Final Paper

Assessment of Metal Containments in Mangrove Leaves in Jobos Bay, Puerto Rico

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Abstract: Mangrove forests provide habitat, sequester carbon, and act as a storm barrier for tropical and sub-tropical coastal regions. Mangroves are also thought to take up excess nutrients and contaminates that would otherwise harm marine ecosystems including seagrass beds and coral reefs. Metal contaminates are of particular concern because they tend to accumulate in marine ecosystems, but there has been relatively little research investigating the potential of mangroves for phytoremediation in metal contaminated environments. In this study, different ages of mangrove leaves were collected from two species of mangroves (*Rhizophora mangle* and *Avicennia germinans*) at three different sites within Jobos Bay, Puerto Rico. The leaf samples were analyzed for metal concentration using an inductively coupled plasma mass spectrometer, and the concentrations were then used to compare variations in metal contamination among sites, species, and leaf ages. Manganese was found in the highest concentration in the leaves followed by aluminum and cadmium. Leaves from *A. germinans* consistently had higher metal concentrations than *R. mangle* suggesting that this species is taking up more metal and may have more potential for phytoremediation. Metal concentrations tended to be highest in middle aged leaves indicating that metals can be translocated within the plants and do not necessarily accumulate at a constant rate in the leaves. The sampling site located between the most inland and the most coastal site tended to have lower metal concentrations suggesting that mangroves are filtering out metal contaminants from both land and ocean sources. This study reinforces the importance of mangrove ecosystems and will help to guide future research on the bioremediation potential of mangroves in Jobos Bay.

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1. Introduction

Mangrove forests are known for their importance as nursery habitats and carbon stores (Donato et al. 2011, Kathiresan et al. 2014). A lesser understood aspect of this ecosystem is its capacity to sequester excess nutrients and contaminants from fresh water inputs before the contaminants enter the marine environment (Nath et al. 2013). Mangroves have been shown to be an extremely effective carbon store because of their ability to store carbon in the biomass of the trees and in the anoxic soil of the mangrove ecosystem (Donato et al. 2011). Because mangroves are extremely effective at sequestering carbon from their environment, it is thought that mangroves could also effectively be used as bioremediators to remove excess nutrients and contaminants from heavily polluted environments (Kathiresan et al. 2014). The presence of pollution in coastal systems is of growing concern because of negative impacts it has on marine organisms and on human communities which come into contact with marine environments (Aldarondo-Torres et al., 2010) Heavy metals are of particular concern because of their many anthropomorphic sources and their ability to accumulate in marine systems (Aldarondo-Torres et al., 2010). Unlike organic contaminants, heavy metals cannot be biodegraded by biological processes (Kathiresan et al. 2014). These contaminants are found to accumulate in marine sediments and in marine organisms, some of which are consumed by humans, thus posing health risks including toxicity to the kidneys, and the reproductive and nervous systems (Aldarondo-Torres et al., 2010).

There is high variability among mangrove forests in their capacity for nutrient and contaminant storage as well their vulnerability to environmental stressors (Chumura et al. 2003). Geomorphology, hydrology, and overall climate are all factors that control nutrient and contaminant uptake by mangrove forests (Rovial et al., 2018). Increased environmental stress such as more frequent hurricanes may negatively impact the function of mangrove ecosystems (Crowl 2017). High winds and storm surges from hurricanes damage the forest canopy and can deposit excess sediment on the forest floor (Ross et al., 2006). One result of this could be large portions of a forest’s biomass being converted into organic debris (Lugo et al., 1974). In addition, hurricanes, saltwater intrusion, and other environmental stressors potentially control the ratio and zonation of mangrove species within the forest (Ross et al., 2006). These impacts could influence the ecosystem's ability to take up pollutants or could even cause the ecosystem to change from a contaminant sink to a source. Pollutants stored in leaves and branches may reenter the system if storms tear off parts of trees and convert them into organic debris. More research on mangrove forests is required to recognize their full potential for phytoremediation as well as threats to ecosystem health from increasing environmental stress.

