environment, e.g., hydrology, to appropriately frame interpretations.

The total mercury concentrations determined in this study for aboveground plant tissue were similar to values detected in other studies of uncontaminated wetlands, such as Moore et al. (1995) 4 to 160 ng/g and Rencz et al. (2003) 5 to 58 ng/g. The total mercury concentrations determined in this study for vegetation was also similar to that reported for the understory of boreal upland forest in Canada (5 to 58 ng/g, Hall and St. Louis 2004; 4 to 52 ng/g, Mailman and Bodaly 2005) and mosses in the arctic (20-112 ng/g, Landers et al. 2005). In general, it does not appear that either above- or below-ground vegetation components represent unusually substantial mercury reservoirs in the Lake Maurepas wetlands.

All wetland soil and soil pore-water variables evaluated were generally similar across sites, with the exception of Turtle Cove, which had a higher pore-water salinity and lower soil organic matter content reflecting its more brackish nature. Pore-water variables determined for the other five sites in this study, including salinity, pH, dissolved nitrate-nitrite, dissolved ammonia, and dissolved sulfate were similar to the results other researchers have found for the Lake Maurepas wetlands (e.g., Hall et al. 2008), and also fall within the range reported for fresh and oligohaline wetlands in Louisiana (Sasser et al. 1991; Hester et al. 2005; Meert and Hester 2009; Swarzenski et al. 2008). It should be noted that soil redox potential was higher for all sampling periods in this study than in other studies of the Lake Maurepas wetlands (Hester et al. 2005), likely a result of water levels in this system being somewhat low during this study period. The soil redox potentials determined during this study are generally corroborated by the essential

absence of sulfides, the main product of microbial sulfate reduction (Delaune et al. 2002). The ratio of oxidized to reduced nitrogen and sulfur compounds, in conjunction with the soil redox data, indicate that at the time these areas in the Lake Maurepas wetlands were sampled, sulfate reduction was not the primary microbial metabolic pathway occurring in these soils (Delaune et al. 2002).

In summary, the concentrations of mercury and methyl mercury in the various environmental compartments measured within the Lake Maurepas wetlands appear to be within the range of uncontaminated wetlands. Interestingly, other recently published data concerning mercury and methyl mercury concentrations in these wetlands report similar levels of total mercury in soils, but higher levels of methyl mercury. This apparent difference may merely reflect the local hydrology at the time of sampling for different studies. Soil characteristics such as the large amount of organic matter with associated reduced sulfur functional groups may provide some substantial capacity to render newly deposited mercury unavailable for microbial activity, thus providing additional protection (Skylberg et al. 2003). However, experimental manipulation of soil mercury levels in a controlled setting coupled with an estimate of microbial bioavailability would be necessary to test this hypothesis. No consistent seasonal variation was detected in methyl mercury levels, or in related soil characteristics, although again this was likely due to the abnormally mild winter and low water levels throughout the study. Results from this study, as well as Yu et al. (2008) and Hall et al. (2008) indicate that methyl mercury levels in the Lake Maurepas wetlands are either equivalent to or elevated in comparison to those of Lake Maurepas itself on average. However, sites with highly elevated levels of methyl mercury in the wetlands were completely absent from this study and rare (2 out of 35) in the study by Yu et al. (2008). Hall et al. (2008) generally found that freshwater and brackish wetlands in the Lake Pontchartrain Basin (e.g., Blind River and Bayou Lacombe) have elevated surface water methyl

mercury levels compared with the surface waters of Pass Manchac and Lake Pontchartrain. This suggests that the surrounding wetlands are likely a source of methyl mercury to adjacent lake waters, which is considered typical for wetland-surface water systems (St. Louis et al. 1994).

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CHAPTER III

GROWTH RESPONSE AND TISSUE ACCUMULATION TRENDS OF HERBACEOUS WETLAND PLANT SPECIES EXPOSED TO ELEVATED AQUEOUS MERCURY LEVELS

Introduction

Phytoremediation of contaminants in aquatic ecosystems has been proposed as a means of reducing a variety of environmental pollutants including industrial organics (Cunningham et al. 1997), pesticides (Xia et al. 2001), petroleum products (Newman et al. 1998), explosives residue (Hughes et al. 1997), and metals (Weis and Weis 2004). The phytoremediation of metals using wetland plants has received the most attention within this area of research (Rai 2008; Williams 2002; Weis and Weis 2004). Investigations into the phytoremediation of aqueous and soil-borne mercury have recently intensified (Moreno et al. 2004; Skinner et al. 2007; Moreno et al. 2008; Su et al. 2008) likely because of the highly toxic and bioaccumulative nature of this metal (Morel et al. 1998). Several variables unique to individual contaminated sites affect the utility of phytoremediation (Weis and Weis 2004), including edaphic characteristics (e.g., pH, redox potential, organic matter content), the phytoremediation approach (e.g., phytoextraction versus phytostabilization), and the nature and concentration of the contaminant in question (EPA 2001; Williams 2002; Sparks 2003; Weis and Weis 2004; Otte and Jacob 2006). Thus, based on site characteristics and the desired approach, appropriate vegetation can theoretically be selected for optimal site restoration efforts (Sparks 2003). However, further basic research on many wetland plant species with regard to their capacity to accumulate and sequester contaminants, particularly metals, would be informative.

The use of wetland plants to reduce mercury levels in various wetland environments has been examined for a number of years (e.g., Breteler et al. 1981; Sundberg-Jones and Hassan

2007). Recent research on the uptake of mercury by various aquatic species has indicated that substantial phytoremediation of mercury-contaminated waters may be possible (Kamal et al. 2004; Skinner et al. 2007). Species that show promise as phytoremediating agents for mercury contamination of aquatic environments include *Azolla carolinia* (Bennicelli et al. 2004), *Myriophyllum spicatum*, *Ludwigia peploides*, and *Mentha aquatica* (Kamal et al. 2004), and *Eichornia crassipes*, *Pistia stratiotes*, *Scirpus tabernaemontani*, and *Colocasia esculenta* (Skinner et al. 2007). In each of the above studies the reduction in the mercury concentration of experimentally contaminated treatments was greater than 90%. Understanding the uptake and partitioning of inorganic mercury in wetland vegetation under various scenarios of elevated mercury concentrations has implications for management of habitats. For example, King et al. (2002) determined that there was a variable removal of mercury from source waters in a wetland planted with *Scirpus californicus*, and that mercury not sequestered by the vegetation became methylated. Incorporation of mercury into vegetative tissue where methylation appears to occur sparingly is an important, if temporary, reservoir of inorganic mercury unavailable for methylation by microbial organisms (Weis and Weis 2004). However, mercury sequestered in plant tissue may still be incorporated into the food chain, even if in a form less toxic and less prone to bioaccumulation. Substantial interspecific variation in the uptake of inorganic mercury by wetland vegetation, as well as the resulting toxicity, has been documented. Descriptions of the tolerance to inorganic mercury by vegetation as well as the patterns of partitioning into above- and below-ground vegetation for individual plant species are needed to better understand mercury cycling in wetland environments. This information will enable the proper management of natural wetlands subjected to elevated mercury inputs as well as provide guidance for the continued refinement of phytoremediation technology. The objectives of this research were to determine the interspecific differences in mercury uptake and localization, as well as the

physiological response and tolerance to elevated mercury levels by common herbaceous fresh marsh plants. The plants chosen for this study are common constituents of freshwater to oligohaline wetlands in the southeastern U.S. and, therefore, are frequently present in treatment wetlands. These species possess several qualities generally regarded as beneficial for application to the phytoremediation of metals, including the potentially high production of aboveground tissue (Cunningham and Ow 1996; Wei et al. 2009) and their capability for clonal growth. Although little information exists regarding the ability of these species to accumulate mercury, the above factors suggest that these species would likely be effectual for phytoremediation if they, indeed, take up sufficient amounts of mercury.

Materials and Methods

I used a completely randomized factorial design to investigate mercury uptake by 5 species: (*Eleocharis parvula*, *Saururus cernuus*, *Juncus effuses*, *Typha latifolia*, and *Panicum hemitomon*) x 3 mercury levels (0- $\mu\text{g ml}^{-1}$ 2- $\mu\text{g ml}^{-1}$ 4- $\mu\text{g ml}^{-1}$) x 4 true replicates (60 experimental units). The experiment was established as a greenhouse container study using 1-gallon polyethylene nursery pots placed within 2-gallon polyethylene buckets as reservoirs. Healthy specimens of the above plant species were collected from the Joyce Wildlife Management Area, LA, placed into 1-gallon nursery pots, and transported back to the University of New Orleans greenhouse facility. Upon returning to the greenhouse facility, plant roots were rinsed of soil material, placed into acid-washed 1-gallon nursery pots with acid-washed sand, and flooded to 2.5 cm above the sand surface using type III deionized water to which nutrients were added to equal 25% Hoaglands solution. Plants were allowed to acclimate for two weeks before the study started. Treatments were randomly assigned and aqueous solutions of mercuric chloride (or type III deionized water for the control) were added to the surface water of appropriate vessels, after which pots were agitated in reservoirs by rapidly lifting them up and

down several times to ensure mixing of treatment solutions throughout the sand matrix. After two months, the net CO₂ assimilation rate, aboveground tissue total mercury, belowground tissue total mercury, total mercury sorbed to sand particles, residual total mercury in pore-water, and biomass partitioning were determined.

The net CO₂ assimilation rate was determined on the two youngest fully expanded leaves for each experimental unit and averaged using a LI-Cor 6400 Photosystem with leaf chamber light intensity set to 1,500 μmol m⁻² s⁻¹, leaf chamber CO₂ set to 370 ppm, and leaf chamber relative humidity maintained between 20 and 60%. At the conclusion of the study, above- and below-ground tissue was clipped with stainless steel scissors, rinsed with type III de-ionized water, placed into paper bags and dried to a constant weight in a drying oven set at 40°C. Subsets of tissue were homogenized with a Wiley Mill and digested using a 1:3 mixture of trace-metal grade nitric and hydrochloric acid (i.e., aqua regia) in Teflon digestion vessels (see appendix to EPA Method 1631, digestion 2 for details and caveats; APHA 2005). Samples of sand were collected using a Teflon coated spatula and extracted using a 1:3 mixture of trace-metal grade nitric and hydrochloric acid (i.e., aqua regia) in Teflon digestion vessels. The residual pore-water was collected by pipetting samples into glass vials with Teflon closures and oxidized overnight with BrCl. Total mercury analysis was accomplished following EPA Method 1631 (Cold Vapor Atomic Fluorescence; APHA 2005) using a 4 unit bubbler setup in conjunction with a Brooksrand amalgamation control module and Model III atomic fluorescence spectrophotometer. The statistical relationships among variables were examined using the appropriate general linear model procedures of PC-SAS 9.1 (SAS 2004).

Results

Tissue and Pore-Water Mercury Concentrations

Species differed significantly in their final total aboveground tissue concentrations of mercury (Fig. 3.1; $F=2.919$, $P=0.341$), with *Eleocharis parvula*, *Panicum hemitomon* and *Typha latifolia* having the greatest concentration in the 4 $\mu\text{g/ml}$ treatment and *Juncus effuses* and *Saururus cernuus* having the lowest concentration in the 4 $\mu\text{g/ml}$ treatment. The mercury treatment level had a significant effect on the final total aboveground tissue mercury concentration, with greater concentrations of mercury accumulating into aboveground tissue with higher mercury addition (Fig. 3.1; $F=3.243$, $P<0.001$). A significant interaction of species and mercury level was detected, resulting from *Eleocharis parvula*, *Panicum hemitomon*, and *Typha latifolia* having total aboveground mercury concentrations that were substantially elevated at 4 $\mu\text{g/ml}$ compared to 0 and 2 $\mu\text{g/ml}$, whereas *Juncus effuses* and *Saururus cernuus* had elevated aboveground total mercury tissue concentrations at both 2 and 4 $\mu\text{g/ml}$ (Fig. 3.1; $F=3.050$, $P=0.047$). Belowground tissue concentrations of mercury displayed similar trends as the aboveground tissue concentrations of mercury with *Eleocharis parvula*, *Panicum hemitomon* and *Typha latifolia* displaying the greatest concentrations in the 4 $\mu\text{g/ml}$ treatment and *Juncus effuses* and *Saururus cernuus* the lowest concentrations in the 4 $\mu\text{g/ml}$ treatment (Fig. 3.1; $F=3.30$, $P=0.0495$). As was found with the aboveground tissue analyses, the mercury added significantly increased the final total belowground tissue mercury concentration (Fig. 3.1; $F=22.096$, $P<0.001$). A significant interaction of species and mercury level was detected, resulting from elevated belowground tissue mercury concentrations in *Eleocharis parvula*, *Panicum hemitomon* and *Typha latifolia* in the 2 $\mu\text{g/ml}$ treatment compared with *Juncus effuses* and *Saururus cernuus*, which had minimal accumulation in this treatment (Fig. 3.1; $F=3.05$, $P=0.0372$). For all species, the accumulation of mercury into belowground tissues was significantly

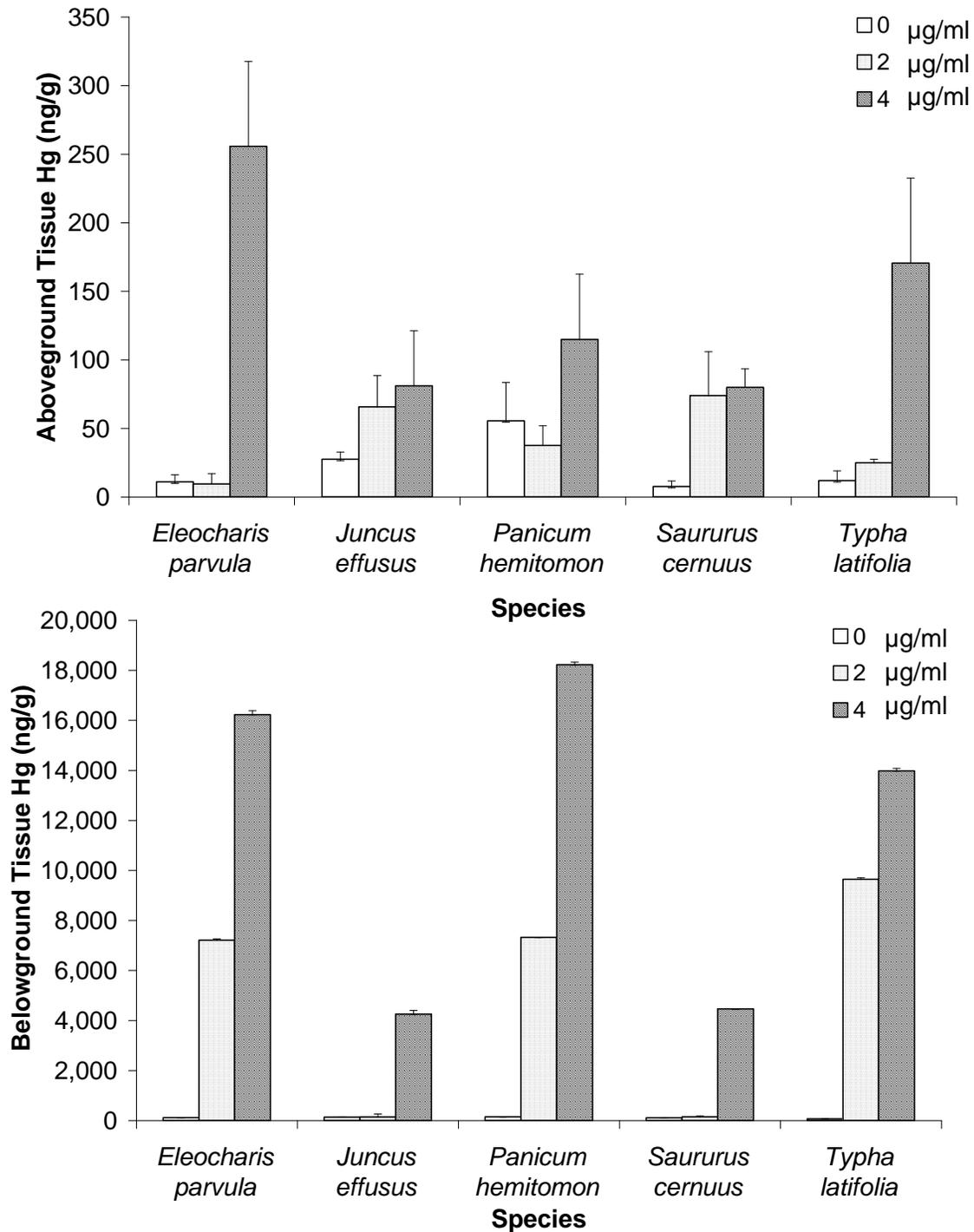


Fig. 3.1. The effect of species and mercury level on aboveground plant tissue mercury concentration (top panel) and belowground plant tissue mercury concentration (bottom panel; mean +/- 1 s.e.).

greater than into aboveground tissues. The final sand-associated concentrations for the 2 and 4 µg/ml treatments did not significantly differ among treatments and were extremely low (overall mean and s.e.= 288.0 ng g⁻¹ +/- 39.9), suggesting minimal binding of mercury to sand. The final pore-water concentrations were substantially reduced in the 4 µg/ml mercury concentration for all species, and in the 2 µg/ml mercury concentration in all species, except *Panicum hemitomon* (Fig. 3.2). However, reductions in final pore-water concentrations in the 2 µg/ml mercury concentration generally tended to be more variable than the reductions in the 4 µg/ml mercury concentration (Fig. 3.2). Importantly, when the mass balance of total mercury added to each experimental unit (14.6 mg and 7.3 mg for 4 and 2 µg/ml treatments, respectively) versus the amount of mercury absorbed into plant tissue is considered, vegetation appears to play a minimal role compared to other effects (e.g., volatilization) in reducing total mercury concentration (Table 3.1).

