6-11-2018

Heavy Metal Accumulation in Seagrasses in Southeastern Florida

Erin Smith
Halmos College of Natural Sciences and Oceanography, erinmsmith91@gmail.com

Thesis of
Erin Smith

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

M.S. Marine Biology

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

June 2018

Approved:
Thesis Committee

Major Professor: Amy C. Hirons

Committee Member: Dimitrios Giarikos

Committee Member: Andre Daniels

HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

Heavy Metal Accumulation in Seagrasses in Southeastern Florida

By

Erin M. Smith

Submitted to the Faculty of

Halmos College of Natural Sciences and Oceanography

in partial fulfillment of the requirements for

the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

June 2018

A Thesis Paper

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science:
Marine Biology

Erin M. Smith

Halmos College of Natural Sciences and Oceanography

Nova Southeastern University

June 2018

Approved:

Thesis Committee Approval

Major Professor: ______________________________
Amy C. Hirons, Ph.D.

Committee Member: ______________________________
Dimitrios Giarikos, Ph.D.

Committee Member: ______________________________
Andre Daniels, M.S.

Table of Contents

Acknowledgements

I would first like to thank my thesis advisor, Dr. Amy C. Hirons, of Nova Southeastern University for all of the help and guidance she has provided me throughout my graduate career. I would also like to thank Dr. Dimitrios Giarikos of Nova Southeastern University and Mr. Andre Daniels of the U.S. Geological Survey for lending their expertise throughout the course of this project. In addition, I would like to extend my gratitude to the many members of the Charismatic Megafauna and Oceanography Lab (CMOL) and NSU undergraduate students that assisted in the methodology creation, sampling process, and laboratory analysis necessary to complete this project.

I would also like to thank The Batchelor Foundation, the South Florida Chapter of the Explorer's Club, Friends of Gumbo Limbo, and the Lerner Gray Fund for Marine Research for providing funds towards the successful completion of this research.

Lastly, I would like to thank my parents, Mark and Rosanne Smith, for all the support and encouragement they have provided me throughout my academic career and my best friend, Holly de Matteo, for the constant daily support. This would not have been possible without all of your love and inspiration to continue following my dreams.

List of Figures

List of Tables

Abstract

Seagrass beds are among the most ecologically important systems in the marine environment. They provide the primary production to nearby coral reef and mangrove communities, and seagrasses comprise a large component of the diets of many marine organisms including fishes, small invertebrate species, and many protected species such as manatees and sea turtles. This consumption provides a pathway for many contaminants to enter the marine food web via the seagrasses. The coastal location of seagrass beds causes them to be especially susceptible to anthropogenic pollution, including accumulation of heavy metals, which has been shown to have many adverse health effects in the seagrasses and marine organisms that feed on them. This study assessed the heavy metal concentrations of seagrasses in three regional locations in South Florida: Port of Miami, Card Sound Aquatic Preserve, and Florida Bay. Three species of seagrasses, *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*, which comprise the majority of South Florida seagrass beds, were collected monthly for a period of one year and analyzed for ten heavy metals: (arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), zinc (Zn)). Concentrations were compared across locations, season, species, and plant part (leaves, shoots, roots, and rhizomes). Concentration ranges, in µg/g (ppm), found in seagrass tissues for all included locations, species, and plant parts were: As (0.02-2.95), Cd (0.09-10.72), Cu (0.38-33.68), Fe (1.52-1877.43), Pb (0.78-156.20), Mn (0.79-300.15), Hg (0.03-16.46), Ni (0.67-87.74), Se (0.01-4.79), Zn (1.48-669.44). Statistical analysis showed significant difference in concentrations among locations, season, species, and plant morphology.

Keywords: Seagrass; Heavy Metals; Florida, *Thalassia testudinum, Halodule wrightii, Syringodium filiforme*

Introduction

Seagrass

Seagrasses are ecologically important, widespread, marine flowering plants that are found along coastlines of tropical and temperate seas around the world at water depths up to 90 meters (Duarte, 1991; Orth et al., 2006; Short et al., 2007). Seagrass communities have been ranked among the most productive systems in coastal waters. They support rich and diverse food webs and fulfill a key role in the structure and functioning of coastal ecosystems (Schlacher-Hoenlinger et al., 1998; Duarte, 2002). Seagrass beds have also been identified as the most productive submerged marine habitat (Whelan et al., 2005). High photosynthetic activity in these systems provides a large amount of primary production necessary to marine ecosystems and helps to support major grazing and detrital food webs (Lanyon et al., 1989; Lewis et al., 2007).

This high production refers not only to the seagrasses themselves, but also to the macrophytes and algae attached to the plants. Leaves of seagrasses greatly increase the amount of surface area available for algal epiphytes to attach and grow (Bell et al., 1984; Schlacher-Hoelinger et al., 1998). Seagrass beds are also of importance to the surrounding habitats, including coral reefs, as they produce much of the organic carbon that is exported to these areas through the foraging activities of reef organisms, generating a substantial contribution to nearby food webs (Behringer et al., 2006).

Seagrass meadows perform extensive and diverse roles in coastal marine ecosystems. They help to promote water quality by filtering the water of contaminants, sequestering carbon, and lessening the turbidity of the waters by trapping sediment (Larkum et al., 2006). This sediment trapping, along with the root system of the grasses, helps to stabilize the sediment and protect the shoreline by preventing erosion. Seagrasses are so important for stabilization because they are the only aquatic plant with a root and a rhizome system found under the sediment. The seagrasses are also a necessary part of the food cycle because of their role in the trapping and cycling of nutrients as well as production of food and oxygen (Larkum et al., 2006; Short et al., 2007).

Dominant Seagrass Species in South Florida

Thalassia testudinum, commonly known as turtle grass, is one of the most abundant and important seagrass species in South Florida waters. It grows in shallow sand or mud up to a water depth of 12 meters. These beds are most successful in clear, high salinity waters around 20-30º C with minimal wave action. They form dense mats of horizontal rhizomes that can be buried up to 25 cm. This species is an important diet component of manatees, sea turtles, fish and invertebrates. Though the population is currently stable, it is threatened by coastal development along with increased sedimentation and eutrophication (Williams, 1987; Dawes and Tomasko, 1988; Fourqurean et al., 1995; Short et al., 2010a).

Halodule wrightii, commonly known as shoal grass, is very abundant locally and widely distributed around the world. It is found growing in sand or mud up to a depth of 5 meters and is often mixed in beds with other species. It is very tolerant of a wide range of environmental conditions including salinity, turbidity, disturbance, and temperature, though the optimal range for temperature is 20-30º C. The population is currently stable and may even be increasing in some areas due to its high tolerance for changing environmental conditions, including water temperature and pH. The major threats to *H. wrightii* are coastal development and mechanical destruction of the beds (Zieman et al., 1989; Fourqurean et al., 1995; Amado Filho et al., 2004; Short et al., 2010b).

Syringodium filiforme, commonly known as manatee grass, is abundant in local waters and is one of the most important habitat builders in the Florida area. It grows in sandy or muddy bottoms to a depth of 20 meters but is often found at deeper depths in clear waters. This species is very sensitive to environmental and water quality changes, and will not grow in low salinity waters, areas with high turbidity, or waters with a temperature below 20º C. It is a major food source for manatees, parrotfish, urchins, and surgeonfish. The population is currently stable but is threatened by sewage discharge, increased sedimentation, and eutrophication (Williams, 1987; Kenworthy and Schwarzschild, 1998; Short et al., 2010c).

Seagrass as a Resource

One of the most significant roles of seagrass meadows is their use as a resource for a wide variety of marine species. They provide protection from predators, habitat, and nursery grounds for many marine species, including fish, benthic organisms, and large herbivorous grazers (Duarte, 2002; Whelan et al., 2005). There are numerous federally listed species that utilize the seagrass communities, including American crocodile, green, leatherback, loggerhead, and Kemp's Ridley sea turtles, roseate tern, wood stork, bald eagle, and Florida manatee (USFWS, 2017). Abundance and biomass of key species were found to be lower in unvegetated areas when compared to nearby seagrass beds. Seagrass environments also serve as critical habitat at some point in the life cycles of many species targeted for recreational and commercial fishing (Connolly, 1995; Orth et al., 2006). These species often settle as larvae in the seagrasses which provide shelter and food during the early life stages when they are more susceptible to predation (Bell and Pollard, 1989). High abundances of prey species living among the seagrasses also lead to greater densities of predators in continuum throughout the food web. In this way, seagrass meadows are extremely important to the trophic structure of the ecosystem (Virnstein et al., 1983; Bell et al., 1984).

One of the most important resources seagrasses provide is as food for marine organisms. Over 154 species are known to feed on living seagrass, which comprised more than 10% of the diets of over half of these species, most of which are fish (Klumpp et al., 1992, Duarte, 2002; Larkum et al., 2006). The nutritive value of seagrasses is comparable to terrestrial vegetation and forage crops, and they provide similar energy levels as algae. Vaslet et al. (2012) showed that organisms that shelter in nearby environments, such as mangroves and coral reefs, will often obtain the majority of their food by grazing in seagrass beds. Food is abundantly available for these grazers in the form of seagrass leaves and periphyton. Heavy grazing on seagrasses is used by a wide variety of species, including macroinvertebrates, numerous species of fishes, and large marine herbivorous grazers such as manatees and sea turtles (Bell et al., 1984; Lanyon et al., 1989; Klumpp et al., 1992). Invertebrates comprise a major part of the marine food web while manatees and sea turtles are ecologically important species that rely on seagrasses for a large portion of their nutritional requirements (Prange and Dennison, 2000).

The primary food source within seagrass communities is epiphytic periphyton, mainly microscopic algae that grow on the surface of the leaves which contributes significantly to the trophic system in terms of photosynthetic activity. The epiphytic community usually begins with rapid colonization by micro-algae and bacteria, and later expands to include large algal species and invertebrates, such as tunicates, sponges, and crustaceans. Seagrasses contain no chemical or physical mechanisms to inhibit this colonization (Borowitzka and Lethbridge, 1989; Klumpp et al., 1992; Duarte, 2002). In one study, epiphytic algae were found to represent 46% of total system primary production, making them the most important productivity component (Moncrieff and Sullivan, 2001).

Faunal Grazing

Invertebrates

Seagrass beds contain large quantities of invertebrate species due to the high habitat complexity. Virnstein et al. (1983) found that seagrass beds contained three times the microbenthic invertebrates and thirteen times the epifaunal species found in surrounding unvegetated areas. Macrofauna also reside in the sediments among the root system where they function as nutrient recyclers and serve as prey items for species at higher trophic levels. Various nematodes, polychaetes, gastropods, isopods, crustaceans, and ostracods are included in this group. Crustaceans and mollusks are especially important contributors to biomass and energy flow and are the major link between primary producers and higher consumers. Not only do seagrasses prove to be an area for heavy grazing, they also trap detritus for utilization by small benthic invertebrates that live among the seagrasses. Decomposition of detritus from seagrass beds is also a main source of production for the coastal planktonic and benthic communities (Bell et al., 1984; Lewis, 1984; Thresher et al., 1992).

The invertebrate faunal community that lives among seagrass beds is very diverse, and studies have shown a high level of activity, even at night. These species, mainly crustaceans and polychaetes, have been shown to serve as the pollinators for the flowers of *Thalassia testudinum*. Invertebrates transfer pollen between the male and female flower organs by carrying the pollen grains on their bodies. This activity improves the reproductive success of the plants as well as helps to maintain genetic diversity within the species (van Tussenbroek et al., 2016).

Fishes

Abundances of small and large fish are greater in vegetated habitats (Connolly, 1995). In the presence or absence of predators, abundances of all fish species decreased in thinned and unvegetated plots, suggesting that predation is not the proximate cause of this habitat preference. Seagrasses provide the physical structure used as habitat, dampen hydrodynamic forces, and create greater stability (Bell and Westoby, 1986; Connolly, 1995).

Numerous species are prevalent in seagrasses, including juvenile and adult snappers, groupers, grunts, butterflyfish, gobies, and surgeonfishes. Different fish species are found at different positions within the seagrass canopy; they dwell above the meadows, on or under the leaves, and among the sediment. Locational preference appears to reflect the feeding mode and morphology of each fish species, and several species may make use of the same microhabitat. Fish reside in the seagrass beds for various lengths of time and at different life stages. Not all fish species that associate with seagrass live there permanently; many species enter the beds as ichthyoplankton and spend their early life within the canopy. The majority of species remain in the beds as juveniles and move on to other habitats as adults; however, most species remain in close proximity to the beds to take advantage of the abundant food (Weinstein and Heck, 1979; Bell and Pollard, 1989). Many of these fishes, especially parrotfishes and surgeonfishes, have been observed feeding directly on large amounts of seagrass tissue. In one case, it made up 95% of stomach contents in one individual (Randall, 1965).

Sea Turtles

The green sea turtle (*Chelonia mydas*) is an endangered, herbivorous reptile that depends predominantly on seagrasses and occasionally algae for its nutritional needs. This species is the only sea turtle in Florida that depends upon seagrasses as a food source (Lanyon et al., 1989). The green sea turtle population is currently in decline and is restricted to tropical regions, limiting their diet. Coastal Florida is an important area for these turtles as they feed and nest along the coastline. Green sea turtles are solitary feeders and it has been shown that seagrass meadows are their chief feeding location. The largest component of the green sea turtle diet is *Thalassia testudinum*, commonly known as turtle grass, which is one of the most common species in Florida seagrass beds (Lanyon et al., 1989; Zug and Glor, 1998).