Isotopic tracing has shown that mangroves do take up excess nutrients and have the potential to be used for waste water treatment (Lambs et al. 2011). Mangroves sequester contaminants by facilitating sedimentation that impede contaminant mobility and by taking up contaminant into the tree's biomass (Rodríguez-Iruretagoiena, et al. 2016). Additionally, mangroves have physiological adaptations including salt glands, and waxy cuticles that make them more resilient to highly polluted ecosystems (Rodríguez-Iruretagoiena, et al. 2016). Metals have been found to be taken up by mangroves, but are variable in terms of which metals are found at higher concentrations, where in the plant the metals accumulate, and the concentrations found in different mangrove species (Chowdhury et al. 2015). Relatively little past research on how mangroves take up metals and act as a filter between freshwater and marine ecosystems make mangrove forests an area requiring more research.

The Center for Aquatic Chemistry and the Environment within the Center of Research Excellence in Science and Technology (CREST CAChE) is a division of Florida International University and was developed in order to measure and track levels of pollution and create remediation strategies in Southern Florida. This is done by quantifying contaminant levels, tracking their transport and transformation, and modeling their impact. Within this program the Mangrove Supplement Project was created to explore the role of mangroves as a filter between freshwater and marine environments and investigate impacts on mangrove ecosystems from growing environmental stress (Crowl, 2017). Through a partnership between Florida International University and the University of Puerto Rico, the Mangrove Supplement focuses on mangrove forests in South Florida and Puerto Rico.

Jobos Bay National Estuarine Research Reserve (NERR) is situated on the central southern coast of Puerto Rico. The reserve has an area of 2,800 acres and is composed of a mangrove wetland complex and 15 reef-fringed mangrove islands (Laboy et al. 2006). The surrounding land is primarily used for agriculture (Whitall et al. 2011). There is also industrial development in the area that could be contributing to pollution in Jobos Bay (Aldarondo-Torres et al. 2010). While the area has been well researched, relatively little has been done to investigate the uptake of contaminants and excess nutrients by the mangroves. This area is of particular interest as the entire island is under increasing stress from more frequent hurricanes and salt water intrusion.

ICP-MS is generally used for metal analysis and has been previously used to analyze mangrove samples (Kathiresan et al. 2014). Al, As, Cu, and Mn have been found at levels higher than the national median in the sediments of Jobos Bay NERR.(Laboy et al. 2006). These metals are of particular interest for this study because they may also be found in elevated levels in the mangrove trees. Aluminum will be the major metal investigated in this study. Trace metals investigated include Ag, Cd, Cu, Mo, Cr, As, Be, Ni, V, Co, Pb, Mu, and Zn.

For my project, I was involved as an intern within the CREST CAChE program investigating the uptake of major and trace metals by mangroves in Jobos Bay National Estuarine Research Reserve. This was part of a pilot study to investigate the role of mangroves as a filter in this area. For my internship, I collected mangrove leaves and used an Inductively Coupled Plasma- Mass Spectrometer (ICP-MS) to detect the levels of various metals in the leaves. I used the data from the ICP-MS to compare metal concentration in the leaves between three different locations, two different species of mangroves (*Rhizophora mangle* and *Avicennia germinans*), and different ages of leaves.

2. Research Objectives

The main objectives of this study were to determine the following:

- The difference in metal contamination of mangrove leaves between three sample sites in Jobos Bay
- The difference in the uptake of metal containments by two different species of mangrove (*Rhizophora mangle* and *Avicennia germinans*)
- The difference in levels of metal contaminants in different ages of leaves from individual trees

3. Methods

3.1 Study sites

This study took place in the Jobos Bay National Estarine Research Reserve (NERR) located on the southeast side of the island of Puerto Rico in the Caribbean Sea. The boundaries of Jobos Bay NERR are shown using outlines (Fig 1). The three sample sites within Jobos Bay are indicated using white stars (Fig 2). Each sites is accessible by land. The sample sites were chosen along a gradient of tidal interaction with the southernmost site being covered by high tide on a daily basis and the most inland site very rarely being covered resulting in a hypersaline environment. The middle site is set back from the shoreline of the bay, but is adjacent to the Mar Negro lagoon giving it an intermediate tidal influence. The Aguirre power plant marked with a triangle is located to the East of the sample sites. The surrounding area is predominantly agricultural plots with some residential area.