Photosynthetic Response

A significant effect of species on net CO₂ assimilation rate was detected (Fig. 3.3; F=77.596, P < 0.001), with *Juncus effuses*, *Saururus cernuus*, and *Typha latifolia* having greater net CO₂ assimilation rates than *Eleocharis parvula* and *Panicum hemitomon* overall, however *Typha latifolia*, and especially *Panicum hemitomon*, displayed significant depressions in CO₂ assimilation rate when exposed to the highest mercury levels (Fig. 3.3). Interestingly, no significant effects of mercury level, or the interaction of mercury level and species, were detected. A significant effect of species on stomatal conductance was detected, with *Eleocharis parvula* displaying much greater stomatal conductance than the other species (Fig. 3.3; F= 10.832, P < 0.001).

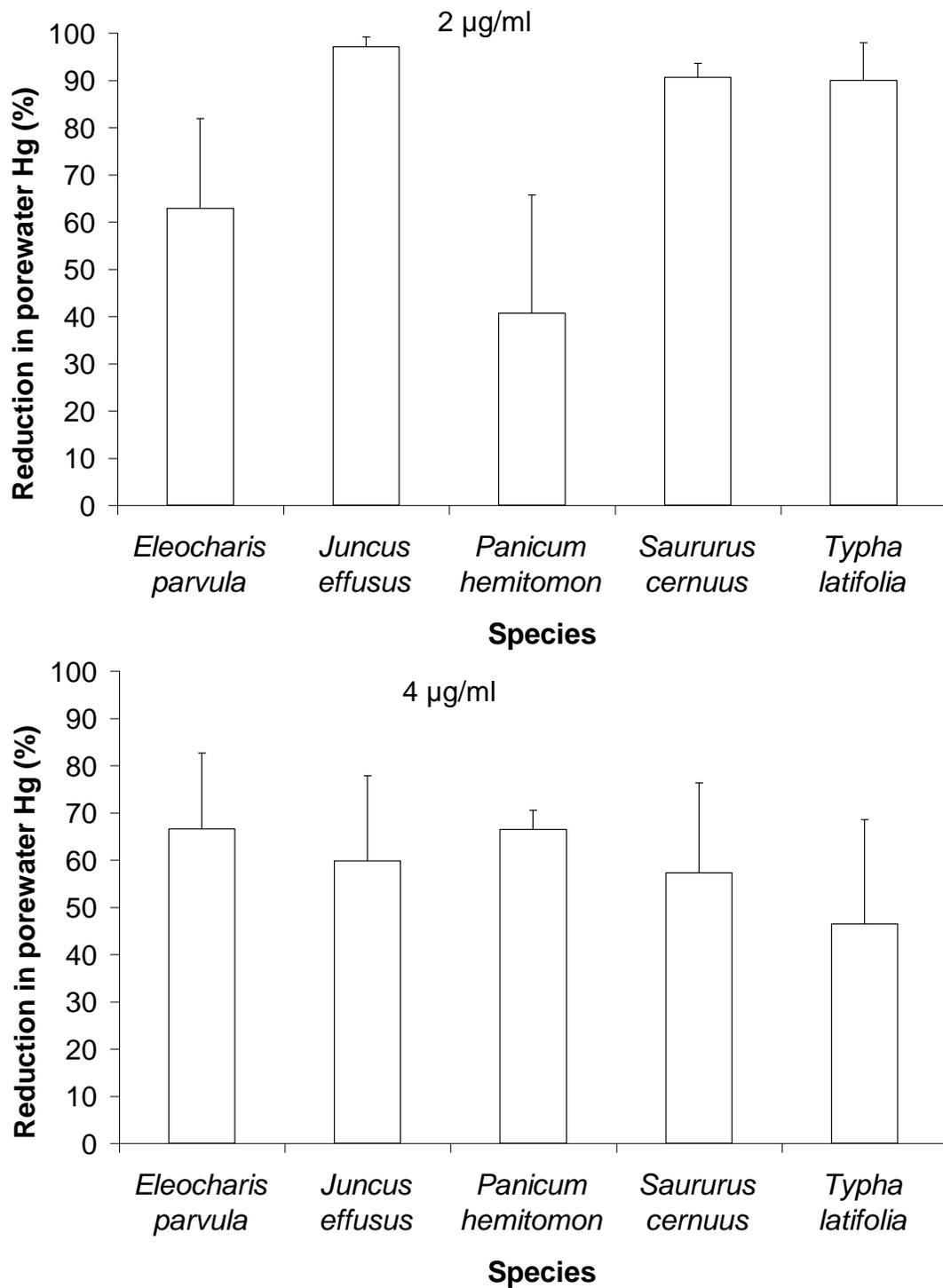


Fig. 3.2. The effect of species on reduction of total mercury in final pore-water concentration in the 2 µg/ml (top panel) and 4 µg/ml (bottom panel) treatments. (mean \pm 1 s.e.).

Table 3.1. Aqueous mercury reduction through plant tissue accumulation (%) and relevant metrics

Treatment	Species	Belowground tissue mercury (µg/g)	Belowground biomass (g)	Aboveground tissue mercury (µg/g)	Aboveground biomass (g)	Total mercury accumulated into biomass (µg)	Total initial aqueous mercury (µg)	Aqueous mercury reduction through plant tissue accumulation (%)
2 µg/ml	<i>Eleocharis parvula</i>	7.21	36.7	0.01	8.4	264.8	7,300	3.63
2 µg/ml	<i>Juncus effusus</i>	0.14	27.5	0.07	7.3	4.3	7,300	0.06
2 µg/ml	<i>Panicum hemitomon</i>	7.32	45.2	0.04	19.2	331.9	7,300	4.55
2 µg/ml	<i>Saururus cernuus</i>	0.15	24.1	0.07	4.8	4.0	7,300	0.05
2 µg/ml	<i>Typha latifolia</i>	9.64	32.2	0.02	8.0	311.2	7,300	4.26
4 µg/ml	<i>Eleocharis parvula</i>	16.23	26.5	0.26	4.4	431.0	14,600	2.95
4 µg/ml	<i>Juncus effusus</i>	4.26	37.1	0.08	8.6	158.4	14,600	1.08
4 µg/ml	<i>Panicum hemitomon</i>	18.23	33.3	0.11	10.6	608.0	14,600	4.16
4 µg/ml	<i>Saururus cernuus</i>	4.46	23.6	0.08	5.4	105.5	14,600	0.72
4 µg/ml	<i>Typha latifolia</i>	13.98	55.6	0.17	16.0	779.3	14,600	5.34

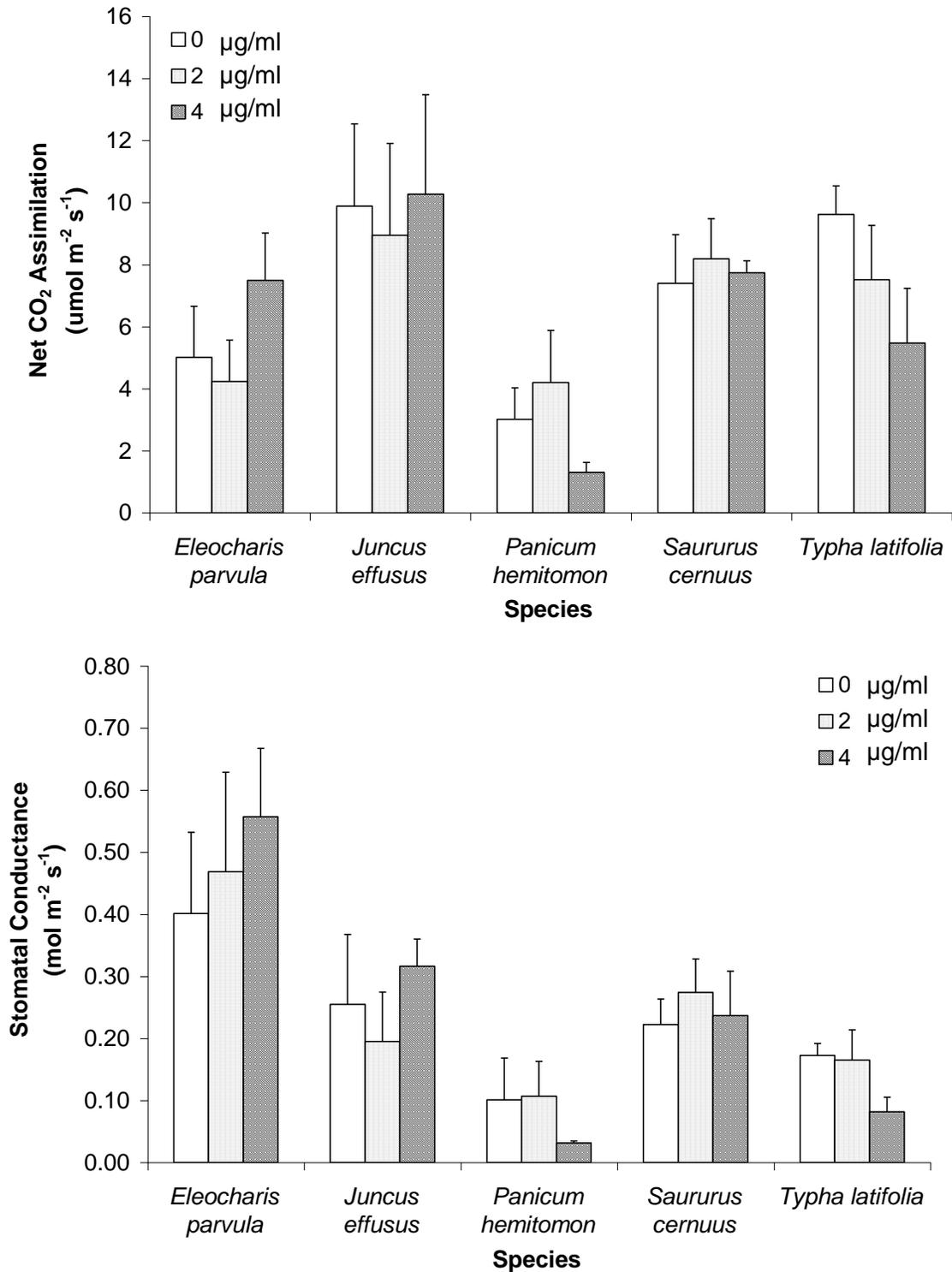


Fig. 3.3. The effect of species and mercury level on net CO₂ assimilation (top panel and stomatal conductance (bottom panel; mean +/- 1 s.e.).

Biomass Response

A significant effect of species on aboveground biomass was detected (Fig. 3.4; $F=4.954$, $P=0.002$). *Panicum hemitomon* and *Typha latifolia* aboveground biomass production was greater than *Juncus effuses*, *Saururus cernuus*, and *Eleocharis parvula*, although no significant effect of mercury level on aboveground biomass production was evident. A marginally significant interaction of species and mercury level was detected (Fig. 3.4; $F=1.970$, $P=0.073$), in which *Eleocharis parvula* produced less aboveground biomass at higher mercury levels, but all other species had equivalent or greater aboveground biomass at higher mercury levels. A significant effect of species on belowground biomass was detected that closely mirrored the aboveground biomass response (Fig. 3.4; $F=3.447$, $P=0.015$), but with belowground production of *Panicum hemitomon* being depressed at both levels of mercury addition. No significant effect of mercury level or interaction of mercury level and species was detected for belowground biomass. The total biomass demonstrated trends consistent with the species effect on aboveground biomass ($F=4.749$, $P=0.003$), with no significant effect of mercury level or the interaction of mercury level and species.

Discussion

All species investigated were able to survive and sustain reasonable levels of metabolic function allowing for growth at elevated aqueous mercury concentrations (up to 4 $\mu\text{g/ml}$) while accumulating substantial amounts of mercury in the belowground tissues. However, although extremely high reductions in pore-water mercury concentrations were noted by the end of the study (up to 97%), mercury accumulation into vegetative tissue appears to play a relatively small role. Interestingly, species differences in mercury accumulated into tissues were found, with *Eleocharis parvula*, *Panicum hemitomon*, and *Typha latifolia*, generally having greater

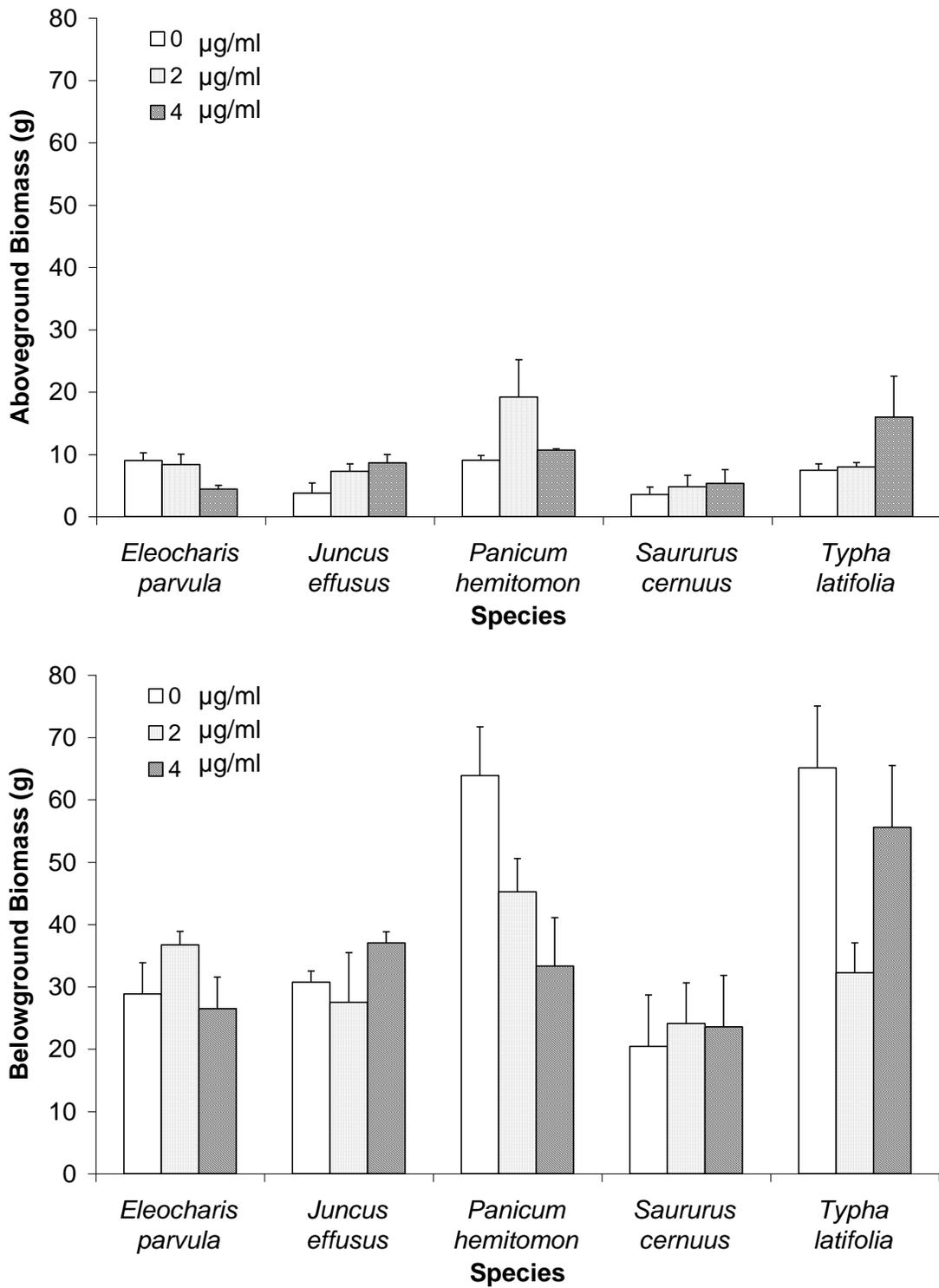


Fig. 3.4. The effect of species and mercury level on aboveground biomass (top panel) and belowground biomass (bottom panel; mean +/- 1 s.e.).

concentrations of mercury in their belowground tissues than *Juncus effusus* and *Saururus cernuus*. Inherent differences between species in growth characteristics which are relevant to phytoremediation, such as biomass production and stomatal conductance, were also elucidated and are discussed below.

The net CO₂ assimilation rates of *Typha latifolia* and particularly *Panicum hemitomon* were decreased from the controls at 4 µg/ml mercury addition, but not at 2 µg/ml mercury addition. The net CO₂ assimilation rates of the remaining species were not significantly decreased from the controls at either mercury addition, and, in fact, for *Eleocharis parvula* a stimulation of net CO₂ assimilation at the 4 µg/ml mercury concentration was discerned. As net CO₂ assimilation is an instantaneous indicator of current growth status, these results indicate that, other than *Typha latifolia* and *Panicum hemitomon*, these species are capable of maintaining normal metabolic growth functions at highly-elevated mercury concentrations after two months. The decrease in net CO₂ assimilation rate for *Typha latifolia* and *Panicum hemitomon* occurred only at the 4 µg/ml mercury addition and was modest, suggesting that these plants would survive and possibly even expand at this elevated concentration, but at a slower rate than under control conditions. Stomatal conductance was highest for *Eleocharis parvula*, and generally similar for the remaining species. Although transpiration is known to be an important component of mercury removal through phytovolatilization (Hussein et al. 2007; Moreno et al. 2008), it does not appear to be a primary driver in this study because reductions in aqueous mercury were not correlated with stomatal conductance rates.

The total biomass at the end of the experiment was generally comparable between the control and elevated mercury treatments with the exception of *Typha latifolia* and *Panicum*

hemitomon. This is in contrast to Du et al. (2005) who reported that the biomass production by *Oryza sativa* decreased when plants were exposed to aqueous mercury loads as low as 0.5 µg/ml for 20 days. As was noted with net CO₂ assimilation rates, the decrease in total biomass for these species are relatively small, indicating that these plants would likely be able to survive at these elevated mercury concentrations, but would grow at a reduced rate compared with control conditions. *Typha latifolia* and *Panicum hemitomon* produced the greatest total biomass overall, with the remaining species having fairly similar total biomass. This, in combination with the concentrations of mercury detected in the tissue of *Typha latifolia* and *Panicum hemitomon*, suggests that, of the species examined, these may be preferable for use in a phytoremediation setting.

