# Does Sargassum spp. Compost Impact the Arsenic and Bacteria Levels within the Beach Environment? (FINAL)

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Afeefa A. Abdool-Ghany, Ph.D. Helena Solo-Gabriele, Ph.D., P.E.

University of Miami, College of Engineering, Coral Gables, FL Department of Chemical, Environmental, and Materials Engineering

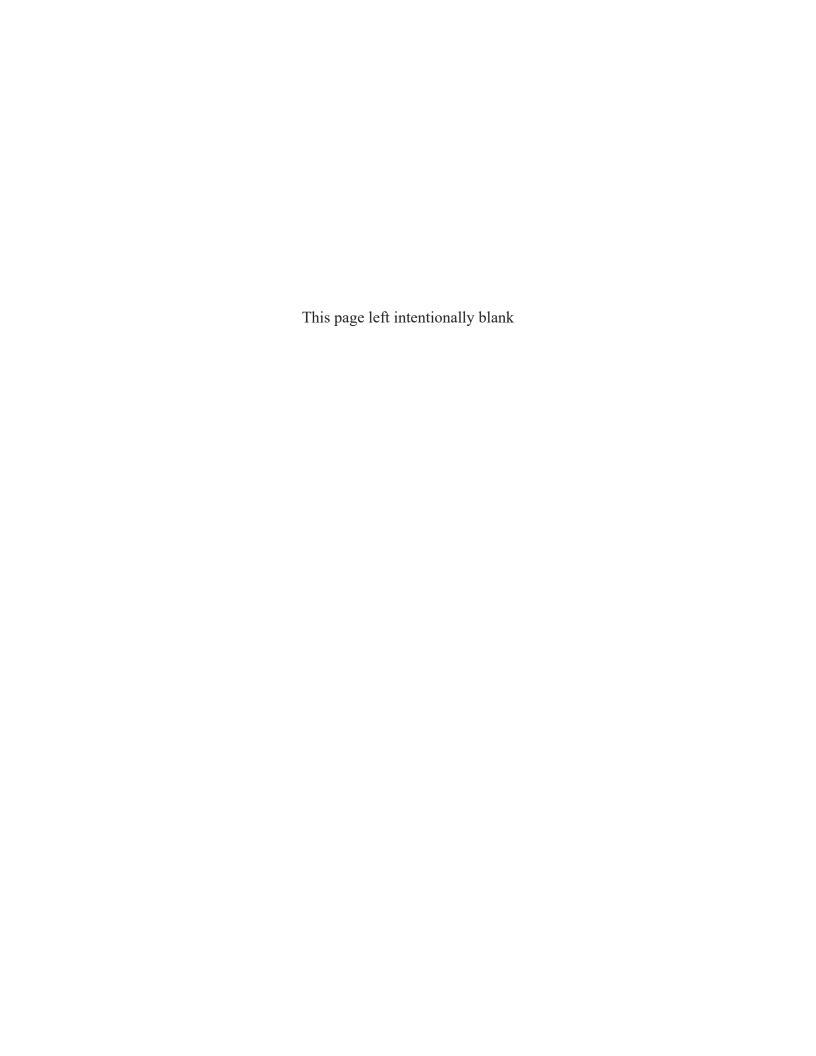
With Arsenic Speciation by

Yong Cai Ph.D. Guangliang Liu Ph.D.

Florida International University, Miami, FL Department of Chemistry and Biochemistry

Hinkley Center for Solid and Hazardous Waste Management

University of Florida P. O. Box 116016 Gainesville, FL 32611 www.hinkleycenter.org



# **TABLE OF CONTENTS**

LIST OF FIGURES	Page no iii
LIST OF TABLES	V
LIST OF ABBREVIATIONS AND ACRONYMS	vi
UNITS OF MEAURE	vi
EXECUTIVE SUMMARY	vii
CHAPTER I	9
I.1 Introduction	10
I.2 Objectives	11
CHAPTER II (ENVIRONMENTAL STUDY)	12
II.1 Introduction	13
II.2 Methods	14
II.2.1 Site Description and Wrack Management Practices	14
II.2.2 Sample Collection	16
II.2.3 Enumeration of Enterococci	16
II.2.4 Arsenic Analysis	17
II.2.5 Statistical Analysis II.3 Results	17 17
II.3.1 Impact of Management Style on Beach Quality	17
II.3.2 Enterococci Levels	18
II.3.3 Arsenic Levels	18
II.4 Discussion	26
II.4.1 Enterococci Concentrations in Sargassum and Seagrass	26
II.4.2 Arsenic Concentrations in Sargassum and Seagrass	26
II.4.3 Variability in Arsenic Concentrations Due to Management Practices	27
II.4.4 Limitations	28
II.4.5 Recommendations	28
II.5 Conclusions	29
CHAPTER III (MESOCOSM STUDY)	30
III.1 Introduction	31
III.2 Methods	32
III.2.1 Sargassum Collection	32
III.2.2 Mesocosm Setup	33
III.2.3 Initial XRF Measurements III.2.4 Sample Collection from Mesocosms	35 35
III.2.4 Sample Collection from Mesocosms	33 36
III.2.5 Laboratory Analysis of Arsenic and Arsenic Species III.2.6 Water and Arsenic Mass Balance	36
111.4.0 Watel alle Albeille Wass Dalaille	30

# **TABLE OF CONTENTS (continued)**

	Page no.
III.2.7 Statistical Analyses	38
III.3 Results	38
III.3.1 Concentration Ranges	38
III.3.2 Temporal Trends in Arsenic Concentrations	41
III.3.3 Water and Arsenic Mass Balance	44
III.3.4 Arsenic Speciation	47
III.4 Discussion	50
III.4.1 Arsenic Levels	50
III.4.2 Temporal Trends	50
III.4.3 Water Balance	52
III.4.4 Arsenic Balance	52
III.4.5 Arsenic Speciation	54
III.4.6 Implications for Management and Risk	54
III.4.7 Future Directions	55
III.4 Conclusions	56
CHAPTER IV	57
IV.1 Summary and Conclusions	58
IV.2 Recommendations	58
IV.3 Practical Benefits for End Users	59
REFERENCES AND PERTINENT LITERATURE	60
APPENDIX A, Field Photos and Data Tables for Environmental Study	66
APPENDIX B, Data Tables for Mesocosm Study	71
APPENDIX C, Project Administration	75

### LIST OF FIGURES

- Figure II.1. A. Photographs illustrating beach zones categorized by Sargassum and seagrass management strategies: integration (in-place decomposition), no removal (unmanaged accumulation), and removal (manual or mechanical extraction). B. Images representing the stages of Sargassum and seagrass degradation: fresh (recently deposited), senescent (partially decayed), and decomposing (advanced breakdown).
- Figure II.2. The top panel shows the concentration of enterococci (CFU/100 mL) in ankle-deep water across five sampled beaches. The middle panel depicts enterococci concentrations (CFU/dry g) in three sand zones (supratidal, sand under, and bladed sand) at the same beaches. Beaches 1,3,4, and 5 had supratidal and sand under sample collected, while beach 2 had an additional sample collected in the bladed sand zone. The bottom panel highlights enterococci concentrations in *Sargassum* and seagrass samples. Beaches 1,2, and 3 has *Sargassum* only collected, while Beaches 4 and 5 had *Sargassum* and seagrass samples collected. Beach 5 has one sample of fresh *Sargassum* collected.
- Figure II.3. The top panel illustrates arsenic concentrations (mg/kg) in sand samples from three zones (supratidal, sand under, and bladed sand) across five beaches. The bottom panel displays arsenic concentrations in *Sargassum* and seagrass samples from the same beaches, highlighting significant variability in arsenic accumulation across different wrack types and locations.
- Figure II.4. The top panel illustrates enterococci concentrations (CFU/dry g) in *Sargassum* and seagrass, showing a significantly higher bacterial load in *Sargassum* compared to seagrass. The bottom panel presents arsenic concentrations (mg/kg), with *Sargassum* accumulating more arsenic than seagrass.
- Figure II.5. Enterococci (Panel A) and arsenic concentrations (Panel B) across different decomposition stages—fresh, senescent, and decomposing—for both *Sargassum* and seagrass. Except for one fresh *Sargassum* sample, enterococci levels are consistently above several hundred CFU/g range. Arsenic levels are consistently higher in *Sargassum* compared to seagrass throughout the decomposition process.
- Figure III.1. Field-collected pelagic Sargassum assemblages. Left panel shows a mixture of *S. fluitans* (broad, serrated blades) and *S. natans* (narrow, smooth to lobed blades with clustered vesicles). Samples were collected from the wrack line prior to mesocosm deployment. Right panel shows representative Sargassum natans subsample collected for initial arsenic concentration analysis. The sample displays characteristic spherical pneumatocysts and narrow blades, with no attachment structures, confirming pelagic origin.
- Figure III.2. Top panel: Mesocosm setup on roof of 5-story building in Coral Gables, Florida (McArthur Engineering Building) used to monitor rainfall and arsenic concentration changes in Sargassum, sand and leachate over time). Photo illustrates the six sand

only (SO) and the six Sargassum and sand (SS) mesocosms. Plastic physical and acoustic bird deterrents were added to minimize disturbances from birds. Bottom panel: close up of 3 SO mesocosms (left) and SS mesocosms (right).

- Figure III.3. Box and whisker plots of arsenic concentrations in solid samples, sand (SO and SS mesocosms) and Sargassum (left) and in liquid samples, rain, and leachate (SO and SS mesocosms) (right). Box edges represent the 25and 75% ranges. The circles represent individual data points. The line in the box represents the median. The "×" symbol represents the average. Data points outside the whiskers represent outliers. The photos in the right panel illustrate the difference in sample color between the SS and SO leachates.
- Figure III.4. Time series of rainfall depth (top panel) and arsenic concentrations (bottom three panels) in Sargassum, sand, and in aqueous samples (rainwater and leachates).
- Figure III.5. Summary results from arsenic mass balance analysis emphasizing the initial reservoir of arsenic in the Sargassum in the SS mesocosms and the reservoirs to which the arsenic was found.
- Figure III.4. Arsenic speciation of Sargassum (top panel), sand (middle panel), and leachate (bottom panel) in the SO mesocosms (no Sargassum) and in the SS mesocosms (with Sargassum). Species measured included As(V), MMA(V), DMA(V), As(III), MMA(III), DMA(III), AsB, AsC, and TMAO.
- Figure A.1. Photos of five beach sites taken during sampling, depicting the environmental conditions at the time of sample collection. The images illustrate the physical state of the beaches under different management practices—Integration of beach wrack, Removal of beach wrack, and No Removal—highlighting the variability in wrack presence, sand conditions, and beach management approaches. Two sampling teams collected samples starting at their designated beaches at sunrise. Sampling commenced at Beach 1 at sunrise and then continued to Beach 2. Similarly sampling commenced at Beach 3 at sunrise and commenced to 4 and 5. Beach grooming usually started at beaches 1 through 4 shortly after sunrise and for this reason, beach grooming activities are more obvious for the second beaches (Beaches 2 and 4) visited by each team.
- Figure A.2. Photos of wrack collected from three sampling periods at beaches practicing Integration, Removal, and No Removal management styles.

# LIST OF TABLES

- Table II.1. Beach Management and Wrack Characteristics for the Study Beaches
- Table II.2. Summary of enterococci and arsenic concentrations by beach site and sampling day
- Table II.3. Summary of enterococci and arsenic concentrations by sample type, wrack decomposing status, and beach management style
- Table III.1. Water balance for mesocosm experiments. Water volumes listed below correspond to the cumulative amounts for all samples collected from the Sargassum plus Sand (SS) mesocosm and from the Sand Only (SO) mesocosm for the period from March 13, 2024 through the last day of sample collection on May 22, 2024.
- Table III.2. Arsenic balance for mesocosm experiments. Arsenic masses listed below correspond to the cumulative amounts for all samples collected.
- Table A.1. Raw data from the study, presenting the concentrations of bacteria and arsenic across various management styles—Integration, Removal, and No Removal. All water samples were collected in ankle deep water and are identified as "Ankle Water". Sand identified as "Sand Under", "Supratidal" and "Bladed". Wrack identified as "Sargassum" and "Seagrass".
- Table B.1. Arsenic concentrations measured in Sargassum samples collected between March 13, 2024, and May 22, 2024. The table includes the measured arsenic result (mg/kg dry), the dilution factor (Dil), and the Method Detection Limit (MDL) for each sampling date. Dilutions were adjusted as needed to ensure values fell within the quantifiable range.
- Table B.2. Arsenic concentrations in sand samples (mg/kg dry weight) from control and experimental conditions collected between March 13, 2024, and May 22, 2024. Control samples contained sand without Sargassum, while experimental samples included sand beneath decomposing Sargassum. The data reflect changes in arsenic levels over time potentially due to leaching from Sargassum into underlying sand.
- Table B.3.Elemental concentrations (ppm) in sand and Sargassum samples measured via X-ray fluorescence (XRF). Notably, arsenic (As) was detected in Sargassum (22.33 ppm) but not in sand (ND). Additionally, titanium (Ti), antimony (Sb), and lead (Pb) were only detected in Sargassum, highlighting potential accumulation of certain elements in the biomass.

### LIST OF ABBREVIATIONS AND ACRONYMS

As Arsenic
AsB arsenobetaine
AsB arsenocholine

As(V) Arsenic in the +5 oxidation state, arsenate As(III) Arsenic in the +3 oxidation state, arsenite

DMA Dimethylarsinic acid

EREF Environmental Research & Education Foundation

F.A.C. Florida Administrative Code

FL Florida

FDEP Florida Department of Environmental Protection

HCSHWM Hinkley Center for Solid and Hazardous Waste Management

MMA Monomethylarsinic acid PI Principal Investigator RFP Request for Proposal

RSMAES Rosenstiel School of Marine, Atmospheric, and Earth Science

SCTL Soil Cleanup Target Levels

SO Sand only, in the context of the mesocosms

SS Sand and Sargassum, in the context of the mesocosms UM University of Miami (also abbreviated UMiami)

USCC United States Composting Council

US EPA United States Environmental Protection Agency

TMAO Trimethylarsine oxide

#### UNITS OF MEASURE

% Percent, Parts per hundred

°C Degrees Celsius

CFU/100mL Colony forming units per one hundred milliliters

cm Centimeters kg Kilograms km Kilometers L Liter

mg Milligrams
m³ Cubic meters
mm Millimeters

mg/kg Milligram per kilogram

mL Milliliter

pH Measure of the hydrogen ion activity

μg/L Micrograms per liter

yd<sup>3</sup> Cubic yards

# **EXECUTIVE SUMMARY**

Sargassum, a floating macroalgae composed primarily of *Sargassum natans* and *Sargassum fluitans*, has increasingly inundated Florida's beaches during the spring and summer months. These seasonal strandings are becoming the "new normal," with volumes expected to rise due to global climatic changes that fuel blooms in the Atlantic Ocean. In response to large Sargassum events, municipalities often contract with third-party haulers to remove the material to landfills, a costly approach. Once deposited in landfills, Sargassum decomposes and can emit hydrogen sulfide, posing additional environmental concerns. There is a growing need for sustainable Sargassum management alternatives.