Manatees

Seagrasses are a major component of the diet of the Florida manatee (*Trichechus manatus latirostris*), which is a subspecies of the West Indian manatee. The Florida manatee is a threatened species and is of special interest to researchers and conservationists because it has one of the lowest population numbers among all mammals. Manatees are year-round residents of Florida waters that move between warm and cold-water sites, often returning to the same seasonal foraging locations (O'Shea et al., 1984; Anzolin et al., 2012; USFWS, 2017). Alves-Stanely et al. (2010) found that seagrasses were the most frequently required component of the Florida manatee's diet. Manatees are grazers and will forage on the above ground parts of the seagrasses, including the leaves and shoots. They must consume large amounts of vegetation daily, up to 10% of their body weight, to support their body functions (Haynes et al., 2005).

Though manatees are fairly immune to infectious agents, they are susceptible to diseases and pollution, especially while in large aggregations. These environmental stressors cause the immune systems of the manatees to be suppressed and leaves them vulnerable to pathogens. Manatees often aggregate around municipal outfalls, which also contain potential pathogens such as endocrine disruptors. They can also be indirectly impacted by pollution due to a reduction in their food source. This will decrease the carrying capacity, cause nutritional stress, and reduce reproduction rates (O'Shea et al., 1984; Bonde et al., 2004).

Threats to Seagrass

Though seagrasses currently cover approximately 0.1-0.2% of the global ocean (Duarte, 2002), they are severely lacking in protective regulations, and are presently experiencing worldwide decline which is primarily human caused (Duarte, 2002; Orth et al., 2006). There are currently 247 marine protection areas globally that include seagrasses; however, the seagrasses themselves are rarely, if ever, singled out as the object of protection (Spalding et al., 2003; Erftemeijer and Lewis, 2006). Seagrasses are exceedingly sensitive to anthropogenic threats, and the combined pressures of direct mechanical destruction and the toxic effects of chemical pollutants place seagrasses among the most endangered ecosystems in the world. Widespread loss of seagrasses is caused by direct and indirect human impacts, as well as natural phenomenon such as large storms and grazing pressure. Direct impacts include mechanical damage caused by dredging, fishing, and anchoring, as well as eutrophication, aquaculture, siltation, coastal construction, and food web alteration. Indirect impacts on seagrasses include the effects of climate change, such as sea level rise, increase in storm frequency and intensity, and increases in UV radiation (Schlacher-Hoelinger et al., 1998; Duarte, 2002; Govers et al., 2014).

Heavy Metals

A major concern for seagrasses, especially those near highly industrialized areas, is excessive nutrient loading along with the addition of a wide range of contaminants. The coastal locations of seagrass beds increase their susceptibility to contaminant overload as they receive input from many agricultural and industrial sources via rivers or other waterways, runoff, and atmospheric deposition (Duarte, 2002; Lafabrie et al., 2007).

Heavy metals are defined as trace metals that have a high atomic weight and a density that is at least five times that of water. These metals exist in trace amounts throughout all environmental reservoirs but can cause issues for marine organisms if they are present in excess of tolerance levels (Tovar-Sanchez et al., 2010; Tchounwou et al., 2012). Some heavy metals are needed in various amounts by marine organisms to support a variety of biochemical functions, while others can be toxic in even very small amounts. These two groups are known as the essential and non-essential elements, respectively. Essential elements are generally used in a catalytic role and non-essential, or pollutant, elements do not have a functional biochemical role. These metals normally only reach high concentrations through anthropogenic activities (George, 1982; Tovar-Sanchez et al., 2010). In seagrasses, essential heavy metals include iron, copper, manganese, nickel, selenium, and zinc, while non-essential heavy metals include arsenic, cadmium, lead, and mercury (Ambo-Rappe et al., 2011; Sudharsan et al., 2012).

Heavy metals are exceptionally dangerous in the environment as they do not biodegrade, but persist, causing their concentrations to continually increase (Luy et al., 2012; Zakhama-Sraieb et al., 2016). Uptake occurs through three mechanisms - bioconcentration, bioaccumulation, and ingestion. Bioconcentration is uptake directly from the abiotic environment; bioaccumulation is the uptake from all sources, including abiotic and biotic; and ingestion occurs during feeding. Introduction into the food chain occurs via direct herbivory or indirectly through detritivore food webs (Reinfelder et al., 1998; Gray, 2002; Coelho et al., 2009; Tovar-Sanchez et al., 2010).

Sources of Heavy Metals

Heavy metals are found in the environment in four phases: dissolved, colloidal, particulate, and sedimentary; and they are constantly being cycled (Zakhama-Sraieb et al., 2016). Metals from natural and anthropogenic sources enter the marine environment mainly through runoff and atmospheric deposition. Once in the ocean, some metals can be sequestered in marine sediments and will remain biologically unavailable unless disturbed (Lafabrie et al., 2007; Fathollahzadeh et al., 2015). Those that are not sequestered, however, can be taken up by marine organisms through their gills or can enter marine plants through their roots and leaves, thereby introducing them into the food web. Trace element accumulation and the impact that the metals may have on biological systems is determined by their bioavailability which is controlled by abiotic factors. In the ocean environment these factors may include temperature, salinity, pH, and pressure with increasing depth, and the bioavailability will also vary due to the nature of the element itself, including its stability, dissolved concentration, interactions with other metals, and how it cycles throughout the environment (Reinfelder et al., 1998; Demirezen and Aksoy, 2006; Tchounwou et al., 2012).

Heavy metals are released into the environment from both natural and anthropogenic sources. All heavy metals occur naturally in the environment and many have sources in the ocean; however, they are not homogenously distributed. Anthropogenic activities largely impact their abundance and distribution, causing their concentrations in the marine environment to vary spatially and temporally (Das et al., 2003). Various human activities are known to be sources of heavy metals to the environment, including aquaculture, discharge of sewage, mining and smelting, deforestation, agriculture (herbicide or pesticide use), and various industries including leather production, shipyards, electronics, and paints (Stavros et al., 2008; Tovar-Sanchez et al., 2010; Govers et al., 2014).

Heavy metals can be released by atmospheric deposition, evaporation of metals from water resources, erosion and leaching from soils, metal corrosion, and resuspension of sediments. One of the current major source of heavy metals in the coastal zone is the dredging of contaminated sediments in ports around the world (Erftemeijer and Lewis, 2006; Tchounwou et al., 2012).

Dredging

Dredging is required in many large ports to maintain depth and aid in navigation through channels and harbor entrances. These ports require continuous deepening of the channel to maintain accessibility as a result of sedimentation (Erftemeijer and Lewis, 2006; van Maren et al., 2015). This process causes a large-scale disturbance and can result in a variety of unfavorable changes in the water and seagrass beds, including physical removal or burial of the plants, increased turbidity and sedimentation, increases in nutrient level, reduction of dissolved oxygen, shifts in trophic structure, decreased nutritional quality of associated organisms, and hydrographic changes (Lohrer and Wetz, 2003; Behringer et al., 2006, Erftemeijer and Lewis, 2006). These changes, in turn, cause numerous indirect effects. Increased turbidity inhibits phytoplankton primary production, reduces the feeding success of visual predators, and has many adverse effects on benthic species, particularly filter feeders (Essink, 1999).

Studies looking at adverse effects of dredging projects have focused on seagrass meadows or coral reefs because these areas suffer the most detrimental impacts (Erftemeijer and Lewis, 2006; Erftemeijer et al., 2012; van Maren et al., 2015). The most significant issues related to seagrass environments are mechanical damage to the beds during the project and the lasting effects caused by disturbing the sediments. Dredging of the sea floor has caused physical damage to seagrass beds by uprooting and burying the plants (Fredette and French, 2004). In addition to this damage, many of the dredged sediments are contaminated due to anthropogenic input from urbanization, tourism, agriculture, aquaculture, industries, and marine traffic, and the disturbance of this material can substantially affect water quality and increase the exposure of marine organisms to contaminants (Tovar-Sanchez et al., 2006; Hedge et al., 2009).

Metals in sediments are often considered immobile if they remain undisturbed. Resuspension of sediments leads to remobilization of these metals (Erftemeijer and Lewis, 2006; Hedge et al., 2009; Fathollahzadeh et al., 2015). This remobilization occurs when anoxic sediment is disturbed, and oxidation takes place in the water column (Hedge et al., 2009). Dredging is one of the only processes that allows metals in the solid-phase to be released into the water column as most released metals are in the dissolved phase (Kalnejais et al., 2007). Dredging causes contamination mainly through the resuspension of fine particles, such as silt and clay. Silt load is usually highest in sediments post-dredging. Many nutrients, environmental contaminants, and organic pollutants are known to selectively bind to these fine particles due to their high surface area. The increased turbidity during and post-dredging adds particulates to the waters that can act as scavengers, acquiring metals from the surrounding waters (Caccia et al., 2003; Nayar et al., 2007).

The dredged material becomes suspended during excavation, as overflow from barges and machinery during transport to the disposal site, as overspill from the hopper, and during disposal and deposition of rejected material. The now bioavailable metal ions are absorbed by marine organisms through their gills and taken up by seagrasses through their roots and leaves (Newell et al., 1998; Erftemeijer and Lewis, 2006; Nayar et al., 2007). This process provides a link between dredging-related resuspension of contaminated sediments and transfer into the marine trophic system which results in metal accumulation in marine organisms. Hedge et al. (2009) observed an increase in metal concentrations in oyster tissues with the onset of nearby dredging activities, some showing over a 100% increase. The bioavailability of metals due to dredging is site specific and the amount is dependent on the degree of sediment contamination, the volume of suspended sediment, and the duration of dredging activities (Eggleton and Thomas, 2004).

Sewage Outfalls

The concentrations of heavy metals within raw sewage can be very high, and these concentrations are not lessened through the treatment processes. Sewage has been found to be a significant source of metal dispersion into the environment and can contain a multitude of different metals, including chromium, copper, nickel, lead, and zinc, from a wide variety of sources (Carrondo et al., 1978; Gaber et al., 2011). Ocean outfalls have been used in southeast Florida for over 30 years and are utilized for the disposal of untreated or partially treated sewage into the ocean. It has been found that approximately 10% of the released sewage may remain untreated and contain metals, chemicals, and viruses. The amount of effluent being released into the global ocean can reach almost one billion gallons of sewage and other wastes per day, and though it has been speculated that this effluent does not reach the shore, it is contaminating the marine environment. The effects of this pollution are demonstrated by the presence of diseased fishes and a lack of aquatic vegetation (U.S. EPA, 1973).

Agriculture

Agricultural activities, including pesticide and herbicide use, release several heavy metals into the environment which are sequestered in the soils or incorporated by crops. At the end of the growing season, crop residues left behind can be severely contaminated. Crop residues have been shown to have higher than normal levels of cadmium, copper, nickel, and zinc (Diaz and Massol-Deya., 2003; Lee et al., 2017). In Florida one of the main agricultural pollution concerns is the elevated level of copper from herbicide used for weed control. The copper concentrations found in treated aquatic vegetation in Florida exceed the levels capable of causing negative toxicity effects in mammals (O'Shea et al., 1984).

Seasonality

Seasonality in South Florida presents as the wet and the dry seasons. The wet season occurs from June to October during which approximately seventy percent of the rainfall for the year occurs. The dry season occurs between November and May. Between these two seasons, there will be variations in water temperature and salinity and changes in the amount of runoff entering the ocean. Larger amounts of runoff will increase the pollutants entering the coastal zone. These abiotic factors will also impact the growth rate, and in turn, the biomass of aquatic vegetation (Duever et al., 1994).

Fritioff et al. (2005) found metal concentrations in plant tissues varied with both salinity and temperature. The concentrations of Cu, Cd, Pb, and Zn in two species of submerged aquatic vegetation, *Elodea canadensis* and *Potamogeton natans*, increased with increasing temperature and decreasing salinity. Concentrations were also found to increase with decreasing biomass of plant material. Metal accumulation tends to be higher in early spring when the biomass of vegetation is low, causing any given amount of metal to be assimilated by a smaller number of plants, and each plant to acquire a proportionally larger amount of metals. When biomass is at a maximum, the proportion of each metal taken up by individual plants would be smaller as the same amount would be more widely distributed.

Heavy Metals in Seagrasses

Seagrasses all over the globe, including the three species in this study, show various levels of numerous heavy metals. Seagrasses accumulate toxins both through leaf surfaces and the roots from sediments and interstitial waters. The majority of contaminants are absorbed through the roots and are then translocated to the leaves. Contamination of the seagrasses varies among plant compartments, showing different levels in the roots, rhizomes, leaves, and shoots. The metal content is generally higher in tissues located above the sediment than found below (Amado Filho et al., 2004; Llagostera et al., 2011). Seagrasses are more susceptible to heavy metal accumulation because of the composition of their cellular walls. The walls are composed of polysaccharide groups that act as a binding site for metal and metalloid cations (Brito et al., 2016).

Heavy Metals in Marine Fauna

Ward et al., 1986 concluded that seagrass leaves were likely the major route for transfer of heavy metals into marine fauna. Metals are incorporated into seagrasses can then be transferred into the marine food web through herbivory (Amado Filho et al., 2004). Though many metals are needed in trace amounts by marine organisms for biological processes, they can be toxic above a certain threshold, and at some level, all heavy metals become toxic. Aquatic organisms are subject to chronic and episodic exposure to heavy metals (Rainbow, 1985; Reinfelder et al., 1998). Though the research on toxicity limits and direct consequences of heavy metal contamination is limited, many studies have shown correlation between contamination and adverse health effects (Bryan, 1971; Das et al., 2003; Deforges et al., 2016).