All plants in control treatments were found to have mercury concentrations typical of plants growing in uncontaminated wetlands (Sparks 2003; Mailman and Bodaly 2005). *Eleocharis parvula*, followed by *Typha latifolia*, had the greatest concentration of mercury in the aboveground plant tissue, indicating that, of the species investigated, these two species would be preferable for a phytoextraction approach where aboveground tissues are harvested and belowground tissues are left in place. In the 2 µg/ml treatment, the final pore-water mercury levels were highly variable, suggesting differential removal of mercury, although species differences in physiological response (e.g., stomatal conductance, vegetative uptake of mercury) did not appear to be driving this variation. The pore-water mercury levels in the 4 µg/ml treatment after two months were fairly consistent regardless of species or interspecific differences in uptake, indicating that utilization of any of these species for phytoremediation of aqueous mercury contamination at similar levels would likely have equitable results.

The fact that similar degrees of reduction in final pore-water mercury concentration occurred both in experimental units planted with *Saururus cernuus* (low tissue mercury concentrations and biomass) and experimental units planted with *Typha latifolia* (higher levels of tissue mercury concentrations and biomass), suggests that some other mechanism of mercury removal occurred. This observation is reinforced by the extremely small percentage of mercury absorbed into plant tissue compared to the initial mercury load. Volatilization of mercury directly from experimental water or adherence of mercury to some component of the experimental units could be factors explaining this apparent discrepancy (Skinner et al. 2007).

Skinner et al. (2007), conducted a similar experiment in which *Eichornia crassipes*, *Pistia stratiotes*, *Scirpus tabernaemontani*, and *Colocasia esculenta* were subjected to hydroponic mercury concentrations of 0, 0.5, and 2 µg/ml. The reductions in final pore-water mercury concentrations in this study are lower than in Skinner et al. (2007), however, the final tissue mercury concentrations for the two emergent species (*Scirpus tabernaemontani* and *Colocasia esculenta*) for that study were generally similar to those in this study (except for *Saururus cernuus*). Skinner et al. (2007) reported that the floating aquatic species (*Eichornia crassipes* and *Pistia stratiotes*) accumulated considerably more mercury into their tissues than the emergent species (*Scirpus tabernaemontani* and *Colocasia esculenta*). Similarly, Molisani et al. (2006) in a survey of plant tissue mercury concentrations in Brazil found that free floating vegetation tended to have greater tissue mercury concentrations than emergent species.

Elevated accumulation of mercury into the tissues by floating aquatic plants has also been documented in a number of other hydroponic mercury dosage studies (e.g., *Eichornia crassipes*, Chigbo et al. 1982; *Salvinia natans*, Sen and Mondal 1987; *Lemna minor*, Choi et al. 1989). In a hydroponic study by Moreno et al. (2008), *Brassica juncea* was found to accumulate much

greater tissue concentrations of mercury than plants in this study when subjected to similar aqueous mercury levels. Similarly, *Oryza sativa* was found to accumulate tissue mercury concentrations nearly two orders of magnitude higher than species in this study when subjected to similar mercury loads (Du et al. 2004). Recently, Esteban et al. (2008) examined the uptake of mercury by *Lupinus albus* at 5 μM and 10 μM (approximately 1.0 and 2.0 $\mu\text{g/ml}$, respectively) and also found higher levels of mercury uptake by belowground tissue than reported in this study. An investigation by Rai and Tripathi (2009) also reported high levels of aqueous mercury reduction in experimental vessels containing *Azolla pinnata* and *Vallisneria spiralis* (up to 94% and 84%, respectively) when vessels received mercury concentrations similar to those used in this study.

Results from this study indicate that all species investigated have some capacity to reduce aqueous mercury loads in a hydroponic setting. However, aqueous mercury reduction through uptake into vegetative tissue appears to be less than in some other studies utilizing different species (e.g., Du et al. 2005; Skinner et al. 2007; Moreno et al. 2008). As has been documented for a number of species (e.g., Moreno et al. 2008), translocation of mercury from belowground to aboveground plant partitions was minimal suggesting these plants would be effective for sequestering limited amounts of mercury in belowground material. Nonetheless, when compared to previous studies, other species, particularly free-floating species, may be more efficient in aqueous mercury removal than the species investigated herein.

This study indicates that *Eleocharis parvula*, *Typha latifolia*, and *Panicum hemitomon*, all accumulate mercury into their belowground tissues under elevated concentrations to a greater extent than *Saururus cernuus* and *Juncus effuses*, which demonstrated more modest concentrations of tissue mercury. Overall these levels are lower than have been reported for

other species during screening experiments for the phytoremediation of mercury. This study emphasizes the importance of determining both biomass production and final tissue concentration to fully understand the potential ability of a plant species to remove aqueous mercury.

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CHAPTER IV

A MESOCOSM EVALUATION OF MERCURY CYCLING IN SEVERAL OLIOHALINE MARSH PLANT SPECIES

Introduction

Mercury contamination of the environment as a result of both chronic (e.g., atmospheric deposition) and acute (e.g., industrial incidents) phenomena has become an area of substantial concern in regard to environmental health (Driscoll et al. 2007). The contamination of aquatic and wetland habitats is particularly worrisome given the tendency of these habitats to possess sediment and soil characteristics that promote the production of methyl mercury species (Lacerda and Fitzgerald 2001), which are among the most toxic and bioconcentrating species of mercury (Driscoll et al. 2007). As the world's human population increases, a concomitant increase in the degree of usage of the world's habitats occurs, especially those which are aquatic (Rasch et al. 2005). Detailed studies of the cycling of contaminants in the soil-water interface of habitats likely to experience substantial human impacts may improve the long-term management of these resources (Driscoll et al. 2007).

There is substantial research on the uptake of metals by plants in virtually all habitats known to humans (McLaughlin et al. 1999; Prasad 2004; Weis and Weis 2004). A large body of work has developed in which metal cycling by wetland plants is the focus, largely because of interests in using wetlands for soil and water quality enhancement (Weis and Weis 2004), as well as a general interest in biogeochemical cycling (Valiela et 1973; Breteler et al. 1981). Much of this research has been carried out on dominant salt marsh plant species (see review by Weis and Weis 2004), as a relatively small number of dominant plant species occur in this habitat type (Mitsch and Gosselink 2000), allowing the findings to have wide-ranging applicability. Freshwater wetlands have much greater species diversity and heterogeneity (Mitsch and

Gosselink 2000). Thus, substantial interspecific variation in metal uptake and release has limited generalizations that can be made across species. The uptake of metals by plants is highly dependent not only on the plant species, but also the metal in question and local soil conditions as described below (McLaughlin et al. 1999). The present research was designed to elucidate the fate of mercury introduced into a simulated wetland environment through surface water in terms of partitioning into soil components and plant uptake, as well as physiological impacts to several common oligohaline plant species.

The species selected for this research (*Polygonum punctatum*, *Sagittaria lancifolia*, and *Pontedaria cordata*) are considered important food sources for herbivores (Chabreck and Condrey 1979) and are widespread throughout the southeastern U.S. (USDA PLANTS database) with little data available regarding their role in mercury cycling. Interestingly, several other species in the *Polygonum* genus have been investigated for their role in mercury cycling. *Polygonum densiflorum* has been observed to encourage methylation of mercury by providing a favorable environment for the proliferation of sulfate-reducing bacteria, the dominant biotic mechanism of mercury methylation (Acha et al. 2005). Kim et al. (2003) reported that *Polygonum thunbergii* moderately accumulated cadmium, lead, copper, and zinc from contaminated soil. The consumption by waterfowl of *Sagittaria* spp. growing on metal contaminated soil is considered a significant risk by the US Department of Interior (Ford 2004). Further, a member of the *Sagittaria* genus was found to play a role in mercury cycling in a South American river system (Molisani et al. 2006).

Soil factors have a paramount role in controlling the availability of elements and other soil constituents to adjacent vegetation (Sparks 2003). Total concentration in soils of many elements, particularly metals, has been shown to be a poor predictor of the corresponding concentration in

plant tissues (Jing et al. 2008). This lack of explanatory capacity results from the tendency of metals to form a myriad array of compounds in varying soil types, which differ over the vast range of chemical properties that collectively determine bioavailability (Gambrell 1994). These characteristics can be parsed into those intrinsic to soil composition (e.g., aluminosilicate composition, etc.) or environmentally imposed (e.g., soil redox potential). To address the impact of metals such as mercury in a given environment, the ability to discern critical chemical species or groups of chemical species is necessary (Issaro et al. 2009). This need is frequently addressed through the use of selective (singular extraction to provide one chemical species or group) or sequential (series of extractions to elucidate several chemical species or group from the sample) extraction techniques (Issaro et al. 2009). No single proposed sequential extraction procedure is considered optimal for all species of mercury, but all generally partition mercury into the following operationally-defined groups: exchangeable, weak acid extractable, metal oxide bound, organic matter bound, and residual (Issaro et al. 2009). The characteristics of these groups are further discussed below.

Organomercury species, which include all forms of mercury bonded to carbon chains and/or functional groups, are as a group more mobile than most inorganic mercury species, but vary widely in toxicodynamics and therefore toxicity (Zareba et al. 2007). Thus, extraction of this group of mercury compounds is important in assessing mobile and biologically-available mercury, but not necessarily direct toxicity. Similarly, mercury species extractable by water are also highly mobile, but are comprised of ionic species and complexes such as $\text{Hg}(\text{OH})_2$, HgOHCl , and HgCl_2 . The complexes formed by mercury with clay surfaces, oxides, and oxyhydroxides are generally considered strong, but under reduced soil conditions these complexes release constituent mercury (Boszke and Astel 2007; Issaro et al. 2009). The weak

acid extractable fraction has been demonstrated to be predictive of the uptake of mercury by crop plants from spiked soils (Jing et al. 2008). The bonds formed by mercury with humic materials are of such strength that they are considered semi-mobile and are generally less toxic than organomercury and water-extractable species (Boszke and Astel 2007). In particular, mercury has an extreme attraction to reduced sulfur functional groups such as thiols (Boszke and Astel 2007), with the resulting bonds of sufficient strength to be considered biologically unavailable (Barnett and Turner 2001). The affinity of mercury for reduced sulfides is of such a magnitude that these mercury sulfide compounds are thought to be bound in a permanent, biologically unavailable fashion under strongly reduced soil and sediment conditions.

The intent of this research was to elucidate the fate of elevated aqueous mercury in surficial water in wetland mesocosms with conditions representative of those typical of oligohaline wetlands in southeastern Louisiana. Although no a-priori salinity-based hypotheses were formulated, two salinity levels were included to broaden applicability of results. Specifically, the operationally defined soil fractions to which additional aqueous mercury was partitioned after two months were investigated. Further, the uptake of mercury by vegetation and the resulting impacts on plant physiological status as indicated by specific stress responses were determined. The following hypotheses were tested: 1) concentrations of mercury in contaminated surficial water will be reduced to near ambient levels as a function of mercury transfer into surficial soil, 2) aboveground plant tissue will not demonstrate elevated mercury concentrations regardless of mercury loading rate, 3) no plant species will demonstrate any reduction of photosynthetic integrity, as indicated by net CO₂ assimilation and chlorophyll fluorescence at any mercury loading rate.

Materials and Methods

Experimental Approach

The experimental design for this study was a 3 plant species (*Pontedaria cordata*; *Sagittaria latifolia*; *Polygonum punctatum*) x 3 mercury surficial water concentrations (ambient; 2.5 µg/ml; 5.0 µg/ml) x 2 salinity levels (0 ppt; 3 ppt) completely randomized design with three replicates, yielding 54 total experimental units. Sediment was collected from a local freshwater bayou in Henderson, Louisiana, transported to the Center for Ecology and Environmental Technology at the University of Louisiana at Lafayette and placed in acid-washed 10-gallon aquaria to a depth of 20 cm. Salinity levels were achieved using synthetic sea salt (Coralife) and by mixing soils and surface waters to ensure uniformity of salinity levels. *Pontedaria cordata*, *Sagittaria lancifolia*, and *Polygonum punctatum* were collected as individual plants from a common fresh water marsh at the northern portion of the Joyce WMA and planted into the aquaria. A static flooding level was maintained at 5 cm above the soil surface for all experimental units for the duration of the study using tap water. A one-week acclimation period after planting was allowed prior to the initiation of surficial mercury treatments. The 2.5 and 5.0 µg/ml mercury level treatments were implemented by adding an aqueous solution of mercuric chloride (Sigma Corp.) directly to mesocosm flood waters after the one-week acclimation, thus simulating a mercury-contamination event. The duration of the experiment was two months, with all soil and plant characterizations occurring at the conclusion of the study.

Soil Physico-Chemical Characterization

Soil moisture content was determined by drying surficial soil samples at 65°C until a constant weight was achieved and expressed on a dry weight basis (Soil and Plant Analysis Council 1999). Soil organic matter content was determined by heating dry samples in crucibles

at 500°C for 5 hours (L.O.I. method) and expressed as percentage of weight lost (Soil and Plant Analysis Council 1999). Surficial (1-cm depth) soil redox potential was determined using a single platinum soil redox electrode and a calomel reference electrode per experimental unit as described in Patrick et al. (1996). Surficial (1-cm depth) soil pH was determined using a combination pH electrode.

Photosynthetic Characterization

Net CO₂ assimilation was determined on two representative leaves from each plant, using the first or second fully expanded leaf, using a LI-6400XT photosystem (Li-cor, Lincoln, NE), with leaf chamber settings of 1,500 μmol m⁻² s⁻¹ PAR and 370 ppm CO₂. Dark-adapted chlorophyll fluorescence was also determined using a LI-6400XT photosystem, but with the fluorescence leaf chamber (see Schreiber et al. 1998; Baker 2008 for description of technique and caveats) on fully-expanded leaves that were dark-adapted for a minimum of 4 hours. The LI-6400F autoprogram for chlorophyll fluorescence was employed for this analysis.

Mercury Determination

Above- and below-ground plant biomass collected for total mercury analysis was rinsed with de-ionized water and briefly air dried prior to being refrigerated at 4°C until analysis. A second portion of plant material was collected, dried at 65 °C, and used to express mercury concentrations on a dry weight basis. Vegetative samples were homogenized with acid-clean stainless steel knives and digested in Teflon bombs using trace metal grade nitric acid as described in the appendix to EPA Method 1631 (digestion II; APHA 2005). Surficial soil material (~1 cm) was collected into clean polyethylene bags and refrigerated until extracted following the method of Boszke and Astel (2007) with minor modifications. Briefly, this is a sequential extraction method that results in the following five operationally defined fractions of

soil mercury, namely organomercury species (F1; e.g., methyl-, ethyl- and phenylmercury), water-soluble mercury species (F2; e.g., HgCl_2), weak acid-extractable mercury species (F3; e.g., oxide and oxyhydroxide associated Hg), humic-bound mercury species (F4; humic matter-associated Hg), and residual mercury species (F5; e.g., HgS). The sequential extraction protocol was as follows. Five grams of soil was placed in acid-cleaned 40-ml borosilicate glass vials with Teflon closures with 30 ml of chloroform and agitated for three hours with a benchtop lab shaker. Thereafter, samples were allowed to settle for one hour and the supernatant poured into an additional vial. This supernatant was then additionally extracted with 10 ml of 0.01 M sodium thiosulfate for three minutes then oxidized with bromine chloride prior to analysis as the organomercury fraction (F1). To the extracted soil sample, 30 ml of ultrapure water was added, the sample was agitated as before for three hours, allowed to settle for one hour, and the supernatant poured into a fresh vial. This supernatant was then oxidized with bromine chloride prior to analysis as the water-extractable mercury fraction (F2). To the extracted soil sample was then added 30 ml of 0.5 M trace-metal grade hydrochloric acid, the sample was agitated as before for three hours, allowed to settle for one hour, and the supernatant poured into a fresh vial. This supernatant was then oxidized with bromine chloride prior to analysis as the weak acid-extractable mercury fraction (F3). To the extracted soil sample was then added 30 ml of 0.2 M sodium hydroxide, the sample was agitated as before for three hours, allowed to settle for one hour, and the supernatant poured into a fresh vial. This supernatant was then oxidized with bromine chloride prior to analysis as the humic-associated mercury fraction (F4). To the extracted soil sample was then added 30 ml of aqua regia, the sample was agitated as before for three hours, allowed to settle for one hour, and the supernatant poured into a fresh vial. This supernatant was then oxidized with bromine chloride prior to analysis as the residual mercury

fraction (F5). A second soil sample was collected for all experimental units, dried at 65 °C, and used to express mercury concentrations on a dry weight basis.

Statistical Analysis

Mesocosm data were analyzed as a completely randomized design using the appropriate general linear model procedures of PC-SAS 9.1 (SAS 2004). An *a priori* structural equation model anticipated to represent a latent “phytoavailable” mercury fraction was built using all soil mercury fractions, soil pH, redox potential, and organic matter, as well as below-and above-ground plant tissue mercury concentration and analyzed using PROC CALIS (SAS 2004; see Hair 1998 for discussion of technique). However, the only significant relationship found was between weak acid-soluble soil mercury (fraction 3) and belowground tissue mercury concentration. Therefore, simple linear regressions generated using the regression procedures of PC-SAS 9.1 (SAS 2004) are presented.