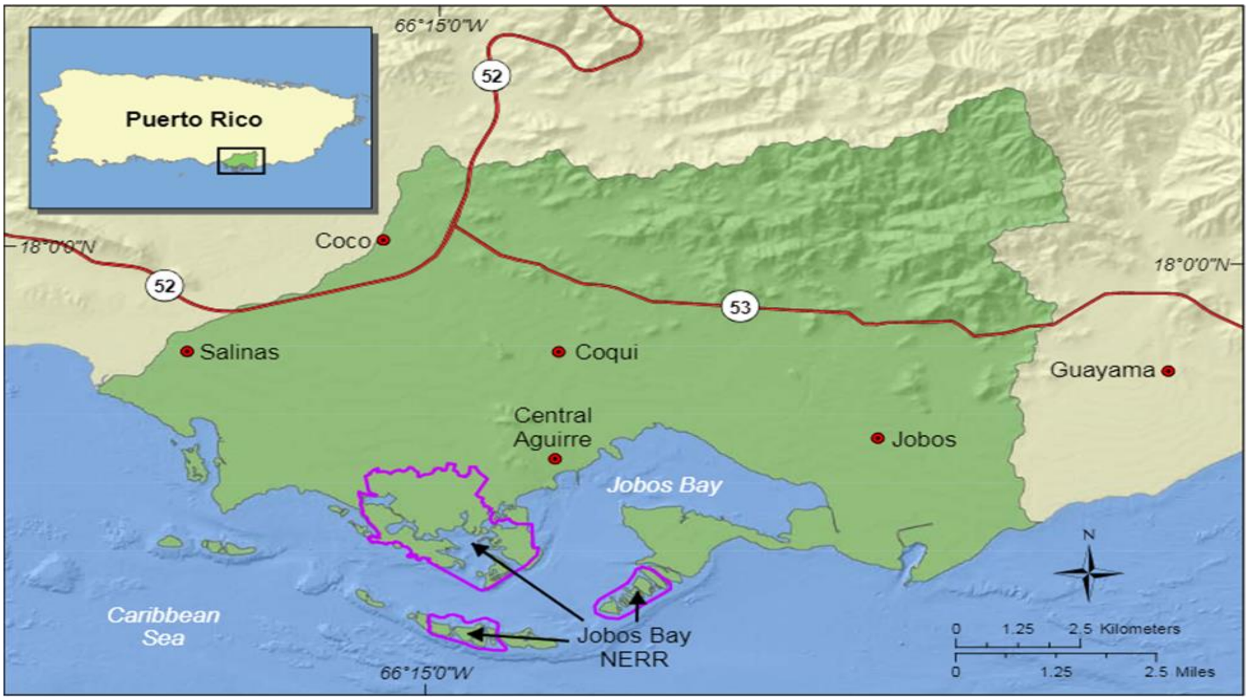

Figure 1: Location of Jobos Bay NERR in Puerto Rico, USA (Zitello et al. 2008)

Figure 2: Sample sites within Jobos Bay NERR, Puerto Rico, USA are indicated by three white stars. The Aguirre power plant is indicated by the white triangle. Sampling took place on June 7-8, 2018.