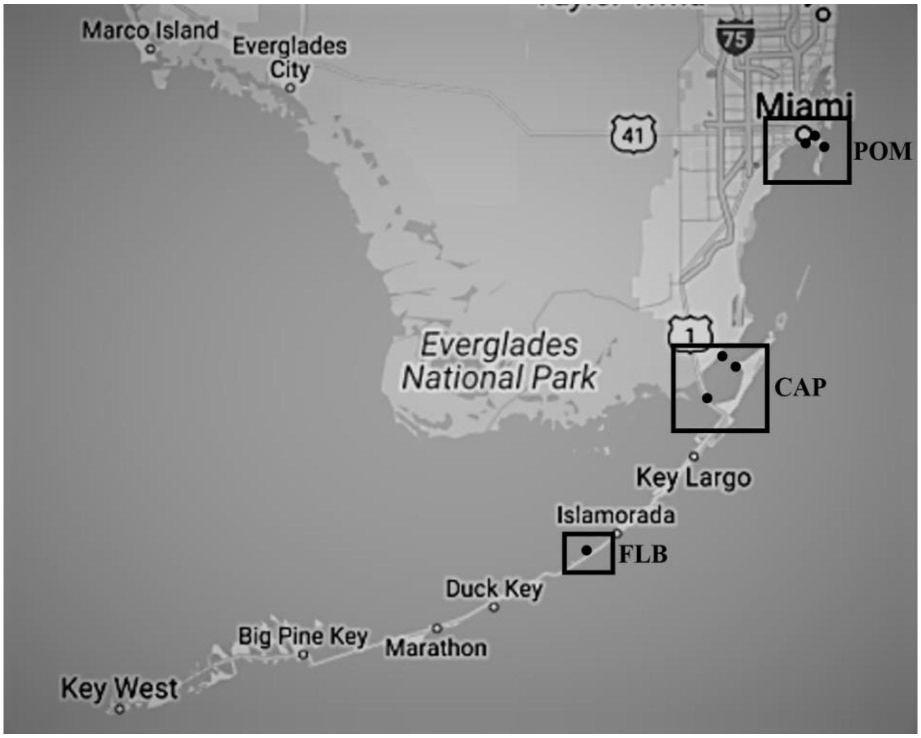
Materials and Methods

Study Area

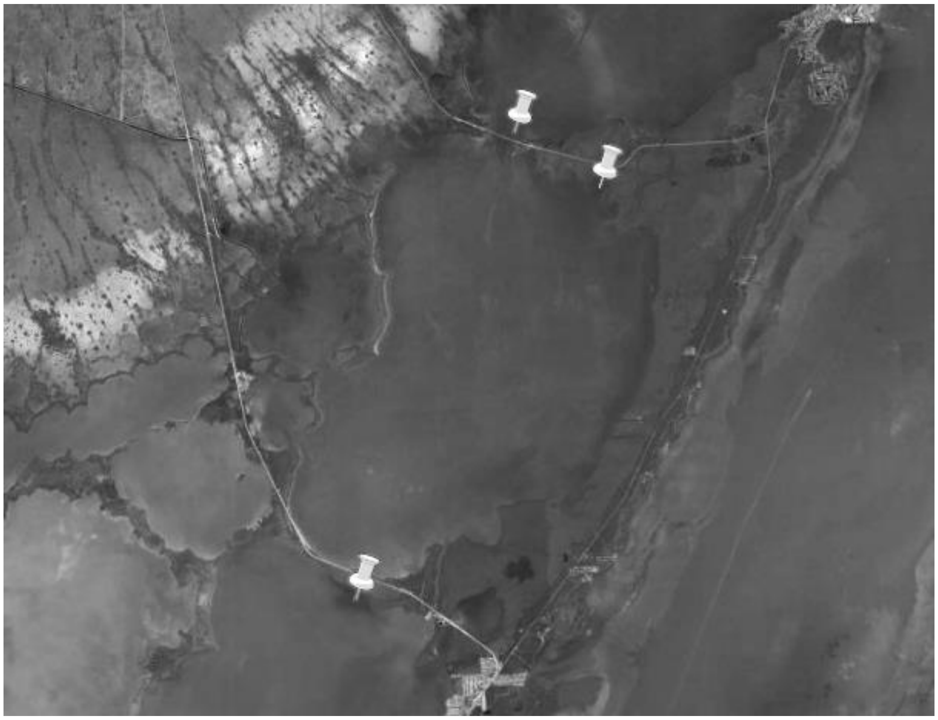
Samples of *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* were collected from three regional locations (Figure 1a). The first location was within two miles of the Port of Miami (POM) (Figure 1b), which is an extremely important seaport and is the second most important economic center in Miami-Dade County. The port is 520 acres and has a depth of approximately 52 feet (16 m). It supports nearly 225,000 jobs and contributes approximately $30 billion to the local economy. It is also known worldwide as one of the most important cruise and cargo centers. The recent PortMiami Deep Dredge project was completed by the U.S. Army Corps of Engineers in September 2015 (PortMiami, 2018). Among environmental concerns about the project was the threat of contaminated sediment disturbance and resuspension, which is known to be a source of heavy metals to aquatic vegetation (Tovar-Sanchez et al., 2006; Hedge et al., 2009). The port is also threatened by high levels of industrial and suburban runoff, which increases during the wet season (Miami-Dade Seaport Business Plan, 2014). Samples were collected from two sites around Virginia Key and one site adjacent to Rickenbacker Causeway. All sites were approximately 2.5 miles from the mouth of the port.

The second location was within the Card Sound portion of Biscayne Bay Aquatic Preserve (CAP) (Figure 1c), which extends from the headwaters of the Oleta River south to Key Largo. The region contains approximately 17,000 acres of lagoon and numerous mangrove islands and was designated a state preserve since 1975. The seagrasses here are major feeding areas for wading birds and nursery ground for juvenile fish and invertebrates, including some of commercial interest (Florida DEP, 2017). Samples were collected from three locations around Barnes Sound, the body of water between Card Sound Road and US Highway 1.

The third sampling location was in Florida Bay (FLB) (Figure 1d). Samples were collected from one site off the north shore of Islamorada. Florida Bay is the body of

a) 
Marco Island
Everglades City
75
41
Miami
POM
Everglades National Park
1
CAP
Key Largo
Islamorada
FLB
Duck Key
Big Pine Key
Marathon
Key West

b) 

c) 

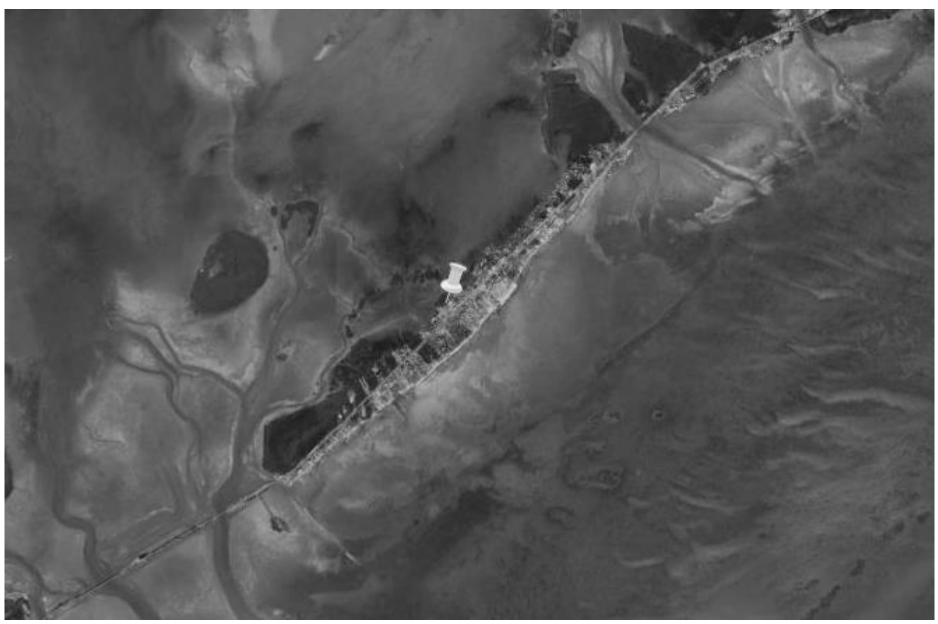
d) 

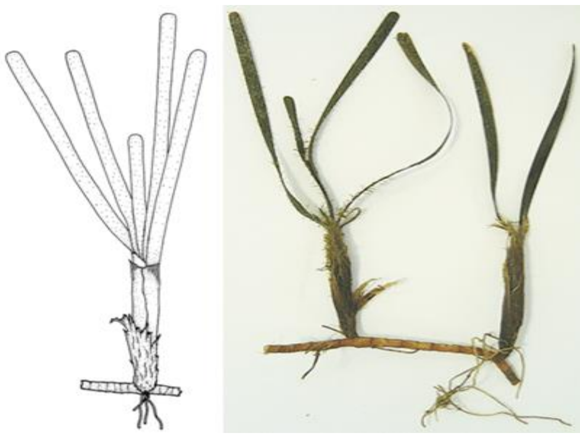
Figure 1. Locations from which samples were collected: a) Full view of all three regional locations, b) Port of Miami (POM), c) Card Sound Aquatic Preserve (CAP), and d) Florida Bay (FLB). White pins represent individual collection sites within each regional location.

water that lies between the Everglades and the Florida Keys, and is approximately 1100 square miles. The water is brackish as a result of Everglades freshwater drainage mixing with salty water from the Gulf of Mexico. The area is composed of seagrass meadows, hard bottom, and mangrove islands, and is home to a wide variety of marine species (USGS, 2013). Florida Bay does not contain large scale industry or shipping operations but is exposed to high volumes of agricultural runoff passing through southern Florida, exposing the area to a wide range of contaminants.

The closest prevailing current in this area is the Florida Current, which is considered the official beginning of the Gulf Stream and carries waters from the Florida Keys northward along the Atlantic Ocean coastline. This transport creates the potential for exogenous contaminants to be incorporated into Florida's seagrasses. The innermost edge of this current in Miami is thought to lie approximately 10 miles offshore with significant meanders that can be miles wide and shift the current daily or weekly (Leaman et al., 1987; Gyory et al., 2013).

Sampling Design

Three species of seagrasses, *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme* (Figure 2), were collected from each site monthly in 2017 and included both the wet (June-October) and dry (November-May) seasons. Sampling was not possible during September 2017 due to the impact of Hurricane Irma. Global Positioning System (GPS) identified each sample site and allowed for identical location sampling each month. Collections took place during ebb to slack low tide resulting in a water depth range of 5 to 15 inches (13-38 cm). Abiotic data, including temperature and salinity, were measured with an environmental YSI meter (model #030130). Multiple individuals of each species were collected from within a randomly placed 0.5m grid using a 25 cm^2 shovel sieve. Entire plants, including leaves, shoots, roots, and horizontal rhizomes, were removed for analysis by shovel (Table 1). Samples were rinsed with seawater in a box sieve, placed in individual plastic bags, and frozen in a standard freezer (-20° C) until processed.



a)

b)

c)

Figure 2. Seagrass species collected: a) *Thalassia testudinum* (turtle grass); b) *Halodule wrightii* (shoal grass); c) *Syringodium filiforme* (manatee grass). Source: http://texasseagrass.org/TxSeagrasses.html

Table 1. Number of samples collected and analyzed for each species and plant part.

	Leaves/Shoots	**Roots/Rhizomes**	**Leaves with Epiphytes**	**Total**
Thalassia testudinum	60	60	60	180
Halodule wrightii	70	70	X	140
Syringodium filiforme	39	39	X	78

In the laboratory, specimens were separated by species and plant part, rinsed three times with deionized water, and cleaned of epiphytes and other particles with the edge of a clean glass slide. Leaves with attached epiphytes were also analyzed to determine epiphyte contribution of metals to species grazing in the seagrass beds. Samples were dried for a minimum of 4 hours in an Isotemp Vacuum Oven (Model 282A) at 75°C and $1x10^{-3}$ torr, manually ground into a composite powder consisting of multiple individual plants, and weighed to approximately 0.2 g of dry weight. They were then digested in 8ml of 99.999% metals basis nitric acid and 2 ml of concentrated sulfuric acid until plant material was dissolved and a clear liquid remained. The solution was then diluted to a total volume of 50 ml with ultrapure (18.2 MOhm-cm) water. Heavy metal analysis was conducted using a Shimadzu AA-6200 Atomic Absorption Flame Emission Spectrophotometer equipped with a Hydride Vapor Generator (Shimadzu, HVG-1) (Table 2). Samples were tested for 10 heavy metals; As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn. These elements were chosen based on their presence in the common contaminant sources in South Florida, including industrial and municipal wastes, sewage, and agriculture. They are also thought to have negative health effects on the marine organisms that feed on seagrasses at high levels (Bryan, 1971; Rainbow, 1985; Das et al., 2003; Deforges et al., 2016).

Permitting

Permits for seagrass collection were provided by NOAA National Marine Sanctuaries- Florida Keys National Marine Sanctuaries (Permit # FKNMS-2016-133), Florida Fish and Wildlife Conservation Commission (Permit # SAL-17-1865-SR), and Crocodile Lake National Wildlife Refuge (Permit # 41581-2017-01).

Results

Average heavy metals concentrations for all specimens ranked as follows: iron > zinc > manganese > lead > nickel > copper > mercury > cadmium > selenium > arsenic. Concentration values for all metals combined ranged from 0.02 to 1877.43 µg/g. Mean, range, and standard deviation of each heavy metal separately are presented in Table 3.

Table 2. Parameters used on the AA-6200 including number of repetitions, maximum number of repetitions, relative standard deviation limit, and standard deviation limit.

	Number of Reps.	Max Number of Reps.	RSD Limit	SD Limit
Blank	2	2	99.90	0.000
Standard	2	3	5.00	0.005
Sample	3	5	7.00	0.008
Reslope	2	3	5.00	0.005

Table 3. Range, mean, and standard deviation (µg/g) of each heavy metal in *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* across all locations, seasons, and plant parts.