Results

As expected, total soil mercury concentrations were significantly greater in treatments receiving elevated mercury loads than the control mesocosms (Fig. 4.1 top panel; $F=14.39$, $P=0.006$). Interestingly, final total surficial mercury tended to be higher in experimental units containing *Polygonum punctatum* than those containing *Pontedaria cordata* and *Sagittaria lancifolia*, although this difference was not significant. The proportion of total mercury occurring as organic species (F1) was consistently low and did not significantly vary by plant species, mercury treatment, or salinity level (Fig. 4.1 bottom panel). Similarly, the proportion of total mercury occurring as water-soluble species (F2) did not differ among treatments, but tended to be higher in the control treatment (Fig. 4.1 bottom panel). No effect of plant species or salinity level or the interaction thereof was detected in regard to the proportion of mercury

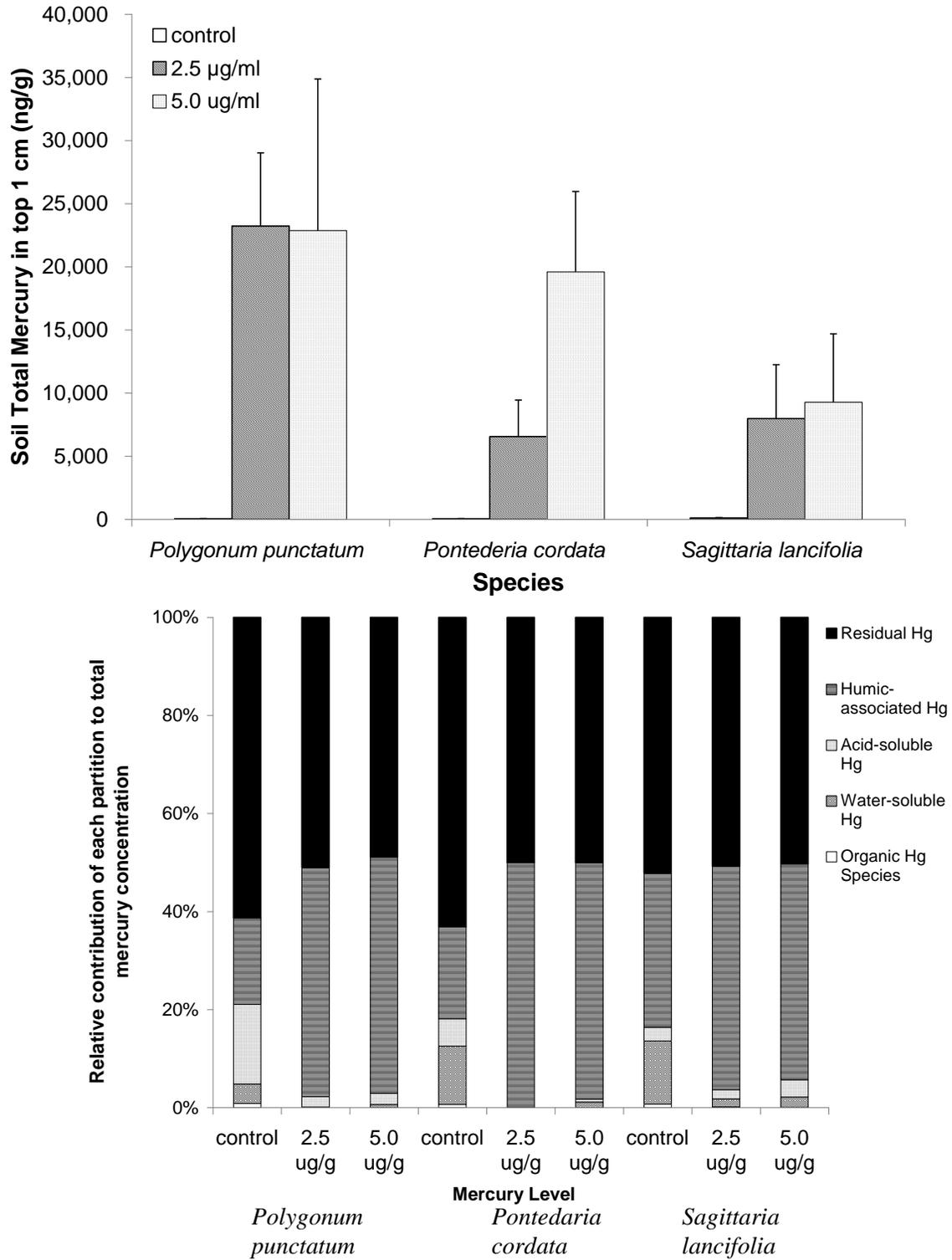


Fig. 4.1. The effect of species and mercury level on surficial soil total mercury (top panel; mean +/- 1 s.e.) and percentage of total mercury in surficial soil partitions (bottom panel), averaged across salinity level.

occurring as water soluble species (Fig. 4.1 bottom panel). Similarly, the proportion of total mercury occurring as weak-acid soluble species (F3) was significantly higher in the control treatment than the two elevated mercury treatments (Fig. 4.1 bottom panel; $F= 2.75$, $P= 0.0204$). Conversely, the proportion of total mercury occurring as humic-bound species (F4) was significantly greater in elevated mercury treatments than control treatments (Fig. 4.1 bottom panel). The proportion of mercury occurring as residual mercury (F5) was not significantly different regarding plant species, mercury treatment, or salinity level (Fig. 4.1 bottom panel).

Marginally significantly greater concentrations of total mercury were found in the aboveground tissues of *Polygonum punctatum* than *Pontedaria cordata* and *Sagittaria lancifolia* (Fig. 4.2 top panel; $F= 3.22$, $P= 0.0806$). Although not significant, a trend towards elevated total mercury in aboveground tissues in *Sagittaria lancifolia* was observed under elevated mercury loadings compared with ambient loads, whereas total mercury in aboveground tissues were not elevated in *Polygonum punctatum* under any mercury treatment (Fig. 4.2 top panel).

All plant species in the 5.0 $\mu\text{g/ml}$ mercury treatment demonstrated significantly higher concentrations of total mercury in belowground plant tissue than the control treatment (Fig. 4.2 bottom panel; $F= 6.26$, $P= 0.0176$). No significant difference was detected between plant species in regard to total mercury in belowground tissues (Fig. 4.2 bottom panel). Salinity level did not significantly influence total mercury concentration in either above- or below-ground tissues (Fig. 4.2 bottom panel). Soil moisture was not significantly different by plant species, mercury treatment, or salinity level (Fig. 4.3 top panel). Similarly, soil organic matter was not significantly affected by plant species, mercury treatment, or salinity level (Fig. 4.3 bottom panel). No significant effect of plant species, mercury concentration, or salinity level was detected in regard to net CO_2 assimilation or chlorophyll fluorescence (Fig. 4.4).

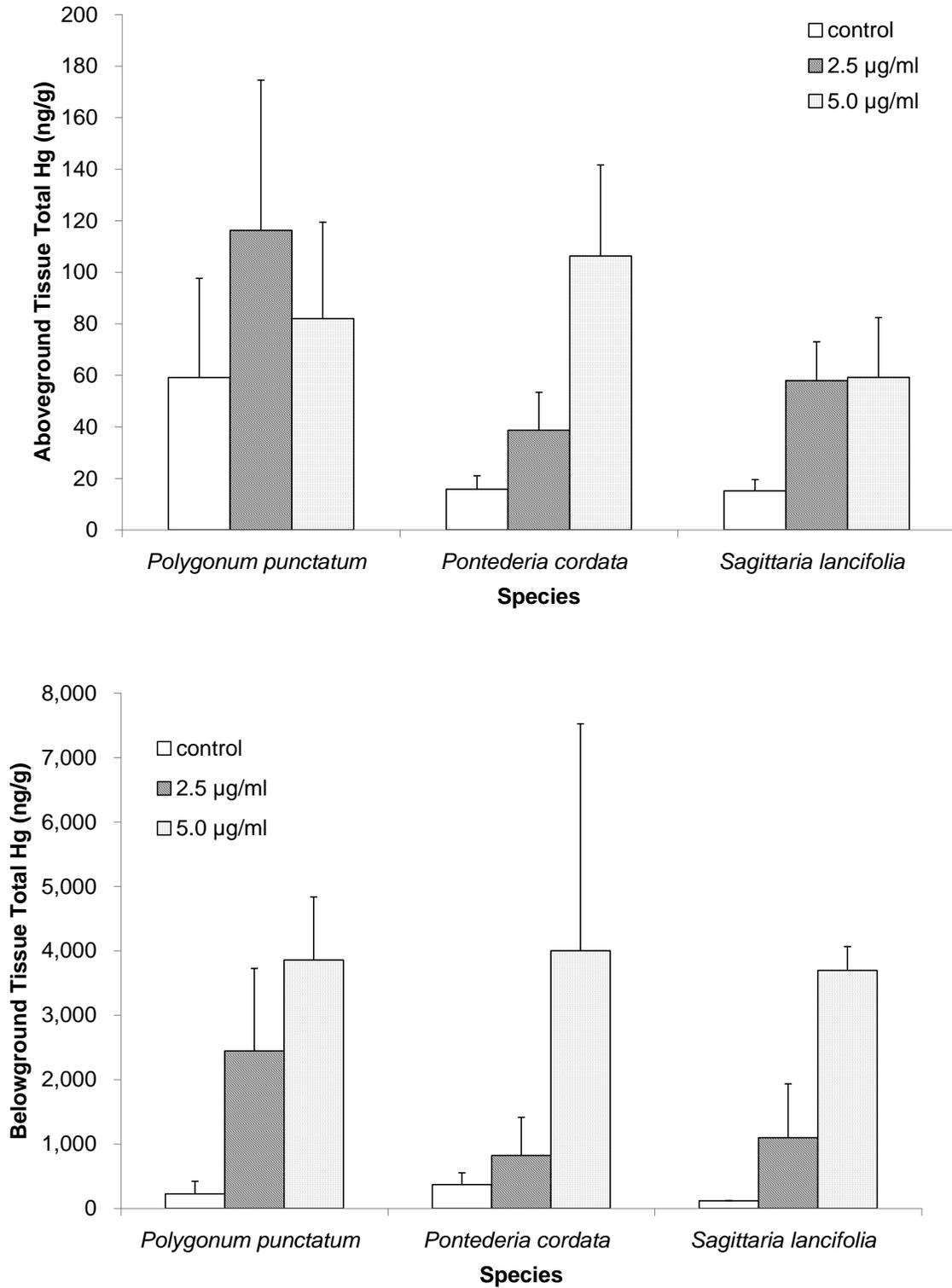


Fig. 4.2. The effect of species and mercury level on aboveground tissue total mercury (top panel) and belowground tissues total mercury (bottom panel), averaged across salinity level (mean +/- 1 s.e.).

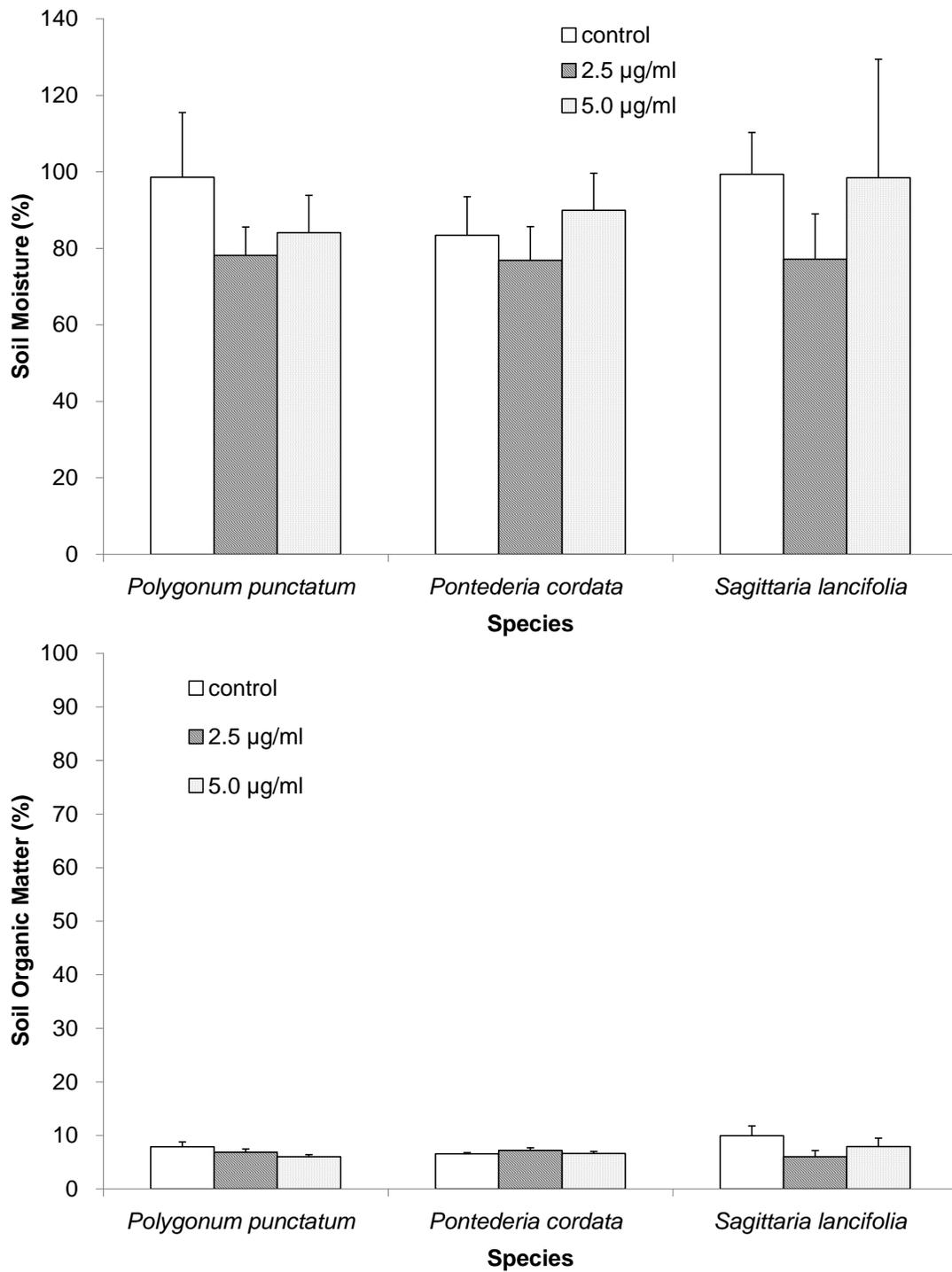


Fig. 4.3. The effect of species and mercury level on soil moisture content (top panel) and soil organic matter content (bottom panel), averaged across salinity level (mean \pm 1 s.e.).

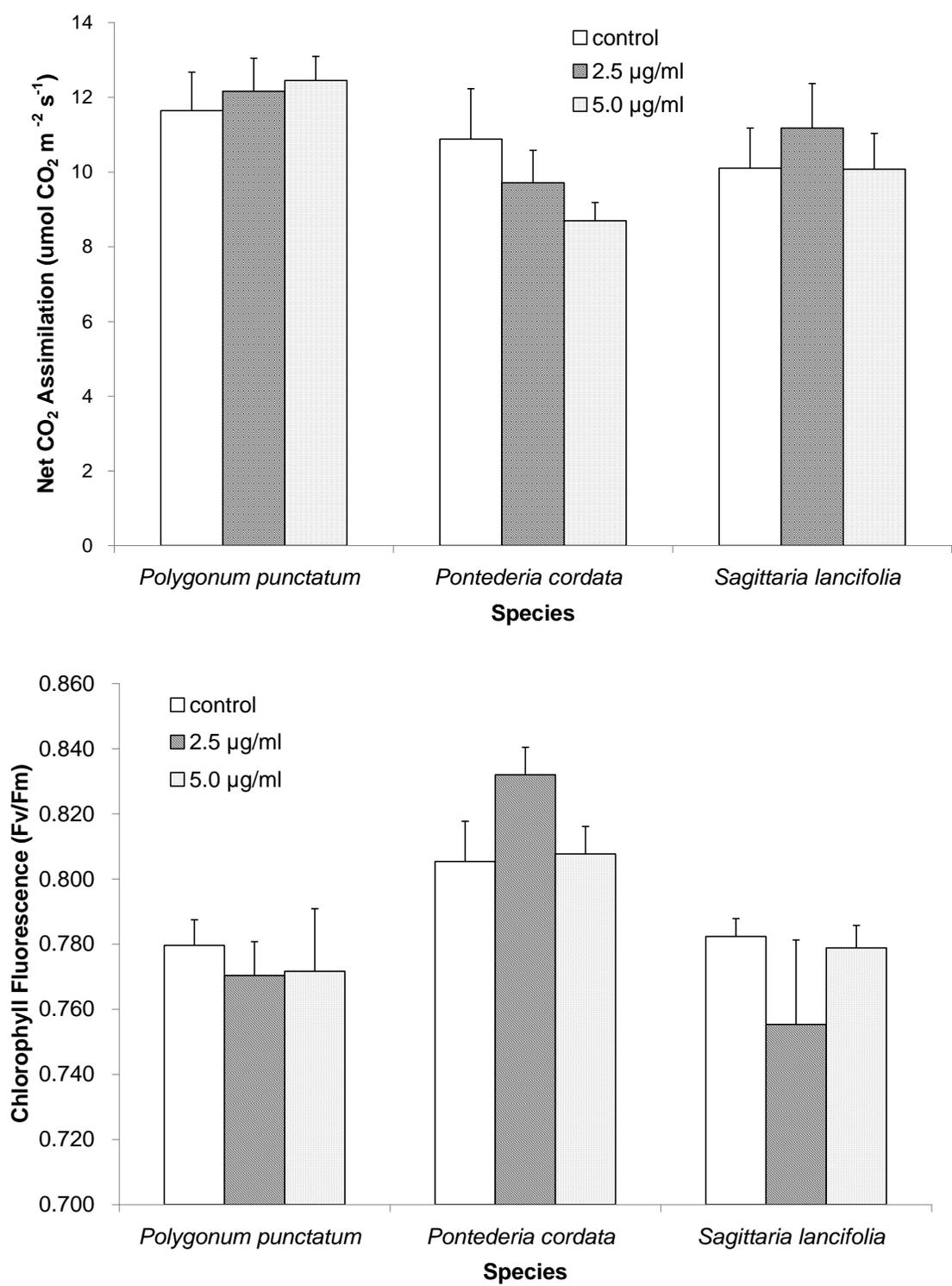


Fig. 4.4. The effect of species and mercury level on net CO₂ assimilation (top panel) and chlorophyll fluorescence (bottom panel), averaged across salinity level (mean +/- 1 s.e.).

