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# Heavy Metal Accumulation in Seagrasses in Southeastern Florida

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**Abstract**

Seagrass beds are among the most ecologically important systems in the marine environment and comprise a large component of the diets of many marine organisms, which provides a pathway for contaminants in the seagrasses to enter the marine food web. In this study, three species of seagrasses, *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme,*were collected monthly, for one year, in three regional locations in South Florida - Port of Miami, Card Sound region of Biscayne Bay Aquatic Preserve, and Florida Bay. These were analyzed for ten heavy metals using Atomic Absorption Spectroscopy. The metal concentrations varied significantly between the three locations, though each location had similar dominant metals (Cu, Fe, Mn, Zn). Significantly higher concentrations of heavy metals were found during the wet season, with the exception of Zn. Metals such as Fe, Mn, and Zn were found to be significantly higher in leaves with epiphytes.

**Keywords**

Seagrass; Heavy metals; South Florida; *Thalassia testudinum; Halodule wrightii*; *Syringodium filiforme*

## Introduction

Seagrasses are ecologically and economically important, widespread, marine, flowering plants that are found along coastlines of tropical and temperate seas around the world at depths up to 90 meters [1-3]. Seagrass beds have also been identified as the most productive submerged habitat globally [4]. These systems provide a large amount of primary production necessary to marine ecosystems and support major grazing and detrital food webs [5,6]. Seagrass meadows perform extensive and diverse roles in coastal marine ecosystems.

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 [4,7]. Seagrass environments also serve as critical habitat at some point in the life cycles of many species targeted for recreational and commercial fishing [2], as well as a major food source in the coastal ecosystem, with over 154 marine species known to feed on living seagrass [8]. A wide variety of species graze heavily on seagrasses, including macroinvertebrates, numerous species of fishes, and large marine herbivorous grazers such as manatees and sea turtles [5,8-10]. Many of the species that rely on seagrass beds are federally listed as threatened or endangered (USFWS, 2017).

Though seagrasses currently cover approximately 0.1%-0.2% of the global ocean, they are severely lacking in protective regulations, and are presently experiencing worldwide decline which is primarily human-caused [1,2]. A major concern for seagrasses, especially those near highly industrialized or populated areas, is excessive nutrient loading along with the addition of a wide range of contaminants, including heavy metals. 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 [7,11].

Concentrations of heavy metals in the environment are impacted by many factors, including proximity to potential sources, seasonal changes, and abiotic characteristics of the environment [12,13]. Various human activities are known to be sources of heavy metals to the environment, including aquaculture, sewage discharge, mining and smelting, deforestation, agriculture (herbicide or pesticide use), and various industries such as leather production, shipyards, electronics, and paints [14-16].

Seagrasses all over the globe, including the three species in this study, *Thalassia testudinum, Halodule wrightii, and Syringodium filiforme*, show various concentrations of numerous heavy metals; however, research on heavy metals in seagrasses in southeastern Florida is extremely limited. Once heavy metals are incorporated into seagrasses, they can be transferred into the marine food web through herbivory or indirectly through detritivore food webs [17-19]. Heavy metals are biologically categorized as essential, meaning they are needed by organisms for some biological process, or non-essential, where they have no biological role in the body. Both of these groups, however, can be toxic above a certain threshold, and at some concentration, all heavy metals become toxic [17,20]. Though the research on toxicity limits and direct consequences of heavy metal contamination is limited, many studies have shown correlations between contamination and adverse health in apex predators [21-23]. This study assessed the heavy metal concentrations in the three main seagrass species found in southeastern Florida seagrass beds.

## Materials and Methods

### Study area, species, and sampling

Samples of *Thalassia testudinum* (n=180), *Halodule wrightii* (n=140), and *Syringodium filiforme* (n=78) were collected from three regional locations: Port of Miami (POM), Card Sound region of Biscayne Bay Aquatic Preserve (CAP), and Florida Bay off the north coast of Islamorada (FLB) (Figure 1 and Table 1). In the Port of Miami, samples were collected from one site adjacent to Rickenbacker Causeway and two sites around Virginia Key; Card Sound samples were collected from three sites around Barnes Sound, and in Florida Bay samples were collected from one site off the north shore of Islamorada. Seagrasses were collected from each site monthly for the entirety of 2017 to include the wet (June-October) and dry (November-May) seasons. Sampling was not possible during September 2017 due to the