Previous studies have identified two main barriers to reusing Sargassum: elevated arsenic concentrations and the presence of bacteria that may exceed regulatory standards. For instance, composted Sargassum has been found to contain arsenic levels ranging from 6.64 to 26.5 mg/kg, surpassing Florida's Soil Cleanup Target Levels, which restricts its use in landscaping or restoration. Additionally, compost produced with tumbler systems has tested above acceptable limits for fecal indicator bacteria such as enterococci and fecal coliform.

One promising strategy is localized composting on or near the beach, which could reduce hauling costs and allow the compost to be repurposed for dune or mangrove restoration. However, concerns remain about whether Sargassum contributes to elevated arsenic levels at the beach—and whether composting it in place could worsen that burden. This study aimed to answer those questions by evaluating background arsenic and bacterial levels in beach environments, alongside laboratory simulations of Sargassum decomposition.

The study was divided into two phases:

#### Phase I – Environmental Study (Chapter II):

Samples were collected from beaches with varying levels of Sargassum accumulation: minimal, moderate, and heavy. Sargassum, sand beneath it, and beach water were analyzed for arsenic and enterococci (a fecal indicator bacteria). Results showed no significant difference in enterococci levels between Sargassum and seagrass wrack types (p = 0.30), with a maximum of 9,600 CFU/g. However, arsenic levels were significantly higher in Sargassum (up to 64.3 mg/kg) compared to seagrass (2.18 mg/kg) (p < 0.001). Sand beneath Sargassum that was managed through integration also exhibited elevated arsenic levels (average 4.92 mg/kg). These findings underscore the importance of wrack type in evaluating potential health risks.

# Phase II – Mesocosm Study (Chapter III):

Controlled lab experiments simulated Sargassum decomposition over time in mesocosms containing either Sargassum and sand (SS) or sand only (SO). In the SS mesocosms, arsenic levels in Sargassum peaked at 66.7 mg/kg during drying and declined to 7.2 mg/kg by day 70. Of the arsenic lost from Sargassum, 41% was recovered in leachate, 5% was absorbed into the underlying sand, and 54% was presumed volatilized. Sand in the SS mesocosms showed a slight but statistically significant arsenic increase (p = 0.003), while the SO controls did not. Leachate arsenic concentrations from the SS mesocosms were significantly higher than rainfall arsenic levels (322  $\mu$ g/L vs. 2.19  $\mu$ g/L; p = 0.0067), whereas SO leachates were not significantly

different from rainfall (p = 0.97). Arsenic speciation analysis revealed that inorganic As(V) was the dominant form, followed by dimethylarsinic acid [DMA(V)].

#### **Conclusion:**

This study confirms that Sargassum can serve as a significant source of arsenic to coastal environments—primarily through leaching and volatilization—with a smaller fraction retained in the sand. These findings highlight the complex role of Sargassum in arsenic cycling and its potential to increase exposure risks for beachgoers. As Sargassum influxes intensify, new management strategies are needed to avoid costly landfilling while addressing the public health and environmental implications of arsenic release during decomposition.

# CHAPTER I INTRODUCTION & OBJECTIVES

## **CHAPTER I**

### **INTRODUCTION & OBJECTIVES**

This chapter focuses on describing the introduction (Section I.1) and objectives (Section I.2) for this study.

#### I.1 Introduction

This proposal is building upon two previous Hinkley research projects. The first research project focused on composting of *Sargassum* using six different recipes (unwashed *Sargassum*, washed *Sargassum*, but grass clippings, *Sargassum* plus mulch, outdoor *Sargassum*, outdoor *Sargassum* plus vegetative waste). Results from this first project suggest that arsenic levels are elevated, and the compost does not pass the Florida Soil Cleanup Target Levels (SCTL) for residential use. In addition, when radishes were grown using the *Sargassum* compost, the arsenic concentrations exceeded guidelines for human consumption as outlined by the Food and Agriculture Organization and the World Health Organization (FAO/WHO). Similarly fecal indicator bacteria levels were also observed to exceed guidelines for the compost samples made for one set of samples, those using smaller scale tumbler systems. Bacteria concentrations met guideline levels within the large-scale compost piles. It is therefore recommended that the compost process take place outdoors in a large-scale setting to avoid excessive levels of fecal bacteria.

One very important benefit of composting is the 90% reduction in weight of the *Sargassum* once it is decomposed. This reduction in weight translates into lower costs for hauling. Aside from hauling the *Sargassum* to a staging area to decompose, there are costs associated with the removal of the *Sargassum* from the beach. To minimize these costs as well as remove the *Sargassum* from the beach, composting closer to the beach (within or near the back dune areas) can be a potentially sustainable solution.

There have been several news articles released that warn of a giant seaweed bloom that is making its way to beaches across the Caribbean and Florida in 2023 (Chow, 2023; Rivero, 2023; Tucker, 2023). This seaweed bloom is so large, that it can be seen from space and spans 5,000 miles (Tucker, 2023). Oceanographers who track the blooms have been showing that the blooms have been increasing over the years (Wang et al, 2019). Oceanographers warn that the bloom this year is one of the largest and depending upon the currents and wind direction, the summer 2023 can be record-breaking in terms of *Sargassum* volumes. As these large strandings of *Sargassum* make their way onshore, there is potential for arsenic contamination (Cipolloni et al., 2022). *Research is therefore needed to understand the impacts that these inundations have on beaches and given this understanding, what are the sustainable management options for these large influxes of macroalgae.* 

To minimize the costs that are associated with the large inundations to the beach, a potentially sustainable solution to *Sargassum* inundations would be to compost it along the upper reaches of the beach. This would minimize the need for hauling the *Sargassum* to an off-site staging area or landfill. Once composted on the beach site, the *Sargassum* compost can then potentially be applied to the dune areas to provide nutrients for plant growth that would in turn reinforce coastal dunes providing protection against beach erosion and storm surge. However, when discussing the possibility of beneficially reusing *Sargassum* compost on beach dunes the first questions regulators asked (specifically from representatives from the FDEP) is, "What is the background level of

arsenic at the beach?" and "What would be the impact of *Sargassum* compost on the arsenic levels within the beach environment?"

The purpose of this study to was address these questions. This study assessed three types of samples (water, sand, and *Sargassum*) for arsenic and bacteria to measure the background levels at the beach under natural conditions. In addition to analyzing samples for arsenic, samples were analyzed for enterococci. Another component of this study was to examine the effect of rain on the *Sargassum* as it decomposes under natural conditions onshore. This was completed in the laboratory through mesocosm experiments which simulated *Sargassum* stranded onshore and subject to rainfall conditions. These experiments were designed to measure the arsenic levels in all compartments (*Sargassum*, sand, runoff, simulated rainfall) over time to assess the movement of the arsenic among the compartments. Through the field and laboratory studies, our objective was to answer the questions posed by FDEP. Answering these questions are important to evaluating beneficial uses of *Sargassum* within the beach environment, which is the most convenient location for composting *Sargassum* and for using this compost.

# **I.2 Objectives**

The objective of this proposal was to assess the fate of arsenic and bacteria in seaweed strandings within the beach environment. The ultimate purpose of this assessment was to evaluate whether composting *Sargassum* on the beach is feasible thus minimizing the need for hauling offsite. This information is necessary to evaluate the use of *Sargassum* compost within the beach environment (e.g., within the dune area) as an alternative to landfilling. To evaluate this objective, we:

- 1) Collected and analyzed samples from beaches to document the levels of arsenic and enterococci in water, sand, and *Sargassum* under natural conditions. (Phase I, Environmental Study, described in Chapter II).
- 2) Collected and examined samples in a controlled laboratory environment to measure how arsenic and bacteria levels change overtime in *Sargassum* as it decomposes and with the introduction of natural rain and sunlight irradiation (Phase II, Mesocosm Study, described in Chapter III). Specifically, through this phase of study we answered the question of whether the arsenic from the decomposing *Sargassum* accumulates in the sand or is readily washed out during rainfall events.

# CHAPTER II ENVIRONMENTAL STUDY

## **CHAPTER II**

#### **ENVIRONMENTAL STUDY**

#### **II.1 Introduction**

Beach wrack, organic plant and algal matter stranded on beach shores through the action of tides, wind, and waves (Graca et al., 2022), has been increasing worldwide due to the impacts of climate change and eutrophication (Wang et al., 2019; Robledo et al. 2021; Theirlynck et al., 2023). The primary components of wrack at beaches that border the Atlantic Ocean are seagrasses and Sargassum. Seagrasses are marine flowering plants, possessing leaves, flowers, seeds, roots, and connective tissue (Nordlund et al., 2018) that grow submerged under water in soft sediment beds near coastlines. Sargassum, on the other hand, is a free-floating macroalgae that includes over 300 species. Although in "normal" stranding scenarios, the Sargassum plays an important role in maintaining ocean ecosystem health (Fourqurean et al., 2012), when the influxes are very large, coastal systems are overwhelmed, threatening ecosystems, fisheries, tourism, and public health.

When the quantities of beach wrack are overwhelming, the wrack is managed at beaches using different strategies. In the Yucatan Peninsula of Mexico, the material is removed as it approaches the shore and after stranding (Chávez et al., 2020). In South Florida wrack is managed at beaches using burial or integration (mixing into sand). When quantities are excessive, the Sargassum is hauled off-site to landfills or to compost facilities (Abdool-Ghany et al., 2023a, 2023b).

The impacts of beach wrack when in excess are far-reaching, including contamination of the beach environment. Studies have started to document the contribution of wrack towards the proliferation of fecal indicator bacteria, enterococci (Abdool-Ghany et al., 2022). However, there is a new concern which has not gained much attention until recently: the impacts of metals in particular arsenic, due to the ability of Sargassum to hyperaccumulate this metalloid.

Sargassum species and most brown macroalgae are known to bioaccumulate trace metals from their surroundings, especially arsenic (Devault et al., 2020; Devault et al., 2021; Dassié et al. 2021; Cipolloni et al., 2022.). Brown macroalgae bioaccumulate arsenic through passive and active uptake mechanisms. Arsenate (As<sup>5+</sup>), structurally similar to phosphate, is absorbed via phosphate transporters, while arsenite (As<sup>3+</sup>) diffuses directly across cell membranes. Within the algal tissues, arsenic can be transformed into organic forms like arsenosugars, which may become environmentally reactive when the algae decompose, potentially leaching toxic inorganic arsenic into beach sand and coastal waters (Francesconi, 2010; Xue et al., 2020).

The concern about arsenic is driven by its toxicity at low levels. Regulatory guidelines for arsenic are dependent upon intended use of a product. The US EPA has established a guideline level of 75 mg/kg for land application of sewage sludge. States such as Florida have established Soil Cleanup Targe Levels (SCTL), which provide guideline level for excessive potential risks in residential settings (2.1 mg/kg) and in industrial settings (12 mg/kg) (FDEP, 2013). No studies have systematically evaluated the levels of arsenic at beaches (in water, sand, and Sargassum) with and without beach wrack nor with the intentional separation of the impacts from Sargassum versus seagrasses and wrack management styles. This is a major gap in our understanding of the risks of Sargassum strandings on beach quality and subsequent ecological and human health.

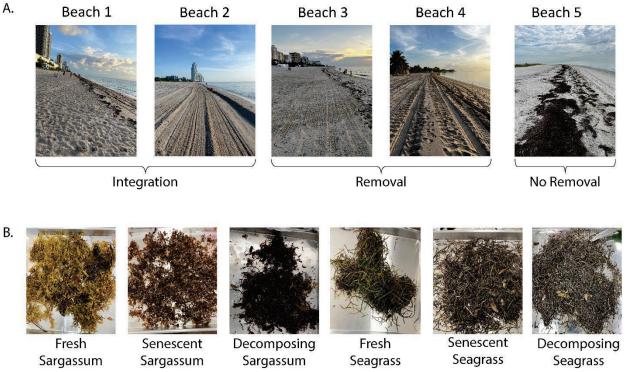
This gap is critical because poorly optimized management practices may inadvertently exacerbate bacterial contamination (Kinzelman et al., 2004) or increase trace metal bioavailability in sand and water (Ofori & Rouleau, 2021). Furthermore, the balance between managing Sargassum influxes for ecological health and public safety remains poorly understood.

The objective of this current study was to evaluate the impacts of stranded wrack on enterococci and arsenic levels in the beach environment in the context of different beach management practices and different wrack types. No studies have considered the differences in wrack type and beach management on the levels of enterococci and arsenic in the beach environment. Levels of enterococci and arsenic in beach water, sand, and wrack were measured at five beaches. The analysis documented the composition of the wrack between Sargassum and seagrasses and documented beach wrack management practices. The knowledge gained from this study can be used to develop management strategies for beach wrack in terms of its composition (Sargassum versus seagrass) and provide information about the potential effectiveness of grooming practices to decrease exposures to enterococci and arsenic at beaches impacted by the increasing volumes of Sargassum.

#### II.2 Methods

#### II.2.1 Site Description and Wrack Management Practices

The five beaches evaluated were located in Southeast Florida (identified as B1 through B5), four were located in Miami-Dade County and one was located in Broward County. The selection of each beach was informed by its wrack management approach, which included no removal (one beach), integration (two beaches), and removal (two beaches). Wrack was removed from the two beaches using a beach chariot (Surf Rake by Barber) connected to a tractor. The chariot removes the wrack by lifting the wrack over a conveyor belt with perforations that allows the sand to fall back onto the beach while capturing the wrack. For the two beaches that managed wrack through integration, two different integration processes were used. The first integration method utilized a "pull bar" which was composed of a large wooden pole attached to a tractor that was dragged across the beach sand at the wrack line, pressing the wrack into the sand. The second integration method consisted of a rear-mounted blader connected to a tractor. The rear mounted blader consisted of an attached blade that cut wrack into smaller pieces and then smoothed and mixed it with the underlying sand. The practice of "no removal" allowed for the accumulation of wrack along various wrack lines letting the wrack to dry onshore and occasionally be carried back into the water depending upon the stranding location and tide (Figure II.1, Table II.1).



