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Evaluating Relationships between Mercury Concentrations in Air and in Spanish Moss (*Tillandsia Usneoides* L.)

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EVALUATING RELATIONSHIPS BETWEEN MERCURY CONCENTRATIONS IN AIR AND IN SPANISH MOSS (*TILLANDSIA USNEOIDES* L.)

by

KATHRYN T. SUTTON

(Under the Direction of Risa A. Cohen)

ABSTRACT

Mercury is a potent neurotoxin that is transported globally in vapor form. A major source of mercury contamination to soil, water, and biota is atmospheric deposition. Therefore, comprehensive monitoring of atmospheric concentrations is important. Limitations of conventional atmospheric measurement techniques include high cost and lack of temporal or spatial integration. Bioindicators, however, may serve as an integrative tool to add to conventional mercury measurement techniques. Spanish moss (*Tillandsia usneoides* L.) is a potential bioindicator of atmospheric mercury concentration in the southeastern United States because it is an abundant epiphyte that absorbs and accumulates atmospheric pollutants. A study was conducted in southeastern Georgia and northern Florida to test the hypotheses that 1) Spanish moss absorbs and retains atmospheric mercury in tissue, and 2) atmospheric mercury concentrations differ geographically due to nonpoint emission sources, and the concentration of mercury in Spanish moss tissue reflects these differences. To determine if Spanish moss exhibits uptake and retention of mercury, an experiment was conducted in which I transplanted Spanish moss saturated with mercury vapor in the laboratory to a field site unimpacted by mercury emissions and measured tissue mercury concentration over time. In addition, to determine if mercury concentrations in Spanish moss are reflective of atmospheric concentrations, I conducted

two field studies in which the mercury concentrations of both resident and transplanted Spanish moss were compared to atmospheric concentrations at sites with different anthropogenic land use. In all studies, tissue was analyzed for mercury concentration using Inductively-Coupled Plasma Mass Spectrometry. Results suggest Spanish moss absorbs and retains atmospheric mercury, and mercury concentrations in Spanish moss tissue are associated with atmospheric concentrations over both small and large geographic scales. Thus, Spanish moss may serve as a useful measurement tool to add to existing monitoring protocols.

INDEX WORDS: Bioindicator, Atmospheric Pollutant, Epiphyte, Bromeliad, Southeastern United States

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CHAPTER 1

INTRODUCTION

Atmospheric mercury has the potential to detrimentally impact the health of fish, wildlife, and humans. Two thirds of atmospheric mercury is of anthropogenic origin, and lake sediment records indicate that anthropogenic inputs to the atmosphere have tripled in the last 150 years (Morel et al., 1998). The majority (95%) of atmospheric mercury is in the gaseous, elemental form, Hg^0. Global transport of Hg^0 through the atmosphere is efficient, thus remote areas are polluted with mercury from anthropogenic sources (Schroeder and Munthe, 1998; Steffen et al., 2005). Chemical processes in the atmosphere convert Hg^0 to an oxidized, gaseous form, Hg (II), and a form bonded to particulates, Hg (p) (Schroeder and Munthe, 1998). Hg (II) is removed from the atmosphere via wet deposition through dissolved Hg (II) in precipitation, and Hg (p) is removed via dry deposition of particulates (Martin et al., 1981). After deposition onto surfaces such as wetlands and water bodies, elemental mercury is converted by sulfate-reducing bacteria to methylmercury (King et al., 2001; Steinnes et al., 2003).

Both gaseous mercury and methylmercury are toxic substances. On land, 80% of inhaled mercury vapor is retained by organisms, with high doses having deleterious effects on the nervous system (WHO, 2000; Bastos et al., 2004). Mercury concentrations of 300 $\mu g\ l^{-1}$ in urine of humans who have had chronic occupational exposure to mercury vapor (> 30 $\mu g\ m^{-3}$) display reversible neurological symptoms including short-term memory loss, tremors, and social withdrawal (WHO, 2000). In water, methylmercury enters aquatic food webs and has the potential to become increasingly concentrated in

organisms with increasing trophic level (Wagemann et al., 1995). This biomagnification is a concern since methylmercury is the most toxic form of mercury (Mason et al., 2000). Humans and other mammals, birds and fish with diets high in methylmercury can suffer from detrimental and irreversible neurological and nervous system effects (WHO, 2000; Mergler et al., 2007). For example, high concentrations of methylmercury in fish muscle (6-20 mg kg^{-1}) from contaminated environments such as Minamata Bay, Japan, have been associated with suppression of gonad development, egg production, and spawning (Scheuhammer et al., 2007). Lower methylmercury concentrations ($\geq$ 1 mg kg^{-1} tissue) can cause adverse neurological effects (including lethargy, decreased muscle coordination, limb paralysis, tremors, and convulsions) and death in adult mink and otter (the mammalian species in which the most information exists regarding toxicity), and in birds can reduce hatchability of eggs and increase mortality of embryos (Scheuhammer et al., 2007). In humans, long-term exposure to methylmercury through diet can cause irreversible neurological effects in individuals in which blood mercury levels reach 200 µg l^{-1}. In addition, prenatal poisoning can occur by exposure to methylmercury through the placenta, and infants may be born with symptoms similar to cerebral palsy (Mergler et al., 2007). Due to the human health risk of methylmercury exposure, many water bodies and fish species have been placed under advisories for high levels of methylmercury (Morel et al., 1998; Mason et al., 2005). Because elemental mercury is ultimately converted to methylmercury, it is important to accurately quantify and monitor changes in elemental mercury concentrations in the atmosphere, particularly in areas

where methylmercury is readily formed so that potential impacts to organisms and ecological systems can be assessed (Morel et al., 1998; Mason et al., 2005).

Typically, atmospheric mercury is measured using mechanical technology. However, the number of measurements required to represent pollution levels at multiple sites and over long periods of time can be laborious and cost prohibitive (Calasans and Malm, 1997; Mason et al., 2005). In addition, the concentration of atmospheric mercury is not static; it depends upon the chemical forms present, proximity to emissions sources, and air circulation and precipitation patterns (Schroeder and Munthe, 1998). Therefore, mechanical measurement technology usually provides instantaneous readings of atmospheric mercury concentration.

Using a plant bioindicator that is spatially and temporally integrative may be a beneficial tool to add to conventional monitoring programs to assess changes in atmospheric mercury concentrations. The purpose of this study was to assess relationships between mercury concentrations in air and in the tissue of Spanish moss (*Tillandsia usneoides*) to determine if this plant can be used as an effective bioindicator of atmospheric mercury concentrations.

CHAPTER 2

USING SPANISH MOSS (*TILLANDSIA USNEOIDES* L.) TO EVALUATE ATMOSPHERIC MERCURY CONCENTRATIONS FROM NONPOINT SOURCES

INTRODUCTION

A major area of uncertainty in biogeochemical cycling of mercury is the linkages among atmospheric mercury emission, deposition, and changes in methylmercury concentrations in food webs (Schroeder and Munthe, 1998; Mason et al., 2005). Mercury enters the atmosphere from emission point sources, including outgassing from volcanoes, wildfires, the industrial burning of fossil fuels, and waste incinerators, and from nonpoint sources, such as urban and industrial centers (Schroeder and Munthe, 1998; Davis et al., 2007; Wiedinmyer and Friedli, 2007; Soerensen et al., 2010). Approximately 95% of mercury in the atmosphere is in the gaseous, elemental form (Hg^0), which is stable and does not readily combine with other atmospheric compounds (Morel et al., 1998; Schroeder and Munthe, 1998). Therefore, Hg^0 has a residence time in the atmosphere of about 1 year. While this form of mercury can be transported globally, it is generally more concentrated around emission sources (Morel et al., 1998; Schroeder and Munthe, 1998; Carballeira and Fernández, 2002). Hg^0 can be slowly oxidized to the mercuric state, Hg (II), with ozone as the primary oxidizing agent, or become associated with airborne particulates, Hg (p) (Morel et al., 1998). Mercury is removed from the atmosphere to land and water surfaces, primarily by Hg (II) dissolved in precipitation, although both wet and dry deposition may act on Hg (II) and Hg (p) (Morel et al., 1998; Schroeder and Munthe, 1998). Atmospheric mercury concentrations in an area are

dependent on the physicochemical properties of the different forms of mercury present, as well as environmental characteristics, such as the concentration of ozone and precipitation patterns (Morel et al., 1998; Schroeder and Munthe, 1998).

Once deposited, mercury can be converted to methylmercury, the most toxic form of mercury that can bioconcentrate in food webs. Environments that are particularly reactive with deposited mercury include both freshwater and marine systems, and wetlands (including fresh, tidal, and salt marsh). After deposition onto water surfaces and sediments, mercury forms numerous complexes with organic matter and sulfide compounds which are methylated by bacteria (Williams et al., 1994; Benoit et al., 1999; King et al., 2001; Sunderland et al., 2004). These complexes may be stored in sediments, taken up by biota (Williams et al., 1994; Canário et al., 2007) or re-released to the air through transpiration by plants or volatilization of the gaseous form, Hg^0 (Lindberg et al., 2002; O'Driscoll et al., 2007). That aquatic systems may serve as both a source and sink of gaseous mercury underscores the importance of being able to estimate delivery of atmospheric mercury to these systems.