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impact of Hurricane Irma. Sampling took place during ebb to slack low tide resulting in a water depth range of 5 to 15 inches (13 cm-38 cm). Multiple individuals of each species were collected from within a randomly placed 0.5 m grid using a 25 $cm^2$ shovel sieve. Entire plants, including leaves, shoots, roots, and horizontal rhizomes of each species, were removed for analysis. Samples were rinsed with seawater, placed in individual plastic bags, and frozen in a standard freezer (-20°C) until processed. Salinity and bottom water temperatures were measured with an environmental YSI meter (model #030130) at each site.

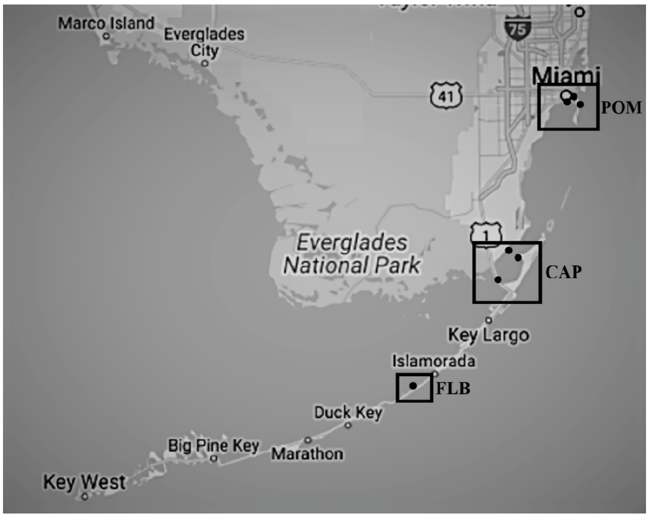


**Figure 1:** Locations from which samples were collected: Port of Miami (POM), Card Sound region of Biscayne Bay Aquatic Preserve (CAP), and Florida Bay (FLB). Black dots represent individual collection sites within each regional location.

## 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).

## Metals analysis

In the laboratory, specimens were separated by species and into above (leaves and shoots) and below (roots and rhizomes) sediment plant parts, rinsed three times with 100 mL deionized water, and cleaned of epiphytes and other particles with the edge of a clean glass slide. *Thalassia testudinum* leaves with attached epiphytes were also analyzed to examine the epiphytes' contribution to the total heavy metal concentrations of the plant. Samples were dried for a minimum of 4 hours in an Isotemp Vacuum Oven (Model 282A) 75°C and 1 × $10^{-3}$ torr, manually ground into a composite powder of multiple individual plants and weighed to approximately 0.2 g of dry weight. They were then digested with 8 ml of 99.999% metals basis nitric acid and 2 ml of concentrated sulfuric acid until the plant material was totally dissolved with no particulate. The solution was then diluted to a total volume of 50 ml with ultrapure (18.2 MOhm-cm) water. The heavy metal analysis was conducted using a Shimadzu AA-6200 Atomic Absorption Flame Emission Spectrophotometer equipped with a Hydride Vapor Generator (Shimadzu, HVG-1). Each sample (Reps=3, RSD Limit=7.00, SD Limit-0.008) was run with a blank (Reps=2, RSD Limit=99.90, SD Limit-0.000) and a standard (Reps=2, RSD Limit=5.00, SD Limit-0.005). Samples were tested for 10 heavy metals: Arsenic (As), Cadmium (Cd), Copper (Cu), Iron (Fe), Total Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), and Zinc (Zn).

**Table 1:** Number of samples (n) tested for each heavy metal in the wet/dry seasons by location, species, and plant part. The numbers shown include samples from all sites and months. Differences in the sample size for each group are due to field availability of each species and plant part for collection and testing.