**Figure II.1**. A. Photographs illustrating beach zones categorized by *Sargassum* and seagrass management strategies: integration (in-place decomposition), no removal (unmanaged accumulation), and removal (manual or mechanical extraction). B. Images representing the stages of *Sargassum* and seagrass degradation: fresh (recently deposited), senescent (partially decayed), and decomposing (advanced breakdown).

**Table II.1**. Beach Management and Wrack Characteristics for the Study Beaches

Beach	Wrack Management	Amount of Wrack by			Wrack Composition by			
ID	Style	Sampling Date			Sampling Date			
		Jul. 6	Aug. 3	Sep. 7	Jul. 6	Aug. 3	Sep. 7	
B1	Integration: Pull Bar	Н	M	L	Sargassum	Sargassum	Sargassum	
B2	Integration: Blader	L	L	L	Sargassum	Sargassum <sup>a</sup>	Sargassum	
В3	Removal: Beach Chariot	Н	L	L	Sargassum <sup>a</sup>	Sargassum <sup>a</sup>	Sargassum	
B4	Removal: Beach Chariot	M	Н	L	Seagrass	Sargassum	Seagrass	
B5	No Removal	L	Н	Н	Seagrass	Sargassum <sup>a</sup>	Seagrass	

<sup>&</sup>lt;sup>a</sup>The sample was composed primarily of *Sargassum* with small amounts of seagrass estimated at 95% *Sargassum* and 5% seagrass mix.

Samples were collected in the early morning shortly after sunrise prior to beach grooming activities. Field data collection included weather conditions (air temperature and humidity from iPhone weather app for the location) plus temperature of the water, sand, and wrack (MT Raytek® laser thermometer). On average the air temperature and humidity at the time of sample collection was 27.2 °C and 83%, respectively. Average water, sand, and wrack temperatures were 29.9 °C, 26.9 °C, and 27.2 °C, respectively. Upon arrival at the beach, the amount of wrack was visually identified as low (L), medium (M), or high (H) with confirmation from photographs of the beach intertidal zone (Appendix, Figure A.1). The wrack samples that were collected were either predominantly Sargassum or seagrass. The Sargassum was characterized as either *S. natans I, S. natans VIII*, and *S. fluitans III* (Iporac et al. 2022). At the laboratory the wrack samples were placed on a sterile tray and photographed. These photographs were used to confirm the wrack composition (usually either predominantly Sargassum or seagrass). Photographs and additional details about the field data are available in the supplemental text.

#### II.2.2 Sample Collection

Samples were collected in sterile Whirlpak bags at each of the five beaches on three different dates (July 6, 2023, August 3, 2023, and September 7, 2023). The early morning sampling time was important for beaches that practiced daily wrack removal, as it facilitated the collection of wrack that may have stranded overnight. At each beach, water, sand, and wrack samples were collected. One water sample was collected in ankle-deep water per beach per sampling day by scooping the water surface with the Whirlpak bag from an area upstream and undisturbed by the sampler. Similarly, for each beach and each sampling day, three sets of sand samples were collected from the upper 2 cm of the sand surface using a sterile spoon. One sample was collected above the high tide wrack line, called "supratidal" sand; another sand sample was collected below the wrack. called "under" sand, and for the beach that bladed wrack, a "bladed" sample was collected when observed. Wrack was placed into a sterile Whirlpak bag using clean gloves. During one of the sampling days for Beach 1, two wrack lines were observed, and two wrack samples along with the corresponding sediment "under" samples were collected. Although the samples from each wrack line were analyzed separately, the results from this one sampling day from B1 were averaged together for data analysis purposes. Upon collection, all samples were immediately placed into a cooler with ice and transported back to the University of Miami laboratory for processing.

#### II.2.3 Enumeration of Enterococci

Enterococci were processed immediately upon arrival at the laboratory (within 2.5 hours of collection). Upon arrival at the laboratory, samples were split. Water samples were split two ways, one for enterococci analyses and another for arsenic analyses. For sand and wrack, samples were split three ways for the analysis of enterococci, arsenic, and moisture content (MC). MC was used to normalize the results by mass of dry sediment or mass of dry wrack. In addition, MC was used to categorize the samples composed predominantly of *Sargassum* into fresh (MC  $\geq$  75 %), senescent (74%<MC<55%), and decomposing (MC $\leq$ 54%) as per prior studies (Abdool-Ghany et al., 2022). Moisture content was determined gravimetrically (110 °C after 24 hours).

Enterococci measurements followed standard membrane filtration protocols (U.S. EPA 2014, Method 1600). The process involved filtering a known volume of sample through a sterile membrane filter (0.45- $\mu$ m mixed cellulose filters, Pall Industries GN-6) and placement of the filter onto Enterococcus indoxyl- $\beta$ -D-glucoside (mEI) agar (Aquaplates). Samples were incubated at 41  $\pm$  0.5 °C for a duration of 24 hours. After incubation, colonies were counted. Any colonies that displayed a blue color or presented a blue halo were counted as positive.

For water, 100 mL of sample were filtered. Enumeration of sand and wrack required the elution of microbes from the sediment (Boehm et al., 2009) or wrack (Abdool-Ghany et al., 2022) into a sterile phosphate-buffered saline solution. In brief, for sand, a measured weight of sediment (about 10 g) was placed into a sterile 100 mL bottle. Then, 100 mL of sterile phosphate buffer saline (PBS) was added, and the bottle was shaken for 2 min. The solution was allowed to settle for 2 minutes, and the supernatant was drawn (2 and 20 mL) and filtered through the membranes. For wrack, 200 mL of sterile PBS was added to a measured weight (about 10 g) of aseptically cut seaweed placed within a sterile Whirlpak bag. The bacteria were eluted by rubbing the bag between the thumb and fingertips for 2 minutes, followed by a 2-minute settling period. The supernatant was drawn (1 mL and 10 mL) and filtered through the membranes. Since there were 2 dilutions used for sediment and wrack, the average of the two values was used for subsequent data analysis.

#### II.2.4 Arsenic Analysis

Water sample splits for arsenic analysis were placed into high-density polyethylene (HDPE) bottles containing nitric acid. For sand and wrack, splits for arsenic analysis were placed into 60 mL glass wide-mouth jars. Once samples were placed in their respective containers, they were then placed in the refrigerator and shipped to the analytical laboratory (Florida Spectrum Environmental Services) within 2 days of collection and analyzed within 7 days of collection. Standardized methods were used for the digestion of water samples (US EPA 1992, Method 3010) and for the digestion of sand and wrack samples (US EPA 1996, Method 3050). Inductively Coupled Plasma, Atomic Emission Spectroscopy (ICP-AES) was used to analyze the digestates (US EPA 2018 Method 6010D).

#### II.2.5 Statistical Analysis

The Shapiro-Wilks test was used to assess the normality of the enterococci and arsenic data. The results of this analysis found that neither the enterococci nor arsenic concentrations were normally distributed; thus, prompting the use of non-parametric analyses. Kruskal-Wallis H test was used to assess statistical differences in the enterococci and arsenic concentrations across the diverse types of samples collected as well as the various beaches sampled. The Dunn's test was used for pairwise comparisons among sample categories. The one-sample Wilcoxon signed rank test was used to evaluate for potential outliers. Among these statistical tests, the comparison of means with p-values less than 0.05 were considered statistically different.

#### **II.3 Results**

#### II.3.1 Impact of Management Style on Beach Quality

For enterococci, no significant differences were observed between management style and concentrations in water (p=0.17), sand (p=0.42), and wrack (p=0.65). For arsenic, no significant differences were observed between management style and concentration in the water due to all levels below the detection limits. In addition, for arsenic, no significant differences were observed between management style and arsenic concentration in the wrack (p=0.56). However, for sand,

management style was statistically significantly associated with arsenic concentrations (p<0.001). Beaches that integrated Sargassum showed higher concentrations than beaches that removed or did not remove Sargassum. Specifically, beaches with integration (e.g., Beach 1, Beach 2) had an average arsenic concentration of 2.75 mg/kg, significantly higher than the no removal sites (1.39 mg/kg, p < 0.001). Beach 1 had the highest arsenic concentration (4.31 mg/kg) compared to others, including Beach 3 and Beach 5, which had lower levels (1.39 mg/kg) (Table II.3).

#### II.3.2 Enterococci Levels

*Water*: Enterococci concentrations in water samples varied across beaches, with Beach 2 (integration by blading) showing the highest levels (126 CFU/100 mL) and Beach 3 (removal) and Beach 5 (no removal) exhibiting lower levels (31.3 and 35.7 CFU/100 mL, respectively) (Table II.2). No significant differences in enterococci levels were observed between beaches (p = 0.36) (Figure II.2A).

*Sand*: No significant differences in enterococci levels were found between sand sample types (supratidal, sand under, and bladed). The average enterococci counts were 198 CFU/dry g for supratidal, 210 CFU/dry g for sand under wrack, and 276 CFU/dry g for bladed sand (Figure II.2B, Table II.2).

*Wrack*: Enterococci concentrations in wrack samples were higher in Beach 3 (3,400 CFU/dry g) and Beach 2 (3,200 CFU/dry g) compared to Beach 1, Beach 4, and Beach 5, which exhibited lower concentrations (Table II.2). No significant differences were observed in wrack enterococci concentrations across beaches (p = 0.46). Moisture content in wrack (fresh, senescent, and decomposing) did not significantly affect enterococci concentrations (p = 0.78) (Figure II.5A).

#### II.3.3 Arsenic Levels

Water: Arsenic concentrations in water samples were all below detection limits (30 µg/L).

Sand: No significant differences in arsenic concentrations were observed between sand sample types (supratidal, under, bladed; p = 0.83). Significant differences were observed between Beach 1 (integrates by pull bar) and other beaches (e.g., B1 vs. B2, B3, B5) (Figure II.3A).

*Wrack*: *Sargassum* had significantly higher arsenic concentrations (36.4 mg/kg) compared to seagrass (1.9 mg/kg, p = 0.004) (Figure II.4). No significant differences were observed in arsenic concentrations when grouped by beach site (p = 0.73) nor by wrack decomposing status (p = 0.88) (Figure II.5B).

Table II.2. Summary of enterococci and arsenic concentrations by beach site and sampling day

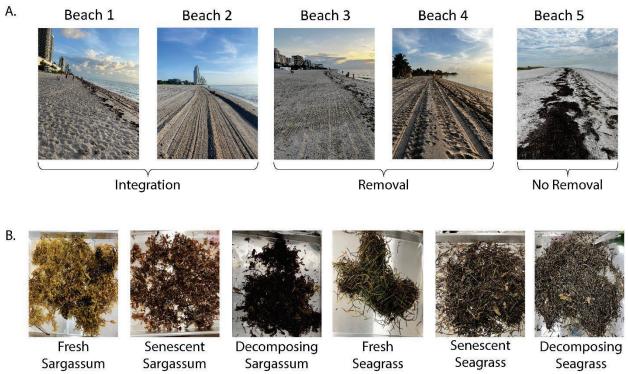
				_						1	1				
Joseph (mar/lea)	(1)	All	Wrack	35.0	32.7	29.9	22.0	17.4	20.7	28.5	33.1	27.8			
	Wrack (mg/kg)		Sand Seagrass Sargassum Wrack	35.0	32.7	29.9	62.5	48.2	25.4	28.5	53.7	36.4			
	Wr		Seagrass				1.79	1.98	1.66		2.11	1.88			
Arsenic		All		4.31	1.71	1.39	2.14	1.39	2.08	2.13	2.23	2.19			
Y	ng/kg)		Bladed		1.76				2.02	1.73	1.54	1.76			
	Sand (mg/kg)		Under	3.16	1.77	1.12	2.13	1.23	2.05	2.18	2.05	2.19			
			Supra	4.41	1.60	1.66	2.15	1.55	2.12	2.16	2.55	2.27			
	W/2+0W	water (1/2/L)	hg/L)	$ND^a$	ND	ND	ND	ND	ND	ND	ND	ND			
		All ,	Wrack	2075	3236	3424	2715	2199	1270	5268	1651	2614			
	Wrack (CFU/g)		Seagrass Sargassum Wrack (µB/L) Supra Under Bladed	2075	3236	3424	2742	68.7	314	5268	578	2491			
	Wra		Seagrass				2701	3264	2704	N/A	3260	2982			
Enterococci		All		180	171	187	435	129	140	284	224	211			
Ente	Sand (CFU/g)		3laded		276				196	625	9.00	276			
	Sand ((	Sand (	Sand (	Sand (		Under]	139	9.77	232	631	27.6	73.6	343	247	210
			Supra	221	158	142	240	231	195	156	245	198			
	Water	(CFU/100	mL) Supra Under Bladed Sand	81.7	126	31.3	71.7	35.7	39.0	106	62.8	69.3			
Category				B1	B2	B3	B4	B5	B1-B5, Inly 6	B1-B5 August 3	B1-B5 September	Overall			

 ${}^{a}\!ND=Not\,Detected.\,$  Detection limit for water samples was 30  $\mu g/L.$ 

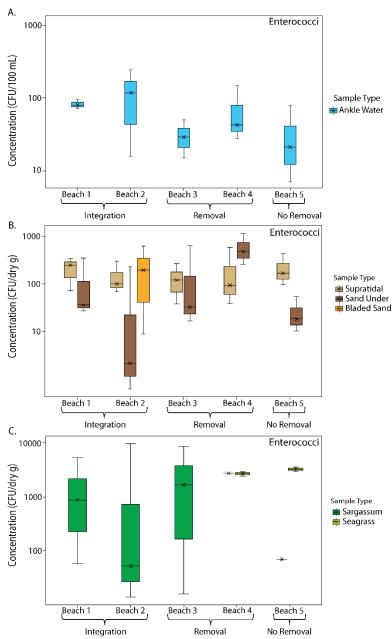
**Table II.3.** Summary of enterococci and arsenic concentrations by sample type, wrack decomposing status, and beach management style

	decomposing status, and beach management style							
Category	Enterococci	Arsenic						
	(CFU/dry g or	$(\mu g/g \text{ or } \mu g/L)$						
	CFU/100 mL)							
	Sample Type							
Seagrass	2982	1.88						
Sargassum	2868	36.0						
Wrack, Overall	2925	19.0						
Supratidal Sand	198	2.27						
Under Sand	221	2.09						
Bladed Sand	276	1.76						
Sand, Overall	211	2.19						
Water	69.3	ND <sup>a</sup>						
	Wrack Decor	mposing Status						
All: Fresh	1252	24.9						
All: Senescent	3556	26.5						
All: Decomposing	2258	29.2						
Seagrass: Fresh	2435	1.55						
Seagrass: Senescent	2973	1.77						
Seagrass: Decomposing	3260	2.11						
Sargassum: Fresh	68.7	48.2						
Sargassum: Senescent	3653	30.7						
Sargassum: Decomposing	1756	42.8						
	Management Style							
	(Sargassum only)							
No Removal	68.7	48.2						
Integration	2655	33.8						
Removal	3253	38.1						
	Management Style							
	(Seagrass only)							
No Removal	2704	1.66						
Integration	NA <sup>b</sup>	NA						
Removal	3260	2.11						
	Management Style							
	(Sand only)							
No Removal	129	1.39						
Integration	174	2.75						
Removal	311	1.77						