	As	**Cd**	**Cu**	**Fe**	**Pb**	**Mn**	**Hg**	**Ni**	**Se**	**Zn**
Range (µg/g)	0.02-2.95	0.09-10.72	0.38-33.68	1.52-1877.43	0.78-156.20	0.79-300.15	0.03-16.46	0.67-87.74	0.01-4.79	1.48-669.44
Mean (µg/g)	0.59	1.48	9.49	222.38	19.40	32.14	1.96	11.15	0.61	76.03
Std. Deviation (µg/g)	0.51	1.19	6.83	249.83	26.01	40.23	2.64	14.25	0.84	57.55

Heavy metals were detected in 79% of samples overall, 71% contained arsenic, 73% contained cadmium, 96% contained copper, 100% contained iron, 62% contained lead, 97% contained manganese, 74% contained mercury, 62% contained nickel, 48% contained selenium, and 100% contained zinc. In this study, no correlation existed between heavy metal concentrations and salinity or water temperature; however, freshwater input into the coastal environmental all along the Florida margin did show correlation with changes in metal concentrations. Significant differences in heavy metal concentrations were seen across location, season, species, and plant part (Table 4). The non-parametric Kruskal-Wallis and pairwise Wilcoxon rank sum tests were used to investigate these differences.

Correlation among Heavy Metals

Significant correlations at the $p<0.05$ and $p<0.001$ levels existed among all ten metals for at least one metal (Table 5). Concentrations of arsenic, copper, iron, and zinc were significantly correlated ($p<0.001$) with each other while a strong correlation existed solely between cadmium and lead concentrations. Significant correlation between metals at $p<0.05$ occurred for cadmium, copper, iron, nickel and lead. The only significant relationship for mercury existed with selenium ($p<0.05$). Correlated metal relationships are likely due to the metals originating from similar sources or similar accumulation rates in the seagrasses.

Location

Significant differences among regional locations were seen in copper ($X^2 = 108.0$, $df = 2$, $p < 0.001$), iron ($X^2 = 14.4$, $df = 2$, $p<0.001$), manganese ($X^2 = 44.3$, $df = 2$, $p<0.001$), and zinc ($X^2 = 73.4$, $df = 2$, $p < 0.001$). Copper concentrations were higher in FLB than POM ($p<0.001$) or CAP ($p<0.001$) and were higher in POM than CAP ($p<0.001$). Iron concentrations were higher in CAP ($p=0.01$) and POM ($p<0.001$) than FLB, but no difference existed between CAP and POM. Manganese concentrations were higher in CAP than FLB ($p<0.001$) or POM ($p<0.001$); no difference existed between

Table 4. Significance for each heavy metal among the four main variables: location, season, species, and plant morphology (NS- not significant, $p<0.05$, or $p<0.001$).

	As	**Cd**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
Location	NS	NS	<0.001	<0.001	NS	<0.001	NS	NS	NS	<0.001
Season	NS	<0.001	<0.05	<0.001	<0.001	<0.05	<0.001	<0.05	<0.001	<0.001
Species	NS	NS	NS	<0.05	NS	<0.001	NS	NS	NS	NS
Plant Morphology	NS	NS	<0.001	<0.001	NS	<0.001	NS	NS	<0.05	<0.001

Table 5. Correlation coefficients (r) among the concentrations of the 12 heavy metals in all combined seagrass tissues. (*significant at p<0.05; **significant at p<0.001).

	As	**Cd**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
As	X	-0.02	0.01	-0.06	-0.01	-0.02	-0.01	-0.01	-0.01	0.13**
Cd		X	0.07*	0.09*	0.02	0.06	-0.03	0.12	0.00	-0.02
Cu			X	0.07*	0.02	0.04	0.15**	0.04	0.01	0.30**
Fe				X	0.01	0.01	0.09*	0.09*	0.04	0.13**
Hg					X	0.03	0.06	0.00	0.08*	0.03
Mn						X	0.09*	0.02	0.00	0.02
Ni							X	0.09*	0.03	0.07
Pb								X	0.09*	0.01
Se									X	0.03
Zn										X

concentrations in FLB and POM. Zinc concentrations were higher in FLB than POM ($p<0.001$) or CAP ($p<0.001$) and higher in POM than CAP ($p<0.001$). Annual mean and standard deviation of each heavy metal for all tissue samples by location are presented in Table 6. The inclusion of multiple variables likely explains the large deviations.

Season

Arsenic was the only metal not significantly affected by season. During the wet season, significantly higher concentrations of cadmium ($X^2 = 21.3$, df = 1, $p<0.001$), copper ($X^2 = 6.6$, df = 1, p = 0.009), iron ($X^2 = 66.4$, df = 1, $p<0.001$), lead ($X^2= 9.04$, df = 1, p = 0.002), manganese ($X^2 = 7.54$, df = 1, p = 0.006), mercury ($X^2 = 49.9$, df = 1, $p<0.001$), nickel ($X^2 = 16.9$, df = 1, $p<0.001$), and selenium ($X^2 = 62.1$, df = 1, $p<0.001$) were found. Zinc was the only heavy metal with significantly higher concentrations during the dry season ($X^2 = 41.8$, df = 1, $p<0.001$) (Table 7).

Seagrass Species

Eight of the ten heavy metals tested showed no significant variability among the three seagrass species. Species had a significant effect on two heavy metals, iron ($X^2 = 8.13$, df = 2, p = 0.01) and manganese ($X^2 = 46.7$, df = 2, $p<0.001$). Iron concentrations were significantly higher in *H. wrightii* than *T.testudinum* (p=0.01) but no other significant differences existed between either species and *S. filiforme*. Manganese concentrations were significantly higher in *T.testudinum* than *H.wrightii* ($p<0.001$) and *S.filiforme* ($p<0.001$) and significantly higher in *H.wrightii* than *S.filiforme* (p=0.004). A comparison of each heavy metal among the three seagrass species collected is shown in Figure 3.

Morphology

Five metals demonstrated no significant difference among seagrass parts but those that did included copper ($X^2 = 21.6$, df = 2, $p<0.001$), iron ($X^2 = 24.3$, df = 2, $p<0.001$), manganese ($X^2 = 232.7$, df = 2, $p<0.001$), selenium ($X^2 = 6.51$, df = 2, p = 0.03), and zinc ($X^2 = 9.10$, df = 2, p-value = 0.01). Copper concentrations were higher in leaves with or without epiphytes than roots ($p<0.001$), but there was no difference in concentrations

Table 6. Annual mean and standard deviation (µg/g) of each heavy metal in the Port of Miami (POM), Card Sound Aquatic Preserve (CAP), and Florida Bay (FLB).

	As	Cd	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Port of Miami	0.58 ± 0.55	1.68 ± 1.51	10.8 ± 6.11	258.6 ± 288.6	2.22 ± 6.14	19.1 ± 18.1	9.16 ± 6.95	15.9 ± 14.0	0.66 ± 0.94	86.3 ± 51.7
Card Sound Aquatic Preserve	0.59 ± 0.43	1.27 ± 0.72	6.13 ± 4.76	199.6 ± 207.2	2.36 ± 3.25	49.1 ± 55.5	14.7 ± 21.6	22.8 ± 32.5	0.59 ± 0.75	54.2 ± 32.1
Florida Bay	0.61 ± 0.60	1.26 ± 0.52	21.2 ± 7.01	97.0 ± 68.5	2.32 ± 2.56	17.7 ± 18.5	9.21 ± 11.0	22.9 ± 38.3	0.28 ± 0.09	145.2 ± 122.5

Table 7. Mean and standard deviation (µg/g) of each heavy metal in the wet (June-October) and dry (November-May) seasons.

	Wet	**Dry**
As	0.57 ± 0.44	0.59 ± 0.53
Cd	1.87 ± 1.55	1.20 ± 0.73
Cu	10.17 ± 6.30	9.08 ± 7.09
Fe	336.67 ± 301.54	155.36 ± 183.14
Hg	4.08 ± 3.82	1.60 ± 5.02
Mn	42.72 ± 57.04	26.52 ± 29.19
Ni	19.56 ± 23.62	7.07 ± 3.77
Pb	31.02 ± 37.83	12.20 ± 8.53
Se	1.80 ± 1.16	0.29 ± 0.20
Zn	55.26 ± 30.92	87.84 ± 65.61

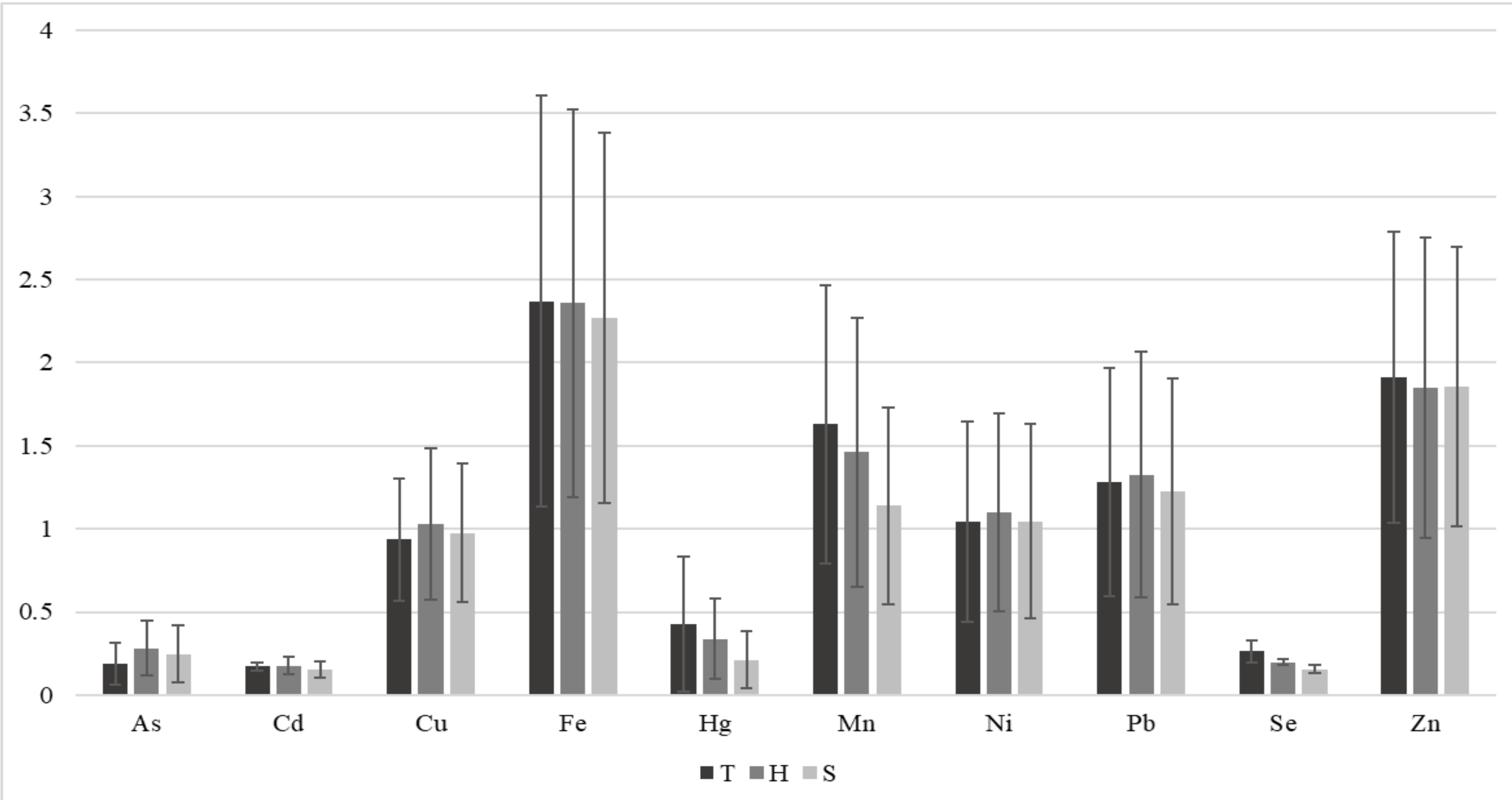


Figure 3. Mean and standard deviation (µg/g) of each heavy metal in *Thalassia testudinum* (T), *Halodule wrightii* (H), and *Syringodium filiforme* (S). Due to the wide range in values, the concentrations were plotted on a logarithmic scale for comparative purposes.

between leaves with and leaves without epiphytes. Iron concentrations were significantly higher in leaves with epiphytes than cleaned leaves ($p<0.001$) or roots ($p=0.005$) and higher in cleaned leaves than roots ($p<0.001$). Manganese concentrations were significantly higher in leaves with epiphytes than cleaned leaves ($p<0.001$) or roots ($p<0.001$), and concentrations in cleaned leaves were higher than those found in roots ($p<0.001$). Selenium concentrations were significantly higher in roots than leaves with epiphytes ($p=0.03$), but there was no difference between leaves with or without epiphytes or cleaned leaves and roots. No significant difference in zinc concentrations between cleaned leaves and those with epiphytes was found, though both had higher concentrations than roots ($p= 0.02$ and 0.03, respectively). Annual mean and standard deviation of each heavy metal in cleaned leaves, leaves with epiphytes, and roots and rhizomes in the three species analyzed is shown in Table 8. Iron, manganese, and zinc were the only heavy metals to have significantly higher concentrations in leaves with attached epiphytes (Figure 4).

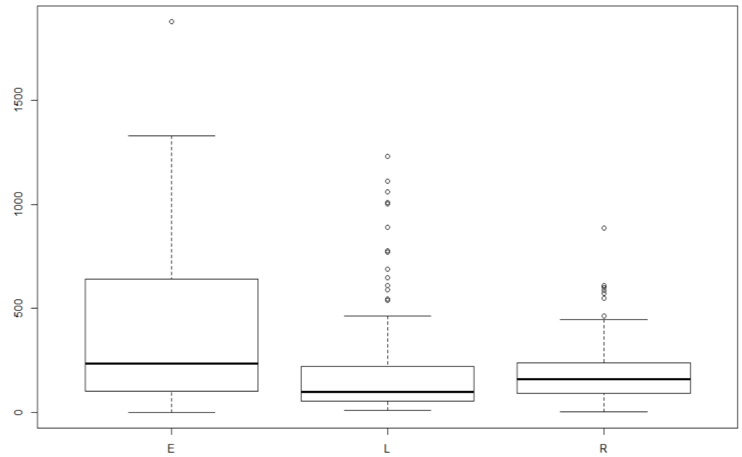
Interactions

No significant heavy metal relationship existed between seagrass species and location or species and plant part. Metal concentrations among the three species did vary between wet and dry season. Se concentrations ($F=3.69$, $p=0.027$) were higher during the wet season in *T.testudinum* ($F=63.1$, $p<0.001$), *H.wrightii* ($F=99.55$, $p<0.001$), and *S. filiforme* ($F=27.8$, $p<0.001$).