| | | As | Cd | Cu | Fe | Pb | Mn | Hg | Ni | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Card sound region of Biscayne bay aquatic preserve** | | | | | | | | | | | |
| ***Thalassia testudinum*** | Leaves/Shoots | 4/13 | 9/15 | 11/19 | 11/20 | 8/11 | 11/20 | 9/11 | 9/10 | 3/8 | 11/20 |
| | Leaves with Epiphytes | 3/15 | 7/11 | 10/20 | 10/20 | 5/11 | 10/20 | 8/14 | 9/16 | 1/9 | 9/19 |
| | Roots/Rhizomes | 4/17 | 7/13 | 9/16 | 10/18 | 6/14 | 9/18 | 6/14 | 6/11 | 2/11 | 10/17 |
| ***Halodule wrightii*** | Leaves/Shoots | 7/19 | 10/14 | 9/19 | 11/20 | 7/15 | 11/21 | 8/18 | 7/11 | 2/14 | 11/21 |
| | Roots/Rhizomes | 4/17 | 9/13 | 11/18 | 11/19 | 8/11 | 11/20 | 6/17 | 7/11 | 1/6 | 11/20 |
| ***Syringodium filiforme*** | Leaves/Shoots | 1/5 | 4/5 | 3/6 | 4/8 | 2/3 | 4/7 | 2/6 | 3/3 | 3/3 | 4/7 |
| | Roots/Rhizomes | -/7 | 2/5 | 4/6 | 4/8 | 4/3 | 2/7 | 3/6 | 2/2 | 1/3 | 4/7 |
| **Florida Bay** | | | | | | | | | | | |
| ***Thalassia testudinum*** | Leaves/Shoots | 1/1 | 1/- | 1/1 | 1/1 | 1/1 | 1/1 | -/1 | 1/1 | -/1 | 1/1 |
| | Leaves with Epiphytes | 1/1 | 1/- | 1/1 | 1/1 | -/1 | 1/1 | 1/1 | 1/1 | -/- | 1/1 |
| | Roots/Rhizomes | -/1 | -/1 | -/1 | -/1 | -/1 | -/1 | -/1 | -/1 | -/1 | -/1 |
| ***Halodule wrightii*** | Leaves/Shoots | 2/5 | 3/3 | 4/6 | 4/6 | 3/3 | 4/6 | 1/6 | 4/5 | -/2 | 4/6 |
| | Roots/Rhizomes | 3/6 | 3/5 | 4/6 | 4/6 | 4/2 | 3/6 | 1/5 | 4/3 | -/3 | 4/6 |
| **Port of Miami** | | | | | | | | | | | |
| ***Thalassia testudinum*** | Leaves/Shoots | 7/13 | 10/15 | 11/18 | 11/18 | 7/12 | 10/18 | 5/17 | 6/17 | 3/8 | 11/18 |
| | Leaves with Epiphytes | 7/16 | 9/12 | 11/18 | 11/18 | 7/11 | 11/18 | 6/17 | 5/15 | 1/7 | 11/18 |
| | Roots/Rhizomes | 5/15 | 10/11 | 10/17 | 10/17 | 5/10 | 10/16 | 4/13 | 5/9 | 3/5 | 10/17 |
| ***Halodule wrightii*** | Leaves/Shoots | 4/15 | 10/10 | 10/16 | 11/17 | 7/9 | 10/16 | 3/13 | 5/9 | 2/11 | 11/17 |
| | Roots/Rhizomes | 5/13 | 9/10 | 10/17 | 11/17 | 5/12 | 11/16 | 5/15 | 5/10 | 4/8 | 11/17 |
| ***Syringodium filiforme*** | Leaves/Shoots | 6/13 | 10/12 | 10/17 | 11/17 | 10/10 | 11/17 | 7/13 | 6/10 | 3/8 | 11/17 |
| | Roots/Rhizomes | 5/13 | 6/11 | 10/16 | 10/17 | 4/10 | 9/15 | 3/14 | 3/11 | 2/7 | 10/17 |

### Statistical analysis

Statistical analysis was performed in program software R (2018) and Primer 7 [24] with a significant level of α=0.05. As data were not normally distributed, non-parametric Kruskal-Wallis analysis of variance (ANOVA) was used to test for significant differences in heavy metal concentrations among the three locations, between wet and dry season, among the three collected species, and among plant parts, followed by pairwise Wilcoxon rank-sum tests to investigate differences among levels of each factor.