Atmospheric mercury concentrations and deposition patterns are typically estimated using mechanical methods. For example, the Mercury Deposition Network of the National Atmospheric Deposition Program uses automated collectors throughout the United States to measure patterns of total mercury deposited by precipitation over land (NADP, 2011). Measurements of gaseous mercury can be conducted using trap systems that consist of glass tubes filled with gold sand, which amalgamates mercury from air pumped through the tube (Calasans and Malm, 1997), and the Differential Absorption Lidar technique which measures atmospheric mercury concentrations along a laser beam

(Grönlund et al., 2005). However, obtaining mercury measurements that are spatially comprehensive and temporally integrated using the above methods requires many replicate samples, which can be both laborious and costly (Carballeira and Fernández, 2002; Figueiredo et al., 2007). A potential solution is the use of bioindicators, which offer an integrative method to estimate atmospheric concentrations and deposition patterns (De Temmerman et al., 2004).

Plants are often used as bioindicators of heavy metal air pollution (e.g., Brighigna et al., 1997; Steinnes et al., 2003; Figueiredo et al., 2004; Davis et al., 2007) largely because they have a high capacity to accumulate heavy metals into their tissue over long time intervals (Calasans and Malm, 1997; Alves et al., 2008). Bioindicators can be sampled from a resident population, or be transplanted to an area (Falla et al., 2000; Fernández et al., 2000; Carballeira and Fernández, 2002). Ideal characteristics of bioindicators in a resident population include having a large geographical distribution and ease of collection, and examples include mosses, lichen, ferns, leafy vegetables, trees, shrubs, and grasses (Gailey and Lloyd, 1985; Carballeira and Fernández, 2002; Manning et al., 2002; Moraes et al., 2002; Szczepaniak and Biziuk, 2003; Fernández et al., 2004; De Temmerman et al., 2009; Kono and Tomiyasu, 2009). Alternatively, transplanted bioindicators can be used to estimate deposition patterns in areas where no suitable bioindicator plants are present, and to establish baseline measurements of deposition in areas where there is no historical data (Falla et al. 2000). Mosses and other bryophytes are often used as transplanted bioindicators of atmospheric pollutants because of their hardiness in handling and transport, and their ability to acclimate to areas where they are not present naturally (Falla et al., 2000; Fernández et al., 2000; Balarama Krishna et al.,

2004). For a plant to be an effective bioindicator of atmospheric heavy metals, whether from a resident population or transplanted, the plant must not only be able to take up metal pollutants but also retain pollutants in tissue for a period of time necessary for tissue concentrations to be representative of average atmospheric concentrations (Baker, 1981; Falla et al., 2000).

Spanish moss (*Tillandsia usneoides* L.), an epiphytic bromeliad, is a potential bioindicator of atmospheric mercury concentration (Husk et al., 2004; Figueiredo et al., 2007). Spanish moss accumulates heavy metals from the environment in its tissue due to high surface area, the absence of a cuticle, and high cation exchange capacity (Calasans and Malm, 1997; Alves et al., 2008). In addition, roots of Spanish moss are virtually non-existent, or are used only as hold-fasts, meaning nutrients, water, and atmospheric pollutants are absorbed directly from the air through scales on the plant surface (Amado Filho et al., 2002; Wannaz et al., 2006; Alves et al., 2008). Therefore, pollutants measured in tissue may be related to atmospheric sources without complication due to uptake from soil or other media (Carballeira and Fernández, 2002). This plant also has a low sensitivity to heavy metal exposure, likely because metals are retained in its outer scales and is not translocated to internal mesophyll fibers (Amado Filho et al., 2002; Bastos et al., 2004; Figueiredo et al., 2007; Alves et al., 2008).

Spanish moss has been shown to take up atmospheric mercury when transplanted close to emission point sources. Calasans and Malm (1997) transplanted Spanish moss inside a chlor-alkali plant with mercury levels at 1-64 μg m^{-3}, and after 15 days found tissue mercury levels up to 13,500 times greater than control plants. In addition, Malm et al. (1998) transplanted Spanish moss inside and around gold shops, which are a mercury

emission source due to the burning of mercury and gold amalgams that are the product of gold prospecting. The mercury levels in indoor transplants reached 26 ppm, which was 300 times higher than control plants, and plant concentrations decreased with increasing distance, up to 20 m from the shops (Malm et al. 1998). Although Spanish moss accumulates mercury when transplanted either indoors or in very close proximity to direct sources of excessive mercury emission (Calasans and Malm, 1997; Malm et al. 1998), it is unclear if Spanish moss can take up mercury from nonpoint emissions sources over wide geographic areas in concentrations reflective of atmospheric concentrations in those areas.

It is also unknown if Spanish moss retains mercury in tissue after exposure. Variability in atmospheric mercury concentrations in an area occurs due to proximity to emission sources, and whether emissions are constant or sporadic, as well as changing wind and precipitation patterns (Schroeder and Munthe, 1998; Conti and Cecchetti, 2001). Therefore, Spanish moss may be exposed to varying atmospheric mercury concentrations over time. The tissue concentration measured in a bioindicator that loses mercury taken up after intermittent changes in atmospheric concentration would therefore not be representative of average air concentrations. Mercury must be retained in tissue to effectively use Spanish moss as a biomonitor of atmospheric mercury.

The purpose of this study was to determine whether Spanish moss can be used as an indicator of atmospheric concentrations of mercury in a range likely to be encountered in the field. I hypothesized that: 1) mercury taken up by Spanish moss is retained in tissue following uptake; and 2) mercury concentrations in Spanish moss tissue are associated with atmospheric concentrations in a wide geographic range. To test my

hypotheses, I conducted field studies in southeastern Georgia and northern Florida, USA. I predicted air mercury concentrations would differ geographically due to nonpoint emission sources, and that concentration of mercury in Spanish moss tissue would reflect these differences. Results of this study address the capability of Spanish moss for temporal integration of atmospheric mercury from point and nonpoint sources, and utility as a tool to augment existing atmospheric mercury monitoring protocols.

METHODS

2.1. Retention Experiment

To determine how long mercury is retained following uptake in Spanish moss tissue, plants with high tissue concentrations of mercury were allowed to depurate in a low mercury environment. First, plant segments to be tested in the experiment were collected haphazardly at the Salt marsh Ecosystem Research Facility (SERF) at Skidaway Island, Georgia, USA (31° 55′ 39″ N, 81° 2′ 33″ W) from an established population with low background tissue mercury levels (0.05 - 0.16 μg Hg g^{-1} tissue). Segments were 10-20 cm of healthy growth from distal ends of each plant at least 1 m from the ground to ensure plants did not have contact with soil. In the laboratory, plants were washed with mercury-free tap water to remove any particulate mercury on the plant surface. Segments were mixed together so individual replicates would be selected at random, and a subsample (n=6) was analyzed for background mercury concentration.

Next, Spanish moss segments (200g) were placed in two closed glass containers and onto latticed cardboard over a pool of liquid elemental mercury and exposed to mercury vapor for one week, resulting in treatment groups 10x (1.3 ± 0.09 μg g^{-1}) and 100x (12.2 ± 0.26 μg g^{-1}) that of ambient Spanish moss concentrations (0.13 ± 0.003 μg

g^{-1}). Individual segments of Spanish moss from the two treatment groups were haphazardly tied to individual trees at least 1 m apart along a 100 m transect in the forest at the SERF site. At 2, 7, and 14 days, 5 segments of each treatment were collected and analyzed for tissue mercury concentration.

2.2. Resident population study

A field study was conducted to determine if the mercury concentration in resident Spanish moss populations reflects air concentrations. Spanish moss from established, resident populations was collected from sites in southeastern Georgia and northern Florida between March and September 2011 from areas that I categorized as urban, coastal, inland, and industrial (Figure 1). The urban sites of Jacksonville, FL and Savannah, GA were expected to have high mercury levels in air and in Spanish moss because these are urban centers with large human populations (>1,000 people mi^{-2}; US Census, 2011). The LCP Chemicals EPA Superfund Site in Brunswick, GA was also predicted to have high mercury levels because this site has a history of mercury pollution in soils and sediments from industrial activity (Windom et al., 1976; Gardner et al., 1978; Winger et al. 1993; US EPA, 2011). In contrast, Georgia coastal barrier islands (Cumberland Island, Ossabaw Island, and Sapelo Island) were predicted to have lower mercury concentrations than urban and industrial sites due to reduced influence of anthropogenic emission sources; these sites have restricted access, relative lack of development, and are located > 30 km from urban centers. However, due to location east of urban centers, it is possible that prevailing winds could transport mercury from urban and industrial sites to the coast. Therefore, Spanish moss was also sampled from the rurally located inland sites of Magnolia Springs and George L. Smith state parks. These

sites also have limited infrastructure and access. At each field site, three air samples were collected, and twenty 2.5 g Spanish moss segments of 10-20 cm of healthy growth on distal ends of plant strands were collected haphazardly, each from individual trees. Sampling 10-20 cm of plants was determined to be appropriate for resident population evaluation based on a study of Spanish moss growth rates by Martin et al. (1981), who found the maximum growth rate (in July and August) to be approximately 270 μm day-1 (standardized per number of leaves). This slow growth rate, in addition to the estimation that 70-80% of a Spanish moss plant is dead in January and February, followed by only 15% in March (Martin et al. 1981), led us to determine that 10-20 cm of distal growth is representative of new plant growth that has been exposed to the atmosphere within the growing season.

2.3. Transplant studies

To determine the range over which Spanish moss tissue mercury concentration changes in response to atmospheric concentrations, two transplant studies were conducted: at a site in which mercury pollution is present, and at sites in without a direct emission source. All Spanish moss plants were collected for transplanting from the SERF site following the collection protocol previously described (section 2.1.).