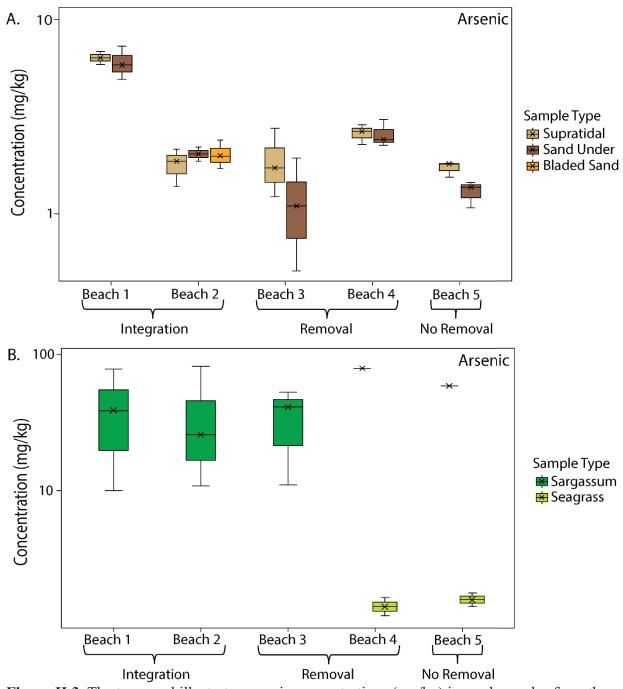
aND=Not Detected. All water samples were below the detection limit (30 μg/L).
bNA=Not Available



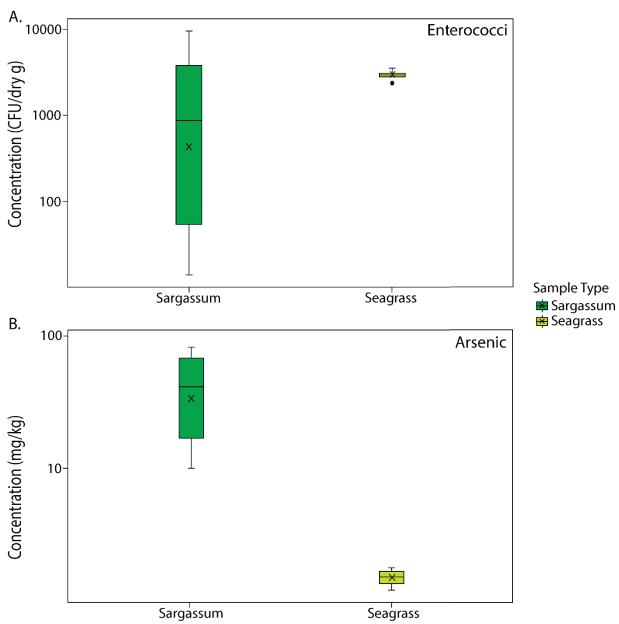
**Figure II.1**. A. Photographs illustrating beach zones categorized by *Sargassum* and seagrass management strategies: integration (in-place decomposition), no removal (unmanaged accumulation), and removal (manual or mechanical extraction). B. Images representing the stages of *Sargassum* and seagrass degradation: fresh (recently deposited), senescent (partially decayed), and decomposing (advanced breakdown).



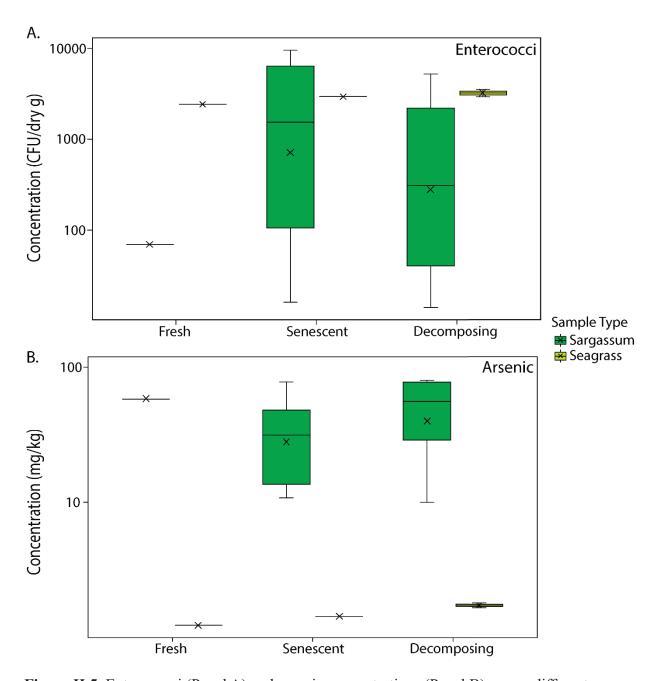
**Figure II.2**. The top panel shows the concentration of enterococci (CFU/100 mL) in ankle-deep water across five sampled beaches. The middle panel depicts enterococci concentrations (CFU/dry g) in three sand zones (supratidal, sand under, and bladed sand) at the same beaches. Beaches 1,3,4, and 5 had supratidal and sand under sample collected, while beach 2 had an additional sample collected in the bladed sand zone. The bottom panel highlights enterococci concentrations in *Sargassum* and seagrass samples. Beaches 1,2, and 3 has *Sargassum* only collected, while Beaches 4 and 5 had *Sargassum* and seagrass samples collected. Beach 5 has one sample of fresh *Sargassum* collected.



**Figure II.3**. The top panel illustrates arsenic concentrations (mg/kg) in sand samples from three zones (supratidal, sand under, and bladed sand) across five beaches. The bottom panel displays arsenic concentrations in *Sargassum* and seagrass samples from the same beaches, highlighting significant variability in arsenic accumulation across different wrack types and locations.



**Figure II.4**. The top panel illustrates enterococci concentrations (CFU/dry g) in *Sargassum* and seagrass, showing a significantly higher bacterial load in *Sargassum* compared to seagrass. The bottom panel presents arsenic concentrations (mg/kg), with *Sargassum* accumulating more arsenic than seagrass.



**Figure II.5**. Enterococci (Panel A) and arsenic concentrations (Panel B) across different decomposition stages—fresh, senescent, and decomposing—for both *Sargassum* and seagrass. Except for one fresh *Sargassum* sample, enterococci levels are consistently above several hundred CFU/g range. Arsenic levels are consistently higher in *Sargassum* compared to seagrass throughout the decomposition process.

#### **II.4 Discussion**

#### II.4.1 Enterococci Concentrations in Sargassum and Seagrass

Of interest was that although arsenic levels were statistically different between Sargassum and seagrass, the enterococci levels were not different statistically. This similarity in enterococci levels may be due to the similarity in which *Sargassum* and seagrass allow for the persistence and regrowth of enterococci. Studies have found associations between seaweed presence and enterococci levels (Anderson et al., 1997, Kelly et al., 2018). Abdool-Ghany et al. (2022) found that elevated levels of enterococci were confined to the beach wrack zone during periods of beach closures induced by the COVID-19 pandemic. They hypothesized that decomposing seaweed provides an additional substrate for enterococci to grow. The results from the current study support that both Sargassum and seagrass serve as possible growth substrates and sources of nutrients for growth, as observed for other macroalgae in freshwater environments (Verhougstraete et al., 2010, Badgley et al., 2011). The management practices of beach wrack whether integration, removal, or no removal—did not significantly affect the persistence of enterococci on either substrate, indicating that other environmental factors such as moisture retention and organic matter could play a more significant role. It is also possible that the Sargassum and seagrass may facilitate persistence by retaining moisture and possible protection against UV light (Beckinghausen et al., 2014). Collectively, the results from the current study support that fecal indicator bacteria persist and grow indiscriminately on organic substrates that accumulate in coastal zones.

#### II.4.2 Arsenic Concentrations in Sargassum and Seagrass

Arsenic was elevated in *Sargassum* compared to seagrass. Two mechanisms are believed to impact arsenic uptake by *Sargassum*. First, *Sargassum*, like many other brown seaweeds, accumulate arsenic and other trace elements (Neff, 1997; Fauser et al., 2012; Alleyne et al., 2023) through its cell-wall polysaccharides, particularly alginate and fucoidan (Ortega-Flores et al., 2022). Alginate contains carboxylic groups that serve as primary active sites for cation uptake, while sulfated fucoidans, with sulfonic acid groups, act as secondary active sites for sequestering divalent cations (Percival & McDowell, 1990; Fourest & Volesky, 1997; Davis et al., 2004). Seagrasses cell walls are composed of cellulose and sulfated polysaccharides (Pfeifer and Classen 2020) which may not have the same sorptive ability as *Sargassum*. *Sargassum* has a documented capacity to uptake arsenic and other metals through sorption, whereas work is needed to evaluate whether seagrasses have the same capacity.

The second mechanism is due to the similarity between arsenate and primary nutrient phosphate. *Sargassum* originates in the nutrient-poor Sargasso Sea, where it evolved mechanisms for hyperaccumulating nutrients. Elevated arsenic levels in *Sargassum* from this subtropical gyre correspond with low phosphorus levels in the oceanic surface waters, reflecting the region's phosphorus scarcity. The high arsenic-to-phosphorus ratio in *Sargassum* arises from its uptake of arsenate, which chemically resembles the phosphate ion that aquatic plants utilize as a source of phosphorus (McGillicuddy et al., 2023). Unlike seagrasses, which absorb nutrients through their roots and leaves, *Sargassum* transports nutrients through its tissues by diffusion, leading to distinct patterns of arsenic accumulation. This difference in nutrient absorption mechanisms results in significantly higher arsenic levels in *Sargassum* compared to seagrass, which does not uptake or sorb arsenic as effectively (Raize et al., 2004). As a result, wrack accumulations along

beaches will have significantly different arsenic composition depending upon wrack composition, with beaches using integration practices (e.g., B1) likely seeing higher arsenic concentrations due to the inclusion of *Sargassum* in the sand during mixing.

#### II.4.3 Variability in Arsenic Concentrations Due to Management Practices

In this study, the coefficient of variation (COV) for arsenic concentrations was highest at beaches practicing integration (49%), followed by removed (32%), and no removal (15%). The higher COV observed at sites utilizing integration suggests greater variability in arsenic levels, indicating inconsistent distribution within the samples. This variability is likely due to the mixing of sand and Sargassum during integration practices, which introduces a wider range of arsenic sources and concentrations. Such variability underscores the risks associated with integrating organic matter like Sargassum into beach sands, where arsenic from different sources may accumulate unevenly. In contrast, beaches where no removal was practiced exhibited the most consistent arsenic levels, with the lowest COV, reflecting more stable and predictable conditions. The removal approach demonstrated intermediate variability, likely reflecting reduced, but not eliminated, arsenic sources compared to integration. These findings highlight the influence of beach management practices on arsenic distribution and underscore the potential risks associated with integration, where mixing could enhance variability in contaminant exposure. Of significance was that the average arsenic concentration in sand was higher at the beaches that practiced integration. The mean sand arsenic concentration for these beaches (2.8 mg/kg) was slightly above the Florida SCTL (2.1 mg/kg). Compared to beaches that did not practice integration (1.8 mg/kg for removal and 1.4 mg/kg for no removal), the differences were not large suggesting that beach sand does not retain arsenic to a great extent. The lack of sorption of arsenic by beach sand is consistent with studies that have found limited sorption under conditions of low organic and iron content (Fauser et al., 2013).

The beach that showed the highest sand arsenic concentrations (B1, 4.3 mg/kg) practiced integration by pull bar. The pull bar compacts the wrack into the sand likely limiting the ability of rainwater to infiltrate thereby resulting in less flushing through rainfall infiltration and greater retention of the arsenic. There is also the possibility that the pull bar utilized by B1 could have been composed of a common wood preservative used for utility poles, known as chromated copper arsenate, which contains very high levels of arsenic (Shibata et al. 2007, Dubey et al. 2007, Jones et al. 2019). This highlights the need for further examination of the materials used in integration equipment and their potential contribution to arsenic contamination. Of interest was that the beach that practiced no removal (B5) had one of the lowest concentrations (1.4 mg/kg), lower than other beaches practicing removal, equal to one beach that practiced wrack removal and lower than another that also practiced wrack removal. It appears that wrack removal does not provide advantages over non-removal in terms of sand arsenic concentrations. Similar to the compaction hypothesis with the pull bar method of integration, it is possible that the equipment used to remove the arsenic from the beach can be compacting the sand, thereby limiting flushing by rainwater infiltration, or the equipment itself can also be contaminating the beach environment. More research is needed to evaluate these hypotheses and to confirm the relative sand arsenic levels between beaches.

#### II.4.4 Limitations

The limited beach sample size may have reduced the statistical power of the study, making it difficult to detect significant differences or trends across different locations or conditions. A larger dataset would enhance the ability to draw more reliable conclusions and potentially uncover subtle variations in arsenic levels, moisture content, and other relationships between beach wrack and enterococci and arsenic levels. Additionally, a more extensive geographic range of sampling sites could provide insights into regional differences in *Sargassum* composition and decomposition dynamics, contributing to a more comprehensive understanding of its environmental impacts.

#### II.4.5 Recommendations

To further understand the decomposition of *Sargassum* wrack, we recommend mesocosm experiments designed to evaluate how different environmental conditions influence the breakdown of Sargassum and the release of arsenic and other trace elements. As Sargassum decomposes and dries out on beaches, it would be valuable to determine if arsenic is released or remains within the biomass. This type of experiment could provide insights into whether the drying process leads to a loss of arsenic, potentially due to desorption or volatilization. Such findings could have significant implications for managing Sargassum wrack on shorelines, particularly in areas where arsenic contamination poses environmental or public health concerns. Additionally, we recommend incorporating chlorophyll measurements into the analysis of Sargassum wrack. Such measurements could offer a more detailed understanding of its decomposition state beyond measurements of moisture content. Chlorophyll is a key indicator of the health and vitality of algal material. By measuring chlorophyll content alongside other parameters, the stages of decomposition and how these stages correlate with changes in arsenic levels, nutrient content, and other trace elements can be evaluated. This approach could help identify when enough arsenic may be lost from the Sargassum, aiding in the development of management strategies for Sargassum wrack on beaches.