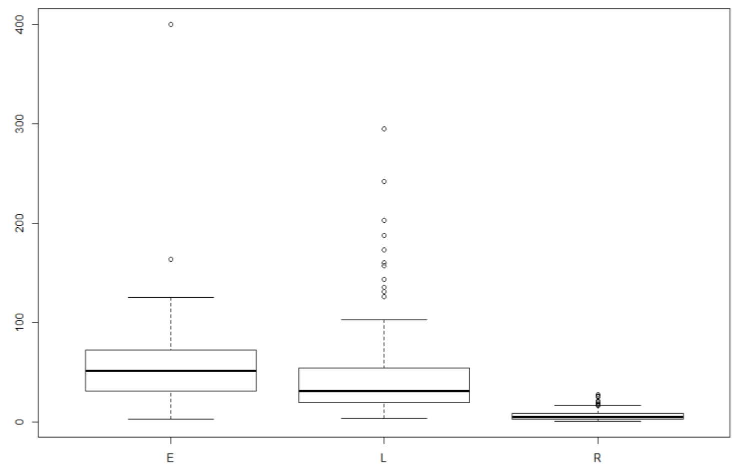
Metal concentrations for all species combined at each location and season had a significant impact on iron ($F= 6.45$, $p=0.001$), lead ($F=8.22$, $p=0.0003$), nickel ($F=6.93$, $p= 0.001$), and zinc ($F=7.55$, $p=0.0006$). Concentrations of iron were higher in the wet season in POM ($F=48.4$, $p<0.001$), CAP ($F=8.92$, $p=0.003$), and FLB ($F=12.7$, $p=0.001$). Concentrations of lead were higher during the wet season in POM ($F=5.1$, $p=0.02$) and CAP ($F=29.3$, $p<0.001$). Concentrations of nickel were higher during the wet season in POM ($F=13.95$, $p<0.001$) and CAP (26.1, $p<0.001$). Concentrations of zinc were higher in the dry season in POM ($F=44.8$, $p<0.001$) and CAP ($F=7.34$, $p=0.007$).

Table 8. Mean and standard deviation (µg/g) of leaves/shoots and roots/rhizomes of *Thalassia testudinum, Halodule wrightii*, and *Syringodium filiforme*, and leaves with epiphytes of *Thalassia testudinum*.

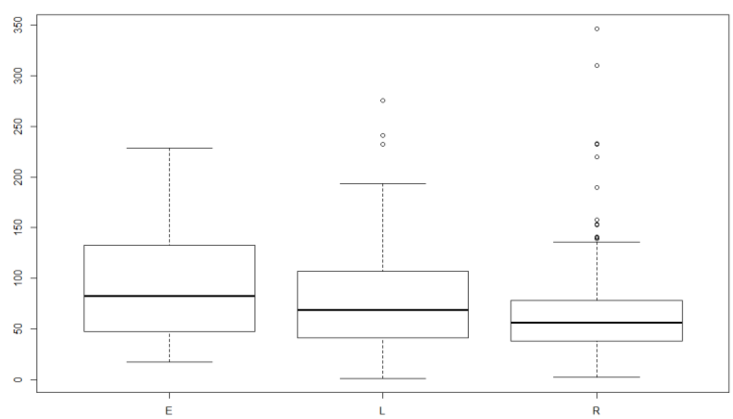
Species		**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
Thalassia testudinum	Leaves/ Shoots	0.51 ± 0.40	1.56 ± 0.92	9.55 ± 5.53	110.35 ± 167.59	2.44 ± 5.58	54.50 ± 43.32	12.33 ± 18.41	20.23 ± 22.18	0.64 ± 0.97	86.68 ± 53.61
	Roots/ Rhizomes	0.75 ± 0.71	1.42 ± 1.14	6.92 ± 4.74	171.15 ± 114.62	1.83 ± 8.97	7.76 ± 5.86	11.23 ± 16.87	17.69 ± 20.12	0.67 ± 0.26	79.88 ± 64.97
	Leaves with Epiphytes	0.66 ± 0.48	1.47 ± 1.31	9.20 ± 5.38	416.78 ± 410.40	3.55 ± 2.10	61.93 ± 53.72	9.82 ± 12.63	19.23 ± 27.82	0.28 ± 0.68	99.65 ± 48.20
Halodule wrightii	Leaves/ Shoots	0.54 ± 0.50	1.53 ± 0.97	12.85 ± 8.70	256.70 ± 260.03	2.37 ± 3.15	50.21 ± 49.74	12.29 ± 14.90	21.67 ± 31.24	0.56 ± 0.96	79.23 ± 81.87
	Roots/ Rhizomes	0.50 ± 0.42	1.50 ± 1.49	8.52 ± 7.10	198.73 ± 147.66	1.96 ± 2.90	7.26 ± 5.31	12.87 ± 16.15	20.66 ± 28.79	0.73 ± 0.83	61.92 ± 34.04
Syringodium filiforme	Leaves/ Shoots	0.58 ± 0.41	1.31 ± 1.03	11.11 ± 7.54	178.01 ± 191.71	1.61 ± 2.15	21.48 ± 16.46	12.03 ± 17.04	17.09 ± 16.96	0.81 ± 0.86	82.36 ± 52.67
	Roots/ Rhizomes	0.55 ± 0.49	1.59 ± 1.48	7.80 ± 5.69	193.84 ± 142.46	1.63 ± 2.24	4.68 ± 4.41	9.88 ± 11.38	16.53 ± 28.68	0.55 ± 0.88	61.35 ± 41.01



a)



b)



c)

Figure 4. Boxplots displaying the mean concentrations (µg/g) of a) iron, b) manganese, and c) zinc in each plant part including leaves with attached epiphytes (E), cleaned leaves (L), and roots and rhizomes (R).

Location and plant part had a significant impact on iron ($F=2.55$, $p=0.03$), manganese ($F=9.46$, $p<0.001$) and zinc ($F=4.23$, $p=0.002$). Concentrations of iron were higher in leaves with epiphytes in POM ($F=16.3$, $p<0.001$) and CAP ($F=10.8$, $p<0.001$). Concentrations of manganese were higher in leaves with epiphytes in POM ($F=85.9$, $p<0.001$), CAP ($F=6.05$, $p=0.008$), and FLB ($F=43.5$, $p<0.001$). Concentrations of zinc were higher in leaves with epiphytes in POM ($F=9.16$, $p<0.001$) and FLB ($F=6.15$, $p=0.002$).

The interaction between season and plant part had a significant impact on iron ($F=6.23$, $p=0.002$) and manganese ($F=3.76$, $p=0.02$). Concentrations of iron were higher during the wet season in cleaned leaves ($F=51.7$, $p<0.001$), leaves with epiphytes ($F=8.50$, $p=0.004$), and roots ($F=19.5$, $p<0.001$). Concentrations of manganese were higher during the wet season in leaves ($F=12.3$, $p<0.001$) and roots ($F=13.1$, $p<0.001$).

Discussion

The heavy metals analyzed in this study were chosen due to their commonality in coastal communities. All ten metals were found in detectable concentrations in all three species of seagrass tested. Heavy metal concentrations did not show any correlation to the abiotic factors collected; neither water temperature nor salinity appeared to have an impact on the accumulation of heavy metals in seagrasses. Though previous studies have found opposite results (Fritioff et al., 2005), the variation in environmental factors in this study were very small by comparison. In this study bottom water temperature ranged from 22.9º C in January to 33.9º C in July with a range of 11º C. Salinity showed more variation, ranging from 22.5 to 38.8‰ with a range of 16.3‰. We can only speculate that these differences were not large enough to enact changes in the accumulation rates.

Results of this study were compared to finding from various other locations and seagrass species (Table 9). Concentrations of arsenic, cadmium, iron, and manganese were generally lower than those found in previous studies, especially when compared to concentrations in *H.ovalis* from Jordan or *Z.capricornii* from Australia. Zinc concentrations were lower than those found along the Australian coast, the Mediterranean

Table 9. Means and standard deviation (μg/g) of heavy metal concentrations (μg/g) reported from various locations and species compared to results from this study.

	Species	As	Cd	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
This Study (Florida, USA)	*T. testudinum*	0.65 ± 0.56	1.49 ± 1.12	8.62 ± 5.37	234.0 ± 298.4	2.65 ± 6.47	42.7 ± 47.0	11.1 ± 16.1	19.1 ± 23.5	0.55 ± 0.74	81.9 ± 55.8
This Study (Florida, USA)	*H. wrightii*	0.52 ± 0.46	1.51 ± 1.25	10.67 ± 8.22	229.58 ± 214.91	2.16 ± 3.04	28.9 ± 41.5	12.6 ± 15.5	21.2 ± 30.1	0.64 ± 0.92	70.8 ± 63.4
This Study (Florida, USA)	*S. filiforme*	0.57 ± 0.46	1.43 ± 1.25	9.46 ± 6.88	183.53 ± 168.22	1.67 ± 2.19	13.8 ± 15.2	11.1 ± 14.8	16.8 ± 23.1	0.68 ± 0.87	72.0 ± 48.4
Barwick & Maher (2003) (Australia)	*Z.capricornii*	1.20 ± 0.1	10.0 ± 0.5	9.40 ± 0.5	-	-	-	-	1.70 ± 0.2	0.38 ± 0.08	133 ± 20
Brito et al., 2016 (Brazil)	*H. wrightii*	2.78	0.57	6.50	-	-	-	5.65	2.68	-	20.1
Campanella et al. (2001) (Mediterranean)	*P. oceanica*	-	2.22 ± 0.75	11.6 ± 6.1	-	-	-	-	0.91 ± 0.23	-	112 ± 5.0
Lafabrie et al. (2007) (Mediterranean)	*P. oceanica*	-	5.38 ± 0.14	-	-	0.13 ± 0.00	-	60.30 ± 3.67	1.8 ± 0.00	-	-
Wahbeh (1984) (Jordan)	*H. ovalis*	-	5.1 ± 0.6	-	29125.9 ± 6865.4	-	244.5 ± 62.2	-	-	-	217.9 ± 53.5

Sea, and Jordanian waters. Concentrations of copper and selenium were comparable to those found in these studies, while nickel concentrations were higher than those found off Brazil but much lower than those from the Mediterranean Sea. Lead and mercury concentrations were consistently higher than those found in other studies (Wahbeh, 1984; Barwick & Maher, 2003; Lafabrie et al., 2007; Brito et al., 2016).

Correlation between metals showed a significant positive correlation relationship between mercury and selenium, which are known to have strong antagonistic effects in mammals. Metal handling strategies can be very different between taxa, and this difference may be due to plants containing different lipid or protein classes than mammals and would therefore bioaccumulate these metals differently (Nichols and Johns 1985; Khan and Wang, 2010). The two major heavy metal-binding protein classes in plants are phytochelatins and metallothioneins. Phytochelatins are peptides that have been identified across various plant species. Though a small number of microorganisms have also been found to contain these proteins, they appear to be more functional in plants than animal tissues. Metallothioneins are polypeptides that were originally thought to be animal-specific ligands. It is now apparent that various categories of metallothioneins are found in plants, though they do not appear to serve the same purpose as they do in animals (Steffens, 1990; Cobbet and Goldsbrough, 2002). This difference in binding ligands may also help to explain the variation in heavy metal behavior in plant tissues.

Location

Seagrasses in the Port of Miami presented with high levels of iron. Iron can originate from natural sources, such as erosion and leaching of the Earth's crust. However, the main sources of iron in this area are anthropogenic, specifically sewage discharge, fertilizers, and herbicides. Of the three sampling locations, Miami is the most highly populated, with a total population of approximately 400,000 people in 2017. Miami-Dade County also contains approximately 158,000 homes with yards and over 81,000 acres of agricultural land (USDA, 2012). The prominent heavy metals found in seagrasses in Card Sound Aquatic Preserve were manganese followed by iron and arsenic. All three of these metals are related to agricultural activities, including fertilizer, herbicides, and pesticides. (Zhang et al., 1997; He et al., 2004). The seagrasses in Florida Bay had high concentrations of copper and zinc, both of which are also related to agricultural activities. Drainage through this area begins in the Everglades Agricultural Area (EAA), just south of Lake Okeechobee, which is an area of over 700,000 acres of farmland. Water flow from the EAA down through the Everglades is currently controlled by a series of manmade canals (Guardo et al., 1995). The crops in this area consist mainly of sugarcane, but seasonal vegetables are also grown. Agricultural runoff that originates in the EAA croplands would transport fertilizers and herbicides down through the Everglades and into Florida Bay and the Card Sound area (Snyder and Davidson, 1994).