2.3.1. Transplants to a mercury-contaminated site

A field study was conducted in which Spanish moss with low background levels of mercury was transplanted to the vicinity of a site historically contaminated with mercury. The LCP Chemicals EPA Superfund site in Brunswick, GA is a 200-250 ha area that is mostly salt marsh and in which multiple industrial activities have operated since 1919, including an oil refinery, a paint manufacturing company, a power plant, and

a chlor-alkali plant (U.S. EPA, 2011). These operations have contaminated the soils and groundwater with PCBs, volatile organics, and mercury (U.S. EPA, 2011). Mercury was discharged from the chlor-alkali plant at a rate of 1 kg Hg day^{-1} for six years until 1972 (Windom et al., 1976; Gardner et al., 1978). Two studies in the 1970s measured high mercury levels in the marsh sediments surrounding the site; Windom et al. (1976) and Gardner et al. (1978) found mercury concentrations up to 1.7 ppm in the top 0-5 cm of sediments in the marsh approximately 2 km from the site. In addition, in 1989 Winger et al. (1993) measured 1-27 ppm in sediments throughout the marsh, with the highest concentrations closest to the LCP site, and the lowest concentrations near the mouth of the creek. While 25,000 tons of contaminated soil were removed from the upland portion of the site in 1998, mercury is still present in the marsh adjacent to the site; Frischer et al. (2000) measured mercury in marsh sediments adjacent to the site to be around 10 μg g^{-1}. The presence of elevated mercury concentrations in wetland sediments long after the source of contamination has ceased has also been noted by Marvin-DiPasquale et al. (2003) who found evidence that a salt-marsh in San Pablo Bay, California, still had elevated mercury concentrations in sediments due to mercury loading from hydraulic mining in the late 1800s. Therefore, mercury may still be present surrounding the LCP Chemicals site and emitting gaseous mercury over a broad area.

In July 2011, three 170 m transects were established in the salt marsh, west of and parallel to the LCP Chemicals site at distances of 0.4, 0.6, and 1.3 km (Figure 2). Along each transect, 15 Spanish moss transplants were tied to PVC poles and placed 12.5 m apart. Each transplant consisted of four 5 g bundles of Spanish moss suspended approximately 2.5 m above sediment to avoid inundation by tides. Transplants were

collected after two weeks. Although transplanting Spanish moss to a salt marsh environment may impose stress on plants that could affect uptake of gasses through stomata, it is unlikely since Spanish moss has been demonstrated to resist water loss and be adapted to xeric environments (Penfound and Deiler, 1947). During both transplant placement and collection, six air samples were collected: three air samples were collected at the closest transect (0.4 km from the LCP Chemicals site) and three were collected at the farthest transect (1.3 km from the site). All air samples were obtained in the center of each transect.

2.3.2. Transplants to coastal and inland sites

To test the efficacy of using transplanted Spanish moss to detect differences in air concentration due to nonpoint sources over a wide geographic scale (encompassing approximately 14,000 km^2), a field study was conducted from June to September 2011 in which transplants were placed at both coastal and inland sites in southeastern Georgia, USA (Figure 1). Coastal sites were of particular interest due to the large area of salt marsh present, and the high abundance of bacteria in these environments increases the potential for rapid mercury methylation (Williams et al., 1994; King et al., 2001). At coastal sites (Sapelo Island, Ossabaw Island, and Cumberland Island) transplants were placed near salt-marshes. For comparison, transplants were also placed at the rurally located inland sites of Magnolia Springs and George L. Smith state parks, and a 45 ha, undeveloped tract of private forested land. At each field site, 20 Spanish moss transplants (5 g) were tied to individual trees 10 m apart along a 200 m transect. After two weeks, 2.5 g of each transplant were collected (excluding Magnolia Springs state park and Ossabaw Island, which were collected after three and four weeks, respectively).

Simultaneously, at each site samples from the established, natural population were also collected for comparison (with the exception of the private land, which does not have a resident Spanish moss population) and three air samples were collected in the center of the transect at times of transplant placement and collection. Air concentration data could not be collected during transplant placement at Cumberland Island due to equipment malfunction.

2.3.3. Spanish moss and air sample collection

For each study, 2.5 g Spanish moss samples were placed into pre-weighed teflon vials with 6 ml nitric acid (HNO_3) in the field to fix mercury into solution and vials were kept on ice and in the dark so that mercury concentrations would not change in transit to the laboratory (Southworth et al. 1958). Air samples were collected using a diaphragm air pump to force 10 L of ambient air over 10 minutes onto a glass tube filled with gold sand to amalgamate mercury (Sutton, unpublished data).

2.4. Sample Analysis

2.4.1. Tissue

In the laboratory, 0.5 ml of ^{201}Hg stable isotope spike solution was added to solutions of Spanish moss and HNO_3 and microwaved (CEM-MDS-2100) at 71.1˚C and a pressure of 170 lbs in^{-2} to break down and liquefy (digest) tissue. An 0.5 ml aliquot of each sample was then passed through an Inductively-Coupled Plasma Mass Spectrometer, and the ratio of the added ^{201}Hg to the more abundantly occurring ^{202}Hg stable isotope was compared to determine the total mercury concentration (μg Hg g^{-1}) in each tissue sample (Smith 1993). NOAA CRM 2976 mussel tissue standards with a

known mercury concentration of 61.0 μg kg^{-1} were also used for comparison and protocol standardization (Smith, 1993).

2.4.2. Air

A nichrome wire coil was placed around each gold sand-filled glass tube and heated, which re-volatilized the amalgamated mercury. The re-volatilized mercury was swept by argon gas into a Cold Vapor Atomic Fluorescence Spectrophotometry detector (Tekran 2500), and an integrator (HP 3394) displayed peak fluorescence from each air sample, which was compared to a standard curve of water with known mercury concentrations to calculate the air mercury concentration of each sample (adapted from Bloom and Fitzgerald, 1988).

2.5. Statistical Analyses

Data were tested for equality of variance using Levene's test and for normality using the Shapiro-Wilk W test. Data not meeting parametric assumptions were either log transformed or nonparametric tests were used. For the retention experiment, Spanish moss tissue data were log transformed and an ANCOVA was conducted with treatment group (starting tissue mercury concentration) and sampling time as factors. The Pearson correlation coefficient was calculated to correlate mean mercury concentrations of resident populations of Spanish moss at sites with differing human influence (urban, coastal, inland, industrial) to air concentrations for each site. In addition, the final mercury concentration of Spanish moss transplanted to the polluted LCP Chemicals site after two weeks was compared to initial concentrations before transplanting and to the resident population at the site using one-way ANOVA. Air mercury concentration at the LCP site was compared across transects and at times of transplant placement and

collection using two-way ANOVA. Final tissue mercury concentration of transplants to coastal and inland sites were compared to initial levels and to resident populations present at the sites using either one-way ANOVA or Kruskal-Wallis, and the mean percent change in transplant tissue mercury concentration was compared between sites nested within category (coastal and inland) using nested two-way ANOVA. Air mercury concentrations at each sampling time (of transplant placement and collection) were compared using t-tests for each coastal and inland field site.

RESULTS

3.1. Retention Experiment

Following removal from a source of mercury vapor, Spanish moss plants retained mercury for up to 15 days (Figure 3). Further, we found a strong trend of plants in the higher tissue concentrations treatment (100x ambient levels) exhibiting an increase in tissue concentration, while plants in the lower concentration treatment (10x ambient levels) maintained starting tissue concentration (ANCOVA; $F_{3,24}$=3.03; p=0.05). After placement in the field, the 100x ambient treatment had a mean increase in tissue mercury concentration of 74.1 ± 17%, while the 10x ambient treatment exhibited a mean increase of 13.7 ± 11%.

3.2. Resident population study

Among field sites, resident Spanish moss mercury concentrations trended toward an increase with increasing air concentration (Pearson correlation; r_p= 0.70; n=8; p= 0.05; Figure 4). The resident population present at the industrial site of LCP Chemicals had the highest mercury concentration both in air and in Spanish moss, while urban sites had the lowest mercury levels. Variability in air data within locations was high. Coefficient of

variation (CV) of air mercury concentration data ranged from 32.9-105.3 across field sites, while the CV of Spanish moss concentration ranged from 0.2- 0.6.

3.3. Transplant Studies

3.3.1. Transplants to a mercury-contaminated site

Mercury levels in transplanted Spanish moss increased by 62% after two weeks (one-way ANOVA; $F_{4,56}$= 20.7; p< 0.001; Figure 5), and was similar to the resident population (Tukey-Kramer HSD, 0.4 km distance, p=0.14; 0.6 km distance, p= 0.08; 1.3 km distance, p= 0.08). There was no difference in air mercury concentration due to sampling time (two-way ANOVA; $F_{1,8}$= 0.53; p= 0.49) or location (two-way ANOVA; $F_{1,8}$= 1.16; p= 0.31). The average combined air mercury concentration (including initial and final air samples) was 0.05 ± 0.007 μg m^{-3}, approaching the World Health Organization air quality guidelines for limit of acceptable occupational exposure, 1 μg m^{-3} (WHO, 2000).