## Results

### Heavy metals

All ten analyzed metals were detected in seagrass tissues. 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 μg/g to 1877.43 μg/g. Mean, range, and standard deviation of each heavy metal are presented in Table 2. 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. No correlation existed between heavy metal concentrations and salinity or bottom water temperature. Metals with the highest overall concentrations were Fe, ranging from 1.52 μg/g-1877.43 μg/g, Zn, ranging from 1.48 μg/g-669.44 μg/g, and Mn, ranging from 0.79 μg/g-300.15 μg/g. Metals with the lowest overall concentrations were As, ranging from 0.02 μg/g-2.95 μg/g, Se, ranging from 0.01 μg/g-4.79 μg/g, and Hg, ranging from 0.03 μg/g-16.46 μg/g. The annual mean and standard deviation of each heavy metal by location, season, species, and plant part are presented in Supplementary Tables 1-10. The values presented include data points from all sites and months. The high standard deviations seen are due to the inclusion of multiple variables in the data. Differences between specific groups are displayed in the p-values returned during ANOVA and Wilcoxon testing.

### 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 FLB than 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 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).

### Season

During the wet season, there were 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). Zinc was the only heavy metal with significantly higher concentrations during the dry season ($X^2$=41.8, df=1, p<0.001) (Table 3).

### 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 or *S. filiforme*. Manganese concentrations were significantly higher in *T. testudinum* than *H. wrightii* (p<0.001) and *S.filiforme* (p<0.001), and its concentration was 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 Table 4.

### Morphology

Five metals exhibited no significant difference among seagrass parts. Those that did include, 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=0.01) (Table 3). Copper concentrations were higher in leaves with or without epiphytes than in roots (p<0.001), but there was no difference in concentrations 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 differences in zinc concentrations between cleaned leaves and those with epiphytes were found, though both leaf types had higher concentrations than roots (p=0.02 and 0.03). Iron, manganese, and zinc were the only heavy metals to have significantly higher concentrations in leaves with attached epiphytes (Figure 2).

## Discussion

These results show that the seagrasses in southeastern Florida waters do contain various concentrations of heavy metals. 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 the opposite results [13], the variation in environmental factors in this study were very small by comparison. Annually, bottom water temperature

**Table 2:** 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 | 19.4 | 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 |

ranged from 22.9°C in January to 33.9°C in July with a range of 11°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.

The results of this study were compared to finding from various other locations and seagrass species (Table 5). 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 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 in Brazil but much lower than those from the Mediterranean Sea. Lead and mercury concentrations were consistently higher compared to the other studies [11,25-27]. Correlation between mercury and selenium had a significant positive correlation relationship, which is known to have strong antagonistic effects in mammals. This difference may be due to plants containing different lipid classes than mammals and would, therefore, bioaccumulate these metals differently [28,29].

**Table 3:** 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 |

These data showed that seasonality had a significant impact on heavy metals concentrations in all locations. Seasonality (dry vs wet) is related to 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 [30]. Eight of the ten heavy metals presented had significantly higher