Major Sources

Runoff

These data showed that seasonality had a significant impact on heavy metals concentrations in all locations. Impacts of seasonality are related to the change from the dry season to the wet season and the amount of run-off entering the coastal zone. Runoff is known to collect and transport potential pollutants such as sediment, pesticides and herbicides, metals, and petroleum by-products (USGS, 2016). Eight of the ten heavy metals presented had significantly higher concentrations during the wet than dry season. This is presumably related to the increase in run-off transport of pollutants and contaminants that accumulate on the ground and in soils and delivering them to the ocean, either directly or through other waterways. The creation of manmade canals throughout South Florida facilitates this transport. Data from the closest South Florida Water Management District (SFWMD) rain gauge in Miami-Dade County show a major increase of approximately 480% in rainfall in June 2017 which continues until a drop begins in November 2017. This pattern correlates to the spikes in concentrations of cadmium, copper, iron, lead, manganese, mercury, nickel, and selenium (Figure 5). These results also show that seasonality had less of an impact in FLB than POM and CAP. This

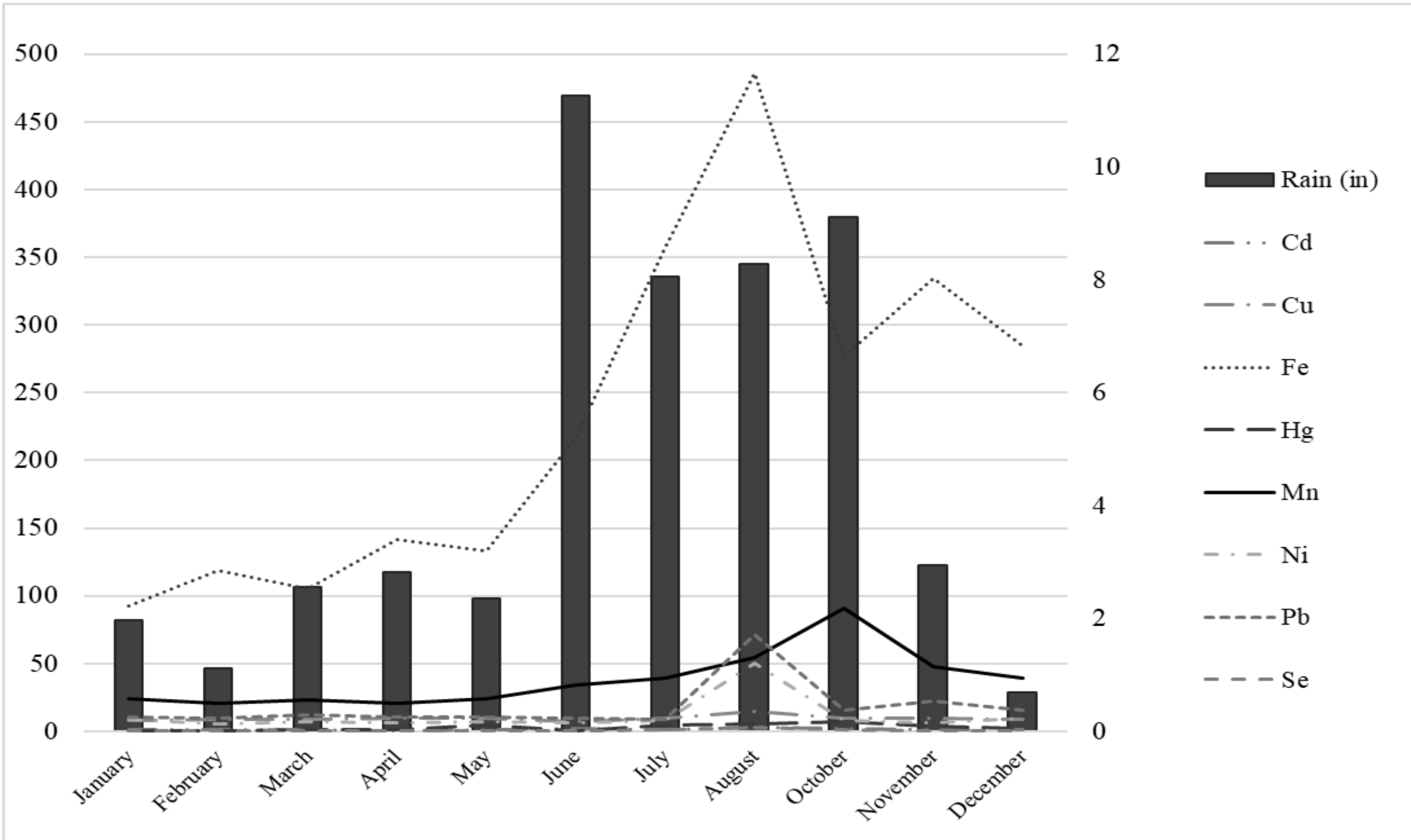


Figure 5. Comparison of rainfall between the wet (June-October) and dry (November- May) seasons in inches from the nearest SFWMD rain gauge in Miami-Dade (Gauge ID: Dade) to the mean concentrations (µg/g) of the eight metals significantly impacted by seasonality. The most noticeable difference with the seasons was seen in iron concentrations, which were also higher overall than all other metals.

could be due to the lower amount of land in close proximity to this site, leading to a lesser volume of contaminants entering the waterways.

Agriculture

Agriculture is an important part of Florida's economy, with cropland reaching 2,744,064 acres according to the USDA 2012 census. Exports from these lands include corn, cotton, sugarcane, peanuts, and citrus. Farming practices in Florida include spraying of copper, pesticides, and fertilizers, and application of compost or sewage sludge, potentially increasing the heavy metal content of the soils (Zhang et al., 1997). The soils used for citrus groves are often sandy, which easily lose metals with heavy rains, as they are not resistant to erosion (Zhang et al., 2004). He et al. (2004) found higher concentrations of copper, iron, manganese, and zinc in agricultural soils than forest soils in South Florida. They also found that the concentration of these metals in soils of citrus and vegetable fields correlated with the amounts measured in surface runoff, showing how agricultural contaminants can be deposited in the coastal zone. Schuler et al. (2008) noted levels of Cu in South Florida surface waters and biota had increased at the same time copper herbicide use for growth control of fungi and weeds increased.

Tourism

Only one heavy metal, zinc, presented with higher concentrations during the dry season, suggesting that the main source of this metal to the coastal zone is not run-off. One possible explanation is that anthropogenic activities involving zinc products increase during the dry season. Many of these sources, such as the use of sacrificial zinc anodes on recreational watercraft and zinc oxides in sunscreen, increase with the spike in tourism to Florida during the drier months. Sacrificial zinc anodes are used to prevent corrosion on submerged structures. This induces dissolution of the anodes into the seawater and can lead to zinc contamination. A study performed by Rousseau et al. (2009) showed that dissolution of zinc anodes did raise the zinc concentration in the seawater and surface sediments nearby. With the large population and growing tourism along the coast of Florida, sunscreen is a widely used product. Tovar-Sanchez et al. (2013) found high levels of zinc in the nearshore surface waters of a highly populated beach in the western

Mediterranean Sea. Zinc is one of the major chemicals used in sunscreens, and concentrations in seawater were highest when the maximum number of beachgoers were likely present and when sunlight radiation peaked, which would correspond to the highest sunscreen application rates. These results suggest that sunscreen has the potential to pollute coastal waters and impact marine organisms.

Morphology

Heavy metal concentrations were generally highest in blades with attached epiphytes and lowest in the underground complex of roots and rhizomes. This finding is in agreement with a previous study by Llagostera et al. (2011) which determined that heavy metal concentrations in seagrasses were generally higher in tissues located above the sediment than below. Three heavy metals - iron, manganese, and zinc - were found to have significantly higher concentrations in leaves with attached epiphytes than leaves that were cleaned or roots and rhizomes (Figure 4).

Epiphytes are sessile organisms that settle and grow on plants. On seagrasses common epiphytes include micro- and macro-algae, bacteria, tunicates, sponges, crustaceans, and mollusks. These epiphytes are directly consumed by seagrass grazers and, therefore, any heavy metals in the epiphytes combined with metal concentrations in the seagrass leaves would be reflected in the overall increased metal concentration. Patrick and Loutit (1977) found that epiphytes on the freshwater plant *Alisma plantago-aquatica* contained significant amounts of Cu, Cr, Fe, Pb, and Zn, which contributed to the overall heavy metal concentrations of the plant. Thus far, no studies have assessed the heavy metal concentrations of seagrass epiphytes in Florida waters. Abundance of epiphytes on seagrass leaves is impacted by various parameters such as water temperature, light, nutrient availability, and seasonal changes. They are usually most abundant on the oldest leaves as they accumulate over time. Nutrient loading near a seagrass meadow can lead to excessive growth of epiphytes on the leaves (Larkum et al., 2006). Iron, manganese, and zinc had the highest concentrations of tested metals overall, suggesting that epiphyte growth due to nutrient loading may be a major source of heavy metals to marine organisms during seagrass grazing. The epiphytes in this study were not identified to species before processing, though they consisted of both flora and fauna. Insufficient mass prevented epiphyte analysis to confirm or refute our hypothesis that epiphytes contained significant concentrations of heavy metals.

Toxicity

Data on heavy metal toxicity thresholds and health consequences are very limited for seagrasses, though many studies have worked to determine possible associated health impacts for various heavy metals (Lyngby and Brix 1984; Brackup and Capone, 1985; Malea, 1994; Malea et al., 1995a; Hamoutene et al., 1996; Ralph and Burchett,1998; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2004) (Table 10). Some heavy metals are more toxic than others, and concentrations of essential to non-essential metals may impact the toxicity to the organism of concern. Of the elements in this study, copper, iron, manganese, nickel, selenium, and zinc are essential elements, while arsenic, cadmium, lead, and mercury are non-essential elements (Ambo-Rappe et al., 2011; Sudharsan et al., 2012). It is worth noting that the heavy metals with the highest concentrations in this study were essential heavy metals and would presumably pose less of a threat even at high concentrations than low concentrations of non-essential metals.

Seagrasses

Studies on the phenology and morphology of seagrasses have shown that they are highly sensitive to pollution and will show many adverse effects with continued exposure (Bucalossi et al., 2006, Ralph et al., 2007). Heavy metal contamination can inhibit their metabolic processes and vital pathways which may cause reduction of photosynthetic activity and can lead to decreased growth rates and slower development (Prange et al., 2000). Due to their role as a pathway for metal incorporation into the biota, along with the fact that they are widespread and sensitive to environmental changes, seagrasses are considered a good bioindicator of ecosystem contamination and environmental variability (Tovar-Sanchez et al.,2006; Coelho et al., 2009; Govers et al., 2014).

Table 10. Biological processes impacted by various heavy metals in laboratory and in situ studies.

	Biological Impacts	References
Cadmium	• Oxidative metabolism • Leaf cell viability • Growth rate • Photosynthetic process	Lyngby and Brix, 1984; Brackup and Capone, 1985; Malea, 1994; Hamoutene et al., 1996; Ralph and Burchett, 1998
Copper	• Leaf cell viability • Growth rate • Amino acid concentrations • Photosynthetic activities • Browning/loss of leaves	Lyngby and Brix 1984; Malea et al., 1995a; Ralph and Burchett, 1998; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2004
Iron	• Amino acid concentrations	Prange and Dennison, 2000
Lead	• Growth rate • Nitrogen fixation • Photosynthetic processes	Lyngby and Brix 1984; Brackup and Capone, 1985; Ralph and Burchett, 1998
Mercury	• Growth rate • Nitrogen fixation	Lyngby and Brix 1984; Brackup and Capone, 1985
Nickel	• Nitrogen fixation	Brackup and Capone, 1985
Zinc	• Leaf cell viability • Growth rate • Photosynthetic processes	Lyngby and Brix 1984; Malea et al., 1995b; Ralph and Burchett, 1998

Marine Fauna

Toxicity in an organism is observed when vital processes are disturbed. The effects of heavy metal toxicity range from minor health impacts and morphological changes to severe health effects and even death through direct and indirect exposure. The concentration of a heavy metal needed to cause mortality depends upon the metal in question and the organism, though these specific levels are not known for many metals and many organisms. Fishes have been shown to suffer morphological changes in the gills, and their larvae exposed to heavy metals may present with numerous structural abnormalities (Bryan, 1971; George, 1982; Zeitoun and Mehana, 2014).

Beyond these physical defects, heavy metals cause issues with growth and development. Marine organisms, including crustaceans, fish, phytoplankton, and bacteria, have shown slow growth in the presence of heavy metals. One aspect of diminished growth is the inability to feed and digest food properly. They also suffer from delayed or a lack of sexual maturation and the inability to spawn successfully. Some of the most detrimental impacts are the sub-lethal health effects occurring inside the body. Exposure to heavy metals has been associated with necrosis of the liver, enzyme inhibition, hypoxia in tissues due to impaired gas exchange, diminished protein formation, improper cell division, and a variety of metabolic issues. Some heavy metals can take the place of other essential metals in the body and can ultimately block metabolic pathways when they do not perform correctly (Bryan, 1971; Rainbow, 1985). The avenue of heavy metal loss from the body occurs mainly through excretion (Reinfelder et al., 1998).

High levels of metals in green sea turtles from the southern Atlantic Ocean have been linked to fibropapillomatosis, which is known to cause severe health impacts and death in many cases. The disease was found to be most closely associated with copper, iron, and lead contamination (Carneiro da Silva et al., 2016). Studies of heavy metal impacts on marine mammals have also linked them to reproductive impairments, organ damage and disease, neurological deficits, impaired development, population declines, and even large-scale die-offs. Most of these health effects can be traced back to immunosuppression caused by the high levels of metals (Das et al., 2003; Deforges et al., 2016; Romero-Calderon et al., 2016). Copper concentrations found in the livers of manatees in Florida have been higher than those of many other marine mammal species, though the health effects of this are undetermined (O'Shea et al., 1984). A recent study also found elevated levels of copper, manganese, and zinc in whole blood samples of Florida manatees (Takeuchi et al., 2016). Manatees may also have elevated levels of arsenic, zinc, lead, and cadmium in various tissues, though it is not known what impacts this may cause (Siegal-Willott et al., 2013).

Conclusion

These results demonstrate that seagrasses in southeastern Florida contain a variety of heavy metals and that the concentrations of these metals vary by location, season, seagrass species, and morphology of the plant. The heavy metals with the highest concentrations were those found in high amounts in leaves with attached epiphytes, suggesting epiphytes may be a major source of contaminants to marine grazers. Contamination spikes during the wet season suggest that run-off contributes significantly to heavy metal accumulation in seagrasses.