3.3.2. Transplants to coastal and inland sites

Transplants to coastal and inland sites differed in percent change in tissue mercury concentration (nested ANOVA; $F_{1,4}$= 29.95; p= 0.005), with inland sites having a higher mean percent increase (152.52 ± 13.2 %) than coastal sites (29.6 ± 4.6 %; Tukey-Kramer HSD, p= 0.005; Figure 6). The inland sites of Magnolia Springs (one-way ANOVA; $F_{2,46}$= 44.22; p<0.01) and George L. Smith (Kruskal-Wallis; H_2= 17.44; p<0.01) had final tissue mercury concentrations similar to the resident population (Tukey-Kramer HSD; MS, p= 0.15; GLS, p= 0.99; Figure 7). At the private inland site, in which no resident population was present, transplants increased from initial levels (t-test; t_{27}= -6.97, p < 0.01). Response of transplants at coastal sites was mixed; tissue

mercury concentrations transplants to Sapelo Island (one-way ANOVA; $F_{2,46}$= 24.57; p<0.01) and Cumberland Island (Kruskal-Wallis; H_2= 19.17; p <0.01) did not reach tissue mercury concentration of the resident population (Tukey-Kramer HSD; SI, p<0.001; CI, p<0.001). In contrast, transplants to Ossabaw Island exhibited no difference among initial and final tissue mercury concentrations of transplants and tissue concentration of the resident population (Kruskal-Wallis; H_2= 3.67; p= 0.16).

Air concentrations at inland sites were more variable than at coastal sites (Table 1). At Magnolia Springs, the CV differed by 0.62 between times of transplant placement and collection, while the CV differed by 10.55 at George L. Smith and by 0.51 at the private land site. In contrast, at coastal sites of Sapelo and Ossabaw Islands, the difference in air concentration CV was 0.12. The air concentration at inland sites of Magnolia Springs (t-test; t_5=4.16; p= 0.009). and George L. Smith (t-test; t_4= -3.43; p= 0.03) was higher during transplant collection, while the air concentration at the private inland site was lower during transplant collection (t-test; t_6= 4.49; p= 0.004). At coastal sites, air concentrations were not different between times of transplant placement and collection at Sapelo Island (t-test; t_6= -1.49; p= 0.19) and Ossabaw Island (t-test; t_6= -0.63; p=0.55).

DISCUSSION

In this study, we found that Spanish moss retains mercury in tissue, an essential characteristic for a time-integrative bioindicator of atmospheric mercury concentrations. Not only did Spanish moss retain mercury in tissue, but this plant was still taking up more mercury after two weeks. The increase in mercury concentration in the 100x ambient treatment after placement in the field suggests this plant can continue to take up

mercury even after reaching a high tissue concentration; therefore, saturation of mercury in tissue is unlikely to occur when exposed to field conditions with lower, more ecologically relevant concentrations of atmospheric mercury. Spanish moss may be similar to other epiphyte species and be capable of retaining mercury for longer than two weeks, such as the moss species *Sphagnum girgensohnii* shown by Lodenius et al. (2003) to exhibit a strong retention of mercury in tissue for up to 4 weeks after removal from a source of mercury vapor.

Areas with different land use, and thus different emission sources, had differing atmospheric mercury concentrations that corresponded to the concentrations in resident Spanish moss present in those areas. Spanish moss and air mercury concentrations at field sites may not have been more strongly related due to the higher variability of air mercury concentration data than of tissue mercury concentration data. High variability in air concentration was expected and was likely due to the discrete sampling method used, in which replicate one-time "snapshot" measurements of air mercury concentration were collected. Wind patterns, circulation, and precipitation can impact the presence of gaseous mercury in an area (Schroeder and Munthe, 1997; Morel et al., 1998; Rothenberg et al., 2010). Therefore, a measurement taken at one point in time will likely not be representative of average atmospheric mercury concentrations in an area.

However, the correlation trend between air and Spanish moss revealed patterns in mercury concentration present across field sites. Mercury concentrations in resident Spanish moss and in air were the highest at the Brunswick LCP site, which was expected given the history of mercury contamination at the site. Our finding that urban sites had the lowest concentrations of mercury in air and in resident Spanish moss was unexpected

and could be due to wind patterns. Prevailing wind patterns affect the concentration of atmospheric mercury by transporting gaseous mercury to or from a target sampling area (Kellerhals et al., 2003; Rothenburg et al., 2010). Soerensen et al. (2010) compared gaseous mercury measurements in air at 15 coastal cities worldwide and found that the concentration of gaseous elemental mercury in the atmosphere generally increased with increasing human population. However, concentrations were variable over time, and affected by location and type of nearby emission sources, wind direction, and wind speed (Soerensen et al., 2010). In the present study, prevailing winds during the time Spanish moss and air samples were collected from urban areas (March) in coastal Georgia and northern Florida are east- and northeastward (Weber and Blanton, 1980). Therefore, mercury emissions emanating from urban areas may have been transported away by winds at the time of sampling. Winds may have also transported sources from surrounding industry offshore, as well. The largest regional mercury-emission source is from coal fired powered plants, producing an estimated 22-33% of mercury in rainfall in the United States (Landing et al., 2010). Several coal fired power plants are located in Georgia and northern Florida; Savannah is located 9 and 20.4 km southeast of two coal-fired power plants, and Jacksonville is located 7.9, 12.18, and 13.4 km southwest of three coal-fired facilities (US EPA, 2011). Therefore, it is likely that prevailing winds transported emissions from the coal fired power plants off shore and away from urban areas.

Wind patterns cannot explain why rural inland and coastal sites had higher mercury concentrations in air and in Spanish moss populations than urban sites. Rural inland sites were not downwind from urban sites, but were, however, situated downwind

of coal fired power plants. Soerenson et al. (2010) determined there is a 2-10x reduction in gaseous elemental mercury concentration in the atmosphere at distances of 40-120 km from emission sources. In addition, Carballeira and Fernández (2002) collected a moss species (*Scleropodium purum*) 10-50 km from a coal-fired power plant and found the lowest tissue mercury concentrations at 20-30 km from the plant, and no difference in tissue concentration at all other distances. Inland sites in this study were no closer than 76 km to a coal-fired power plant; therefore, gaseous mercury concentrations measured at these sites may not have been heavily influenced by the burning of coal.

A more likely explanation for the elevated mercury concentrations observed at the inland and coastal sites was the presence of wetlands. Mercury deposited from the atmosphere onto sediment surfaces or through river or tidal inundation reacts with organic matter (Williams et al., 1994). In sediments, mercury can become sequestered by organic or sulfidic material, be released through vegetation transpiration in the form of Hg^0, or become labile (Langer et al., 2001; Lindberg et al., 2002). Labile mercury can be reduced to Hg^0 (volatile), or methylated by bacteria (Langer et al., 2001). The high populations of bacteria present in the humic environments of both freshwater and salt marshes have been shown to increase the rates in which mercury is methylated or forms complexes with sulfide compounds in which the predominant form is HgS^0 (Benoit et al., 1999; King et al., 2001; Canário et al., 2007). Methylated species are then taken up by organisms, or de-methylated to Hg (II) and Hg^0. Therefore, wetlands can accumulate and re-release mercury to the atmosphere (Zillioux et al., 1993; Williams et al., 1994), increasing atmospheric mercury concentrations around wetlands on the barrier islands relative to urban areas.

Resident populations with a long exposure time to atmospheric concentrations make it difficult to determine the most prevalent emission source influencing tissue concentrations, due to variability in wind patterns and environmental characteristics over time (Schroeder and Munthe, 1998; Mason et al., 2005). However, because transplants are exposed to atmospheric concentrations for a shorter, known time period, it may be easier to determine emission sources that have a short-term influence on mercury levels in air and in Spanish moss tissue. Spanish moss transplant concentrations were associated with air concentration both at the site with a known mercury pollution source (Brunswick LCP site) and at sites without a constant emission source. At the contaminated Brunswick LCP site, we found mean air mercury concentration in the marsh surrounding the site to be 2.5 times greater than levels found within 1 km of an active chlor-alkali plant by De Temmerman et al. (2009). In addition, we found levels in both transplanted and resident population of Spanish moss at the LCP Chemicals site to be above background levels reported in the literature. For example, at rural control sites Calasans and Malm (1997) found mercury concentrations in Spanish moss tissue to be 0.1 μg g^{-1}, and Rinne and Barclay-Estrup (1980) found background mercury levels of 0.06-0.09 μg g^{-1} in a moss species (*Pleurozium schreberi*). Therefore, the elevation in mercury concentration of Spanish moss tissue above background levels after placement at the Brunswick LCP site after only two weeks indicates this plant is capable of rapidly responding to a constant, but diffuse source in the field. Further, there was no difference in mercury concentration in transplants or air measurements due to distance (0.4-1.3 km) from the site. These findings contrast those of Malm et al. (1998), who found that transplants of Spanish moss located 20 m from gold shops had a 65% reduction in tissue

mercury concentration from transplants located 5 m from the shops, as well as Fernández et al. (2000) who noted a 4x reduction in mercury concentration of a transplanted moss species (*S. purum*) within 400 m of an active chlor-alkali plant. The elevated air and transplanted Spanish moss tissue mercury concentrations measured up to 1.3 km from the LCP site suggest mercury pollution is present throughout the marsh and this area may serve as a constant source of gaseous mercury emission, and Spanish moss had sufficient sensitivity to take up the gaseous mercury that was present in the area.

Transplants of Spanish moss at coastal and inland sites show that this plant is also sensitive to increased levels of mercury from environments without a constant source of mercury emission. Spanish moss transplanted to inland and coastal sites, with the exception of Cumberland and Ossabaw Islands, had increased tissue mercury concentration. The site that was most likely to be influenced by emission sources transported by August northeastward winds was Sapelo Island. This site is located 30.5 km northeast of a coal fired power plant, and 35.5 km northeast of the Brunswick LCP Chemicals site (US EPA, 2011). However, inland sites less likely than Sapelo Island to be influenced by major emission sources carried by prevailing winds also showed increased transplant mercury concentration. Coastal sites did not have larger concentrations of mercury in air and in Spanish moss than inland sites, as seen in the resident population study. Both coastal and inland sites had wetland habitat that was present at all sites; therefore, release of gaseous mercury from wetland sediments may have influenced transplant mercury concentrations over the short time period tested.