In summary, results from this study emphasize the importance of documenting the characteristics of the wrack (Sargassum versus seagrass) when evaluating beach management strategies that address fecal indicator bacteria and arsenic levels. Enterococci were not sensitive to beach wrack type. However, arsenic was. Due to the higher levels of arsenic in Sargassum compared to seagrass, arsenic mitigation may be needed for beaches characterized by excessive Sargassum inundations. For the study beaches, those that managed wrack by integrating Sargassum had statistically higher levels of arsenic in the sand compared to beaches that did not remove Sargassum. More beaches impacted by Sargassum should be evaluated to confirm trends. Risk assessments are also needed to determine if the increase in arsenic in sand and the presence in Sargassum wrack is of health concern to beach goers who recreate at coastal beaches. Such data can be used to determine at what point Sargassum wrack needs to be removed to maintain arsenic within acceptable levels.

#### **II.5 Conclusions**

The increasing accumulation of Sargassum on global coastlines presents significant environmental challenges, particularly concerning arsenic and bacteria dynamics and ecosystem health. Sargassum's capacity to bioaccumulate heavy metals, such as arsenic, and its potential to influence microbial contamination raise concerns about the ecological and public health impacts of current beach management practices. Understanding how wrack composition and handling contribute to environmental pollution is critical for informing sustainable coastal management. This study contributes to the broader field of environmental monitoring and pollutant risk assessment by evaluating how different wrack types and management strategies affect contaminant levels in coastal environments. While both seagrasses and Sargassum contributed towards excess fecal indicator bacteria, the impacts of each were different for arsenic. Elevated levels of arsenic were found in beach sand and wrack when the wrack was dominated by Sargassum, but not when dominated by seagrass. Such findings from this research support the development of evidence-based approaches to mitigate pollutant exposure allowing for different strategies for managing seagrass inundations versus inundations composed mainly of Sargassum. Such information can be used to protect both ecosystem integrity and public health, aligning with global efforts to address pollution and promote sustainable environmental practices.

# CHAPTER III MESOCOSM STUDY

### **CHAPTER III**

# **MESOCOSM STUDY**

#### **III.1 Introduction**

Sargassum blooms have become increasingly frequent along coastal regions, leading to large accumulations on beaches. These accumulation events are driven by a combination of nutrient pollution, rising ocean temperatures, and changing ocean currents (Wang et al. 2019, Theirlynck et al. 2023). While Sargassum plays an important ecological role in marine environments, excessive beach-cast accumulations present significant environmental (Fourqurean et al. 2012), economic (Chávez et al., 2020, Tonan et al. 2022), and public health (Rodríguez-Martínez et al. 2024) challenges.

Environmentally, large volumes of decomposing Sargassum can disrupt coastal ecosystems by depleting oxygen levels in nearshore waters, smothering seagrass beds, and affecting marine biodiversity. Economically, the removal and disposal of beach-cast Sargassum places a significant financial burden on coastal communities (Rodríguez-Martínez et al. 2023), tourism industries (Mohan and Strobl 2024) and local governments (Oxenford et al. 2021,). Public health concerns also arise, particularly from the release of hydrogen sulfide gas during decomposition, which may cause respiratory issues (Resiere et al. 2021), and the potential presence of pathogenic bacteria, including Vibrio spp., which dominate the microbial community (Abdool-Ghany et al., in review).

Additional risks are associated with arsenic. Sargassum is known to bioaccumulate arsenic from seawater (Devault et al. 2020, Ortega-Flores et al. 2022, McGillicuddy et al. 2023, Gobert et al. 2022). The speciation of arsenic—whether in organic or inorganic forms—determines its toxicity, mobility, and environmental impact. Inorganic arsenic species (arsenate As(V) and arsenite As(III)) are particularly concerning due to their high toxicity and potential health risks. In the natural environment, As(V) and As(III) are known to methylate under the action of microbes. The toxicity of methylated species is governed by their oxidation state, with methylated forms (monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) being less toxic in the +5 oxidation state and more toxic in the +3 oxidation state (Di et al., 2019). Methylated forms of arsenic are also known to volatilize (Planer-Friedrich et al. 2006), a process that could lead to airborne contamination.

Additional species of arsenic, such as arsenobetaine (AsB) and arsenocholine (AsC), are commonly found in shellfish and are considered non-toxic. Trimethylarsine oxide (TMAO), an intermediary for the biosynthesis of arseno-organic compounds like DMA and MMA, exhibits toxicity comparable to As(III) (Yong and Liu, personal communication). In terms of the transport and fate of arsenic during decomposition once stranded on shore, no studies are available to our knowledge that systematically evaluate the transport of arsenic out of the Sargassum tissue through leaching and volatilization, nor are there studies that evaluate the transformation of arsenic species as Sargassum decomposes. A study by Cipolloni et al. 2023 documented the loss of arsenic from the Sargassum as it degraded in cages offshore. A study by Alleyne et al. 2023 measured the distribution of arsenic between

organic and inorganic forms within pelagic Sargassum and found up to 62% of the total arsenic in inorganic forms. Datta et al. 2024 maintained Sargassum cultures in natural seawater and observed the transformation of As(V) to DMA over a period of 14 days. No studies to our knowledge evaluate the arsenic species in Sargassum as it naturally decomposes along the shore. Specifically, there is limited understanding of whether arsenic from stranded Sargassum is leached from the macroalgae, captured by the underlying sand layers, or volatilized into the atmosphere. This lack of data creates significant uncertainty regarding potential risks to beachgoers and the development of effective management strategies for stranded Sargassum beach-cast.

This study aims to address these knowledge gaps by utilizing mesocosms to measure the loss of arsenic from Sargassum and arsenic speciation during Sargassum decomposition. The study assessed the distribution and transformation of arsenic in Sargassum using a mass balance approach to quantify the fraction of arsenic volatilized from Sargassum and sand systems. Samples were collected of Sargassum, sand, rainwater, and leachates and quantified for total arsenic and arsenic species (As(V), As(III), MMA(III), DMA(III), AsB, AsC, and TMAO). The results of this research provide critical insights into the potential environmental and public health impacts of stranded Sargassum at beaches, and contributes to the development of effective management strategies for coastal areas impacted by Sargassum.

#### III.2 Methods

#### III.2.1 Sargassum Collection

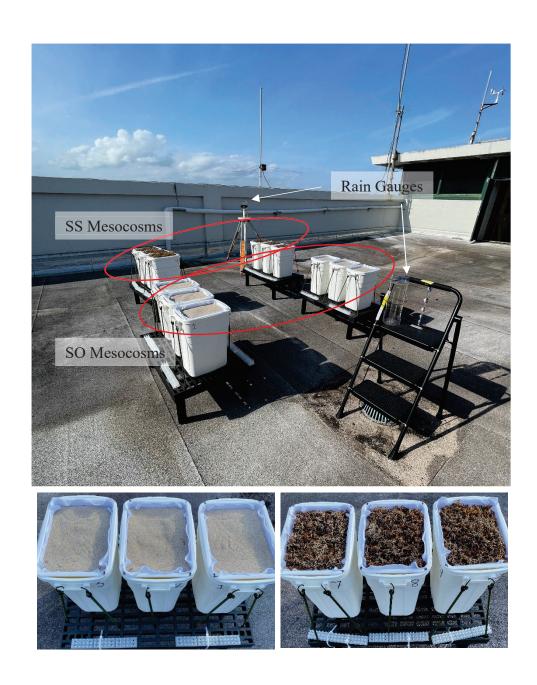
Fresh Sargassum was collected on March 13, 2024, from an ocean facing beach located in southeast Florida (26° 11' 31.29N, 80° 05' 40.18W) following a systematic remote monitoring process using the Citizen Science Epicollect tool (Iporac et al. 2022), which is used by beach goers to report the locations of fresh Sargassum strandings. Samples (reported through Epicollect at 9 am that morning) were collected between 11 and 12 noon. At the beach shore, samples of stranded Sargassum (Sargassum natans and Sargassum fluitans) were fresh and golden in color typical of the freshly stranded macroalgae (Vázquez-Delfin et al. 2021) (Figure III.1). Samples were collected using gloves into clean plastic lined coolers. The Sargassum was carefully gathered by hand, ensuring that only freshly deposited material was selected, free from visible sand, debris, or signs of decomposition. In addition to the Sargassum, sediment from the supratidal zone was also collected in a separate plastic lined cooler. Upon collection the Sargassum was protected from sunlight and immediately transported to the laboratory for mesocosm setup within 1.5 hours of collection (by 1:15 pm).



**Figure III.1.** Field-collected pelagic *Sargassum* assemblages. Left panel shows a mixture of *S. fluitans* (broad, serrated blades) and *S. natans* (narrow, smooth to lobed blades with clustered vesicles). Samples were collected from the wrack line prior to mesocosm deployment. Right panel shows representative *Sargassum natans* subsample collected for initial arsenic concentration analysis. The sample displays characteristic spherical pneumatocysts and narrow blades, with no attachment structures, confirming pelagic origin.

#### III.2.2 Mesocosm Setup

To simulate the natural processes occurring in beach environments for Sargassum stranded at or above the high tide line, the mesocosms were set up outdoors on the flat roof of a five-story building (McArthur Engineering Building of the University of Miami) to allow for natural rainfall and sunlight, and to avoid contamination from street level activities. A total of twelve mesocosms were set up, six controls (collectively called the SO for sand only mesocosms) and six experimental (collectively called the SS for sand and Sargassum mesocosms). Each mesocosm consisted of a plastic tray strainer (27.5 cm x 21.5 cm x 4.2 cm) that was lined with felt. Beach sand was placed on top of the felt (2 kg each) to a depth of 3 cm in all 12 mesocosms. For the six experimental mesocosms, 0.5 kg Sargassum (57% initial moisture content) was additionally overlayed on the sand. Each plastic tray strainer was placed on top of a reservoir to capture rainfall that infiltrated through the sand in the SO mesocosms and through the layered sand and Sargassum in the SS mesocosms. The strainer fit the top of the reservoir snugly so that infiltrated rainwater must pass through the Sargassum and/or sand before its collection in the bottom of the reservoir (See Figure III.2).



**Figure III.2**. Top panel: Mesocosm setup on roof of 5-story building in Coral Gables, Florida (McArthur Engineering Building) used to monitor rainfall and arsenic concentration changes in Sargassum, sand and leachate over time). Photo illustrates the six sand only (SO) and the six Sargassum and sand (SS) mesocosms. Plastic physical and acoustic bird deterrents were added to minimize disturbances from birds. Bottom panel: close up of 3 SO mesocosms (left) and SS mesocosms (right).

#### III.2.3 Initial XRF Measurements

Immediately upon receipt in the laboratory, Sargassum and sand samples received from the beach site were homogenized by mixing and analyzed by X-ray Fluorescence Microscopy (XRF) (Innov-X, alpha 2000) to confirm the presence of arsenic. XRF analysis consisted of placing a piece of thin, clean plastic over the Sargassum and recording the levels of the metals detected (Abdool-Ghany et al. 2023b, Block et al. 2007). The anticipated levels of arsenic in the Sargassum were near the detection limits of the instrument so multiple analysis were taken. Arsenic was detected in 3 of the 22 measurements confirming the presence of arsenic. Additional details of this analysis including a list and levels of arsenic and other metals detected (e.g., lead) is provided in the appendix, Table B.3

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### III.2.4 Sample Collection from Mesocosms

The mesocosms were in operation for 70 days, from March 13, 2024, through May 22, 2024 with measurements taken between 9 am and 10 am local time. To account for rainfall volumes, rainfall depths were recorded daily at two standard rain gauges, which were placed on each side of the 12 mesocosms. Rainfall was detected in the gauges during 10 days of the 70 days. The total rainfall depth for these 10 rain days was 19.36 cm (range from 0.24 cm to 9.13 cm per day). Of these 10 days, sufficient rainfall fell to generate leachate for 9 of the 10 days. During these nine days, the leachate volumes captured in the reservoirs were measured volumetrically for small volumes (<100 mL per reservoir) and by weight for large volumes (>100 mL). All water samples (rainfall and leachate) were composited and placed into metal-free 250 mL plastic bottles with 1 mL of pre-dispensed nitric acid. The rainwater composites consisted of equal volumes from each rain gauge (pre-acid washed plastic). For leachates two composite samples were prepared per sampling event, one consisting of a composite from each of the six SO mesocosms and another consisting of a composite from each of the six SS mesocosms. Once leachate volumes were determined and composite samples were collected, the additional water within the bottom reservoirs were discarded to eliminate carry over of arsenic from prior rainfall days.

Sargassum and samples were collected in quadruplicate at the beginning of the experiment (March 13), plus in quadruplicate (for Sargassum, SO sand, and SS sand) at the end of the experiment (May 22). To monitor the progression of arsenic losses, single composite samples were collected of Sargassum and sand (SO sand and SS sand) from each of the mesocosms each Sunday and Thursday, plus after each day rainfall was recorded. The only exception was the sample collection only after rainfall days from May 4 to May 22.

Sand aliquots were collected using a stainless-steel spoon and Sargassum was clipped using stainless steel scissors. The aliquots were then composited and homogenized by mixing within their respective collection Whirlpak bags followed by weighing each sample to document the mass removed. The composite samples included the six aliquots (one from each mesocosm) corresponding to: a) sand collected from the SO mesocosms, b) sand collected from the SS mesocosms, and c) Sargassum also collected from the SS mesocosms. All sand and Sargassum composites were then split at least two ways. One for water content measurements by gravimetric analysis (100 °C for 24 hours), and one for total arsenic measurements. The water

content was defined as the mass of the evaporated water divided by the wet weight. Each time Sargassum and samples were harvested from the mesocosms their masses were weighed.

A subset of samples was targeted for arsenic speciation analysis (As(V), As(III), MMA(III), DMA(III), AsB, AsC, and TMAO). These samples were analyzed using an additional split of the sand aliquots (SO sand and SS sand) and Sargassum aliquots collected the first day (March 13) and last day of the mesocosm experiment (May 22), plus aliquots of sand (SO and SS sands) and Sargassum collected on April 30, May 2. Splits of rainfall and leachates (from SO and SS mesocosms) were analyzed for arsenic species on April 30, May 2, and May 22.

## III.2.5 Laboratory Analysis of Arsenic and Arsenic Species

All samples were analyzed for total arsenic by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent Technologies Model 5110) using standard protocols [Methods 200.7, US EPA 1994, and 6010D, US EPA 2018) for water and Method 3010A (US EPA 1992) for sand and Sargassum]. Samples were digested with nitric acid, hydrochloric acid, and hydrogen peroxide (Method 3050B, US EPA 1996) prior to ICP-OES analysis.