3.2 Sample collection

At each of the three sample locations, three individual trees of both red mangroves (*Rhizophora mangle)* and black mangroves (*Avecennia germinans*) were selected for sampling. At the bay fringe site only *R. mangle* were present, so samples could not be collected from *A. germinans*. Each tree sampled was tagged and its GPS coordinates taken. Trunk diameter at approximately 1.5 m height was measured and the height of the tree visually estimated. Leaf samples were collected using a pruning pole to retrieve a small branch. The samples were then be taken back to the field station where leaves were cleaned with deionized water and removed in pairs along one stem from each branch, with the oldest leaf pair being located furthest down the stem and the youngest pair at the tip of the stem. The leaf pairs were then dried in a drying oven at 40 °C in preparation for transport to Florida and further processing.

3.3 Sample Digestion and Analysis

The dried samples were homogenized using an electric mortar. 2mL of 50% nitric acid and then 5mL of concentrated nitric acid were added to 5g of each sample in a polypropylene digestion vessel and allowed to sit for 12 hours to start the digestion process. The samples were then placed in a hot block at 35°C for 1 hour. The samples were then allowed to cool for 20 minutes before adding an additional 5 ml of concentrated nitric acid to each and heating for an additional hour. After an hour the samples were cooled again for 20 minutes before adding 0.5 mL of 30% hydrogen peroxide to each sample. The samples were returned to the hot block for 1 more hour and then allowed to cool. Deionized water was used to dilute each sample up to 25 mL. Samples were then further diluted by taking 500 µL of sample solution, 100 µL of an internal standard solution, and 9400 µL of 3% nitric acid. These dilutions were then analyzed for manganese, aluminum, cadmium, copper, molybdenum, chromium, arsenic, beryllium, silver, nickel, vanadium, and cobalt using an ICP-MS.

3.4 Data analysis

Once concentrations are determined for each sample, manganese and copper levels were used to compare the three sites, two species, and leaf ages. A One-Way ANOVA was used to determine significant differences between factors for data that are normal and evenly distributed. A Kruskal-Wallis was used in the case that the assumptions of normality and equal variability weren't met. All statistical analysis was done using Minitab 17.

4 Results

Estimated tree height ranged from 2-5 m at the interior site, 3-13 m at the Mar Negro site, and 5-15 m at the bay fringe site. The trees at the interior site were found to have the lowest range of diameter from 2-6 cm. Tree diameter ranged from 6-13 cm in the Mar Negro site and 8-16 cm in the bay fringe site.

The following metals were found to be present in the leaf samples in order of highest mean concentration to lowest: manganese>aluminum>cadmium>copper>molybdenum>chromium> arsenic>beryllium>silver>nickel>vanadium>cobalt. Lead, mercury, and zinc were also found to be present in the samples, but their concentrations were not determined.

Only *R. mangle* was used to compare metal concentrations among sites since this species was the only one present in all three sites. Copper concentrations were observed to be highest at the Bay Fringe site, however no significant variation was observed in among sites (H=3.47, P=0.177; Fig. 3). Similarly, no significant variation was found among the sites for manganese although concentrations were observed to be higher at the interior site (F=5.35, P=0.057; Fig. 4). Both copper and manganese were found to be lowest in the Mar Negro site, although this trend was not found to be significant.