In this study we found Spanish moss may make an effective bioindicator of atmospheric mercury concentrations. Retention of mercury by Spanish moss is a

valuable characteristic of a bioindicator, and tissue concentrations of both resident populations and transplants with a long exposure time may be used to assess typical atmospheric concentrations of an area. The trend between mercury concentration in resident Spanish moss and air concentrations indicates that this plant can detect patterns in atmospheric concentration across a wide geographic scale. Resident Spanish moss populations have a prolonged exposure to atmospheric concentrations where they reside. Therefore, tissue mercury levels proportionate to atmospheric levels may be used to estimate typical long-term atmospheric concentrations, particularly in locations where there is no historical data. Further, the transplant studies suggest Spanish moss may be effective in monitoring changes in atmospheric mercury concentrations from diffuse sources on the order of weeks, to determine atmospheric concentrations during short time periods. The increased mercury concentration in Spanish moss when exposed to the elevated atmospheric concentrations at the polluted Brunswick LCP site indicates Spanish moss responds rapidly to changes in environmental mercury concentrations due to a nonpoint emission source. In addition, the increased tissue concentrations in Spanish moss transplants at inland sites and at Sapelo Island indicates that Spanish moss is capable of taking up mercury that is present in the atmosphere in low, ambient concentrations at areas not influenced by an emission source. Thus, a Spanish moss bioindicator that is sensitive and effective in integrating mercury concentration patterns temporally and reflective of atmospheric concentrations in contaminated and non contaminated sites offers a valuable and cost-effective tool to supplement existing monitoring programs.

Table 1. Total air mercury concentration (μg m^{-3}) ± one standard error of the mean (SEM) and n=4 measured during Spanish moss transplant placement and collection at each field site.

Site	Initial	Final
Sapelo Island	0.007 ± 0.00	0.011 ± 0.00
Cumberland Island	--	0.012 ± 0.00
Ossabaw Island	0.004 ± 0.00	0.006 ± 0.00
Private Land	0.075 ± 0.02	0.000 ± 0.00
Magnolia Springs	0.003 ± 0.00	0.041 ± 0.02
George L. Smith	0.000 ± 0.00	0.006 ± 0.00

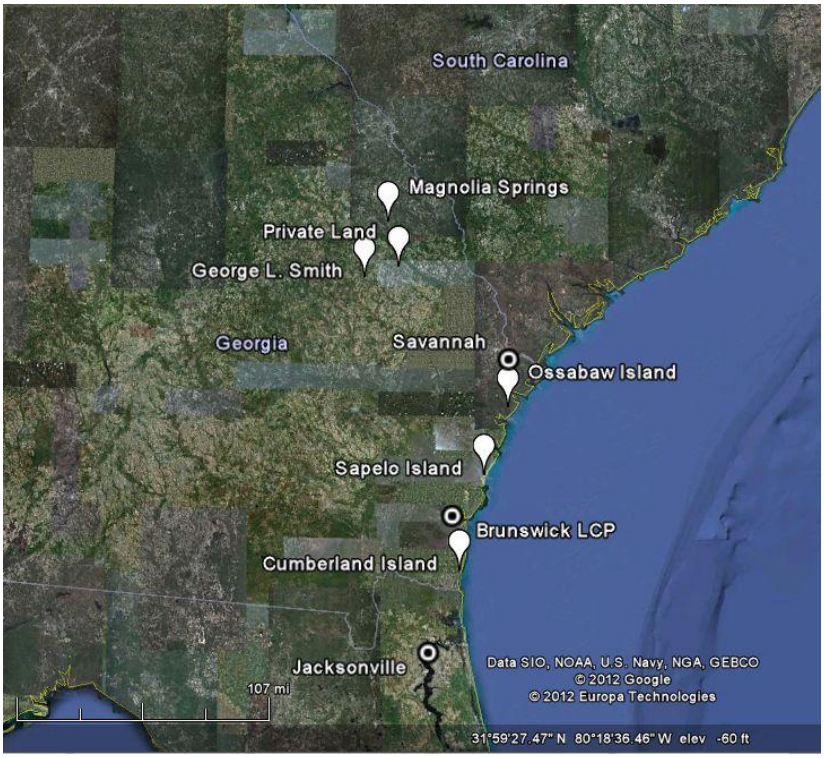


Figure 1. Field sites of resident population and coastal vs. inland transplant studies, located in southeastern Georgia and northern Florida.

Figure 2. Three 170.7 m transects, represented by white lines, established west of and parallel to the LCP Chemicals EPA Superfund site in Brunswick, Georgia.

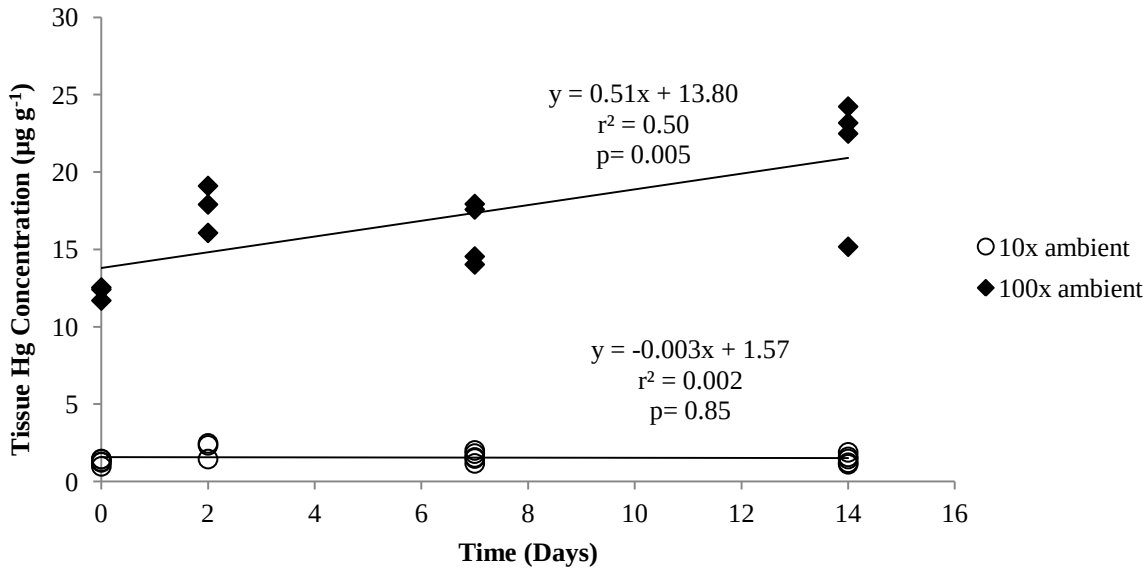


Figure 3. Total mercury concentration of Spanish moss tissue in 100x and 10x ambient concentrations over two weeks following removal from mercury vapor (n=5).

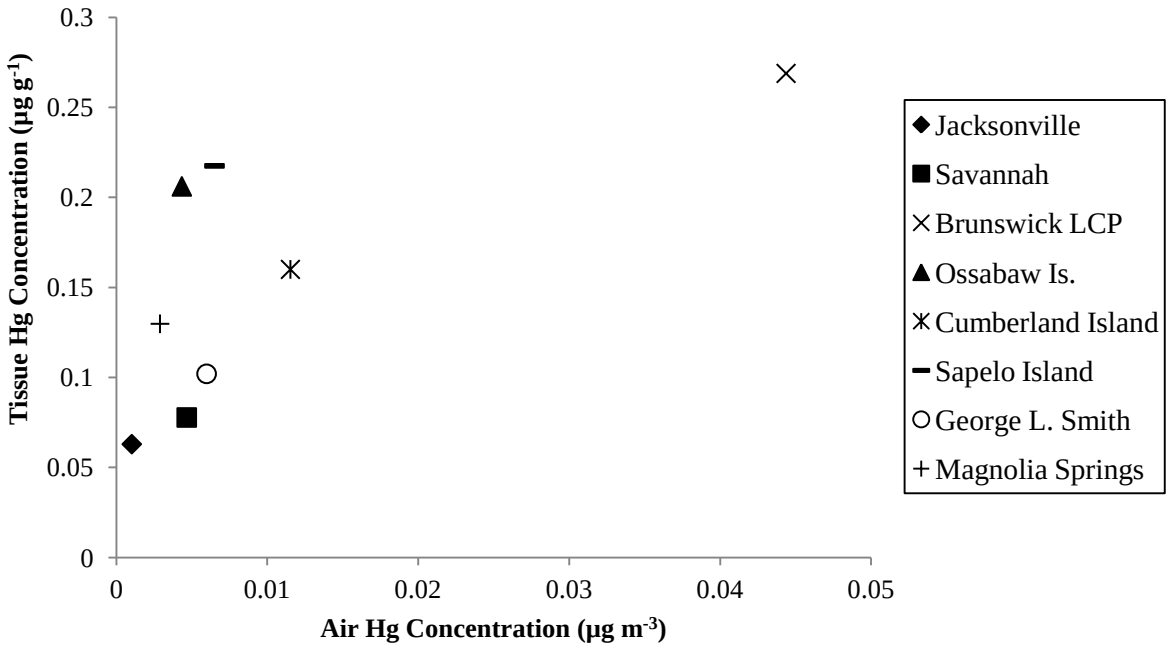


Figure 4. Relationship between mean air and tissue mercury concentration in resident Spanish moss plants at each urban (Jacksonville, ♦; Savannah, ■), industrial (Brunswick LCP, ×), coastal (Ossabaw Island, ▲; Cumberland Island, ⁎; Sapelo Island, ▬), and inland (George L. Smith state park, ○; and Magnolia Springs state park, +) site.