Arsenic speciation was conducted by high performance liquid chromatography (HPLC, HPLC, Perkin Elmer Series 200) coupled with ICP Mass Spectroscopy (ICP-MS, Perkin Elmer NexION 2000) at the chemistry laboratories of Florida International University. The HPLC was fitted with a Waters Spherisorb C8 column (150 x 4.6 mm in dimension and 5 µm particle size) with the mobile phase consisting of 0.1% formic acid and acetonitrile in gradient mode at a flow rate of 1 mL/min. Water samples were preserved by the addition of 3 mL of pretested 6 M HCl per liter of sample, while solid samples (0.02 g, dried and ground) were extracted using an ethanolwater (DI) solution (1:3 ratio) using a probe sonication method. The sonication method utilized a sonic dismembrator (Fisher Scientific Model 100) with triple pulses each for four seconds, while the centrifuge tube was dipped in an ice bath to prevent overheating. Following sonication, samples were centrifuged at 4000 RPM for 10 minutes (Thermo Scientific Sorvall ST 40 centrifuge). This sonication and centrifugation process was repeated two more times for each sample. The extracted samples were transferred into 10 mL centrifuge tubes after each round of sonication and centrifugation, and ethanol-water was added back into the 5 mL tubes containing the solids. All samples were filtered through a 0.45 µm sterile nylon syringe filter (Whatman, USA) followed by the collection of the filtered extract into pre-labeled 1 mL vials for HPLC-ICP-MS analysis. Arsenic species detected using this method included As(V), MMA(V), DMA(V), As(III), MMA(III), DMA(III), AsB, AsC, and TMAO.

Since samples collected for arsenic speciation were also analyzed for total arsenic, the data set for total arsenic analysis was augmented. As a result, the initial and final total arsenic concentrations in the sand (SO and SS mesocosms) and Sargassum were available in quadruplicate.

#### III.2.6 Water and Arsenic Mass Balance

Mass balances were conducted for water and for arsenic. For the water balance, the main goal was to compute the percent of the water volume that was evaporated (% $V_E$ ,) from each set of mesocosms. To estimate % $V_E$ , the evaporated (E) volume,  $V_E$ , was estimated by water balance (equation A). The water balance was based upon the recorded rainfall I volumes,

 $V_R$ , and the volumes of leachate (L) produced,  $V_L$ , the wet weights (subscript w) of the sand (S),  $M_{S,w}$ , and Sargassum (G),  $M_{G,w}$ , within the mesocosms along with their water content,  $WC_S$  and  $WC_G$ , for sand and Sargassum, respectively. The dry weights (subscript d) of the sand ( $M_{S,d}$ ) and Sargassum ( $M_{G,d}$ ) were then computed as the product of the wet weight and the water content minus 1. To obtain  $V_R$ , the average of the rainfall depth measured from the two rain gauges was multiplied by the surface area of the mesocosms.  $V_E$  was then estimated as follows for the SS mesocosm:

$$V_{E} = \sum_{n=1}^{n_{S}} V_{R,i} - \sum_{n=1}^{n_{S}} V_{L,i} - M_{S,w,initial} (WC_{S,final} - WC_{S,initial}) - M_{G,w,initial} (WC_{G,final} - WC_{G,initial})$$
Equation A

Where  $n_s$  corresponds to the number of samples collected and additional subscripts "final" and "initial" correspond to values at the final (May 22) and initial measurements times (March 13), respectively. For the SO mesocosm, the same equation A was used to estimate  $V_E$  except that the last term, the term corresponding to the water in the Sargassum, wase omitted. The % $V_E$  was then computed for the SS mesocosm and the SO mesocosm from the ratio of  $V_E$  and  $V_R$ , which was then multiplied by 100%. For comparison, the percent attributed to leachate, % $V_L$ , and the change in matrix moisture, % $\Delta WC$ , were also computed.

The arsenic balance was like the water balance with an objective of estimating the percentage of arsenic mass ( $M_{As}$ ) lost through volatilization (E),  $\%M_{As,E}$ . Two sets of percentages were computed, one normalized to the total arsenic in the system (indicated by a subscript "total") and the second normalized to the arsenic initially in the Sargassum (indicated by a subscript "Sargassum). Arsenic loss by volatilization ( $M_{As,E}$ ) was estimated by arsenic mass balance (equation B). The arsenic balance required, in addition to the water balance (described above), measurements of arsenic concentrations (As) in each of the mesocosm compartments. Arsenic concentrations were measured for each: a) rainfall sample I,  $As_{R,i}$ , b) leachate sample i,  $As_{L,i}$ , c) Sargassum sample i,  $As_{G,i}$ , and d) sand sample i,  $As_{S,i}$ . For the solid samples, As values were normalized to a dry mass basis (mg of arsenic per kilogram of dry sand or dry Sargassum). The product of the corresponding volume (for liquids) or mass (for solid samples) times concentration was then computed as the arsenic mass.  $M_{As,E}$  was then estimated as follows for the SS mesocosm:

$$M_{As,E} = \sum_{n=1}^{n_S} V_{R,i} A s_{R,i} - \sum_{n=1}^{n_S} V_{L,i} A s_{L,i} - M_{s,d,initial} (A s_{S,final} - A s_{S,initial}) - M_{G,d,initial} (A s_{G,final} - A s_{G,initial})$$
Equation B

The initial and final arsenic concentrations in the sand ( $Ass_{final}$ ,  $Ass_{finitial}$ ) and Sargassum ( $Asg_{final}$ ,  $Asg_{finitial}$ ) were measured in quadruplicate and the average value was utilized for mass balance computations. Similar as for the water balance, for the SO mesocosm, the same equation B was used for the arsenic balance except that the last term, the term corresponding to Sargassum, was omitted. The  $\%M_{As,E,total}$  was then computed for the SS mesocosm and the SO mesocosm from the ratio of  $M_{As,E}$  and the total mass of arsenic within the system (As in sand for the SO mesocosms and As in both Sargassum and sand). The mass within the system,  $M_{As,total}$ , corresponded to the arsenic mass from rainfall ( $M_{As,R}$ ), the mass of arsenic initially in the sand

 $(M_{As,S,initial})$ , and for the SS mesocosm plus the mass of arsenic initially in the Sargassum  $(M_{As,G,initial})$ . Similarly,  $\%M_{As,E,Sargassum}$  was then computed from the ratio of  $M_{As,E}$  and the mass of arsenic within Sargassum.

For comparison, the percent of arsenic in additional reservoirs were computed including the percent of the arsenic lost as leachate ( $\%M_{As,L}$ ), the percent lost to the sand below the Sargassum ( $\%M_{As,S}$ ), and the percent that remained the sand,  $\%M_{As,S}$ , or Sargassum,  $\%M_{As,G}$ . Percentages were computed from the mass of the corresponding compartment divided by the corresponding  $M_{As,total}$  or  $M_{As,G,initial}$ , for normalization by arsenic in the entire system or arsenic within the Sargassum. Additional mathematical explanatory expressions are provided in the second columns of Tables III.1 and Table III.2 for the water and arsenic mass balance, respectively.

#### III.2.7 Statistical Analyses

Descriptive statistics including mean, standard deviation, and coefficient of variation were calculated to summarize arsenic concentrations across sample types. All statistical analyses were conducted using R (version 4.4.3) and relevant base and tidyverse packages.

Normality of arsenic concentrations in sand and leachate samples was assessed using the Shapiro-Wilk test. Both the SS and SO sand datasets were found to be approximately normally distributed (SS: W = 0.956, p = 0.468; SO: W = 0.951, p = 0.382), supporting the use of parametric statistical tests. Accordingly, Welch's t-test was used to evaluate differences in mean arsenic concentrations between SS and SO sand, as well as between Sargassum and the combined sand dataset. For leachate comparisons, SO and SS leachate arsenic concentrations were evaluated against measured rainfall arsenic values using one-sample t-tests. To evaluate the trend in arsenic concentrations in Sargassum over time, a linear least-squares regression was applied to the log10-transformed arsenic concentrations versus time in days. The Pearson  $R^2$  value was used to assess the strength of the correlation, with  $R^2$  values greater than 0.6 interpreted as indicating a strong relationship.

#### **III.3 Results**

#### III.3.1 Concentration Ranges

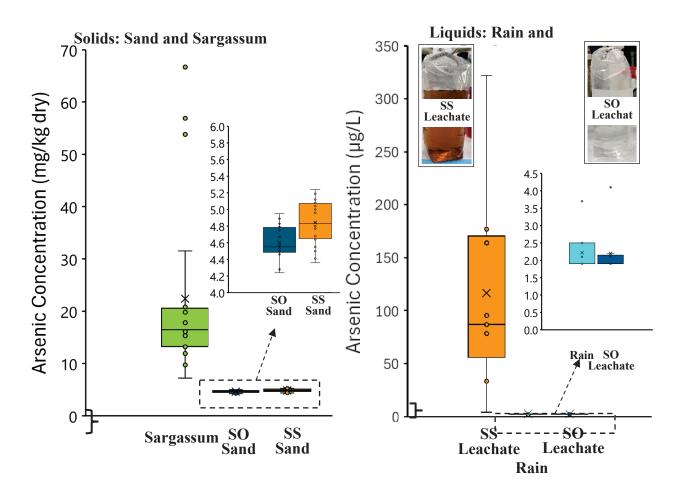
Results show that among the solid samples (Figure III.3, left panel), Sargassum had the highest concentrations of arsenic, significantly higher than sand in the SO and SS mesocosms (p <0.001). The median concentration of the Sargassum throughout the 70-day mesocosm experiment was 16.5 mg/kg, with a maximum of 66.7 mg/kg and a minimum of 7.2 mg/kg. The coefficient of variation (COV) of the Sargassum concentration was 74.8%, reflecting wide variability due to drying and decomposition processes. The Sargassum concentrations contrast with the much lower concentrations observed in the sand.

Among the sand samples, the sand from the SO mesocosm had the lowest median arsenic concentration (4.6 mg/kg) compared to the SS mesocosm (4.8 mg/kg). Although the increase in sand arsenic concentrations in the SS mesocosms relative to the SO mesocosms was relatively small (only 0.2 mg/kg), the difference was statistically significant (p = 0.003) due to the low

variability in sand values (COV = 4.5% for SO and 5.4% for SS) (Figure III.3, inset to left panel).

For the leachates, the arsenic concentration of the SO mesocosm was statistically not different from that of rainwater (median below the detection limit for both) (p = 0.97). The maximum arsenic concentration in the SO leachate was 4.1  $\mu$ g/L, only slightly higher than the maximum in rainwater (3.7  $\mu$ g/L). This contrasts sharply with the SS leachate, which had a significantly higher arsenic concentration (p = 0.0067) with a median of 86.9  $\mu$ g/L and a range from 4.0  $\mu$ g/L to 322  $\mu$ g/L. The COV for the SS leachate was also much higher (81.2%) compared to the SO leachate (32.8%). The impact of the Sargassum on leachate composition was also visually evident from the release of tannins into the SS leachate (see upper inset to Figure III.3, right panel).

In summary, the impact of Sargassum was most pronounced in the leachates but was also statistically significant in the sand. The presence of Sargassum over the sand resulted in an approximate 6% increase in arsenic concentrations in the sand, whereas a much greater increase by a factor of about 50 (5,000 %) was observed in the leachates.



**Figure III.3**. Box and whisker plots of arsenic concentrations in solid samples, sand (SO and SS mesocosms) and Sargassum (left) and in liquid samples, rain, and leachate (SO and SS mesocosms) (right). Box edges represent the 25and 75% ranges. The circles represent individual data points. The line in the box represents the median. The "×" symbol represents the average. Data points outside the whiskers represent outliers. The photos in the right panel illustrate the difference in sample color between the SS and SO leachates.

# III.3.2 Temporal Trends in Arsenic Concentrations

The temporal variation in arsenic concentrations across the Sargassum, sand, and leachate within the mesocosms revealed complex interactions between these components and rainfall (Figure III.4). For Sargassum, the initial (Day 0) arsenic concentration was 34.00 mg/kg. Notably, arsenic concentrations tended to rebound in the Sargassum following major rainfall events. During the dry period in which the first four samples were collected, arsenic concentrations increased and peaked at 66.7 mg/kg. Immediately after the first large rainfall event on Day 10 (9.3 cm), the arsenic concentration in Sargassum dropped to 13.3 mg/kg, then rebounded to a smaller peak of 20.8 mg/kg by Day 20 (April 2). On Day 40 (April 23), the second-largest rainfall event occurred (3.4 cm), and the arsenic concentration again dropped to 9.7 mg/kg before rebounding to 13.7 mg/kg. After Day 40, concentrations continued to decline, reaching 7.3 mg/kg by Day 70. These fluctuations suggest a dynamic relationship between the Sargassum biomass and environmental conditions, likely involving arsenic concentration increases due to tissue drying and loss of Sargassum tissue through volatilization. In other words, during rain events, the arsenic is released via leaching from the decomposing Sargassum. Additional drying then results in loss of Sargassum tissue resulting in an increase in the arsenic concentration. A portion of the arsenic is then released during subsequent rain events.

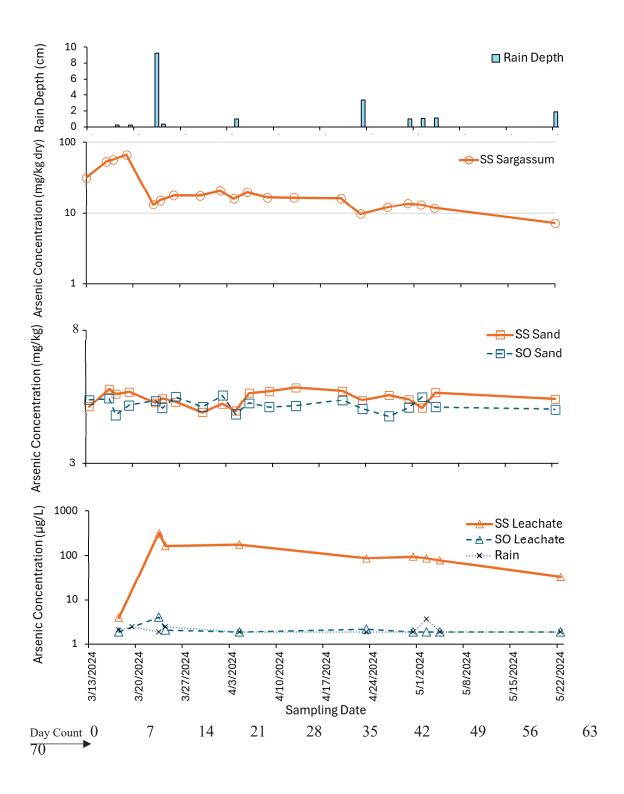
In contrast, arsenic concentrations in sand remained relatively stable throughout the experiment. At the start of the study, the SS and SO mesocosms had initial sand arsenic concentrations of 4.11 mg/kg and 4.17 mg/kg, respectively, both considerably lower than Sargassum. By Day 70, sand in the SS mesocosm had slightly increased to 4.27 mg/kg, while the SO mesocosm decreased marginally to 4.11 mg/kg. Although the absolute difference between the SS and SO sand samples was small (~0.2 mg/kg), the difference was statistically significant (p = 0.003, Welch's t-test). This result is supported by low variability in both groups (coefficient of variation of 5.4% for SS and 4.5% for SO). The statistical significance indicates that the presence of Sargassum contributed to measurable arsenic accumulation in the underlying sand. While the magnitude of change in sand concentrations was modest compared to the leachate, these findings underscore the importance of including the sand compartment in assessments of arsenic fate and transport in coastal environments.