Surficial soil redox potential was not significantly affected by plant species, mercury treatment or salinity level. Experimental vessels containing *Pontedaria cordata* and *Sagittaria lancifolia* were significantly more acidic than experimental vessels containing *Polygonum punctatum* (Fig. 4.5 bottom panel; $F= 7.78$, $P=0.0016$). However, average pH values for all treatments ranged from ~6.5 to 7.0, suggesting relatively little impact on most biological processes.

Of the five soil mercury fractions extracted, only the weak-acid extractable fraction (F3) demonstrated a significant relationship with total mercury concentration in belowground plant tissue (Fig. 4.6; F1: $P= 0.897$, $R^2= 0.001$; F2: $P= 0.309$, $R^2= 0.038$; F3: $P<0.001$ $R^2= 0.591$; F4: $P= 0.155$, $R^2= 0.094$; F5: $P= 0.112$, $R^2= 0.114$). A significant relationship was detected between total soil mercury and belowground tissue total mercury (Fig. 4.6; $P=0.0039$, $R^2= 0.215$), but with a much lower degree of explanatory power than the weak-acid extractable (F3) soil fraction. Further, as has generally been reported in similar studies, little relationship was observed between belowground tissue total mercury and aboveground tissue total mercury (Fig. 4.6; $P=0.5674$, $R^2= 0.012$).

Discussion

Although substantial reductions (>98%) in surficial water mercury concentrations were noted for both elevated mercury treatments after two months, these concentrations (means of 11.7 and 55.4 $\mu\text{g L}^{-1}$ for the 2.5 and 5.0 mg L^{-1} treatments, respectively) were still well above levels that would be considered typical for southeastern U.S. wetlands (1.62 ng L^{-1} ; Hall et al. 2008). Further, these final aqueous concentrations exceeded the NOAA screening levels for mercury in recreational use waters (1.4 $\mu\text{g L}^{-1}$). It should be noted however, that these results are typical of many experimental phytoremediation studies using similar initial concentrations of mercury in surface waters. For example, Rai and Tripathi (2009) exposed *Azolla pinnata* and

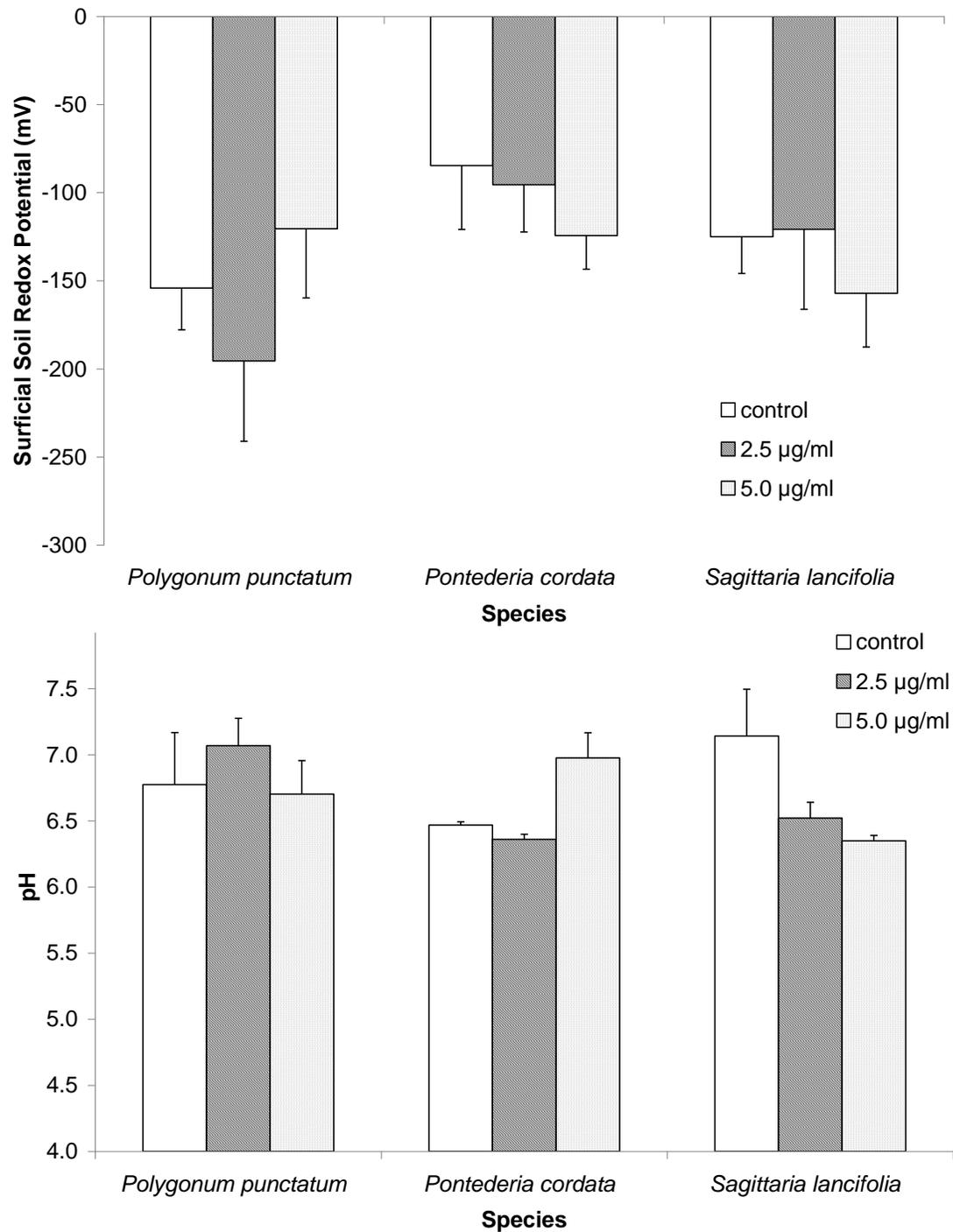


Fig. 4.5. The effect of species and mercury level on surficial soil redox potential (top panel) and surficial soil pH (bottom panel), averages across salinity level (mean +/- 1 s.e.).

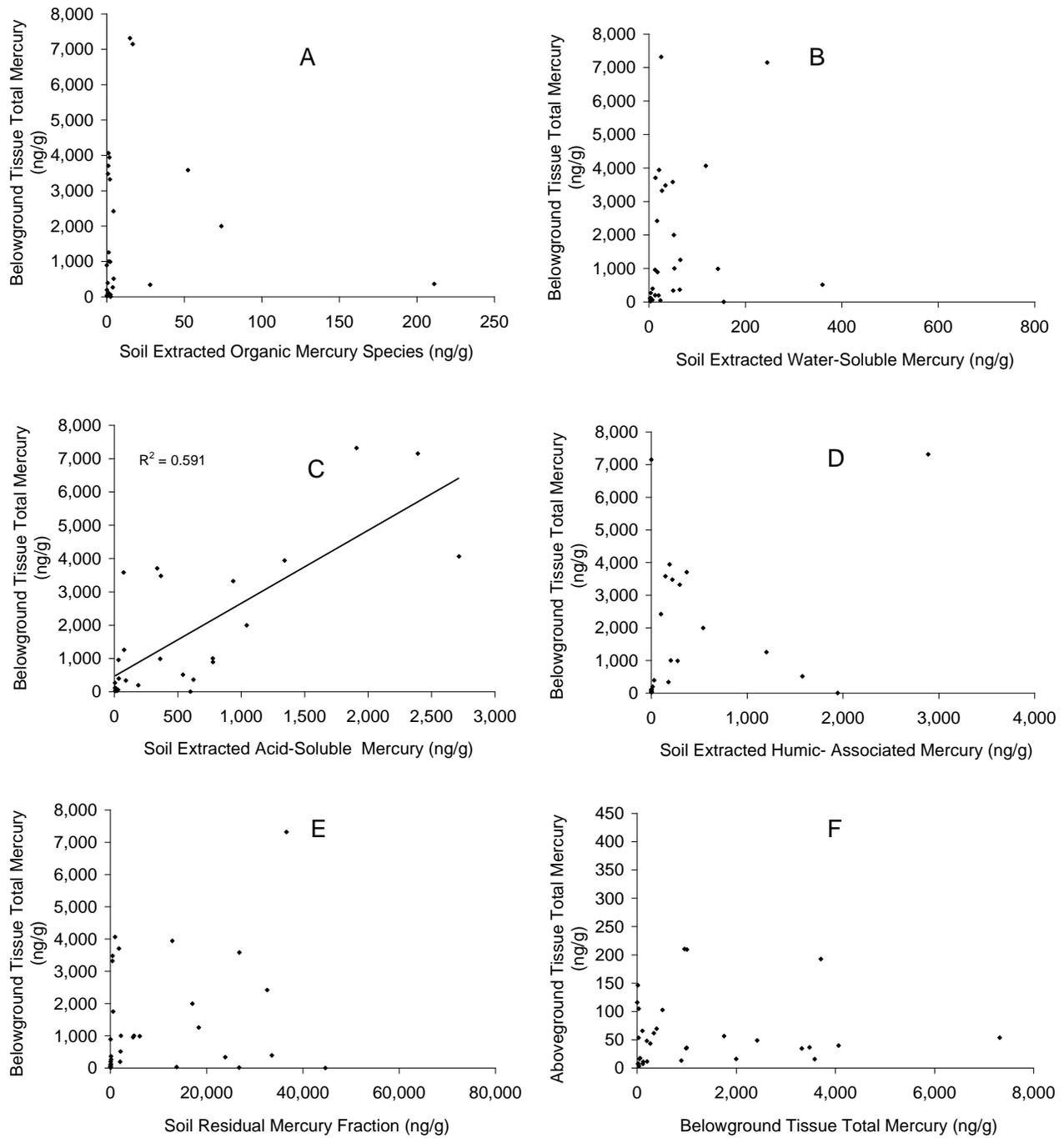


Fig. 4.6. The effect of each soil mercury fraction on belowground plant tissue total mercury concentration. Panel A= F1 (organic mercury); Panel B= F2 (water-soluble mercury); Panel C= F3 (weak-acid extractable mercury); Panel D= F4 (humic-associated mercury); Panel E=F5 (residual total mercury). The effect of belowground total mercury on aboveground total mercury (Panel F).

Vallisneria spiralis to aqueous mercuric chloride levels of 0.1, 0.5, 1.0 and 3.0 mg L⁻¹ in a 6-day aquarium study and demonstrated reduction of aqueous mercury by up to 86%, although final average aqueous mercury concentrations ranged up to 0.6 mg L⁻¹. The typical aqueous species of mercury occurring under oligohaline conditions are generally considered to be sparingly soluble in water (Morel et al. 1998). In this study it is important to note that the majority of mercury in the surficial soil layer appeared to be bound in the minimally available humic-bound and residual soil partitions. Further, mercury translocation from belowground to aboveground tissues was minimal regardless of plant species or mercury loading rate.

As one would expect, total surficial soil concentrations were highly elevated in both mercury addition treatments, whereas soils in control treatments were noted to have total soil mercury levels typical of wetland soils and aquatic sediments in the region (see Delaune et al. 2008; Hall et al. 2008; Yu et al. 2008). Results of the sequential extraction analyses indicate that relatively little of the total mercury in the soil from elevated treatments (<18%) occurred as organomercury species after the two-month study duration and even lower organomercury species portions were detected in control units (< 4%). Boszke and Astel (2007) reported the organic mercury fraction to comprise 11.9% on average of the total sediment mercury in a post-2004 Tsunami sampling effort in Thailand. Of great importance is that no significant increase of organic mercury fraction was detected in the elevated salinity treatment. Given the well documented stimulation of mercury methylation in soils and sediments receiving low to moderate levels of sulfate (Compeau and Bartha 1985; Gilmour et al. 1992), it is encouraging that no increase in organomercury content was found in these experimental scenarios.

As with many metals, water-extractable mercury is generally regarded as biologically available. Interestingly, water-extractable mercury composition was higher in control units (8%)

than either of the elevated mercury units (3% and 4% for the 2.5 µg/ml and 5.0 µg/ml treatments, respectively). Thus, water-extractable mercury proportions in control units for this study are higher than those found by Boszke and Astel (2007), who reported an average percentage of 2.4%, where as the proportions for the elevated mercury treatments are relatively close. A greenhouse study by Han et al. (2006) examined the phytoavailability of mercury to *Pteris mayii* (Chinese brake fern) in spiked sediments using a different sequential extraction procedure than that used in this study. However, the procedure used by Han et al. (2006) did include a similar extraction for water-soluble mercury, with their results indicating a lower overall proportion of water-extractable mercury for 100 mg/kg and control soils (0.05% and below detection respectively) than in this current study.

Weak acid-extractable mercury represents mercury bound to the surface of minerals and organic matter as well as those complexed by the hydroxides and oxyhydroxides of iron and manganese (Boszke and Astel 2007). This was unexpected as soil redox potential measurements indicated that anoxic soil conditions were ubiquitous in all experimental vessels by the conclusion of the study and iron hydroxides and oxyhydroxides are highly soluble under such reduced conditions (Reddy and Delaune 2008). Under these anoxic soil conditions, iron and manganese oxides and oxyhydroxides should dissolve and release any associated mercury (Boszke and Astel 2007; Issaro et al. 2009). Thus, the relatively high values of this fraction through the experimental units may primarily represent mercury that was bound to the surface of minerals and organic matter. Extraction of soil with hydrochloric acid of this strength has been shown to be an effective indicator of soil mercury available to crop plants (rice and radish) in a typical agricultural soil (Jing et al. 2008). Overall, small proportions of the total soil mercury in this study were found to occur in this fraction (4%), with little difference among treatments.

However, the proportions in both control and elevated treatments for this study are still slightly greater than those reported by Boszke and Astel (2007).

Of great interest is that mercury concentrations in aboveground plant tissues in elevated mercury treatments were not increased above the background levels detected in control plant tissues. This suggests relatively little translocation of mercury from below- to above-ground plant tissues, as has been found in many mercury-oriented plant uptake studies (Moreno et al. 2008). Further, this suggests that little of the added mercury in the elevated treatments volatilized into the immediate atmosphere of the plants where it could be absorbed into the aboveground plant tissue through stomatal exchange. This highlights the importance of chemical form in the phytoaccumulation processes, as many studies evaluating the uptake of atmospheric mercury, which is largely elemental in composition, have found higher statistical correlations of aboveground tissue mercury concentrations with atmospheric mercury concentrations than with belowground tissue or soil concentrations (Rea et al. 2002; Ericksen et al. 2003). In this study mercury was added as mercuric chloride, an ionic compound with relatively high water solubility and reactivity, as this was thought to better represent a significant contamination event. The limited volatilization indicated by the minimal concentrations of mercury in aboveground plant tissues in elevated mercury treatments suggests that the added mercury was not reduced to an elemental form, but rather was maintained in a charged reactive state. An additional indicator of this is revealed by the sequential extraction procedure, as relatively large proportions of the total mercury occurred in fractions that form strong, stable bonds, such as the humic-bound and residual fractions.

In conclusion, this research suggests that a mercury contamination event of the magnitude simulated would generate a range of impacts. Surficial soil mercury concentrations

were elevated in 2.5 and 5.0 µg/ml mercury treatments and substantial bioaccumulation into belowground plant tissues occurred. Importantly, little translocation of mercury into aboveground tissue or damage to photosynthetic processes was discerned. As previously noted, surficial water concentrations in elevated mercury treatments were substantially reduced from initial levels, but still highly elevated compared to ambient levels and exceeded NOAA guidelines for recreational water. The proportions of soil total mercury occurring in biologically-available fractions were less than those occurring in the biologically-unavailable fraction, suggesting that much of the added mercury became assimilated into unavailable forms relatively quickly (i.e., within two months). These findings suggest that no substantial impact to plant health will occur within two months of exposure to the mercury levels and environmental conditions of this study. It should be emphasized that this experimental design does not incorporate several key aspects of actual wetland systems, such as bioturbation from fauna or fluctuating water levels that could rapidly alter soil redox status. Also, organic matter content in these soils is lower than that frequently found in many oligohaline wetlands, which could substantially affect mercury partitioning and availability.

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CHAPTER V

THE EFFECTS OF ELEVATED SURFACE WATER NUTRIENTS ON THE METHYLATION OF MERCURY IN A TIDAL FRESHWATER WETLAND SOIL

Introduction

Alteration of the chemical composition of waters entering wetlands are a frequent impact to these systems, and many other aquatic habitats globally (Beeton 2002; Brinson and Malvarez 2002; Kennish 2002; Scavia and Bricker 2006; Corstanje et al. 2007; Nekola et al. 2008). These chemicals include xenobiotic substances, such as pesticides and pharmaceuticals (Kuster et al. 2008), and additions of nutrients and ions (Beeton 2002; Brinson and Malvarez 2002). These alterations of surface waters have pronounced effects on the biogeochemical processes occurring in adjacent soils and sediments (Wright et al. 2008; Wright et al. 2009).

The nutritive limitations of microbial populations in wetland systems have been studied extensively, often with varying results, suggesting that these limitations are frequently site specific (Bowen et al. 2009). For example, nitrogen has been found to be a limiting nutrient for microbial populations in a number of coastal wetland systems, including Atlantic Coast *Spartina patens* marshes (Caffrey et al. 2007) and Gulf Coast *Taxodium distichum* swamps (Jackson and Vallaire 2009). However, phosphorus has also been implicated as the limiting nutrient for microbial populations in Atlantic Coast *Spartina alterniflora* marsh (Sundareshwar et al. 2003) as well as the Florida Everglades (Drake et al. 1996). Microbial cells are regarded as requiring comparatively large amounts of nitrogen because this essential element generally accounts for approximately 10% of the dry weight of bacterial cells (Gottschalk 1986). Virtually all bacteria are capable of directly assimilating ammonia and incorporating it into metabolic activities, with fewer bacteria able to directly assimilate nitrate, which must then be reduced to ammonia in the cell prior to anabolic metabolism (Gottschalk 1986). Phosphorus is required for cellular

components such as nucleic acids, phospholipids, and nucleotides among others (Gottschalk 1986). Although some bacteria are known to assimilate organophosphate compounds, most phosphorus uptake is thought to occur as phosphate, often released via extracellular enzymes (Wanner 1996).