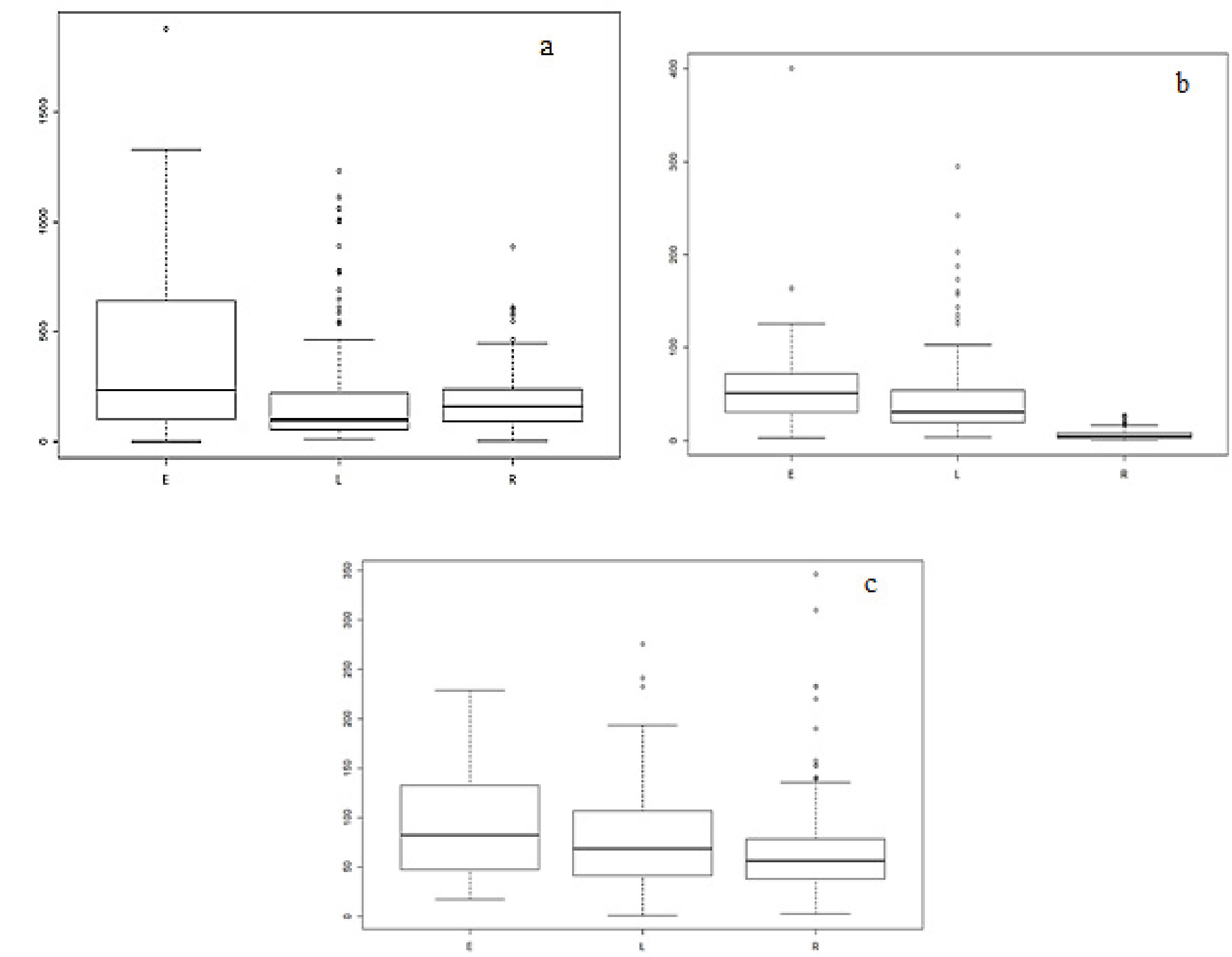


**Figure 2:** Boxplots displaying the mean concentrations (µg/g) of (a) iron, (b) manganese, (c) zinc in each plant part including leaves with attached epiphytes (E), cleaned leaves (L), and roots and rhizomes (R).

concentrations during the wet than the dry season (Figure 3). This is presumably related to the increase in runoff transporting various anthropogenic pollutants and contaminants that accumulate on the ground and in soils and delivering them to the ocean, either directly or via 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 showed a major increase of approximately 480% in rainfall from May to June 2017 which continued until a drop began in November. This pattern correlates to the spikes in concentrations of cadmium, copper, iron, lead, manganese, mercury, nickel, and selenium (Figure 3). These results also show that seasonality had less of an impact in FLB than POM and CAP. The smaller land area in close proximity to this site may explain this finding. Runoff in this area would traverse a much smaller expanse of land before entering the waterways, and may, therefore, encounter a reduced volume of contaminants compared to the other two locations.

Heavy metal concentrations were generally highest in leaves with attached epiphytes and lowest in the underground complex of roots and rhizomes. This finding agrees with a previous study by Llagostera et al. [31] 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 had significantly higher concentrations in leaves with attached epiphytes than leaves that were cleaned (Table 4). Epiphytes are sessile organisms that settle and grow on plants. On seagrasses, common epiphytes include micro-and macroalgae, bacteria, tunicates, sponges, crustaceans, and mollusks. These epiphytes are directly consumed by seagrass grazers and therefore add to the heavy metal load being ingested [32,33]. Nutrient loading near a seagrass meadow, such as from nearby agricultural lands or sewage outfalls, can lead to excessive growth of epiphytes on the leaves [34]. These same three heavy metals were found to have the highest concentrations overall, suggesting that epiphyte growth due to nutrient loading may be the main source of heavy metals to marine organisms during seagrass grazing. The epiphytes in this study were not identified to species before processing, though we know they consisted of both flora and fauna. This increase in certain heavy metals within epiphytes is most likely due to metabolic processes that bioaccumulate more contaminants than the seagrass tissues.