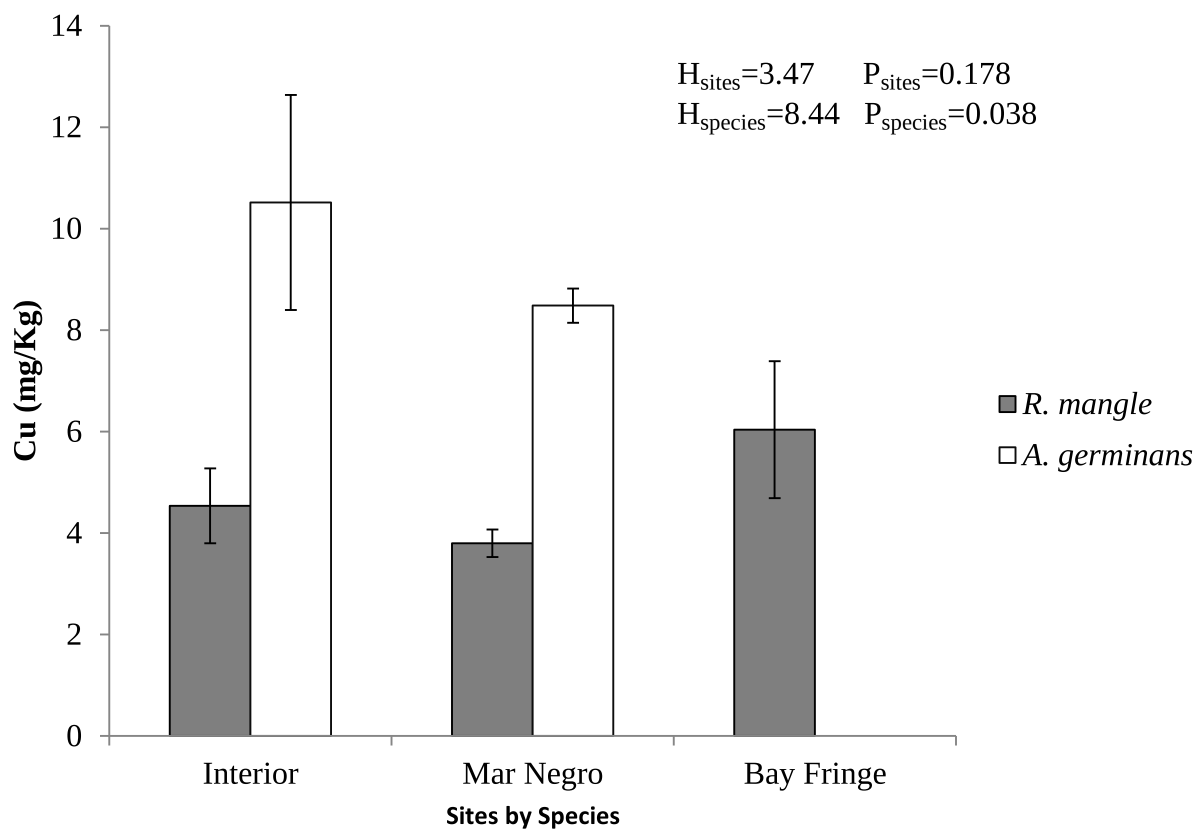

Figure 3: Comparison of mean (±S.E.) copper concentrations among species and sites. Results of the Kruskal Wallis tests are displayed on upper right of graph. Sampling took place on June 7-8, 2018 in Jobos Bay National Estuarine Research Reserve, Puerto Rico, USA.

Comparing the metal concentrations between the two species sampled revealed copper concentrations to be significantly higher in *A. germinans* than *R. mangle* (H=8.44, P=0.038).

Manganese concentrations were also found to be higher in *A. germinans,* but only in the Mar Negro site (F=7.59, P=0.013).