Since baseline data on toxicity thresholds of heavy metals for marine species is limited, more research is needed to determine if the concentrations found in this study pose a health risk to seagrasses or the organisms that feed on them. Future research on the extent of transfer of these heavy metals in grazing organisms would help determine if contaminants in the seagrasses contribute substantially to the heavy metal load in organisms that feed on them. A separate study to investigate the strong correlations among heavy metals would help to identify their sources and which sources pose the greatest threat to the marine ecosystem. Separate analysis of epiphytes would help determine if they contribute substantially to the heavy metal intake of grazing organisms. It would also be useful to increase the time period over which seagrasses are analyzed to determine if heavy metal concentrations are relatively stable or increasing over time.

Literature Cited

Alves-Stanley, C.D., Worthy, G.A.J., Bonde, R.K., 2010. “Feeding preferences of West Indian manatees in Florida, Belize, and Puetro Rico as indicated by stable isotope analysis.” *Marine Ecology Progress Series* 402: 255-267.

Amado Filho, G.M., Creed, J.C., Andrade, L.R., Pfeiffer, W.C., 2004. “Metal accumulation by *Halodule wrightii* populations.” *Aquatic Botany* 80: 241-251.

Ambo-Rappe, R., Lajus, D.L., Schreider, M.J., 2011. “Heavy metal impact on growth and leaf asymmetry of seagrass, *Halophila ovalis*.” *Journal of Environmental Chemistry and Ecotoxicology* 3(6): 149-159.

Anzolin, D.G., Sarkis, J.E.S., Diaz, E., Soares, D.G., Serrano, I.L., Borges, J.C.G., Souto, A.S., Taniguchi, S., Montone, R.C., Bainy, A.C.D., Carvalho, P.S.M., 2012. “Contaminant concentrations, biochemical and hematological biomarkers in blood of West Indian manatees *Trichechus manatus* from Brazil.” *Marine Pollution Bulletin* 64: 1402-1408.

Barwick, M., Maher, W., 2003. “Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic, and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia.” *Marine Environmental Research* 56: 471-502.

Behringer, D., Butler, M., 2006. “Stable isotope analysis of production and trophic relationships in a tropical marine hard-bottom community.” *Oecologia* 148: 334-341.

Bell, J. D., Pollard, D. A., 1989. “Ecology of fish assemblages and fisheries associated with seagrasses.” In: Larkum, A. W. D., McComb, A. J., Shepherd, S. A. (eds.) *Biology of seagrasses*. Elsevier, Amsterdam, pp. 536-564

Bell, S., Walters, K., Kern, J., 1984. “Meiofauna from seagrass habitats: a review and prospectus for future research.” *Estuaries* 7(4): 331-338.

Bell, J.D., Westoby, M., 1986. “Abundance of macrofauna in dense seagrass is due to habitat preference, not predation.” *Oecologia* 68: 205-209.

Bonde, R.K., Aguirre, A.A., Powell, J., 2004. “Manatees as Sentinels of Marine Ecosystem Health: Are They the 2000-pound Canaries?” *EcoHealth* 1: 255-262.

Borowitzka, M.A., Lethbridge, R.C., 1989. “Seagrass epiphytes.” In: Larkum, A.W.D., McComb, A.J. and Shephard, S.A., (eds.). *Biology of seagrasses: a treatise on the biology of seagrasses with special reference to the Australian region*. Elsevier Science Pub., Amsterdam, The Netherlands, pp. 458-499.

Borowitzka, M.A., Lavery, P.S., van Keulen, M., 2006. "Epiphytes of Seagrasses." In: Larkum, A.W.D., Orth, R.J., Duarte, C. (Eds). *Seagrasses, Biology, Ecology and Conservation*. Springer, pp. 441-461.

Brackup, I., Capone, D.G., 1985. "The effect of several metal and organic pollutants on nitrogen fixation (acetylene reduction) by the roots and rhizomes of Zostera marina L." *Environmental and Experimental Botany* 25-2: 145-151.

Brito, G., de Souza, T., Costa, F., Moura, C., Korn, M., 2016. "Baseline trace elements in the seagrass *Halodule wrightii* Aschers (Cymodoceaceae) from Todos os Santos Bay, Bahia, Brazil." *Marine Pollution Bulletin* 104: 335-342.

Bryan, G.W., 1971. "The effects of heavy metals (other than mercury) on marine and estuarine organisms." *Proceedings of the Royal Society of London* 177: 380-410.

Bucalossi, D., Leonzio, C., Casini, S., Fossi, M., Marsili, L., Ancora, S., Wang, W., Scali, M., 2006. "Application of a suite of biomarkers in *Posidonia oceanica* (L.) delile to assess the ecotoxicological impact on the coastal environment." *Marine Environmental Research* 62: S327-S331.

Caccia, V., Millero, F., Palanques, A., 2003. "The distribution of trace metals in Florida Bay sediments." *Marine Pollution Bulletin* 46: 1420-1433.

Campanella, L., Conti, M.E., Cubadda, F., Sucapane, C., 2001. "Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean." *Environmental Pollution* 111: 117-126.

Carneiro da Silva, C., Klein, R.D., Barcarolli, I.F., Bianchini, A., 2016. "Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean." *Aquatic Toxicology* 170: 42-51.

Carrondo, M.J.T., Lester, J.N., Perry, R., Stoveland, S., 1978. "Analysis of Heavy Metals in Sewage Sludge, Sewages and Final Effluent." Final Report, to the Department of the Environment for Contracts DGR/480/66 and DGR/480/240.

Cobbett, C., Goldsbrough, P., 2002. "Phytochelatins and Metallothioneins: Roles in Heavy Metal Detoxification and Homeostasis." *Annual Review of Plant Biology* 53: 159-182.

Coelho, J.P., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2009. "Contribution of primary producers to mercury trophic transfer in estuarine ecosystems: Possible effects of eutrophication." *Marine Pollution Bulletin* 58: 358-365.

Connolly, R.M., 1995. "Effects of removal of seagrass canopy on assemblages of small, motile invertebrates." *Marine Ecology Progress Series* 118: 129-137.

Das, K., Debacker, V., Pillet, S., Bouquegneau, J., 2003. "Heavy Metals in Marine Mammals." In: *Toxicology of Marine Mammals*. Taylor and Francis Publishers, pp. 135-167.

Dawes, C.J., Tomasko, D.A., 1988. “Depth distribution of *Thalassia testudinum* in two meadows on the west coast of Florida; a difference in effect of light availability.” *Marine Ecology* 9: 123-130.

Deforges, J., Sonne, C., Levin, M., Siebert, U., de Guise, S., Dietz, R., 2016. “Immunotoxic effects of environmental pollutants in marine mammals.” *Environment International* 86: 126-139.

Demirezen, D., Aksoy, A., 2006. “Common hydrophytes as bioindicators of iron and manganese pollutions.” *Ecological Indicators* 6: 388-393.

Diaz, E., Massol-Deya, A., 2003. “Trace Element Composition in Forage Samples from a Military Target Range, Three Agricultural Areas, and One Natural Area in Puerto Rico.” *Caribbean Journal of Science* 39: 215-220.

Duarte, C.M., 1991. “Seagrass Depth Limits.” *Aquatic Botany* 40(4): 363-377.

Duarte, C.M., 2002. “The future of seagrass meadows.” *Environmental Conservation* 29(2): 192-206.

Duever, M.J., Meeder, J.F., Meeder, L.C., McCollom, J.M., 1994. “The climate of South Florida and its role in shaping the Everglades ecosystem.” In: Davis, S.M., Ogden, J.C. (Eds) *Everglades: the Ecosystem and its Restoration*. St. Lucie Press, pp. 225-248.

Eggleton, J., Thomas, K.V., 2004. “A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events.” *Environment International* 30: 973-980.

Erftemeijer, P., Lewis III, R., 2006. “Environmental impacts of dredging on seagrasses: A review.” *Marine Pollution Bulletin* 52: 1553-1572.

Erftemeijer, P., Riegl, B., Hoeksema, B.W., Todd, P.A., 2012. “Environmental impacts of dredging and other sediment disturbances on corals: a review.” *Marine Pollution Bulletin* 64: 1737–1765.

Essink, K., 1999. “Ecological effects of dumping of dredged sediments; options for management.” *Journal of Coastal Conservation* 5: 69-80.

Fathollahzadeh H., Kaczala, F., Bhatnagar, A., Hogland, W., 2015. “Significance of environmental dredging on metal mobility from contaminated sediments in the Oskarshamn Harbor, Sweden.” *Chemosphere* 119: 445-451.

Florida DEP, 2017. “Biscayne Bay Aquatic Preserves.” Florida Coastal Office, Aquatic Preserve Program. Available: https://floridadep.gov/fco/aquatic-preserve/locations/biscayne-bay-aquatic-preserves

Fourqurean, J.W., Powell, G.V.N., Kenworthy, W.J., Zieman, J.C., 1995. “The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay.” *Oikos* 72: 349-358.

Fredette, T.J., French, G.T., 2004. “Understanding the physical environmental consequences of dredged material disposal: history in New England and current perspectives.” *Marine Pollution Bulletin* 49: 93-102.

Fritioff, A., Kautsky, L., Greger, M., 2005. “Influence of temperature and salinity on heavy metal uptake by submersed plants.” *Environmental Pollution* 133: 265-274.

Gaber, S.E., Rizk, M.S., Yehia, M.M., 2011. “Extraction of certain heavy metals from sewage sludge using different types of acids.” *Biokemistri* 23(1): 41-48.

George S.G., 1982. “Subcellular accumulation and detoxification of metals in aquatic animals.” In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ, editors. *Physiological mechanisms of marine pollutant toxicity.* Oxford: Academic Press, pp. 3–52.

Govers, L., Lamers, L., Bouma, T., Eygensteyn, J., Brouwer, J., Hendriks, A., Huijbers, C., van Katwijk, M., 2014. “Seagrasses as indicators for coastal trace metal pollution: A global meta-analysis serving as a benchmark, and a Caribbean case study.” *Environmental Pollution* 195: 210-217.

Gray, J.S., 2002. “Biomagnification in marine systems: the perspective of an ecologist.” *Marine Pollution Bulletin* 45: 46-52.

Guardo, M., Fink, L., Fontaine, T.D., Newman, S., Chimney, M., Bearzotti, R., Goforth, G., 1995. “Large-Scale Constructed Wetlands for Nutrient Removal from Stormwater Runoff: An Everglades Restoration Project.” *Environmental Management* 19(6): 879-889.

Gyory, J., Rowe, E., Mariano, A., Ryan, E., 2013. “The Florida Current.” Ocean Surface Currents.

Hamoutene, D., Romeo, M., Gnassia, M., Lafaurie, M., 1996. “Cadmium effects of oxidative metabolism in a marine seagrass: Posidonia oceanica”. *Bulletin of Enviromental Contamination and Toxicology* 56: 327-334.

Haynes, D., Carter, S., Gaus, C., Muller, J., Dennison, W., 2005. “Organochlorine and heavy metal concentrations in blubber and liver tissue collected from Queensland (Australia) dugong (*Dugong dugon*).” *Marine Pollution Bulletin* 51: 361-369.

He, Z.L., Zhang, M.K., Calvert, D.V., Stoffella, P.J., Yang, X.E., Yum S., 2004. “Transport of Heavy Metals in Surface Runoff from Vegetable and Citrus Fields.” *Soil Science Society of America Journal* 68(5): 1662-1669.

Hedge, L.H., Knott, N.A., Johnston, E.L., 2009. “Dredging related metal bioaccumulation in oysters.” *Marine Pollution Bulletin* 58: 832-840.

Kalnejais, L., Martin, W., Signell, R., Bothner, M., 2007. “Role of sediment resuspension in the remobilization of particulate-phase metals from coastal sediments.” *Environmental Science and Technology* 41: 2282-2288.

Kenworthy, W.J., Schwarzschild, A.C., 1998. "Vertical growth and short-shoot demography of *Syringodium filiforme* in out Florida Bay, USA." *Marine Ecology Progress Series* 173: 25-37.

Khan, M.A.K., Wang, F., 2010. "Mercury-selenium compounds and their toxicological significance: Toward a molecular understanding of the mercury-selenium antagonism." *Environmental Toxicology* 28(8): 1567-1577.

Klumpp, D.W., Salita-Espinosa, J.S., Fortes, M.D., 1992. "The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community." *Aquatic Botany* 43: 327-349.

Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., Gonzalez, J.-L., 2007. "Trace metals assessment in water, sediment, mussel, and seagrass species – Validation of the use of *Posidonia oceanica* as a metal biomonitor." *Chemosphere* 68: 2033-2039.

Lanyon, J.M., Limpus, C.J., Marsh H., 1989. "Dugongs and turtles: grazers in the seagrass system." *Biology of Australian Seagrasses An Australian Perspective*: 610-634.

Leaman, K.D., Molinari, R., Vertes, P., 1987. "Structure and Variability of the Florida Current at 27N: April 1982-July 1984". *Journal of Physical Oceanography* 17: 565-583.

Lee, J., Kim, J.R., Jeong, S., Cho, J., Kim, J.Y., 2017. "Long-term performance of anaerobic digestion for crop residues containing heavy metals and response of microbial communities." *Waste Management* 59: 498-507.

Lewis, F.G. III, 1984. "Distribution of microbenthic crustaceans associated with *Thalassia*, *Halodule* and bare sand substrata." *Marine Ecology Progress Series* 19: 101-113.

Lewis, M., Dantin, D., Chancy, C., Abel, K., Lewis, C., 2007. "Florida seagrass habitat evaluation: A comparative survey for chemical quality." *Environmental Pollution* 146: 206-218.

Llagostera, I., Perez, M., Romero, J., 2011. "Trace metal content in the seagrass *Cymodocea nodosa*: Differential accumulation in plant organs." *Aquatic Botany* 95: 124-128.