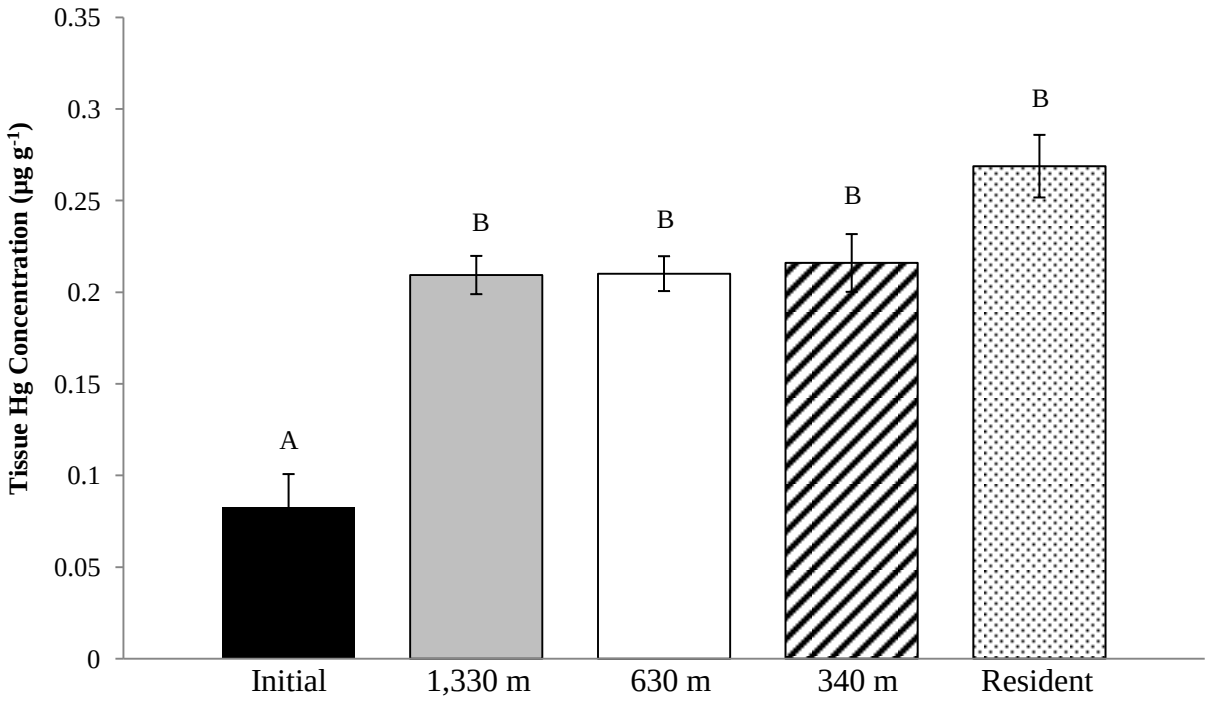


Figure 5. Mean mercury concentration of Spanish moss tissue prior to transplanting (n=10), after two weeks of exposure (n=15) at three distances from the LCP Chemicals Superfund site in Brunswick, GA, of a resident Spanish moss population present at the site (n=7). Error bars are ± one standard error of the mean (SEM).

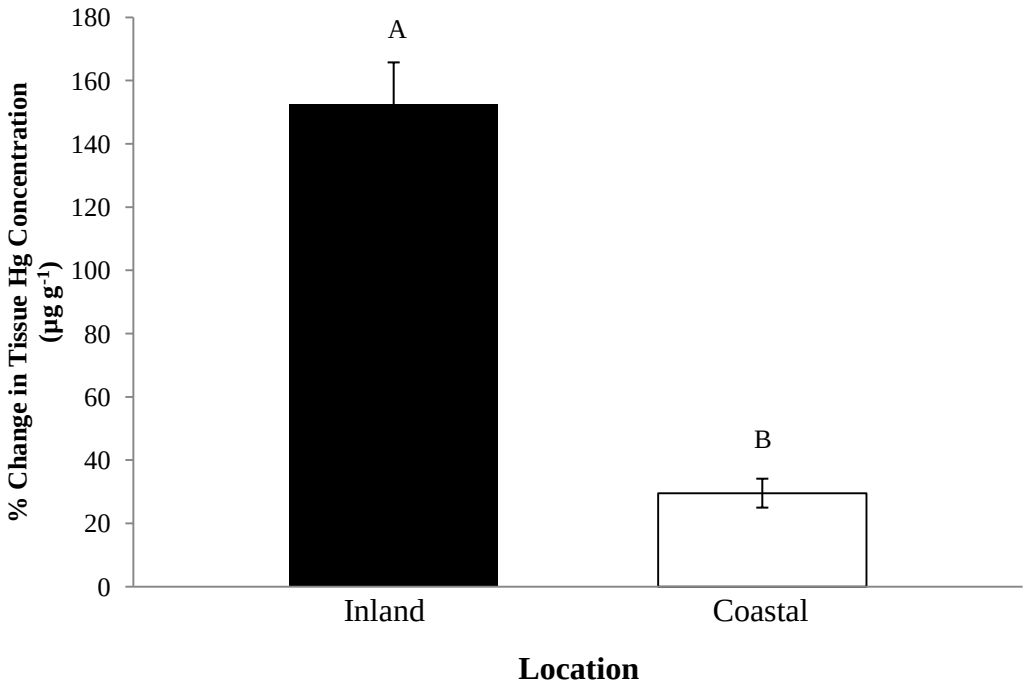
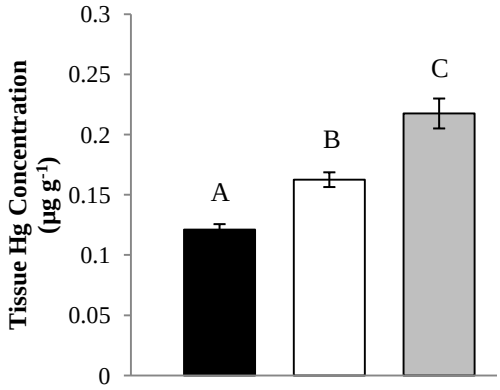
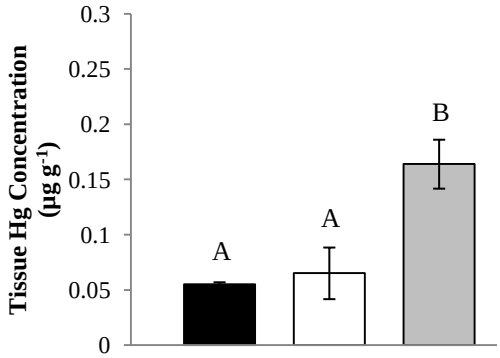


Figure 6. Percent change from initial Spanish moss tissue mercury concentration in transplants to coastal and inland sites. Error bars are ± one SEM, and n=57 and 58 for coastal and inland site category, respectively.

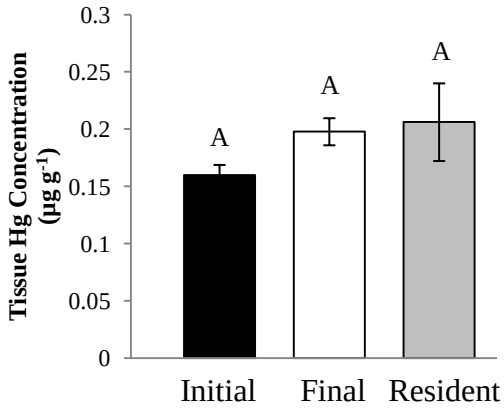
A. Sapelo Island



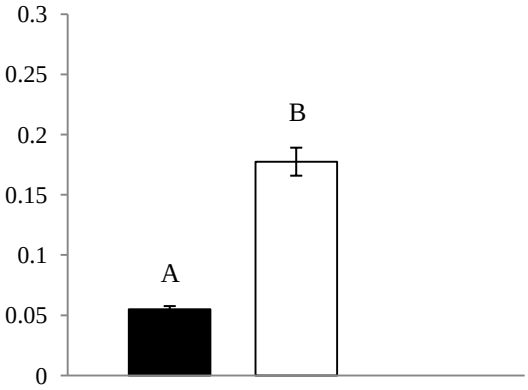
B. Cumberland Island



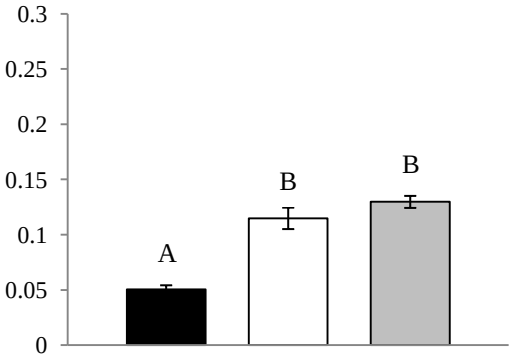
C. Ossabaw Island



D. Private Land



E. Magnolia Springs



F. George L. Smith

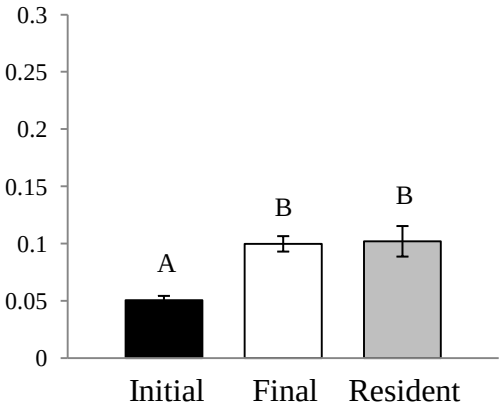


Figure 7. Initial (n=10) and final (n=20) tissue mercury concentration (μg g^{-1}) ± one SEM of Spanish moss transplanted to the coastal sites of Sapelo Island (**A**), Cumberland Island (**B**), and Ossabaw Island (**C**); and the rural inland sites of Private Land (**D**), Magnolia Springs (**E**), and George L. Smith (**F**), in comparison to the resident population concentration (n=20) at each site.