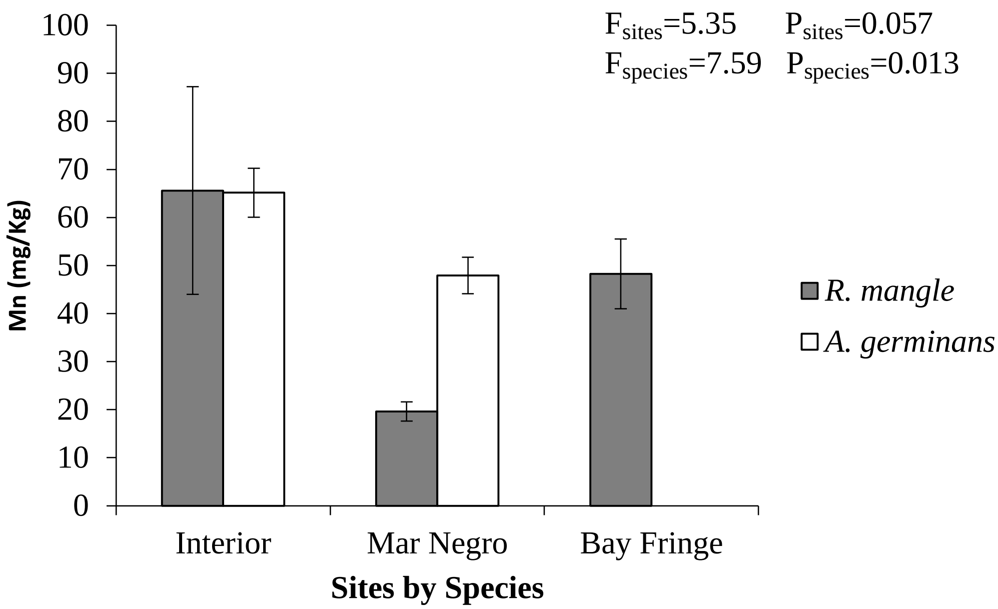

Figure 4: Comparison of mean (±S.E.) manganese concentrations among species and sites. Results of the One Way ANOVA tests are displayed on upper right of graph. Sampling took place on June 7-8, 2018 in Jobos Bay National Estuarine Research Reserve, Puerto Rico, USA.

A general trend was observed where the middle aged leaf of each pair had higher copper concentrations than the younger and older leaf pairs, however there was no significant variation among ages (H=35.58, P=0.669; Fig. 5). Similarly, no significant variation was observed for manganese for different aged leaves (H=0.18, P=0.914; Fig. 6).

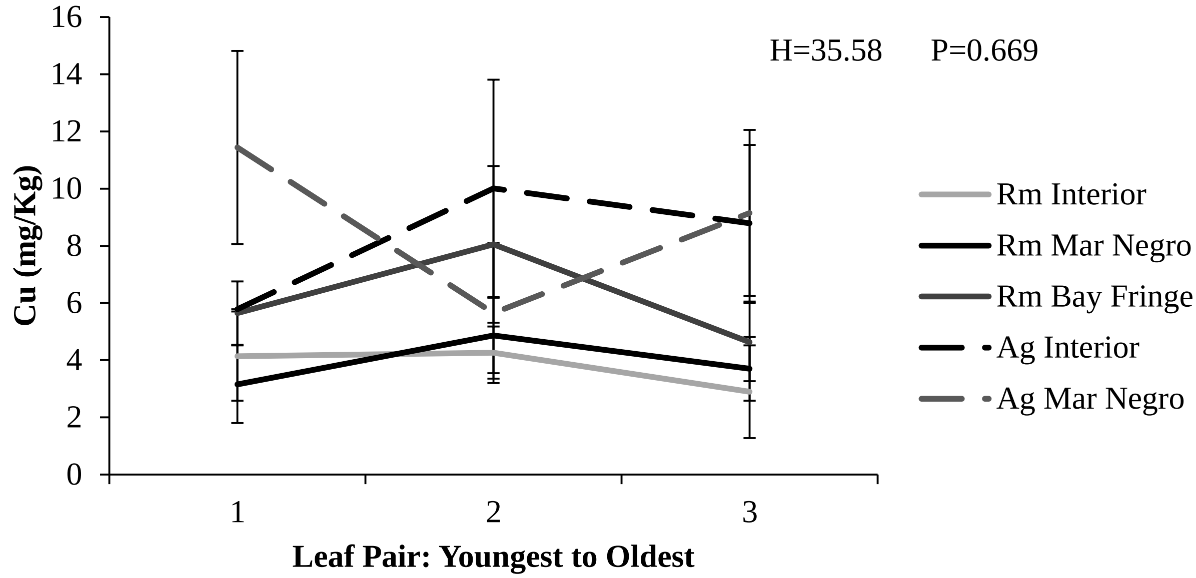

Figure 5: Comparison of mean (±S.E.) copper concentrations among different aged leaf pairs for each species at each site. Results of the Kruskal Wallis test are displayed on upper right of graph. Sampling took place on June 7-8, 2018 in Jobos Bay National Estuarine Research Reserve, Puerto Rico, USA.