Wetlands represent exceptionally interesting systems to study mercury transformations because of the myriad chemical reactions that occur in these systems. In particular, many wetlands soils become sufficiently reduced for the thermodynamically-favorable microbial metabolic pathway to be sulfate reduction (Mitsch and Gosselink 2000), which is often the major route through which mercury becomes methylated in natural systems (Compeau and Bartha 1985; Gilmour et al. 1992). Studies by Branfireun et al. (1996), Harmon et al. (2004), and Jeremiason et al. (2006) have demonstrated that the addition of sulfate to wetland soils tends to increase the methyl mercury concentration of these soils. Because of the energetics of microbial metabolism, it could be anticipated that the addition of alternative electron acceptors that allow for more efficient metabolism may limit sulfate reduction, and thereby mercury methylation, in these soils (see Warner et al. 2003). However, a study by Jackson (1989) found inconsistent results when oxidized iron was added to sediments and methyl mercury concentrations were thereafter evaluated. In contrast, Steffan et al. (1988) found that the addition of nitrate in one of their treatments did reduce mercury methylation. Warner et al. (2003) found that mercury methylation was inhibited under iron-reducing conditions, but appeared stimulated under sulfate-reducing conditions. Mehrotra and Sedlak (2005) determined that addition of reduced iron resulted in a reduction of dissolved methyl mercury, likely due to reduced bioavailability (i.e., non-polar) of dissolved mercury-sulfide compounds. Although it is well established that mercury-sulfide compounds are generally highly insoluble, within a narrow range of sulfide

concentrations and under non-saline conditions, soluble, non-polar mercury polysulfide compounds are formed that are thought to be a major form available to bacteria (Benoit et al. 1999). Despite the lack of clear evidence, it is apparent that shifting concentrations of more energetically favorable alternative electron acceptors could alter local mercury / methyl-mercury cycling.

Many natural wetlands are currently being employed as tertiary wastewater treatment systems (Jeng and Hong 2005), resulting in a substantial change in the balance of many compounds, including oxidized and reduced sulfur and nitrogen compounds, as well as labile carbon concentrations. Changes in the input of these compounds can modify system biogeochemistry both in terms of availability of electron acceptors (e.g., Steffan et al. 1988; Harmon et al. 2004) and rates of microbial metabolism (e.g., Feng and Hsieh 1998; Lloyd et al. 2004; Wiessner et al. 2005). As stated above, shifting the balance of chemical concentrations may substantially alter the mercury cycling in these systems with potentially serious effects to the local environment. Although many proposed large-scale wetland restoration methodologies, (e.g., diversions of river water, dedicated dredge spoil placement) result in the addition of inorganic nutrients into wetland systems, the time scale over which rehabilitation and land building may occur can be vastly different depending on rates of mineral sediment input. Therefore, the elucidation of the potential main and interactive effects of these constituents (e.g., nitrogen, phosphorus, and salinity) whose concentrations are likely to shift and result in changes to local mercury cycling is one key aspect to the management of natural systems that will be exposed to such perturbations. The research detailed herein seeks to expand current knowledge regarding the alteration of oligohaline wetland microbial communities, particularly those

relevant to mercury methylation, to altered surface water concentrations of nitrogen, phosphorus, and salinity.

Material and Methods

Potential shifts in surficial soil methyl mercury concentrations resulting from the alteration of floodwater constituents were assessed using a 3 nitrogen (NO_3 addition, NH_4 addition, none) x 2 phosphorous (PO_4 addition, none) x 2 salinity (0 ppt, 2.5 ppt) completely randomized factorial design in conjunction with flooded soil cores. Soil, and associated interstitial/flood waters, were collected from the Joyce Wildlife Management area (Joyce WMA) to a depth of 7.5 cm with a 7.5-cm diameter soil corer, placed into acid cleaned 400 ml borosilicate glass beakers and transported back to the laboratory. Upon returning to the lab, surface waters were inspected daily and additions of ultrapure water made as necessary to provide a surface water depth of ~3 cm (i.e., approximately 100 ml of surface water in the experimental vessel). This approach ensured that the soil in the experimental vessels was maintained in its original reduced state and that floodwaters with consistent chemical composition were achieved. Experimental treatments were randomly assigned in a cross-classified fashion and applied as described below. Elevated levels of nitrate, ammonium, and phosphorous were established by the addition of either potassium nitrate, ammonium sulfate, or potassium phosphate solutions as appropriate into the surficial waters of the experimental units to generate concentrations of 50 mg N per liter or 5.0 mg P per liter (Jackson and Valliare 2009). Elevated salinity levels of 2.5 ppt were produced by the addition of InstantOcean scientific salt in solution. Surficial soil redox potential (1-cm depth) was determined daily during the study using a mV meter in conjunction with a calomel reference electrode, and brightened redox electrodes. To reduce variation, one redox probe was placed in each experimental beaker at the beginning of

the study and maintained in this vessel with minimal disturbance for the duration of the study. Surficial soil pH was determined daily with a calibrated pH meter. At the end of the two-week experimental period, pore water was collected using an interstitial soil sipper (McKee et al. 1988) and analyzed for dissolved sulfate, total sulfides, dissolved nitrate-nitrite, dissolved ammonium, dissolved phosphate, and dissolved chemical oxygen demand as described below. Pore-water collection was carefully performed such that a composite sample of the upper 5 cm of pore-water was collected, but surficial water was excluded. At the conclusion of the study, total and methyl mercury were extracted from soils following the procedures outlined in EPA 1631 for total mercury (APHA 2005) and Horvat et al. (1993) for methyl mercury. Methyl and total mercury determinations focused on surficial (i.e., top 1 cm of soil) soil because the majority of mercury methylation is believed to occur in this portion of the soil (Han et al. 2007).

Soil redox potential was determined following the methods described in Patrick et al (1996). Porewater pH and conductivity was determined using EPA-approved methods and hand-held meters. Pore-water nitrate-nitrite, ammonium, phosphate, chemical oxygen demand, and sulfate were determined using EPA approved colorimetric techniques (APHA 2005: EPA methods 4500F, 4500G, 4500F, and 4500E, respectively). Total and methyl mercury were determined using EPA methods 1631 and 1630, respectively, in conjunction with a Brooksrand atomic fluorescence spectrophotometer. Data were analyzed as a completely randomized design using the appropriate general linear model procedures of PC-SAS 9.1 (SAS 2004).

Results

A significant effect of time was detected for surficial soil redox potential (Fig. 5.1; $F=9.249$, $P=0.006$), but no significant interactions of time with other factors were detected for redox potential. Surficial soil pH was significantly affected by time and the interaction of time and

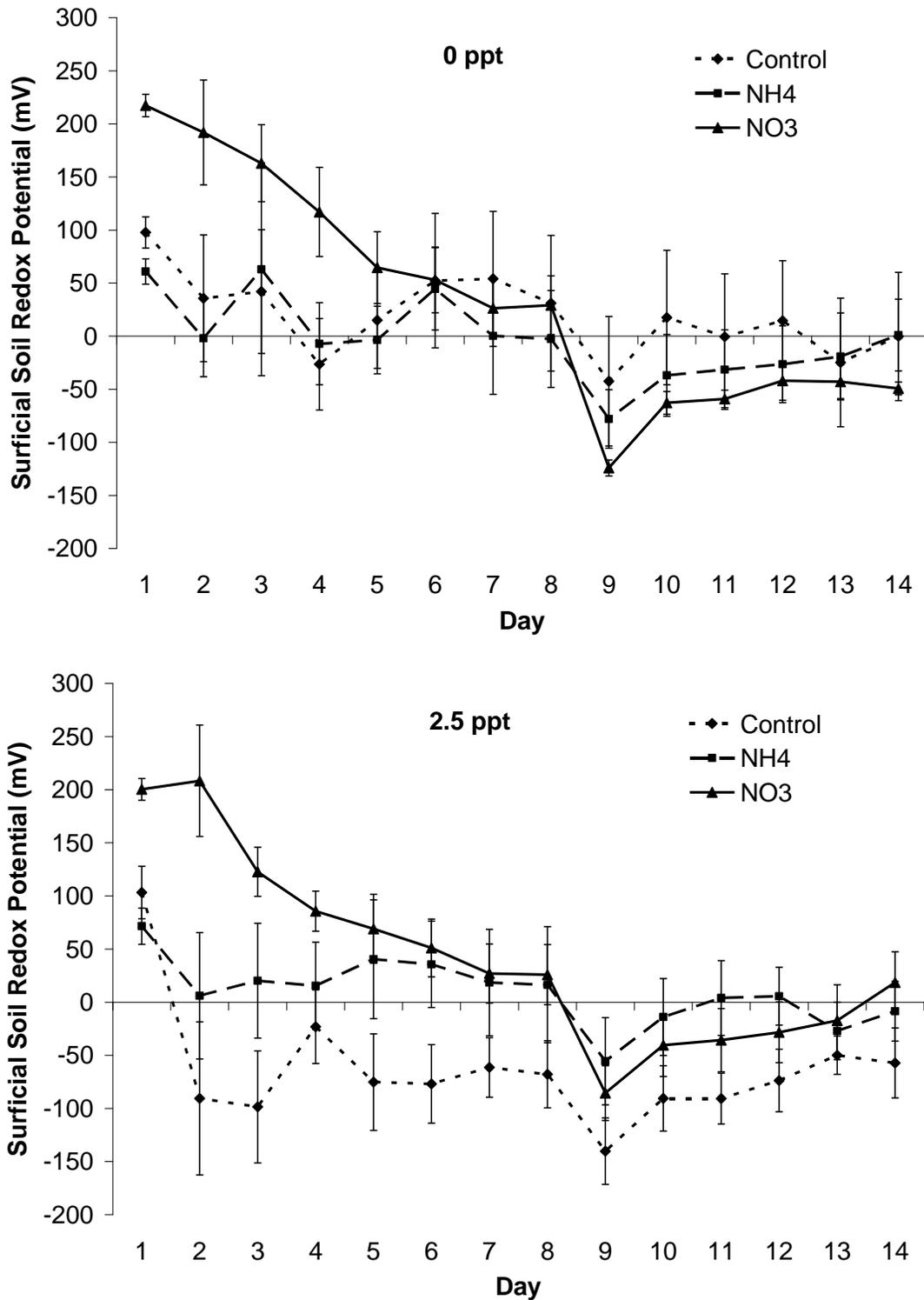


Fig 5.1. The effect of time, nitrogen amendment, and salinity level on surficial soil redox potential, averaged across phosphorus amendment (mean \pm 1 s.e.).

nitrogen (Fig. 5.2; $F= 9.249$, $P=0.050$ and $F= 9.249$, $P=0.050$, respectively). Interestingly, a significant effect of salinity was detected for final pore-water nitrate-nitrite concentrations, with oligohaline salinity level treatments resulting in lower final pore-water nitrate-nitrite concentrations than the fresh salinity treatment (Fig. 5.3; $F= 5.810$, $P=0.024$). As expected, nitrate additions resulted in higher final pore-water nitrate-nitrite concentrations than the ammonium additions or control treatments (Fig. 5.3; $F= 4.994$, $P=0.035$). The final pore-water ammonium concentrations were significantly higher in both nitrate and ammonium amended treatments (Fig. 5.4; $F= 7.368$, $P=0.012$). No significant effects of phosphate amendment, salinity level, or any interactions were detected for final pore-water interface ammonium concentrations.

Interestingly, no significant effects of nitrogen amendment, phosphate amendment, salinity level, or any interactions thereof were detected regarding final pore-water phosphate concentrations. A significant effect of salinity level was detected for final pore-water sulfate concentrations, but unexpectedly not for final pore-water sulfides (Fig. 5.5; top panel; $F=4.475$, $P=0.045$; bottom panel, respectively). No significant effects of nitrogen amendment, phosphate amendment, or any interactions thereof were detected for final pore-water sulfate or pore-water sulfide concentrations. Also, no significant effects of nitrogen amendment, phosphate amendment, or salinity level, or the interaction thereof was detected in regard to final pore-water chemical oxygen demand (5.6). No significant effects of nitrate amendment, phosphate amendment, or salinity level, or the interaction thereof was detected in regard to final soil methyl mercury concentration or final methyl mercury concentrations as a percentage of total mercury (Fig. 5.7 and 5.8, respectively).

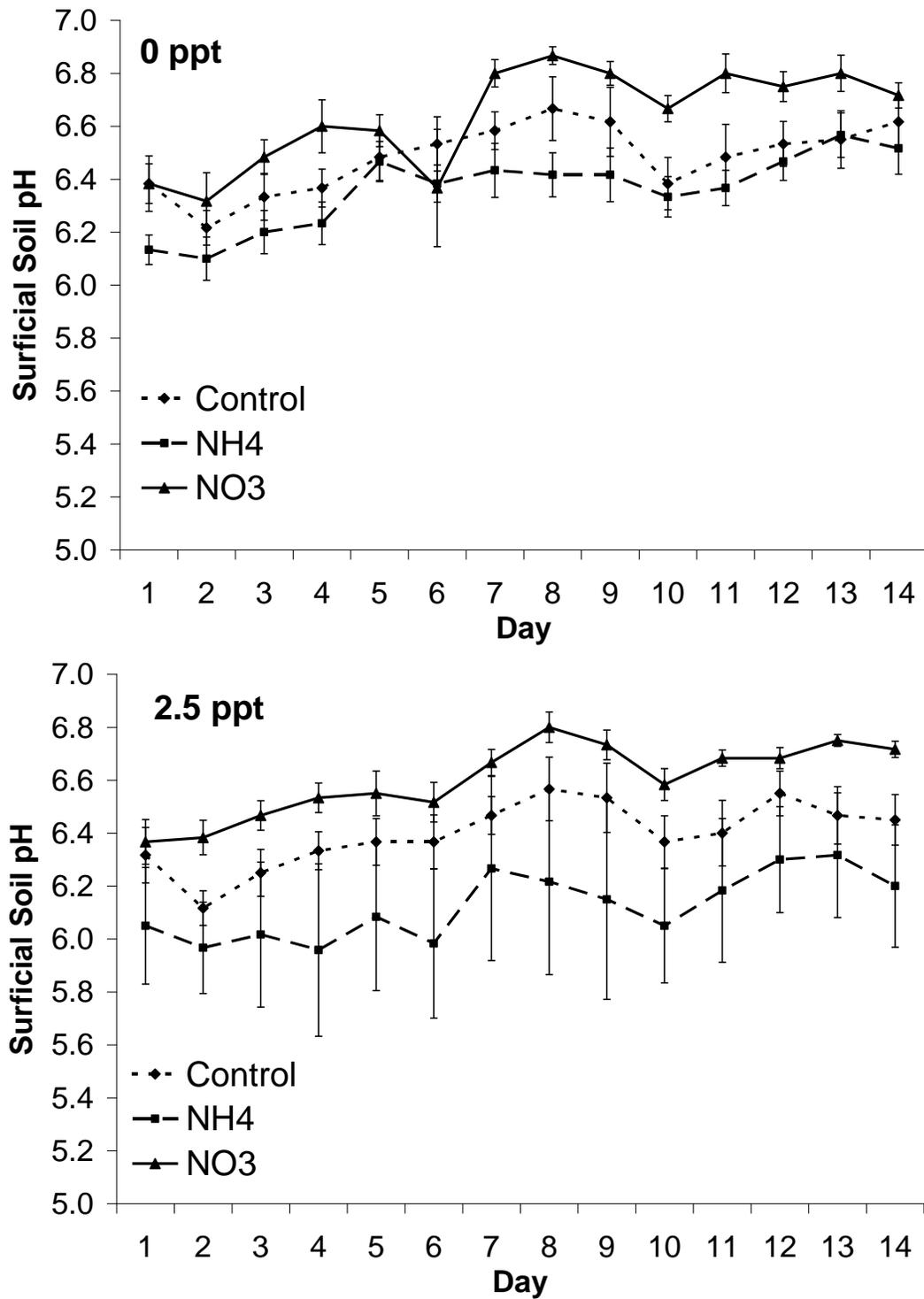


Fig. 5.2. The effect of time, nitrogen amendment, and salinity level on surficial soil pH, averaged across phosphorus amendment (mean \pm 1 s.e.).