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APPENDIX A

EVALUATION OF MERCURY UPTAKE BY SPANISH MOSS IN LABORATORY MICROCOSMS

RATIONALE

It is unknown how exposure to different ecologically relevant air mercury concentrations affects uptake by Spanish moss tissue. To establish the relationship between concentrations of mercury in air and in Spanish moss, it is important to quantify the rate at which Spanish moss takes up mercury from air and if that uptake rate is affected by differing air concentrations. I hypothesized that mercury concentration in Spanish moss would differ in response to external concentration. To test this hypothesis, I conducted a laboratory experiment to determine differences in mercury uptake by Spanish moss when exposed to different ecologically relevant air concentrations of mercury. It was predicted that the uptake rate of mercury in Spanish moss tissue would increase with increasing concentration.

METHODS

Spanish moss was collected haphazardly from a naturally occurring population in the vicinity of the Salt Marsh Ecosystem Research (SERF) site, located adjacent to the Skidaway Institute of Oceanography (SKIO) in southeastern Georgia (31° 55′ 39″ N, 81° 2′ 33″ W). Each plant collected consisted of approximately 10-20 cm of new growth from at least 1 m in height from the ground. In the laboratory, all plants were cut into 5-10 cm segments and mixed to randomize plant assignment to treatments. A subsample was analyzed for background mercury concentration (n=5).

Spanish moss segments (17.5g) were rinsed thoroughly with mercury-free tap water to remove dirt and particulate mercury from the surface, blotted dry, and placed in acid-cleaned (10% HCl) 2 l glass jars for a total of 300 experimental units. Mercury air concentration treatments were then added to each treatment container. The three treatment concentrations were based on World Health Organization guidelines (WHO, 2000). The high treatment concentration (30 µg m^{-3}) is considered to be the highest concentration to result in adverse health effects in workers subjected to long-term mercury vapor exposure (WHO, 2000). To approximate ecologically relevant outdoor concentrations, intermediate (15 µg m^{-3}), low (1 µg m^{-3}), and control (ambient air, $1.6x10^{-3}$ µg m^{-3}) treatments were also tested. Treatments were created by adding mercury-rich air syringed from flasks containing liquid elemental mercury at equilibrium with the flask air. Using the ideal gas law, I derived the volume of air to be syringed from the flasks that would contain the number of moles of mercury to give the desired high, medium, and low treatments. The control treatment (ambient air) was obtained by letting control treatment jars sit outside at the SERF site overnight to equilibrate the air inside the jar with the ambient environment. Control containers were then sealed with a rubber stopper, and only opened quickly to place washed Spanish moss sample inside.

I then randomized the experimental containers by location in an environmental chamber with a mean light intensity of -7.23 µmol m^{-2}, a 12 h light/dark cycle, and a constant temperature of 27˚C (Schlesinger and Marks, 1977; Martin et al., 1981). Daily, for 15 days, five replicates from each treatment were destructively sampled for tissue mercury concentration. This duration was shown to be sufficient to see differences in Spanish moss mercury concentration following exposure to mercury-contaminated air

(Calasans and Malm, 1997). At each sampling time, the plants were weighed to measure growth to be certain any differences measured in tissue mercury concentration over time were not due to growth dilution. In addition, on days 4, 6, 8, and 15, the mercury concentration in air in each treatment was determined.

Tissue Sample Processing

A 2.5 g portion of sample from each replicate was placed in a 100 ml teflon vessel and submerged in 6 ml of trace metal grade nitric acid and 0.5 ml of ^{201}Hg stable isotope spike solution. Tissue was then broken down and liquefied (digested) in a CEM-MDS-2100 microwave oven at maximum temperature (71.1˚C) and pressure (170 lbs in^{-2}). One 0.5 ml aliquot of each digested sample was passed through an Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) and the ratio of ^{201}Hg spike to the most abundantly occurring ^{202}Hg stable isotope was used to determine the total mercury concentration in each sample (µg mercury g $tissue^{-1}$). NOAA CRM 2976 mussel tissue standards with a known mercury concentration of 61.0 µg kg^{-1} were also used for comparison and protocol standardization (Smith, 1993).

Air Sample Processing

Mercury concentration in air was measured using Cold Vapor Atomic Fluorescence Spectrophotometry (CV-AFS). A peristaltic pump forced air for 2 minutes through an input gold trap (so any mercury present in outside air would not affect measurements), then through experimental containers, then through an output gold trap. Gold traps consisted of glass tubes filled with gold sand that amalgamated mercury from the air. The output gold trap was then attached to a nichrome wire coil connected to a voltage regulator that heated the tube and thus volatilized the mercury amalgamated by

the gold sand. The mercury concentration in the volatilized sample was measured using an atomic fluorescence detector (Tekran 2500) and an integrator (HP 3394) (modified from Bloom and Fitzgerald, 1988).

Statistical analyses

Spanish moss tissue mercury concentration data were tested for normality using the Shapiro-Wilk W test and homogeneity of variances using Levene's test. Spanish moss tissue data could not be transformed to meet assumptions of parametric tests so the Kruskal-Wallis test with the Scheirer-Ray-Hare extension was conducted with tissue mercury concentration and sampling day as factors. Statistics could not be conducted on air sample data, as all replicates on all sample days were 0 $\mu g\ m^{-3}$.

RESULTS

Although mercury concentration in Spanish moss samples was affected by treatment (Scheirer-Ray-Hare; $H_{3,\ 228}= 41.61$; $p< 0.001$) and time (Scheirer-Ray-Hare; $H_{14,\ 228}= 54.92$; $p<0.001$) with no interaction effect, patterns in tissue mercury concentration were complex (Figure 1). By day two, the mercury concentration of Spanish moss samples in all three treatment groups increased relative to the control, followed by a general decline in tissue concentration in all treatments. However, all mercury treatments remained elevated 23-37% above control concentrations through day seven. During the second week of the experiment patterns reversed; tissue concentration in all treatments remained similar to concentration of control plants (approximately 0.07 $\mu g\ g^{-1}$) until days 14 and 15, when there was an apparent spike in the mean low (53%) and control (35%) concentrations, respectively. Air concentrations remained at 0 $\mu g\ m^{-3}$

for all replicates on each day sampled, and mass of Spanish moss samples did not change from initial measurements.

DISCUSSION

Spanish moss showed an increase in tissue mercury concentration within two days. The rapid uptake of elevated levels of atmospheric pollutants into the tissue of Spanish moss and other epiphytes is well-supported (Brighigna et al., 1997; Malm et al., 1998; Fernández et al., 2000; Carballeira and Fernández, 2002; Figueiredo et al., 2007). However, a decrease and loss of mercury in tissue after day 2 occurred, which can be explained in several ways. One possibility is that the mercury initially taken up by Spanish moss could have been lost through transpiration. While gaseous elemental mercury is taken up by plants through stomata (Schroeder and Munthe, 1998; De Temmerman et al., 2009), studies have shown that a flux of gaseous mercury occurs between plants and the atmosphere. For example, Poissant et al. (2004) conducted monitoring over 11 days in a lacustrine wetland and found that more gaseous elemental mercury was emitted from the system (32.1 ng m^{-2} h^{-1}) than deposited. A flux of mercury favoring emission over a large stand of wetland plants was also noted by Lindberg et al. (2002) who found more emission over cattail (*Typha domingenesis*) and sawgrass (*Cladium jamaicense*) than open water (30 ng m^{-2} h^{-1}). Further, results of a laboratory experiment conducted by Hanson et al. (1995) suggested that at low air mercury concentrations (0.5-1.5 ng m^{-3}), emission of mercury from tree species occurred (at a rate of 1.7-5.5 ng m^{-2} h^{-1}) indicating the plants served as a source of gaseous mercury emission; at medium air concentrations (9-20 ng m^{-3}) little exchange of mercury occurred between plants and the air, and at high concentrations (50-70 ng m^{-3}), there was an

overall pattern of deposition of mercury onto foliage surfaces (at a rate of 22-38 ng m^{-2} h^{-1}), indicating the plants served as a mercury sink. Similar to the Hanson et al. (1995) study, I found tissue mercury concentration of Spanish moss in the control treatment (with air concentration of $1.6x10^{-3}$ µg m^{-3}) decreased, which may have been due to emission to the container air. However, the low, medium, and high air concentrations tested in the present study were higher than those tested by Hanson et al. (1995), but I did not see retention of mercury in Spanish moss tissue. Lodenius et al. (2003) also tested higher air concentrations of mercury than Hanson et al. (1995) in a laboratory experiment in which the moss *Sphagnum girgensohnii* was exposed to mercury vapor emitted from a liquid elemental mercury pool. It was also found that high mercury concentrations during exposure resulted in a strong retention of mercury in moss tissue (Lodenius et al., 2003). In the present study, the lack of retention and decrease of tissue mercury concentration by Spanish moss in the low, medium, and high treatments was also coupled with air concentrations of 0 µg m^{-3} from day 4 throughout the experiment, indicating a loss of mercury from both tissue and air.