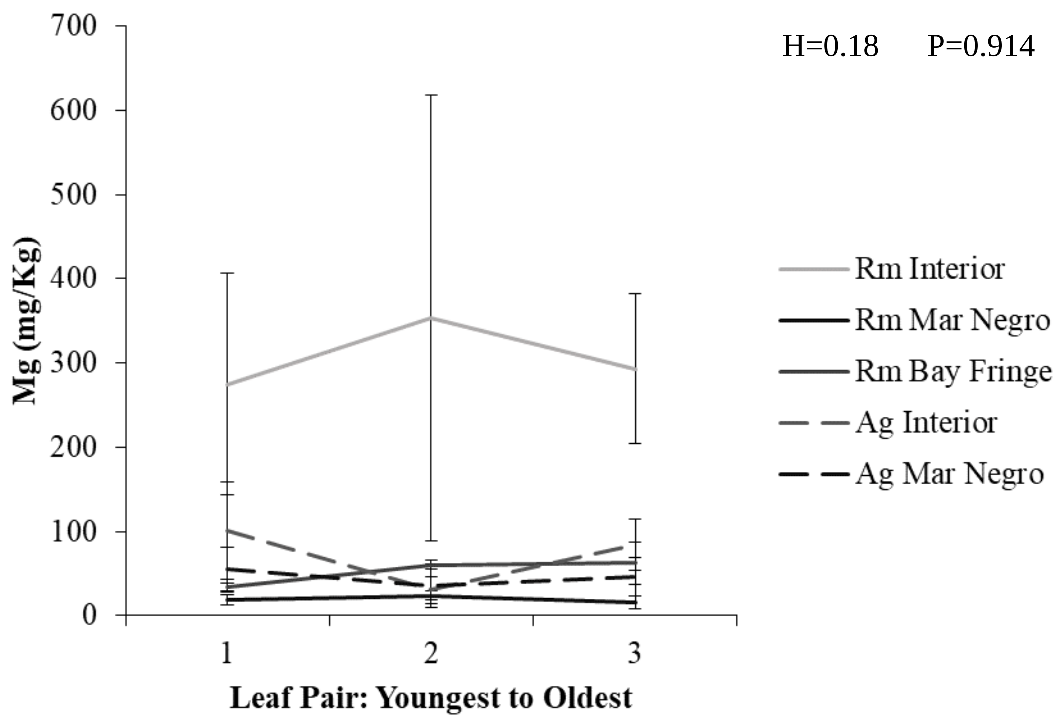

Figure 6: Comparison of mean (±S.E.) manganese concentrations among different aged leaf pairs for each species at each site. Results of the Kruskal Wallis test are displayed on upper right of graph. Sampling took place on June 7-8, 2018 in Jobos Bay National Estuarine Research Reserve, Puerto Rico, USA.

5 Discussion

The general concentration of copper considered healthy for plants is 10 mg/kg. Healthy concentrations of manganese can be up to 25 mh/kg (Agriculture and Forestry). Copper concentrations observed only exceeded this healthy threshold in the *A. germinans* sampled at the interior site suggesting that mangroves in Jobos Bay aren't under stress from copper. On the other hand, the concentrations of manganese observed far exceeded the reported healthy level. Levels of metal tolerance are highly variable between plant species, so it is possible that both species of mangroves sampled have a much higher manganese tolerance than other species (Chumura et al. 2003). The highest levels of manganese were found in the interior site which was also the site with the smallest trees, but the second highest levels were found in the bay fringe site which had the largest, healthies looking trees. Further studies are needed to determine the level at which manganese goes from being a micronutrient to a contaminant in different species of mangrove.