Arsenic dynamics in the leachate showed the most pronounced differences between mesocosms. In the SO mesocosm, arsenic concentrations remained consistently low, with a weighted average of 3.2  $\mu$ g/L and a maximum of 4.1  $\mu$ g/L. Six of the nine leachate samples from SO were below the detection limit of 2  $\mu$ g/L. These concentrations were comparable to those observed in rainfall, which had a weighted average of 2.2  $\mu$ g/L and a maximum of 3.7  $\mu$ g/L. There was no statistically significant difference between arsenic concentrations in SO leachate and rainfall (p = 0.97).

In contrast, the SS mesocosm leachate exhibited substantially higher arsenic concentrations, with a weighted average of 116.4  $\mu$ g/L and a peak of 322  $\mu$ g/L. The highest concentrations were observed on Day 10, coinciding with the largest rainfall event (9.3 cm), suggesting mobilization of arsenic from the Sargassum into the leachate. While arsenic concentrations declined over time, they remained elevated relative to the SO mesocosm, falling to 33.2  $\mu$ g/L by Day 70. The difference between SS leachate and rainfall arsenic concentrations was statistically significant (p

= 0.007), and the declining trend mirrored the reduction in Sargassum arsenic concentrations over the same period.

In summary, arsenic levels in Sargassum and leachate were strongly influenced by rainfall events. Arsenic in Sargassum was concentrated in the Sargassum tissue during dry periods and was released during rainfall, contributing to elevated leachate concentrations early in the experiment. As the Sargassum weathered and its arsenic content decreased, leachate concentrations also declined. By Day 70, arsenic concentrations in the Sargassum had decreased to 7.3 mg/kg, while leachate concentrations in the SS mesocosm remained elevated (33.2 µg/L) compared to consistently low levels in the SO mesocosm (near 2 µg/L).



**Figure III.4**. Time series of rainfall depth (top panel) and arsenic concentrations (bottom three panels) in Sargassum, sand, and in aqueous samples (rainwater and leachates).

#### III.3.3 Water and Arsenic Mass Balance

The water balance indicates that evaporation was larger for the SS mesocosm (7.1% of rainfall) compared to the SO mesocosm (-0.54%), indicating that the presence of Sargassum resulted in a greater evaporation rate of incident rainfall. Sargassum's ability to retain moisture (WC ranging from 9.5% to 69.7%) likely contributed to this enhanced evaporation. The Sargassum at the surface may trap moisture, which is then released through evaporation due to solar heating. In contrast, sand, with a moisture content ranging from 0.08% to 21.5%, did not retain water as effectively, resulting in a lower moisture level at the surface of the SO mesocosm and less evaporation. The negative capture of moisture by the sand was due to the collection of dry sand at the very beginning of the experiment and collection of wet sand at the ending of the experiment the morning after a rainfall event, resulting in a net gain of moisture by the SO sands (Table III.1). The differences in moisture retention explains the overall increase in leachate generation for the SO mesocosm (67.1 L) compared to the SS mesocosm (61.7 L). Overall Sargassum captured more of the rainfall at the surface resulting in greater evaporation and the production of less leachate.

In terms of the sand moisture content, the sand as collected from the beach site was very dry at the initiation of the mesocosm experiment (<0.5%). At the end of the experiment, the moisture content in the sand within the SS mesocosm was higher (21.5%) than the sand within the SO mesocosm (17.3%). This difference in sand moisture was observed for most samples collected with a mean sand moisture for the SS mesocosm at 7.1% and for the SO mesocosm at 5.0%, with these values showing statistical differences (p=0.012). It appears that the presence of Sargassum within the mesocosm tends to increase the moisture content of the sand located below it, compared to the mesocosm without the Sargassum layer.

The arsenic balance shows that most of the arsenic within the mesocosm systems was found in both the Sargassum (48.1%) and interestingly from the sand (51.8%) too. The initial dry mass of Sargassum in the mesocosm (1.3 kg) was small relative to the initial dry mass of sand (11.7 kg) (Table III.1), and yet the Sargassum accounted for an almost equal amount of arsenic in the initial set up (44.7 mg) compared to the sand (48.1 to 49.1 mg) (Table III.2). The initial concentrations of arsenic in the Sargassum was 34 mg/kg (5.79 mg/kg standard deviation), whereas for the sand was 4.11 mg/kg (0.33 mg/kg standard deviation) in the SS mesocosm and 4.17 mg/kg (0.41 mg/kg standard deviation) in the SO mesocosm. By the end of the mesocosm experiment the Sargassum lost 77% (34.6/44.74) of its arsenic, whereas sand below the Sargassum layer in the SS experiment gained arsenic (3.7%, 1.79/48.14) while the sand in the SO mesocosm without Sargassum lost arsenic (1.4%).

Leachates from the SS and SO mesocosms accounted for 14.32 mg and 0.217 mg of arsenic respectively. Statistically the concentrations of arsenic in the SS mesocosm were higher than in the SO mesocosm (p<0.001). Of the overall amount of arsenic in each mesocosm, 15.4% was transferred to the leachate for the SS mesocosm and 0.44% was transferred to the leachate for the SO mesocosm (Table III.2). Although the sand held about half of the arsenic in the system, only a small fraction of the arsenic was lost from the sand. In contrast, for the mesocosm containing Sargasssum, a much larger fraction of arsenic was transferred to the leachate.

**Table III.1**. Water balance for mesocosm experiments. Water volumes listed below correspond to the cumulative amounts for all samples collected from the Sargassum plus Sand (SS) mesocosm and from the Sand Only (SO) mesocosm for the period from March 13, 2024 through the last day of sample collection on May 22, 2024.

	Water Balance				
		Rainfall			
Rainfall Volume (cm)		19.36			
Rainfall Volume (L)	$V_R = \sum_{n=1}^{n_s} V_{R,i}$	68.68			
		SS Mesocosm		SO Mesocosm	
		Sargassum	Sand	Sand	
Leachate Volume (cm)	$V_L = \sum^{n_s} V_{L,i}$	17.39		18.90	
Leachate Volume (L)	$\sum_{n=1}^{r} L_{n}$	61.68		67.06	
		Sargassum	Sand	Sand	
Initial wet weight of Sargassum or Sand (kg)	$M_{G,w,initial}$ and $M_{S,w,initial}$	3.049	11.761	11.810	
Initial dry weight of Sargassum or Sand (kg)	$M_{G,d,initial}$ and $M_{S,d,initial}$	1.316	11.706	11.769	
Sargassum and Sand Moisture Initial (%)	$WC_{G,initial}$ and $WC_{S,initial}$	56.83	0.47	0.35	
Sargassum and Sand Moisture Final (%) <sup>a</sup>	WCG,,final and WCs,,final	46.55	21.46	17.28	
Volume Evaporated, L	$V_E$ (equation A)	4.85		-0.37	
% Evaporated	$V_E$ (equation A) $%V_E = \frac{V_E}{V_R} \times 100\%$ $%V_L = \frac{V_L}{V_R} \times 100\%$	7.06 -0.54%		-0.54%	
% Leachate	$\%V_L = \frac{V_L}{V_R} \times 100\%$	89.81 97.63		97.63	
% Change in Matrix WC	$\mathcal{M}_{-}$		3.59	2.91	

<sup>&</sup>lt;sup>a</sup> The last samples collected on May 22, 2024 were collected immediately after a 1.9 cm rainfall event (7.42 L).

Table III.2. Arsenic balance for mesocosm experiments. Arsenic masses listed below correspond to the

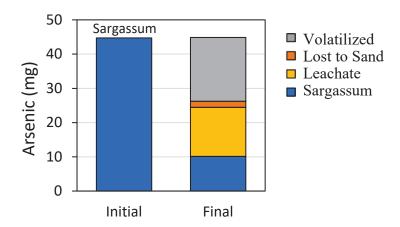
cumulative amounts for all samples collected.

mulative amounts for all sa	Arsenic Balance			
	Arsene Balance		Rainfa	all
Average Rainfall Arsenic Concentration, mg/L	$As_R = \frac{\sum V_{R,i} As_{R,i}}{\sum V_{R,i}}$		a,b	
Arsenic Mass from Rain, mg	$M_{AS,R} = \sum_{i=1}^{n_S} V_{R,i} A s_{R,i}$		0.138	
	π=1	SS Meso	ocosm	SO Mesocosm
Leachate Arsenic Concentration, mg/L	$As_L = \frac{\sum V_{L,i} As_{L,i}}{\sum V_{L,i}}$	0.2322 <sup>b</sup>		0.00324 <sup>b</sup>
Arsenic Mass in Leachate, mg	$M_{As,L} = \sum_{n=1}^{n_s} V_{L,i} A s_{L,i}$	14.322		0.217
	111	Sargassum	Sand	Sand
Arsenic Concentration Initial (mg/kg)	As <sub>G,initial</sub> OR As <sub>s,initial</sub>	34.00	4.11	4.17
Arsenic Mass, Initial (mg)	$M_{As,G,initial} = M_{G,d,initial} \times As_{G,initial}$ OR $M_{As,S,initial} = M_{S,d,initial} \times As_{S,initial}$	44.74 48.14		49.11
Total Mass of Arsenic Initially in System (mg)	For SS, Omit $M_{As,G,initial}$ for SO	93.04		49.26
% Arsenic in Matrix Initially	$\begin{aligned} M_{AS,total} &= M_{AS,G,initial} + M_{AS,S,initial} + M_{AS,R} \\ For SS, Omit &M_{AS,G,initial} for SO \\ &\underbrace{M_{AS,G \text{ or S,initial}}}_{M_{AS,G,initial} + M_{AS,S,initial} + M_{AS,R}} \end{aligned}$	48.09 51.75		99.69
Arsenic Concentration Final (mg/kg)	$As_{G,final} OR As_{s,final}$	7.71	4.27	4.11
Arsenic Mass Final, mg	$M_{As,G,final} = M_{G,d,final} \times As_{G,final}$ $OR$ $M_{As,S,final} = M_{S,d,final} \times As_{S,final}$	10.14 49.93		48.40
Arsenic Mass Gained from Sargassum or Sand, mg	$M_{G,d,initial}(As_{G,final} - As_{G,initial}) \ OR \ M_{S,d,initial}(As_{S,final} - As_{S,initial})$	-34.60 1.79		-0.71
Mass Arsenic Volatilized, mg	$M_{As,E}$ (equation B)	18.63		0.01
% Arsenic Mass in Rainfall	For SS, $\%M_{As,R,total}$ Omit $M_{As,G,initial}$ for SO $\frac{M_{As,R}}{M_{As,G,initial} + M_{As,S,initial} + M_{As,R}} \times 100\%$	0.15 0.28		
% Arsenic Mass in Leachate	For SS, %MAs,L,total, Omit MAs,G,initial for SO $\frac{M_{As,L}}{M_{As,G,initial} + M_{As,S,initial} + M_{As,R}} \times 100\%$	15.4	40	0.44
% Arsenic Remaining in Matrix	For SS, $\%M_{As,G \text{ or } S, remaining total}$ , $Omit M_{As,G, initial}$ for SO $\frac{M_{As,G \text{ or } S, final}}{M_{As,G, initial} + M_{As,S, initial} + M_{As,R}} \times 100\%$	10.90	53.67	98.29
% Arsenic Gained by Matrix	0/ 4 3 4	-37.20 1.92		-1.43
% Arsenic Mass Volatilized from entire system	$\%\Delta M_{As,G, total} \ OR \%\Delta M_{As,S,total}$ $\%M_{As,E,total} = \frac{M_{As,E}}{M_{As,total}} \times 100\%$	20.03		1.27
Net % Arsenic Mass Volatilized from Sargassum	Mass Balance Focused on Sargassum Only $\% M_{As,E,Sargassum} = \frac{M_{As,E,Ss mesocosm}}{M_{As,E,Sargassum}} \times 100\%$	41.64		
% Arsenic Mass Lost from Sargassum to Sediment Below	$\%M_{As,S,Sargassum} = \frac{M_{s,d,initial}(As_{s,final} - As_{s,initial})}{M_{As,G,initial}} \times 100\%$	3.99		
% Arsenic Mass Lost from Sargassum to Leachate	$\%M_{As,L,Sargassum} = \frac{M_{As,L} - M_{As,R}}{M_{As,G,initial}} \times 100\%$	31.70		
% Arsenic Mass Remaining in Sargassum	$\%M_{As,G,remaining\ Sargassum} = \frac{M_{As,G,final}}{M_{As,G,initial}} \times 100\%$	22.66		

<sup>&</sup>lt;sup>a</sup> Ten samples were measured with 4 of the 10 above detection. The 6 that were below detection were set to the detection limit value of 0.0019 mg/L. <sup>b</sup> Weighted average arsenic concentration based upon volume of rainfall or leachate.

The most intriguing feature about the arsenic balance is the apparent loss of arsenic due to volatilization. The arsenic lost (or unaccounted for) in the system was computed as 18.6 mg for the SS mesocosm compared to a much smaller value (0.01 mg) for the SO mesocosm. The volatilization of arsenic from the SS mesocosm represents 20.0% of the arsenic in the SS mesocosm (sum of mass input from rainfall plus mass contained initially in sand and Sargassum). The volatilization of arsenic from the SO mesocosm represented a much smaller fraction 1.3%.

When conducting the mass balance focused on Sargassum only, the proportions are even more intriguing (Figure III.5). When focusing the mass balance on the Sargassum compartment, the net amount of arsenic volatilized is 41.6%, the net amount lost to leachate was 31.7%, and the net loss to the sand beneath the Sargassum was 4%. By the end of the 70-day mesocosm experiment, 22.7% of the arsenic remained in the Sargassum.



**Figure III.5**. Summary results from arsenic mass balance analysis emphasizing the initial reservoir of arsenic in the Sargassum in the SS mesocosms and the reservoirs to which the arsenic was found.