Lohrer, A., Wetz, J., 2003. "Dredging-induced nutrient release from sediments to the water column in a southeastern saltmarsh tidal creek." *Marine Pollution Bulletin* 46: 1156-1163.

Luy, N., Gobert, S., Sartoretto, S., Biondo, R., Bouquegneau, J., Richir, J., 2012. "Chemical contamination along the Mediterranean French coast using *Posidonia oceanica* (L.) Delile above-ground tissues: a multiple trace element study." *Ecological Indicators* 18: 269-277.

Lyngby, J.E., Brix, H., 1984. "The uptake of heavy metals in eelgrass Zostera marina and their effect on growth." *Ecological Bulletin* 36: 81-89.

Macinnis-Ng, C.M., Ralph, P.J., 2004. "Variations in sensitivity to copper and zinc among three isolated populations in the seagrass, Zostera capricorni." *Journal of Experimental Marine Biology and Ecology* 302: 63-83.

Malea, P., 1994. "Uptake of cadmium and the effect on viability of leaf cells in the seagrass Halophila stipulacea (Forsk.) Aschers." *Botanica Marina* 37: 67-73.

Malea, P., Kevrekidis, T., Haritonidis, S., 1995a. "The short-term uptake of copper by the two parts of the seagrass Halophila stipulacea (Forsk). Aschers and leaf cells viability." *Fresenius Environmental Bulletin* 4: 117-122.

Malea, P., Kevrekidis, T., Haritonidis, S., 1995b. "The short-term uptake of zinc and cell morality of the seagrass Halophila stipulacea (Forsk.) Aschers." *Israel Journal of Plant Sciences* 43: 21-30.

Miami-Dade Seaport Business Plan; Attachment B, 2014. Miami-Dade Seaport FY 2012-14 & FY2013-15.

Moncreiff, C.A., Sullivan, M.J., 2001. "Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses." *Marine Ecology Progress Series* 215: 93-106.

Nayar, S., Miller, D.J., Hunt, A., Goh, B.P.L., Chou, L.M., 2007. "Environmental effects of dredging on sediment nutrients, carbon and granulometry in a tropical estuary." *Environ Monit Assess* 127: 1-13.

Newell, R.C., Seiderer, L.J., Hitchcock, D.R., 1998. "The impact of dredging works in coastal waters: a review of the sensitivity to disturbance and subsequent recovery of biological resources on the sea bed." *Oceanography and Marine Biology: an Annual Review* 36: 127-78.

Nichols, P.D., Johns, R.B., 1985. "Lipids of the tropical seagrass *Thallassia hemprichii*." *Phytochemistry* 24(1): 81-84.

Orth, R.J., Carruthers, T., Dennsion, W., Duarte, C., Fourqurean, J., Heck Jr, K., Hughes, R., Kendrick, G., Kenworthy, W.J., Olyarnik, S., Short, F., Waycott, M., Williams, S., 2006. "A global crisis for seagrass ecosystems." *BioScience* 56(12): 987-996.

O'Shea, T., Moore, J., Kochman, H., 1984. "Contaminant Concentrations in Manatees in Florida." *The Journal of Wildlife Management* 48(3): 741-748.

Patrick, F.M., Loutit, M.W., 1977. "The uptake of heavy metals by epiphytic bacteria on *Alisma plantago-aquatica*." *Water Research* 11(8): 699-703.

PortMiami, 2018. PortMiami 2035 Master Plan. Available: http://www.miamidade.gov/portmiami/master-plan.asp

Prange, J.A., Dennison, W.C., 2000. “Physiological responses of five seagrass species to trace metals.” *Marine Pollution Bulletin* 41: 327-336.

R Core Team (2018). “R: A language and environment for statistical computing.” *R Foundation for Statistical Computing*, Vienna, Austria., Available: http://www.R-project.org/

Rainbow, P., 1985. “The Biology of Heavy Metals in the Sea.” *Intern. J. Environmental Studies* 25: 195-211.

Ralph, P.J., Burchett, M.D., 1998. “Photosynthetic response of Halophila ovalis to heavy metal stress.” *Environmental Pollution* 103: 91-101.

Ralph, P., Tomasko, D., Moore, K., Seddon, S., Macinnis-Ng, C., 2007. “Human Impacts on Seagrasses: Eutrophication, Sedimentation, and Contamination.” In: *Seagrasses: Biology, Ecology and Conservation*, 567-593. Springer, Dordrecht.

Randall, J.E., 1965. “Grazing effect on sea grasses by herbivorous reef fishes in the West Indies.” *Ecology* 46(3): 255-260.

Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W.-X., 1998. “Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach.” *The Science of the Total Environment* 219: 117-135.

Romero-Calderon, A.G., Morales-Vela, B., Rosiles-Martinez, R., Olivera-Gomez, L.D., Delgado-Estrella, A., 2016. “Metals in Bone Tissue of Antillean Manatees from the Gulf of Mexico and Chetumal Bay, Mexica.” *Bulletin of Environmental Contamination and Toxicology* 96: 9-14.

Rousseau, C., Baraud, F., Leleyter, L., Gil, O., 2009. “Cathodic protection by zinc sacrificial anodes: Impact of marine sediment metallic contamination.” *Journal of Hazardous Materials* 167: 953-958.

Schlacher-Hoenlinger, M.A., Schlacher, T.A., 1998. “Accumulation, contamination, and seasonal variability of trace metals in the coastal zone- patterns in a seagrass meadow from the Mediterranean.” *Marine Biology* 131: 401-410.

Schuler, L., Hoang, T., Rand, G., 2008. “Aquatic risk assessment of copper in freshwater and saltwater ecosystems of South Florida.” *Ecotoxicology* 17(7): 642-659.

Seigal-Willot, J., Harr, K., Hall, J., Hayek, L., Auil-Gomez, N., Powell, J., Bonde, R., Heard, D., 2013. “Blood mineral concentrations in manatees (Trichechus manatus latirostris and Trichechus manatus manatus).” *Journal of Zoo and Wildlife Medicine* 44(2): 285-294.

Short, F., Carruthers, T., Dennison, W., Waycott, M., 2007. “Global seagrass distribution and diversity: a bioregional model.” *Journal of Experimental Marine Biology and Ecology* 350: 3-20.

Short, F.T., Carruthers, T.J.R., van Tussenbroek, B. & Zieman, J. 2010a. "*Thalassia testudinum*." *The IUCN Red List of Threatened Species* 2010.

Short, F.T., Carruthers, T.J.R., van Tussenbroek, B. & Zieman, J. 2010b. "*Halodule wrightii*." *The IUCN Red List of Threatened Species* 2010.

Short, F.T., Carruthers, T.J.R., van Tussenbroek, B. & Zieman, J. 2010c. "*Syringodium filiforme*." *The IUCN Red List of Threatened Species* 2010.

Snyder, G.H., Davidson, J.M., 1994. "Everglades Agriculture: Past, Present, and Future." In: Davis, S.M., Ogden, J.C., (eds). Everglades. The Ecosystem and Its Restoration. St. Lucie Press, Boca Raton, Florida, pp. 85-117.

Spalding, M., Taylor, M., Ravilious, C., Short, F., Green, E., 2003. "The distribution and status of seagrasses: a global overview." In: E.P. Green, F.T. Short (Eds.) *World Atlas of Seagrasses*, pp. 5-26.

Stavros, H.W., Bonde, R.K., Fair, P.A., 2008. "Concentrations of trace elements in blood and skin of Florida manatees (*Trichechus manatus latirostris*)." *Marine Pollution Bulletin* 56: 1215-1233.

Steffens, J.C., 1990. "The Heavy Metal-Binding Peptides of Plants. Annual Review of Plant Physiology." *Plant Molecular Biology* 41: 553-575.

Sudharsan, S., Seedevi, P., Ramasamy, P., Subhapradha, N., Vairamani, S., Shanmugam, A., 2012. "Heavy metal accumulation in seaweeds and sea grasses along southeast coast of India." *Journal of Chemical and Pharmaceutical Research* 4(9): 4240-4244.

Takeuchi, N.Y., Walsh, M.T., Bonde, R.K., Powell, J.A., Bass, D.A., Gaspard III, J.C., Barber, D.S., 2016. "Baseline reference range for trace metal concentrations in whole blood of wild and managed West Indian manatees (*Trichechus manatus*) in Florida and Belize." *Aquatic Mammals* 42(4): 440-453.

Tchounwou, P., Yedjou, C., Patlolla, A., Sutton, D., 2012. "Heavy Metals Toxicity and the Environment." *National Institutes of Health* 101: 133-164.

Thresher, R.E., Nichols, P.D., Gunn, J.S., Bruce, B.D., Furlani, D.M., 1992. "Seagrass detritus as the basis of a coastal planktonic food chain." *Limnology and Oceanography* 37(8): 1754-1758.

Tovar-Sanchez, A., Seron, J., Marba, N., Arrieta, J.M., Duarte, C.M., 2010. "Long-term records of trace metal content of western Mediterranean seagrass (*Posidonia oceanica*) meadows: Natural and anthropogenic contributions." *Journal of Geophysical Research* 115, G02006.

Tovar-Sanchez, A., Sanchez-Quiles, D., Basterretxea, G., Benede, J.L., Chisvert, A., Salvador, A., Moreno-Garrido, I., Blasco, J., 2013. "Sunscreen products as emerging pollutants to coastal waters." *PLOS One* 8(6): e65451.

USDA, "2012 Census of Agriculture: Florida." Available: https://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_State_Level/Florida/

U.S. EPA, 1973. "Ocean Outfalls, Treated Wastewater Disposal: Environmental Impact Statement. Ocean Outfalls and other Methods of Treated Wastewater Disposal in Southeast Florida.US Fish and Wildlife Service, 2017." *Seagrasses.* South Florida Multi-Species Recovery Plan.

U.S. FWS, 2017. "Seagrasses." *Multi-Species Recovery Plan for South Florida.* Available: https://www.fws.gov/verobeach/ListedSpeciesMSRP.html

U.S. Geological Survey, 2013. "Florida Bay." *South Florida Ecosystem History Project.* Available: https://sofia.usgs.gov/flaecohist/floridabay.html

U.S. Geological Survey, 2016. "Runoff (surface water runoff)." *The USGS Water Science School*. Available: https://water.usgs.gov/edu/runoff.html

Van Maren, D.S., van Kessel, T., Sittoni, C., 2015. "The impact of channel deepening and dredging on estuarine sediment concentration." *Continental Shelf Research* 95: 1-14.

Van Tussenbroek, B., Villamil, N., Marquez-Guzman, J., Wong, R., Monroy-Velazquez, V., Solis-Weiss, V., 2016. "Experimental evidence of pollination in marine flowers by invertebrate fauna." *Nature Communications* 7: 12980.

Vaslet, A., Phillips, D., France, C., Feller, I., Baldwin, C., 2012. "The relative importance of mangroves and seagrass beds as feeding areas for resident and transient fishes among different mangrove habitats in Florida and Belize: Evidence from dietary and stable-isotope analyses." *Journal of Experimental Marine Biology and Ecology* 434-435: 81-93.

Virnstein, R., Mikkelsen, P., Cairns, K., Capone, M., 1983. "Seagrass beds versus sand bottoms: the trophic importance of their associated benthic invertebrates." *Florida Scientist* 46: 363-381.

Wahbeh, M., 1984. "Levels of zinc, manganese, magnesium, iron and cadmium in three species of seagrass from Aqaba (Jordan)." *Aquatic Botany* 20: 179-183.

Ward, T., Correll, R., Anderson, R., 1986. "Distribution of cadmium, lead and zinc amongst the marine sediments, seagrasses and fauna, and the selection of sentinel accumulators, near a lead smelter in South Australia." *Marine Freshwater Research* 37: 567-585.

Weinstein, M.P., Heck Jr., K.L., 1979. "Ichthyofauna of seagrass meadows along the Caribbean coast of Panama and in the Gulf of Mexico: composition, structure, and community ecology." *Marine Biology* 50: 97-107.

Whelan III, T., Espinoza, J., Villarreal, X., CottaGoma, M., 2005. “Trace metal partitioning in *Thalassia testudinum* and sediments in the Lower Laguna Madre, Texas.” *Environment International* 31: 15-24.

Williams, S.L., 1987. “Competition between the seagrasses *Thalassia testudinum* and *Syringodium filiforme* in a Caribbean lagoon.” *Marine Ecology- Progress Series* 35: 91-98.

Zakhama-Sraieb, R., Sghaier, Y., Hmida, A., Cappai, G., Carucci, A., Charfi-Cheikhrouha, F., 2016. “Variation along the year of trace metal levels in the compartments of the seagrass *Posidonia oceanica* in Port El Kantaoui, Tunisia.” *Environ Sci Pollut Res* 23: 1681-1690.

Zeitoun, M.M., Mehana, E.E., 2014. “Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates.” *Global Veterinaria* 12(2): 219-231.

Zhang, M., Alva, A., Li, Y.C., Calvert, D.V., 1997. “Chemical Association of Cu, Zn, Mn, and Pb in Selected Sandy Citrus Soils.” *Soil Science* 162(3): 181-188.

Zhang, M., He, Z., Calvert, D.V., Stoffella, P.J., 2004. “Spatial and temporal variations of water quality in drainage ditches within vegetable farms and citrus groves.” *Agricultural Water Management* 65: 39-57.

Zieman, J.C., Fourqurean, J.W., Iverson, R.L., 1989. “The distribution, abundance and productivity of seagrasses in Florida Bay.” *Bulletin of Marine Science* 44: 292-311.

Zug, G.R., Glor, R.E., 1998. “Estimates of age and growth in a population of green sea turtles (Chelonia mydas) from the Indian River lagoon system, Florida: a skeletochronological analysis.” *Canadian Journal of Zoology* 76(8): 1497-1506.