A. germinans has been found to have a higher phytoremediation capability than other species including *R. mangle* (Maldonado-Román et al. 2016). The results of our study corroborate this since both copper and manganese are found at higher concentrations in the leaves of *A. germinans* than the leaves of *R. mangle*. The higher levels of metal in *A. germinans* could be related to the different methods each species has of dealing with salt stress. *R. mangle* is a salt-excluding species whereas *A. germinans* is a salt excreting species. Through the mechanism of iron plaques, *R. mangle* may prevent the entrance of both salt ions and metals while *A. germinans* transports ions up to the leaves where they can be excreted (Maldonado-Román et al. 2016). These different methods of dealing with salt stress may indicate mangrove forests containing primarily *A. germinans* can be be more effective barriers for preventing metals from reaching the marine environment as the metals are more likely to be sequestered by the trees when they enter the ecosystem.

Sediment samples throughout the bay found that concentrations of most metals increased towards the inner bay (Whitall et al. 2011). Thus, it was anticipated that the metal concentrations in mangrove leaves would follow the same trend. The highest levels of manganese were indeed found in the interior site. On the other hand, copper was found to be highest in the bay fringe site where it was anticipated to be the lowest. As the variation among sites wasn't significant, further research is needed. Copper is a micronutrient and the levels observed didn't exceed what was expected, so the higher concentrations in the bay fringe site could simply indicate a healthy forest. Alternatively the higher concentrations could indicate that metal contamination is actually coming from the ocean rather than being filtered out before it reaches the ocean. A channel in the bay leads directly from the power plant in Aguirre past the bay fringe site (Morelock et al. 2018; Fig.2). The current in the bay typically flows in a direction that would carry contaminants from the power plant to the bay fringe site. The channel and the current suggest that metal entering the environment from the power plant could be carried directly to the bay fringe site.

Other metals such as magnesium have been found at higher concentrations in the older leaves of mangroves and copper concentrations were expected to follow this pattern (Lugo et al. 1998). While no significant pattern was found, it appears that the middle-aged leaves had more copper than both the old and young leaves. One possible reason for this is that the plant is translocating copper from the old leaves to the middle ages leaves in order to retain the copper as the old leaves are lost. Copper is a micronutrient, so it is possible that the plant is passing copper back from the older leaves in order to prevent losing it when the oldest leaves fall off the branch.

The main limitation of this study was the small sample size. Only three trees of each species were sampled from each site. Thus, while some trends were observed, they were mostly insignificant. Collecting samples from a greater number of trees within each location would increase the probability of finding significant variations that answer the questions posed. Additionally, this study is limited in that only leaf samples were analyzed. In order to fully understand if and how mangroves are sequestering contaminants, we need to know the levels of contamination throughout the ecosystem and how those contaminants are distributed through the mangrove itself. Further studies should look at metal concentrations both in the soil at each site and in parts of the mangroves other than the leaves.

6 Conclusion

The most significant result of this study is that the leaves of *A. germinans* have significantly higher concentrations of copper and manganese than *R. mangle*. This indicates that *A. germinans* may have higher potential for phytoremediation use. No significant trends were found among the sample locations and the leaf ages, but this is likely because of the small sample size. The results of this pilot study provide preliminary data on the metal uptake by mangroves in Jobos Bay, Puerto Rico. This data will help direct future research in the area. Future studies may include similar designs, but with larger sample sizes to determine significant trends. Leafe samples will be re-analyzed to determine concentrations of lead, mercury, and zinc. Additionally, looking at metal concentrations in other parts of the mangroves and the soil at the sample sites will aid in determining accumulation rates of metal contaminants. Looking at nutrient levels alongside contaminant levels with in tree tissue will lead to a better understanding of if the mangroves are under stress from contaminants. Mangrove forests are a critical ecosystem and more research is needed to understand and protect them.

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