Agriculture is an important part of Florida's economy; however, the common farming practices in Florida, including spraying of metal-based herbicides, pesticides, and fertilizers, and application of compost or sewage sludge, are potentially increasing the heavy metal concentrations entering the coastal zone [35]. The soils used for citrus groves are often sandy, and easily lose, metals with heavy rains as they are not resistant to erosion [36]. He et al. [37], found higher concentrations of copper, iron, manganese, and zinc in agricultural soils in South Florida, and that the concentration of these metals found in citrus and vegetable field soils correlated with the amounts found in surface runoff. Levels of copper in South Florida surface waters and biota have also been increasing due to its use in agriculture as a fertilizer and to control the growth of fungi and weeds [38]. The copper concentrations found in treated aquatic vegetation in Florida exceed the levels capable of causing negative toxicity effects in mammals [39].

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

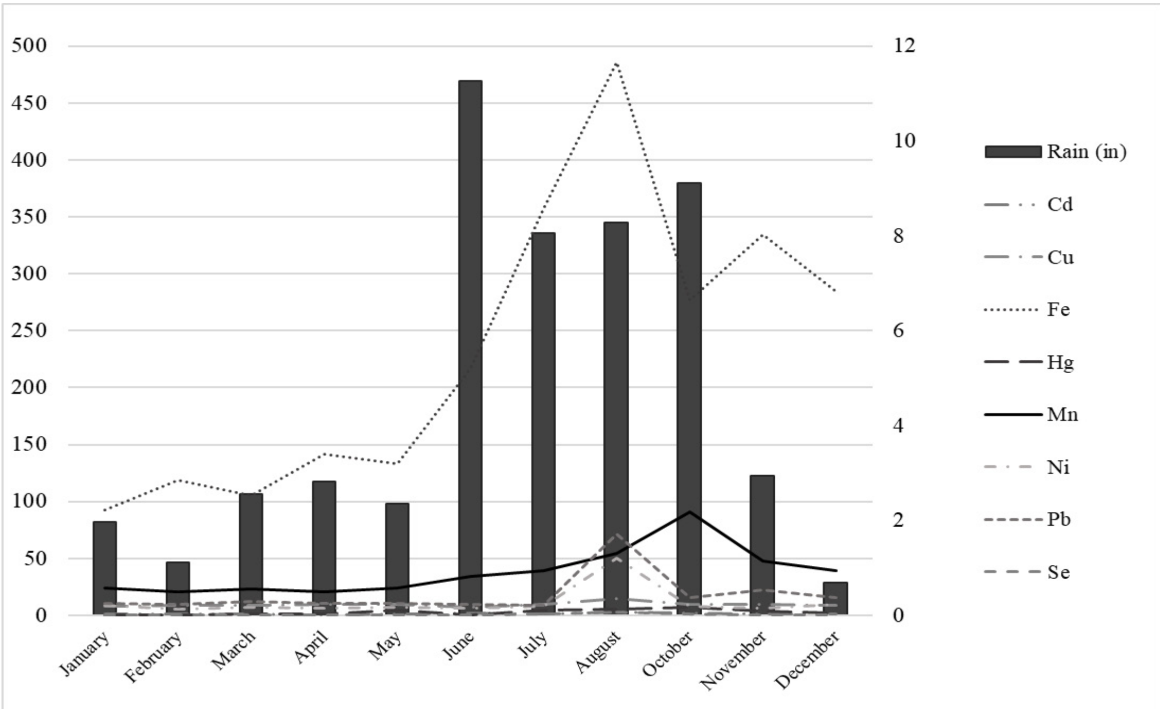


**Figure 3:** Comparison of rainfall between the wet (June-October) and dry (November-May) seasons in inches from the nearest SFWMD rain gauge in Miami-Dade (Gauge ID: Dade) to the mean concentrations (µg/g) of the eight metals significantly impacted by seasonality.