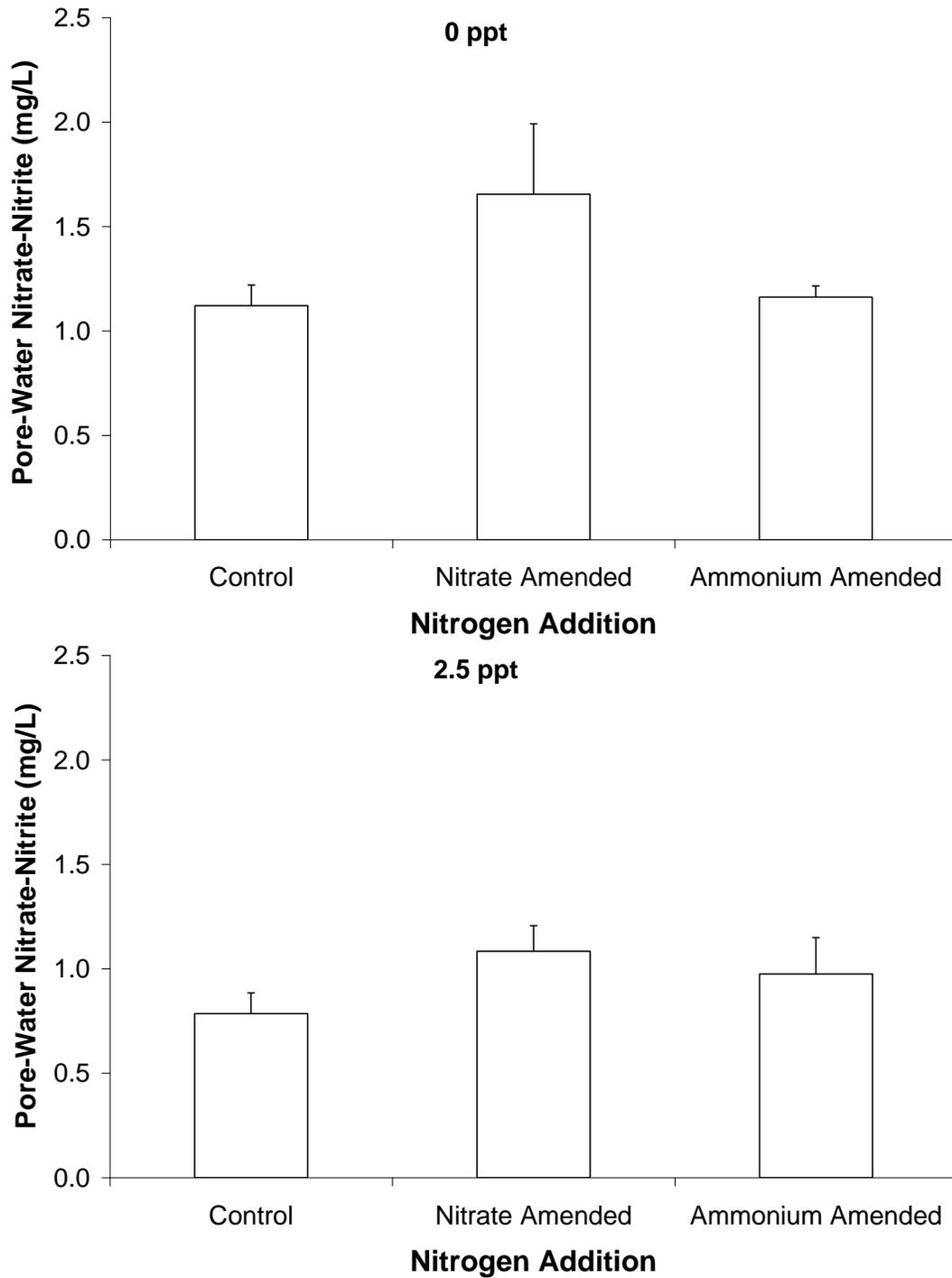


Fig. 5.3. The effect of nitrate amendment and salinity level on pore-water nitrate-nitrite, averaged across phosphorus amendment (mean \pm 1 s.e.).

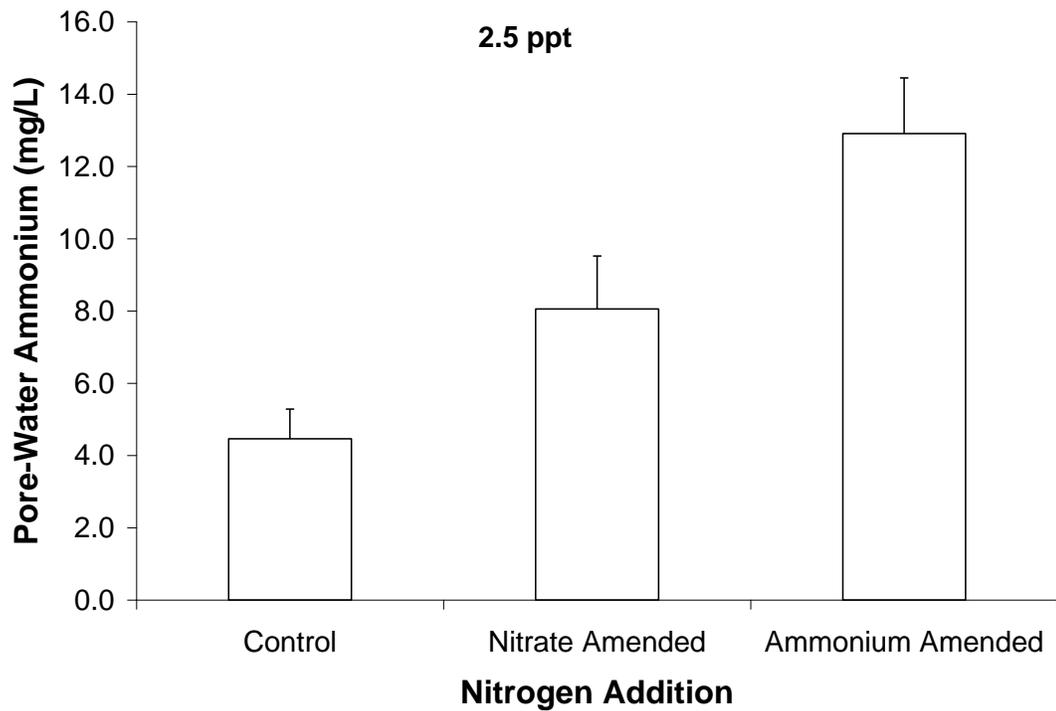
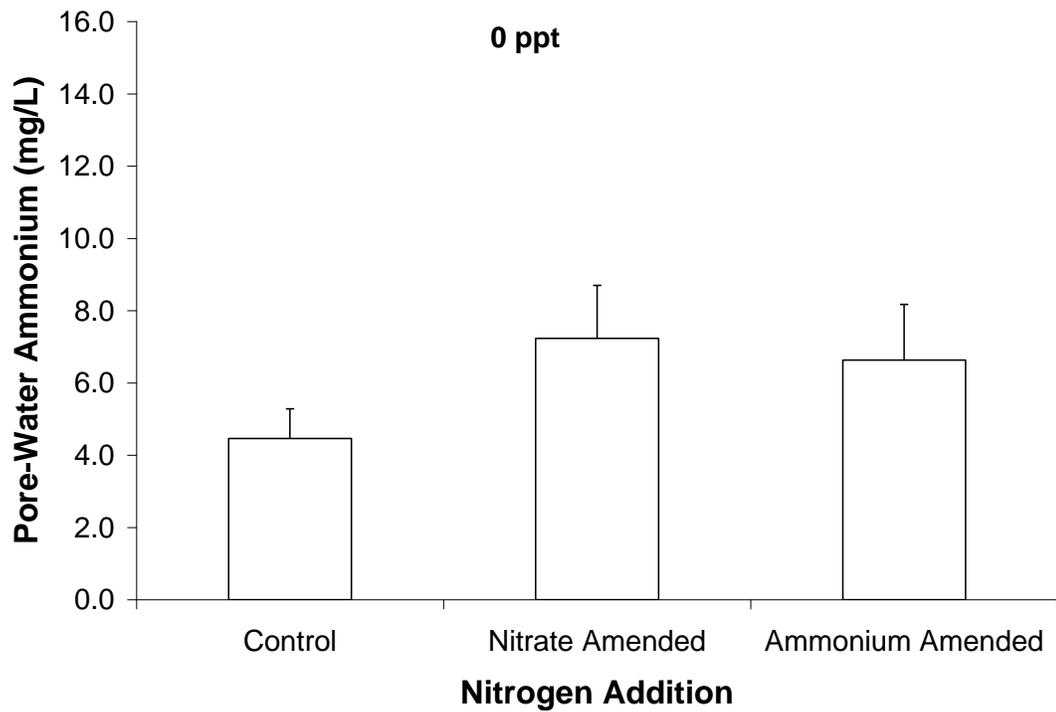


Fig. 5.4. The effect of nitrate amendment and salinity level on pore-water ammonium, averaged across phosphorus amendment (mean +/- 1 s.e.).

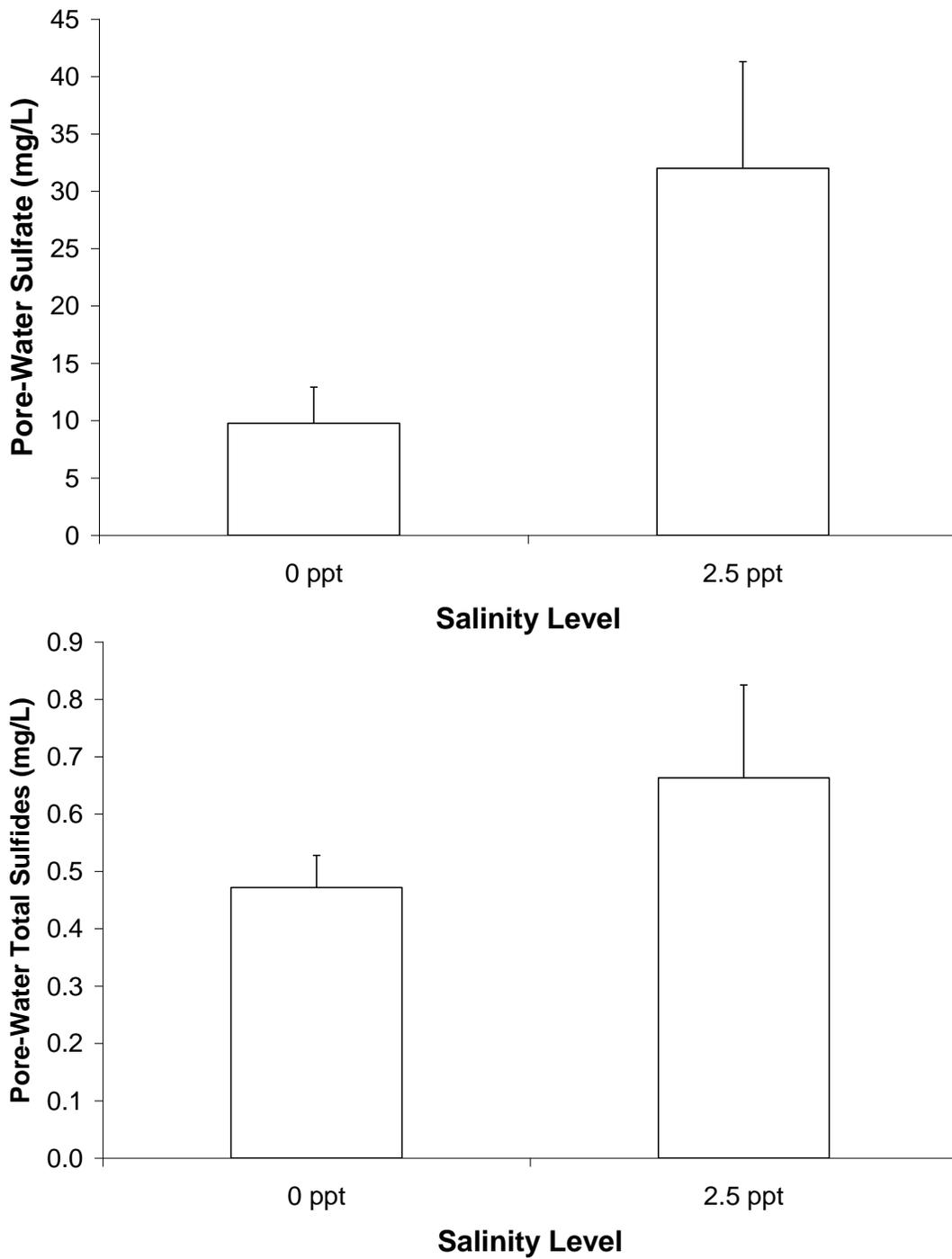


Fig. 5.5. The effect of salinity level on pore-water sulfate (top panel) and pore water sulfides (bottom panel), averaged across nitrogen and phosphorus amendment (mean +/- 1 s.e.).

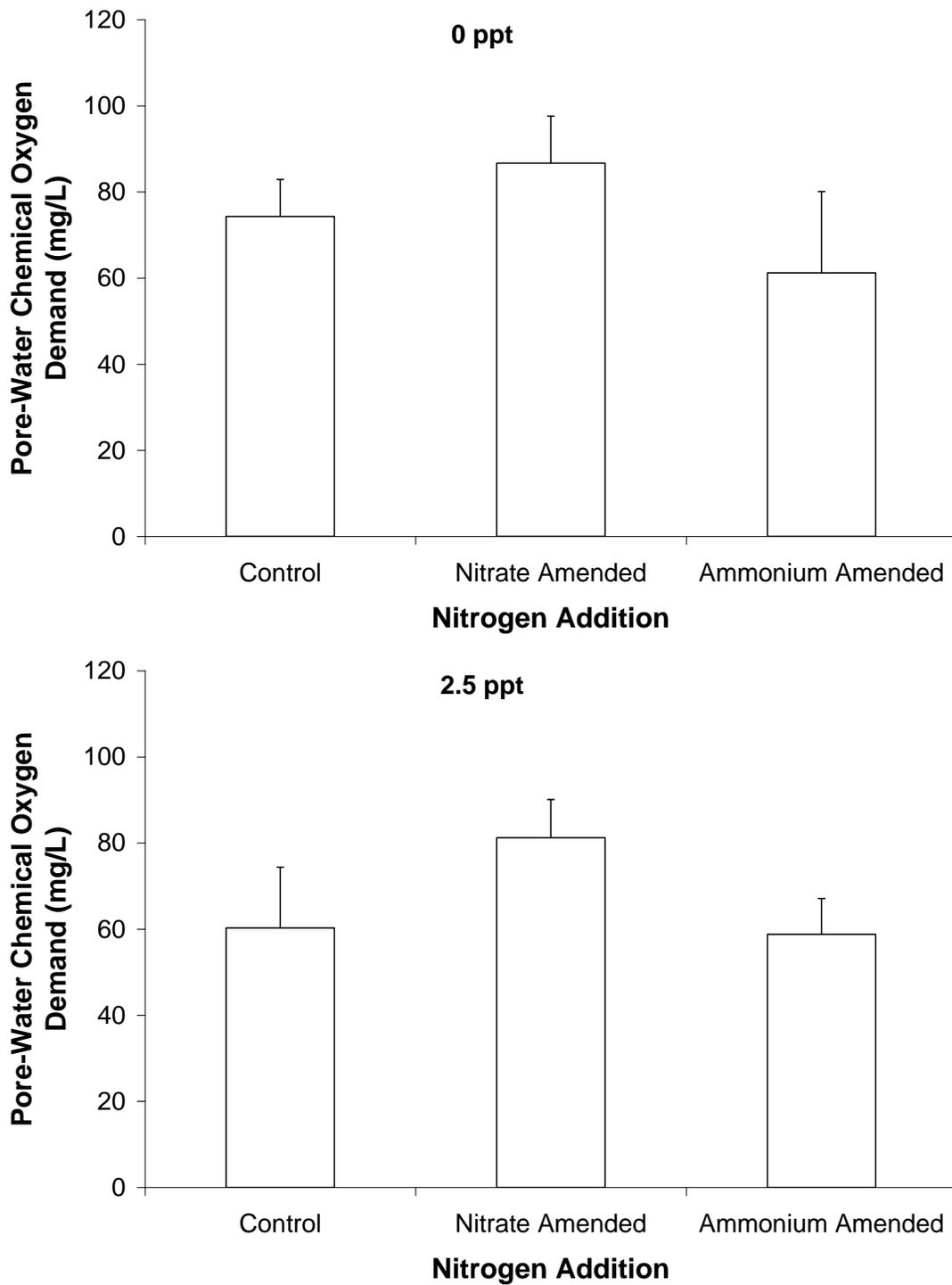


Fig. 5.6. The effect of nitrate amendment and salinity level on pore-water chemical oxygen demand, averaged across phosphorus amendment (mean \pm 1 s.e.).