A second explanation for the decrease in Spanish moss concentration, as well as a reason for air concentrations of 0 µg m^{-3}, is that after initial addition of mercury vapor to treatment containers, gaseous mercury interacted with the crystalline structure of the glass experimental containers. This hypothesis was supported by a subsequent experiment in which empty glass containers were inoculated with mercury. The mercury concentration decreased with time consistent with findings from the uptake experiment (Table 1). Further, a study by Doughty et al. (1995) showed that in fluorescent lamps, mercury binds to bare glass in the form of HgO, and adsorbtion to glass increases with

time. Therefore, if the mercury vapor added to treatment containers adsorbed to the glass sides, Spanish moss would be exposed to less available gaseous mercury and thus influencing uptake patterns. Therefore, it is likely patterns of mercury uptake seen in this experiment are due to the influence of the glass, and are not representative of uptake by Spanish moss in the field. Future studies should use material in treatment containers less reactive with mercury, such as teflon.

Table 1. Mean air mercury concentration ± one standard error of the mean (SEM) in glass exposure jars (ng l^{-1}) over 5 days, following inoculation with mercury vapor. Initial air mercury concentration treatments were either High (30 ng l^{-1}) or the Control (ambient air, ≈ $1.6x10^{-3}$ ng l^{-1}). Mercury concentrations were analyzed using CV-AFS.

Time	High	Control
0	2.04 ± 0.20	0.1 ± 0
1	1.69 ± 0.25	0 ± 01
2	0.78 ± 0.09	0.16 ± 0
3	0.4 ± 0.05	0 ± 0
4	0.57 ± 0.05	0.21 ± 0
5	0.34 ± 0.07	0.13 ± 0

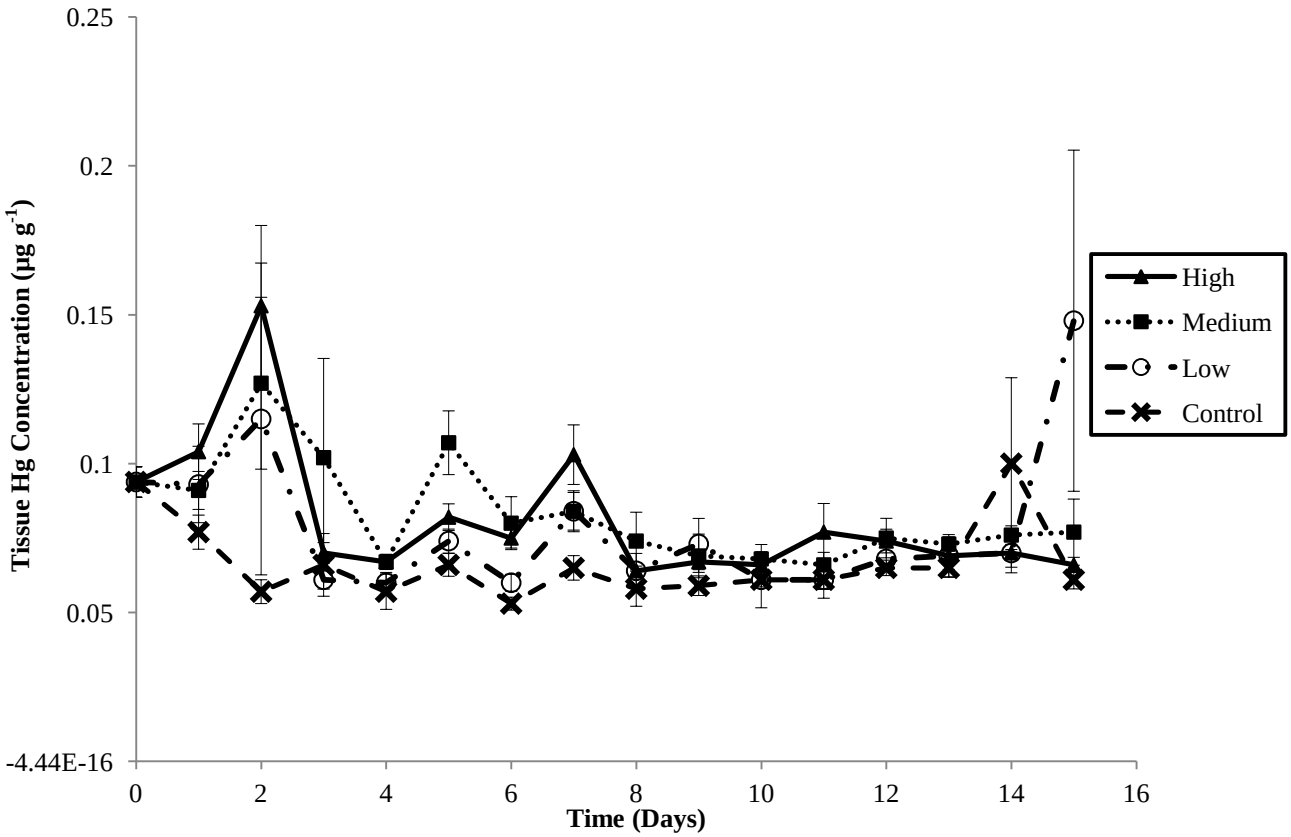

Figure 1. Mean total mercury concentration in Spanish moss samples over 15 days of exposure to elemental mercury vapor in High (30 ng l^{-1}), Medium (15 ng l^{-1}), Low (1 ng l^{-1}), and Control (ambient air, ≈ $1.6x10^{-3}$ ng l^{-1}) treatment concentrations. Error bars are ± one SEM and n=5.

APPPENDIX B

PRELIMINARY METHODS TESTING

RATIONALE

Prior to conducting laboratory and field experiments, I conducted preliminary tests of sample collection and processing protocols for Spanish moss plants. First, I conducted a study to determine if washing Spanish moss with mercury-free tap water after collection from the field would affect the measured mercury concentration in tissue. Mercury has a form, Hg (p) that is associated with atmospheric particulates that can become associated with the surface of Spanish moss plants (Schroeder and Munthe, 1998; Amado Filho et al., 2002). Therefore, washing Spanish moss may remove particulates with the particulate-associated Hg (p) from the surface of the plants, which would affect the total mercury concentration of Spanish moss tissue. I predicted washed Spanish moss would have a lower measured concentration of mercury because particulate mercury would not be included in the measurement.

Second, a test of the proper method to transport Spanish moss samples from the field to the laboratory was conducted. Elemental mercury is volatile (Schroeder and Munthe, 1998), so it is possible that mercury bound to the surface of Spanish moss plants could be volatilized to the atmosphere during transport from the field to the laboratory. However, it has been shown that nitric acid (HNO_3) can remove mercury species from the surface of plants (Rea et. al., 2000). Therefore, I wanted to know if Spanish moss samples lose mercury from tissue during transport when not placed in nitric acid. I predicted the Spanish moss samples placed in acid would have a higher mercury concentration than Spanish moss samples not placed in acid. Results of this test would

indicate the best method in which to transport collected Spanish moss samples from the field to the laboratory.

METHODS

Washing Experiment

Twenty Spanish moss samples (2.5 g each) were collected haphazardly from individual trees at Salt marsh Ecosystem Research Facility on Skidaway Island. Ten samples were washed repeatedly with mercury-free tap water and ten samples were not washed. All twenty samples were analyzed for mercury content using inductively coupled plasma mass spectrometry (ICP-MS).

Acid Treatment Experiment

Spanish moss samples were collected from Jacksonville, FL; Savannah, GA; 0.5 km from the LCP Chemicals EPA Superfund site in Brunswick, GA; Cumberland Island, GA; and Ossabaw Island, GA. At each field site, 15-17 Spanish moss samples were collected haphazardly. Each sample was collected from individual trees, and enough plant material was collected to constitute 2.5 g per sample. Samples were weighed using a field balance. After collection, Spanish moss plant samples were placed immediately into pre-weighed teflon vials with 6 ml HNO_3 (nitric acid). From each location, five additional Spanish moss samples were collected in the same manner and placed in teflon vials without nitric acid. The concentration of mercury in plant samples was analyzed in the laboratory using ICP-MS (Smith, 1993).

Statistical Analyses

Spanish moss mercury concentration data were tested for normality using the Shapiro-Wilk W test and for equal variance using Levene's test. For the washing

experiment, mercury concentration of washed samples was compared to unwashed samples using a t-test. For the acid treatment experiment, data were log transformed to meet assumptions, and then a two-factor ANOVA was conducted with field site and acid treatment as factors.

RESULTS

Washing Experiment

There was no difference in Spanish moss tissue mercury concentration due to washing samples (t_8= -0.37, p=0.71).

Acid Treatment Experiment

Acid treatment did not affect the mercury concentration of transported Spanish moss samples (F= 2.11; df=1, 92; p=0.15). However, there was a difference in mercury concentration due to site (F= 8.79; df= 4, 92; p<.001). There was no interaction effect (F=1.03; df=4, 92; p=0.40).

DISCUSSION

Results of the washing experiment indicated that washing Spanish moss does not affect total mercury concentration of tissue. This is in contrast to Calasans and Malm (1997) who found unwashed samples to be significantly higher than unwashed samples, which was suggested to be due to loss of Hg (p) from washing. However, Calasans and Malm (1997) washed samples using sonication, which may be a more thorough cleansing than the manual rinsing used in the present study. Future studies should explore more rigorous rinsing regimes to remove particles from the surface of Spanish moss.

The acid treatment experiment indicates that placing Spanish moss samples in teflon vials with acid versus teflon vials without nitric acid does not affect the mercury

concentration of sample tissue. However, a larger sample size of untreated Spanish moss may have shown differences in mercury concentration from treated samples.

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