**Table 4:** 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 |

**Table 5:** Means and standard deviation (µg/g) of heavy metal concentrations (µg/g) reported from various locations and species from other studies 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 and Maher** (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.** [27] (Brazil) | *H. wrightii* | 2.78 | 0.57 | 6.50 | - | - | - | 5.65 | 2.68 | - | 20.1 |
| **Campanella et al.** (Mediterranean) | *P. oceanica* | - | 2.22 ± 0.75 | 11.6 ± 6.1 | - | - | - | - | 0.91 ± 0.23 | - | 112 ± 5.0 |
| **Lafabrie et al.** [11] (Mediterranean) | *P. oceanica* | - | 5.38 ± 0.14 | - | - | 0.13 ± 0.00 | - | 60.30 ± 3.67 | 1.8 ± 0.00 | - | - |
| **Wahbeh** [25] (Jordan) | *H. ovalis* | - | 5.1 ± 0.6 | - | 29125.9 ± 6865.4 | - | 244.5 ± 62.2 | - | - | - | 217.9 ± 53.5 |

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 the dissolution of the anodes into the seawater and can lead to zinc contamination. A study performed by Rousseau et al. [40], showed that the 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. [41], 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 were highest when the maximum number of beachgoers was present and during the hours that sunlight radiation peaked, which would correspond to the highest sunscreen application rates. Our results appear to follow the same pattern of zinc concentration spikes with higher tourism rates. Both findings suggest that sunscreen has the potential to pollute coastal waters and impact marine organisms.

Of the elements in this study, copper, iron, manganese, nickel, selenium, and zinc are essential elements, while arsenic, cadmium, lead, and mercury is non-essential, or toxic elements [42,43]. 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 (Table 6). These impacts include decreased growth rates and slowed development, loss of leaf cell viability, reduction of photosynthetic processes, and nitrogen fixation disruption [44-47].

Since many marine species, including numerous fish species and invertebrates, directly consume seagrasses, this contamination may also pose a threat to the health of marine organisms. The effects of heavy metal toxicity in organisms range from minor health impacts and morphological changes to severe health issues and can even lead to death through prolonged exposure. Various fishes and crustaceans have displayed slow growth in the presence of heavy metals, which can lead to the inability to feed and digest food properly, lack of sexual maturation, and failure to spawn [21,48].

Two important species, known to feed on seagrasses in Florida, have already been found to contain high levels of various heavy metals in their body tissues. 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 even death in many cases. The disease was found to be most closely associated with copper, iron, and lead contamination [49]. Manatees in Florida have been found to have higher concentrations of copper in their livers, though the health effects of this are undetermined [39]. A recent study also found elevated levels of copper, manganese, and zinc in whole blood samples of Florida manatees [50].

**Table 6:** Biological processes impacted by various heavy metals in laboratory and *in situ* studies.

| | Biological impacts | References |
|---|---|---|
| **Cadmium** | • Oxidative metabolism<br>• Leaf cell viability<br>• Growth rate<br>• Photosynthetic process | [44,45,47,51,52] |
| **Copper** | • Leaf cell viability<br>• Growth rate<br>• Amino acid concentrations<br>• Photosynthetic activities<br>• Browning/loss of leaves | [44,47,53-55]. |
| **Iron** | • Amino acid concentrations | [54] |
| **Lead** | • Growth rate<br>• Nitrogen fixation<br>• Photosynthetic processes | [44,45,47] |
| **Mercury** | • Growth rate<br>• Nitrogen fixation | [44,45] |
| **Nickel** | • Nitrogen fixation | [45] |
| **Zinc** | • Leaf cell viability<br>• Growth rate<br>• Photosynthetic processes | [44,46,47] |

## Conclusion

The results showed that southeastern Florida seagrasses are accumulating heavy metals. Since baseline data on toxicity thresholds of heavy metals for marine species are limited, more research is needed to determine if the concentrations found pose a health risk to the seagrasses or the organisms that feed on them. A separate analysis of epiphytes would help determine their input to the total heavy metal concentrations of the seagrasses and if they contribute substantially to the heavy metal intake of grazing organisms. Future research on the extent of transfer of these heavy metals in grazing organisms would help to 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 between certain heavy metals would help to identify the sources of these metals, and which sources pose the greatest threat to the marine ecosystem. 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.

## Highlights

- Southeastern Florida seagrasses at three locations are accumulating heavy metals
- Higher concentrations of heavy metals during the wet season
- Zinc in seagrasses was at significantly higher concentrations during the dry season
- Heavy metal highest in leaves with attached epiphytes
- Epiphytes on southeastern Florida seagrasses contribute to heavy metals

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