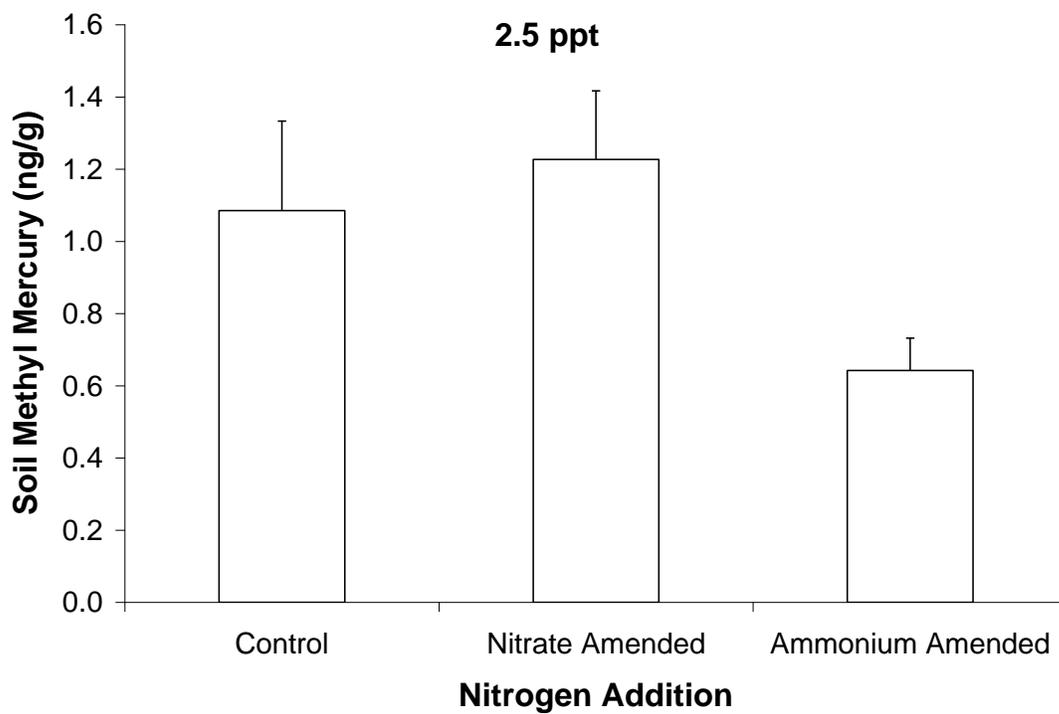
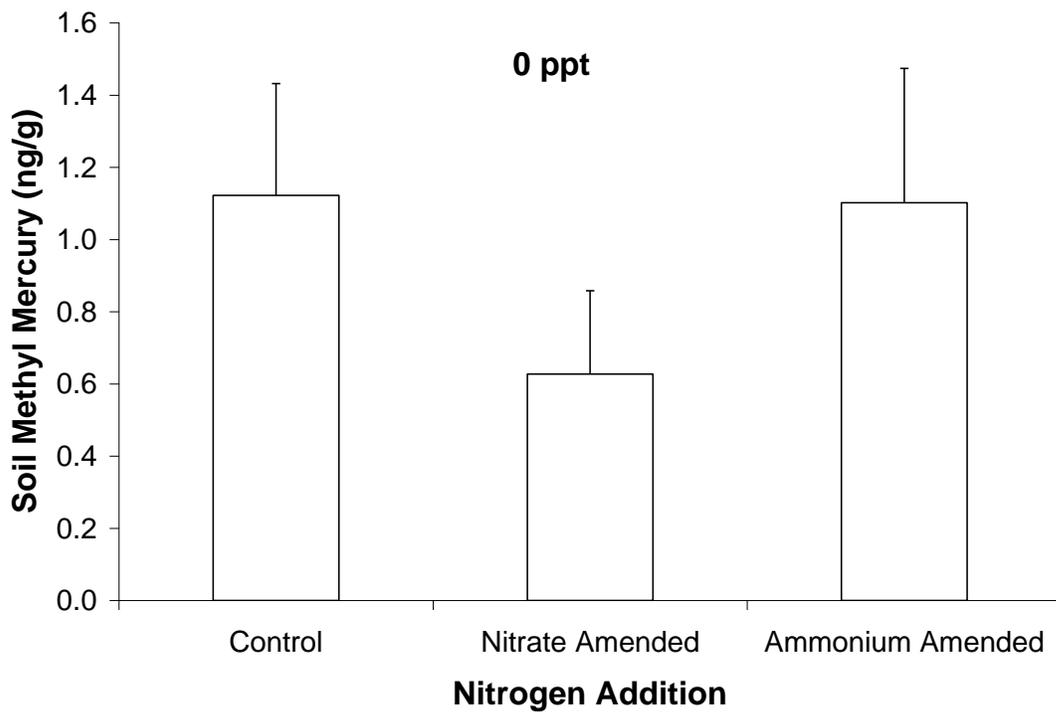


Fig. 5.7. The effect of nitrate amendment and salinity level on soil methyl mercury concentration, averaged across phosphorus amendment (mean +/- 1 s.e.).

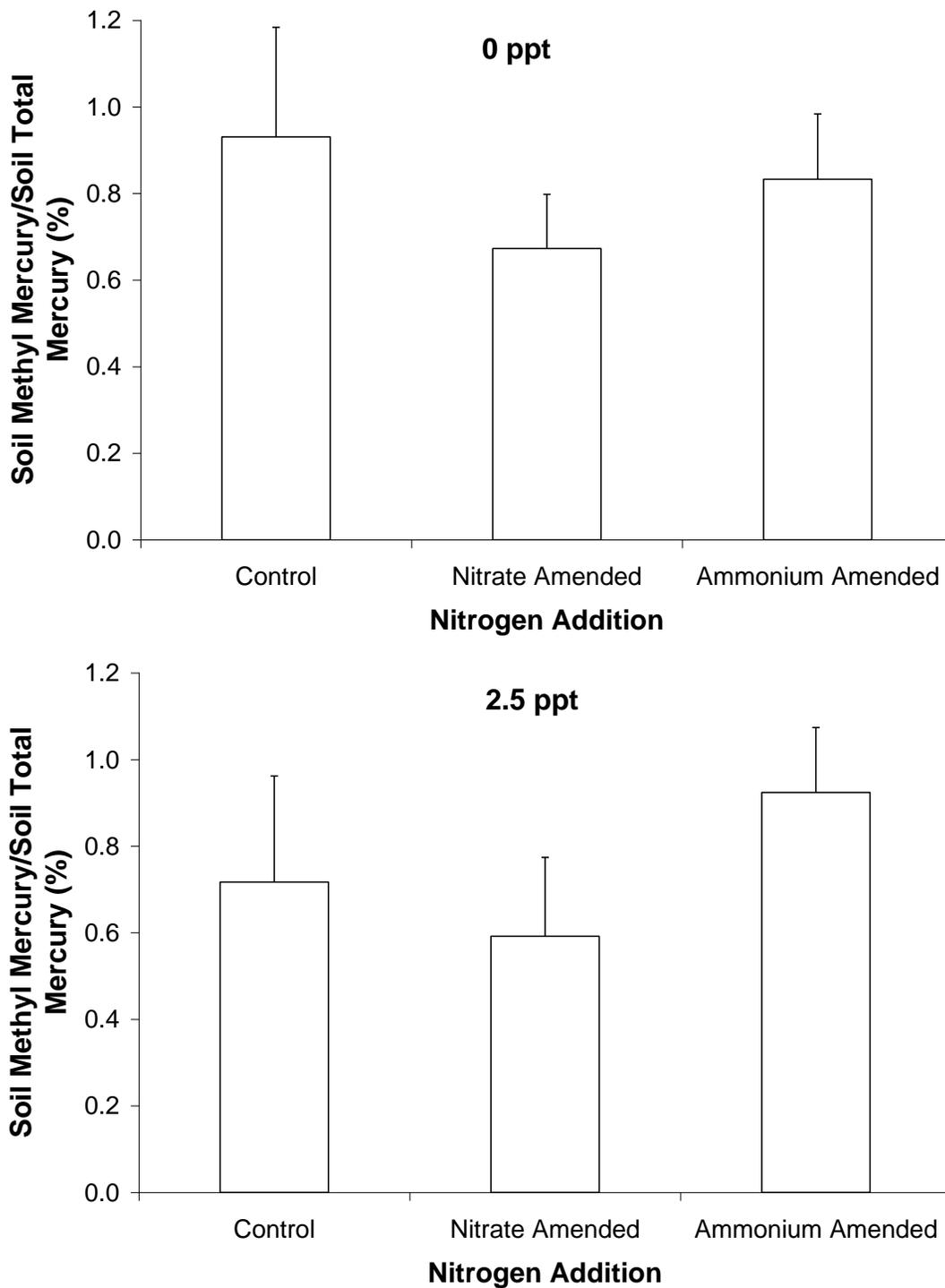


Fig. 5.8. The effect of nitrate amendment and salinity level on the percentage of total soil mercury occurring as methyl mercury, averaged across phosphorus amendment (mean \pm 1 s.e.).

Discussion

The lack of significant differences among the experimental nitrogen, phosphorus and salinity level treatments on surficial soil methyl mercury concentration suggests that, in the short-term, alteration of surface water nutrient composition at these concentrations will not impact mercury methylation. Also, minimal shifts in the final concentrations of the oxidized and reduced forms of nitrogen and sulfur were detected, suggesting that lasting impact to the biogeochemical status of these soils was negligible. This is generally similar to the results of sediment amendment studies conducted by Gilmour et al. (1998), who found that sulfate demonstrated inconsistent effects on mercury methylation, whereas nitrogen and phosphorus were consistently not significant in affecting mercury methylation. Together, these results suggest that microbial availability of mercury, rather than any limitation by nutritive elements, is the controlling factor for mercury methylation in these oligohaline soils. This is in agreement with Delaune et al. (2004) who found that highly reduced conditions in several Louisiana Lake sediments were optimal for the methylation of mercury, but this methylation occurred with substantial addition of a soluble mercury salt.

The surficial soil redox potential was generally above -100 mV in all experimental treatments, with the exception of units receiving no nitrogen amendment, but with salinity levels of 2.5 ppt, in which soil redox potentials close to -100 mV were noted by day 2. Although sulfate reduction to sulfide is known to occur around -100 mV (Mitsch and Gosselink 2000), increases in methyl mercury concentration are often associated with lower soil redox values (Compeau and Bartha 1985; Delaune et al. 2004). Compeau and Bartha (1985) noted greater formation of methyl mercury in estuarine sediments at -200 mV than at 110 mV and under low (4 ppt) salinity levels compared with elevated (25 ppt) salinity levels. Similarly, Delaune et al.

(2004) found elevated levels of methyl mercury in lake sediments maintained at -200 mV, with substantial reduction in methyl mercury level in sediments maintained at 250 mV. The results of research by King et al. (2000), Harmon et al. (2004), and Lambertsson and Nilsson (2006) have indicated that the methylation of mercury is enhanced in reduced sediments with elevated organic matter content.

Harmon et al. (2004) found that sediment methyl mercury concentrations were elevated subsequent to low sulfate amendment compared with control and high sulfate amendment. Importantly, methyl mercury concentrations in surficial soils at the end of this study were similar to those reported in recent field evaluations of the total and methyl mercury soil content in the Lake Maurepas wetlands (Yu et al. 2008) as well as for the sediments of Lake Maurepas (Delaune et al 2008). Also, the percentage of total mercury occurring as methyl mercury in this study is similar to that reported by Delaune et al. (2009) for Louisiana waterbody sediments as well as by Gilmour et al. (1998) for the unimpacted portions of the Florida Everglades. Delaune et al. (2008) found that the average soil redox potential for Lake Maurepas sediments was -163 mV, slightly more reduced than the wetland soils used in this study. Overall final pore-water sulfide concentrations were in the range of 3 to 12.5 μmol per liter and no significant differences existed between experimental units that were maintained as fresh water or oligohaline.

Although pH values significantly varied among treatments and overall became more neutral with time, surface soil pH remained weakly acidic for the duration of the study. This would suggest that mercury methylation would be somewhat less favored as moderate acidity results in a greater proportion of polar mercury-sulfide compounds, which are less bioavailable than non-polar mercury-sulfide compounds due to interactions with biological membranes (Benoit et al. 2003).

No significant effect of nitrate amendment on final surficial methyl mercury concentration was found in this study. Interestingly, Gilmour et al. (1998) reported that addition of 50 μmol nitrate directly into the pore-waters of Florida Everglades sediment cores resulted in a significant decrease in the fraction of mercury methylated per day (~ 0.025 versus 0.003). However, Gilmour et al. (1998) also found that the addition of 100 μmol ammonium directly into the pore-waters of Florida Everglades sediment cores generated a non-significant decrease in the fraction of mercury methylated per day (~ 0.025 versus ~ 0.0225). Gilmour et al. (1998) do not provide a discussion of the effects of nitrogen amendment as their larger focus was sulfate cycling, however, it is possible that the addition of nitrate favored nitrate reducing bacteria in these sediment cores rather than sulfate reducing bacteria, thus reducing sulfate reduction and mercury methylation rates. The results of this study do not show a reduction in final methyl mercury concentration, but this may suggest that mercury methylation is controlled by other factors in the Maurepas wetlands. Interestingly, surficial soil redox potential is lower than the range where nitrate reduction would be dominant (i.e., +250 mV). This may indicate that nitrate amendment did in fact stimulate nitrate-reducing bacteria, but not sufficiently to shift the dominant biogeochemical status as indicated by surficial soil redox potential.

No significant effect, main or interactive, of phosphate amendment was found in regard to final surficial soil methyl mercury concentration or surficial redox potential in this study. This suggests that phosphate amendment had a minimal effect on the microbial flora during the duration of the study. A potential reason for this is would be limitation of the microbial community of the Lake Maurepas system by a resource other than phosphorus. Jackson and Vallaire (2009) also investigated the impacts of altered surface water treatments at the levels used in this study on the microbial community composition of Joyce WMA soils using DGGE

based techniques and concluded that the microbial populations were nitrogen rather than phosphorus limited. A sediment incubation study conducted by Gilmour et al. (1998) found a slight, but non-significant increase in the fraction of mercury methylated per day (~0.025 versus ~0.035) when sediments were amended with 10 μmol phosphate for 6 hours. Although this study was relatively short in duration, similar studies assessing stimulation of mercury methylation by various amendments demonstrate stimulation within this timeframe (Gilmour et al. 1998). Therefore, it is unlikely that phosphate amendment levels evaluated in this study will result in elevated soil methyl mercury concentrations.

In summary, the alterations of the constituents of surficial waters at the level assessed in this study suggest that no shifts in mercury methylation will occur. This is generally consistent with other research of this nature, which suggests that changes in sulfate concentration larger than those occurring in this study, or changes in system characteristics such as microbially available mercury content are necessary to substantially alter methyl mercury concentrations. Substantial impacts of altering nutrient loadings and salinity level in an oligohaline wetland of this character may occur, such as accelerated decomposition of soil components, however, increased methyl mercury production appears unlikely over the short term without additional alterations.

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CHAPTER VI

GLOBAL SYNTHESIS

The research presented in the preceding chapters expound upon a current theme: the importance of mercury bioavailability in ecotoxicological studies. This is particularly apparent when assessing toxicants such as mercury, which is naturally occurring and is present at some trace background level globally. In fact, the importance of chemical form influencing both the bioavailability and toxicity of an agent is likely not more evident in any other well known contaminant. The findings of this dissertation serve to reinforce and expand this premise in regard to herbaceous vegetation and soil microorganisms and provide context to both ecotoxicological theory and application.

This assessment of total mercury concentrations in the above- and below-ground partitions of various herbaceous species in the Lake Maurepas wetlands indicates that mercury levels within this system are typical for an uncontaminated wetland in the eastern portion of North America. The behavior of mercury in vegetation, as revealed in this dissertation's experimental investigations as well as in other studies of similar focus, is generally consistent in that minimal translocation of assimilated mercury between above- and below-ground partitions is noted. This consistent behavior, and the lack of elevated mercury concentrations in either above or belowground plant tissues, suggests that there have been no substantial mercury contamination events to local soils and that local atmospheric mercury levels are not sufficiently high for significant bioaccumulation to be detectable. The field sites sampled during this field assessment experienced low water levels during the study period, resulting in biogeochemical conditions and data that are representative of less flooded conditions than would normally be expected. Similar studies by Yu et al. (2008) and Hall et al. (2008) that captured the same

geographic area, but were performed in different years, reported the same range of soil total mercury as in this study, but higher levels of soil methyl mercury. The lower concentrations of soil methyl mercury found in this study likely due to the low hydrology demonstrates the importance of understanding a local environment's typical condition when interpreting an assessment. Also, this research further highlights the benefit of collecting ancillary biogeochemical data to appropriately frame interpretations, especially when microbially-mediated processes play such a critical role.

The results of the sand culture phytoremediation study are consistent with other similarly designed studies conducted on wetland macrophytes, that the accumulation of mercury from aqueous solution is dependent on aqueous mercury concentration and that the vast majority of this accumulated mercury is retained in belowground tissues. None of the plant species employed in this study were found to be hyperaccumulators of mercury and, at the time of this writing, no macrophytes that hyperaccumulate mercury have been demonstrated. Although several of the species investigated were shown to be beneficial in the treatment of elevated aqueous mercury concentration, none of these species performed at a level exceeding other rooted macrophytes in the peer-reviewed literature. Further, as was shown by Skinner et al. (2007) the level of aqueous mercury phytoremediation exhibited by rooted macrophytes is substantially below that of floating macrophytes. Above- and below-ground concentrations of mercury in control treatments were consistent with the tissue mercury concentrations of other herbaceous species found in the field survey, suggesting the findings of the field study are broadly applicable across species.

The oligohaline contamination study similarly investigated the response of an oligohaline wetland system to elevated mercury loading, but under more realistic soil conditions. An

important distinction regarding this study is that it sought to emulate a direct mercury contamination event of the surface water of an oligohaline wetland, rather than direct mercury contamination of both soil and surface water. Thus the findings of this research effort are more applicable to mercury fate ecotoxicologically, rather than a phytoremediation application. Surface water mercury concentrations at the conclusion of the study were greatly reduced from the initial loading levels likely due to mercury migration into surficial soils, but were elevated and above the NOAA screening levels. Importantly, mercury in the surficial soils as assessed by sequential extraction was largely present as biologically unavailable fractions at the end of the study. Again, the control species for this research effort displayed above- and below-ground tissue mercury concentrations consistent with other herbaceous species in the Lake Maurepas wetlands.

Finally, the results of the soil core incubation study serve to verify the logical interpretation of the field study and provide experimental data on the potential stimulation or limitation of soil methyl mercury concentration under altered surface water composition. Importantly, the soils employed for this research, which were collected from a site adjacent to the permanent plots established for the Joyce WMA site for the field site, displayed total mercury concentrations consistent with this and other field studies for the area (Yu et al. 2009). Further, methyl mercury concentrations in the soils of the control treatments were similar to those reported by Yu et al. (2009), suggesting that these soils had experienced a more typical inundation pattern prior to their collection. Results of soil core amendments suggest that minimal shifts in methyl mercury concentration would occur with these alterations to surface water composition, which are consistent with the findings of Gilmour et al (1998) who conducted a similar study using Everglades soil. However, the biogeochemical measurements

suggest that soil conditions are such that mercury methylation would be favored and it is likely a lack of microbially available mercury that is limiting this process. These findings are consistent with Delaune et al. (2004), who demonstrated substantial accumulation of methyl mercury after spiking Louisiana lake sediments, similar in biogeochemical character to those used in this study, with a soluble mercury salt.

The research presented in this dissertation, as well as contemporary research, has functioned to resolve existing data gaps, but has generated a new series of research questions. In particular, further elucidation of factors controlling the availability of mercury to microbial communities using explicit approaches, such as engineered bacteria, could greatly aid to improve our understanding of mercury cycling. Specifically, a greater understanding of the interactions of mercury with biological compounds produced by vegetation, such as phytochelatins and other secondary compounds, would likely advance the field of phytoremediation. Similarly, further investigation of mercury interactions with particular components of naturally occurring organic matter and their degradation products, could greatly enhance ecotoxicological understanding of mercury cycling. Although progress on this research front has recently been made (e.g., Hall et al. 2008), further investigations would be beneficial.

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VITA

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