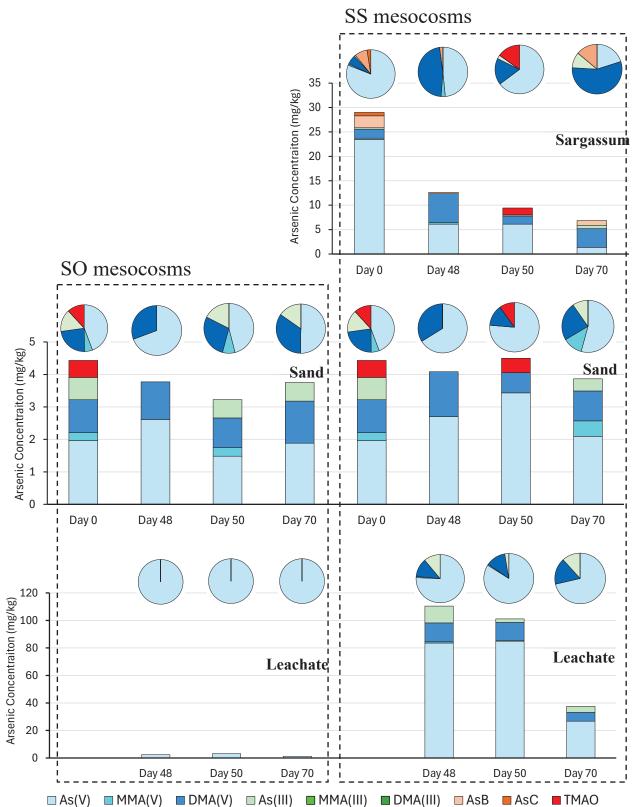
#### III.3.4 Arsenic Speciation

The Sargassum sample from the SS mesocosms showed a diversity of arsenic species with As(V) generally dominating (Figure III.4, top panel). Overall, As(V) was observed to dominate at the initiation of the experiment representing 81% of the total arsenic. By the end of the 70 days, the total amount of arsenic declined and the proportion of As(V) dropped to 21%. A diversity of arsenic species was observed at low proportions in the Sargassum for all time points. At Day 0, low proportions of AsB (8.4%), DMA(V) (6.4%), AsC (2.5%), MMA(V) (0.9%), and As(III) (0.9%) were observed. By Day 48, As(V) still dominated (48%) but DMA(V) represented a significant proportion (47%) followed by MMA(V) (2.7%) and AsB (2.1%). For the Day 50,

four species in addition to As(V) were again observed but, in this case, TMAO and As(III) were observed instead of MMA(V) and AsB. By the last day, for Day 70, the dominant species switched to DMA(V) (56%), followed by As(V) (21%), AsB (15%), and As(III) (7.8%). Overall, averaging the results of all four sampling days together, the average proportions were As(V) (59.5%), DMA(V) (16.8%), AsB (8.2%), TMAO (7.8%), AsC (3.9%), As(III) (2.2%) and MMA(V) (1.5%).

For the sand samples (Figure III.4, middle panel), five arsenic species were observed with As(V) dominating, followed by DMA(V), TMAO, As(III) and then MMA(V). The proportion of these five species were similar between the SO and SS sands. The corresponding proportions were 47%, 23%, 12%, 11%, and 6%, respectively, on average. For samples of the SO and SS sands collected on different days, the detected arsenic species were different. Only two species (As(V) and DMA(V)) were detected on Day 48 and three species were detected on Day 50. However, the species detected differed for the SS sand (As(V), DMA(V), TMAO) and the SO sand (As(V), DMA(V), As(III)). On Day 70, As(V), DMA(V), and As(III) were detected in both SS and SO sands, with the Day 70 SS sand sample also showing the presence of MMA(V). The first day of sample collection (Day 0), all five species listed above were observed (As(V), DMA(V), As(III), MMA(V), TMAO). TMAO has been observed in wet deposition and its source has been identified as atmospheric oxidation of marine derived trimethylarsine (Savage et al. 2017).

For the liquid samples, no arsenic species were detected in the rainfall samples (all below the limit of detection by HPLC-ICP-MS). For the SO leachate, only As(V) was detected at low levels (2.3  $\mu$ g/L) (Figure III.4, bottom panel). In the SS leachate influenced by Sargassum the arsenic species detected were more diverse with the majority as As(V) (65.1  $\mu$ g/L, 78% overall) followed by DMA(V) (11.0  $\mu$ g/L, 13%), As(III) (6.4  $\mu$ g/L, 8%) and MMA(V) (0.8  $\mu$ g/L, 1%), on average.



**Figure III.4**. Arsenic speciation of Sargassum (top panel), sand (middle panel), and leachate (bottom panel) in the SO mesocosms (no Sargassum) and in the SS mesocosms (with Sargassum). Species measured included As(V), MMA(V), DMA(V), As(III), MMA(III), DMA(III), AsB, AsC, and TMAO.

#### **III.4 Discussion**

#### III.4.1 Arsenic Levels

The initial arsenic concentration in the Sargassum used in this study (34 mg/kg) is consistent with previously reported values for Sargassum collected along the Atlantic coastline, which typically range from 10 to 150 mg/kg depending on location, water chemistry, and bloom maturity (Kwon et al., 2021; Almela et al., 2006, Rodríguez-Martínez et al. 2020, Davis et al. 2021, Hatt et al. 2024, Liranzo-Gomez et al. 2023). The decline in arsenic concentration to 7.3 mg/kg over the 70-day period reflects a substantial loss (approximately 77%), highlighting that Sargassum does not retain arsenic indefinitely and functions as a dynamic reservoir releasing arsenic as it decays.

For beach sand, few studies have evaluated arsenic concentrations in pristine environments. Background concentrations generally range between 1 and 5 mg/kg, although levels can be much higher near industrial or mining sites (Smedley and Kinniburgh, 2002). In this study, the initial concentrations of arsenic in the sand (4.11 mg/kg in SS, 4.17 mg/kg in SO) fell within this natural background range. However, the observed statistically significant difference in final concentrations (p = 0.003), with a slight increase in the SS mesocosm, suggests that Sargassum contributes to the transfer of arsenic to the underlying substrate, even if the magnitude of this transfer is relatively small. Of particular concern is that the arsenic sand concentrations exceed the Florida Soil Cleanup Target Levels (SCTLs) for residential use, which set a benchmark of 2.1 mg/kg for total arsenic in soil (FDEP, 2005).

Leachate arsenic levels in the SS mesocosm reached up to 322  $\mu g/L$ , significantly exceeding the U.S. EPA maximum contaminant level (MCL) for drinking water (10  $\mu g/L$ ; USEPA, 2001) although below the threshold (5,000  $\mu g/L$ ) that would classify it as a hazardous waste as per toxicity characteristic leaching procedure (TCLP) (US EPA 1980). Although leachate from Sargassum is not considered a drinking water source, the high arsenic concentrations raise concerns about the potential for contamination of groundwater or coastal waters, especially in regions where decaying Sargassum accumulates near storm drains or shallow aquifers.

Furthermore, if leachate is used for irrigation, disposal, or composting, comparisons to USEPA biosolids standards (e.g., 41 mg/kg for arsenic in exceptional quality biosolids) become relevant and may indicate exceedance depending on concentration and use case. In the current study, the maximum arsenic concentration of Sargassum reached 67 mg/kg. Depending upon where and when the Sargassum is harvested, concentrations can be much higher, on the order of several hundreds of mg/kg (REF). Such results suggest that use of Sargassum as a biosolid amendment to soils may not meet regulatory standards due to arsenic exceedances. The elevated levels of arsenic in the Sargassum and in leachate from Sargassum underscores the need for careful management of decomposing Sargassum and its associated runoff (Mossbauer et al. 2012).

#### III.4.2 Temporal Trends

Arsenic concentrations in Sargassum exhibited a clear temporal pattern, characterized by a rebound effect during drying phases and substantial losses following rainfall events. During dry periods, moisture loss from the Sargassum led to concentration of arsenic in the remaining

biomass, with peak concentrations reaching 66.7 mg/kg. However, after major rainfall events, particularly on Day 10 and Day 40, arsenic concentrations in the Sargassum dropped sharply, followed by smaller rebounds. This suggests that rainfall promotes leaching of arsenic from the biomass, while subsequent drying reconcentrates arsenic in the remaining tissue. These cycles highlight the dynamic exchange of arsenic between the Sargassum and surrounding media, driven largely by environmental conditions. A study of Sargassum accumulations in the Mexican Caribbean documented the arsenic concentrations within "recently arrived" Sargassum to a natural 60 cm high pile formed by prior natural strandings (Chavez-Vergara et al. 2025). Results from this study showed that the arsenic concentration in the recently arrived stranding was highest (88.6 mg/kg) with the lowest levels observed in the most degraded Sargassum at the bottom of the 60 cm pile (10.5 mg/kg), resulting in a 90% decrease in the arsenic concentration. Of interest was that Olguin-Maciel et al. (2022) for this same study evaluated additional metals (Cd, Cr, Cu, Ni, Zn, Cu) and only arsenic demonstrated significant losses. The reduction in arsenic concentration observed by the researchers evaluating Sargassum in the Mexican Caribbean was similar to the 80% reduction observed in the current study (from a concentration of 34 at stranding to 7.4 mg/kg at the end of the 70-day study period). The current study, in addition to documenting the decrease, demonstrated a rebound effect coupled with the capture of the leachates, confirming the connection between the wetting and drying cycles of the Sargassum and its release of arsenic to the environment.

The precise timing and mechanism of volatilization remains uncertain. While leaching is clearly linked to rainfall events, volatilization is more likely associated with dry, warm conditions that favor microbial methylation of arsenic species and subsequent release as volatile compounds (Datta et al. 2025). However, without real-time gas-phase arsenic measurements, it is difficult to pinpoint whether volatilization occurs steadily during dry periods or episodically during transitions in moisture status. This remains an important direction for future research.

A limitation of this study is that it ended after day 70, at which point the Sargassum remained visible on the sand surface and retained a measurable arsenic concentration of 7.3 mg/kg. Using a best-fit regression model based on log-transformed arsenic concentration data over time:

#### Arsenic concentration = $10^{-4}(-0.01(\text{days}) + 1.5334)$ , $R^2 = 0.60$ ,

we estimate that it would take approximately 120 days—about four months—for the arsenic concentration to decline to 2.1 mg/kg, the Florida residential SCTL. This model implies that although arsenic concentrations in Sargassum decline over time, they remain above risk-based thresholds for weeks after visible decay begins. Extending the mesocosm duration to track full disintegration of the Sargassum would be valuable for confirming whether and when it becomes environmentally benign.

Sargassum decomposition also had a clear effect on surrounding media. The statistically significant increase in sand arsenic content, while modest, demonstrates that underlying sediments can serve as a sink. In contrast, the leachate exhibited the most pronounced response, confirming that water percolating through Sargassum layers is a key vector for arsenic mobilization.

#### III.4.3 Water Balance

The results from the water balance experiments indicate that the presence of Sargassum significantly impacts the moisture distribution and evaporation from the shoreline. Sargassum consistently exhibited a much higher water content compared to sand, both in mesocosms with and without the macroalgae layer. On average, Sargassum maintained a water content of 26.45%, whereas sand in the SS mesocosms had an average water content of 7.1%, and sand in the SO mesocosms exhibited an average water content of 4.95%. The coefficient of variation for water content in the sand was similar between the SS and SO mesocosms (110% and 140%, respectively), but the coefficient of variation for Sargassum was notably lower (65%). These results suggest that the presence of Sargassum on the beach shoreline helps to maintain a more consistent and higher moisture level compared to the sand alone, offering a higher and relatively less variable moisture environment, which has environmental benefits in the context of regulating the incubation temperatures of sea turtle eggs (Maurer et al. 2022).

The difference in water retention between Sargassum and sand is of significant ecological importance, especially when considering the role of microbial communities. These communities depend heavily on moisture availability, and the reduced variability in moisture levels in Sargassum could explain why stranded Sargassum has been shown to harbor higher levels of fecal indicator bacteria along the beach shoreline. Previous studies (e.g., Abdool-Ghany et al., 2022) have shown that when Sargassum is integrated with beach sand, it provides an environment conducive for the growth of fecal bacteria. This interaction could be facilitated by the more stable moisture levels that are characteristic of Sargassum-covered sand. Further studies are needed to investigate the relationship between Sargassum moisture content and microbial dynamics, especially regarding pathogen persistence and growth.

When comparing the evaporation rates of rainwater from SS and SO mesocosms, the presence of Sargassum was again associated with higher evaporation rates. This can be attributed to the ability of Sargassum to retain moisture at the surface, preventing rainwater from infiltrating the substrate. In contrast, rainwater in the SO mesocosms had greater infiltration into the sand layer, resulting in lower moisture retention at the surface. Consequently, less rainwater percolated to the underlying groundwater in mesocosms with Sargassum, suggesting that Sargassum can influence groundwater recharge dynamics by reducing water infiltration. This phenomenon could have implications for coastal hydrology, particularly in regions where Sargassum is prevalent.

#### III.4.4 Arsenic Balance

The results from the arsenic balance experiments further highlight the complex interactions between Sargassum, sand, and arsenic in coastal environments. In the SS mesocosms, a substantial amount of arsenic (18.63 mg) was volatilized, while virtually no volatilization occurred in the SO mesocosms (0.01 mg). This significant volatilization of arsenic from Sargassum suggests that the process is likely microbially mediated. Several studies have demonstrated that microbial activity can influence arsenic speciation and volatilization, with certain microbes capable of methylating inorganic arsenic to volatile forms such as trimethylarsine (e.g., Breuninger et al. 2024). The volatilization from Sargassum in our mesocosms may be driven by these microbial processes, where arsenic is transformed into its more volatile methylated forms.

Overall, more than half (54%) of the arsenic lost from Sargassum was attributed to volatilization, while a smaller proportion (41%) was lost via rainwater leachate (Figure III.4). Only a small fraction (4%, or 1.79 mg) of arsenic was transferred to the sand layer. In contrast, the SO mesocosms without Sargassum saw minimal arsenic loss (0.71 mg), with most of the arsenic presumably rinsed out by rainwater infiltration. These findings suggest that volatilization is a key mechanism for arsenic loss in systems with Sargassum and may represent a significant pathway for arsenic release into the atmosphere.

The final concentration of arsenic in the Sargassum was 7.7 mg/kg, considerably lower than the initial concentration of 34 mg/kg. However, even after over two months of drying under ambient conditions, the arsenic concentration remained above the residential risk-based threshold of 2.1 mg/kg and just below the industrial threshold of 12 mg/kg (FDEP, 2005). While this indicates a reduction in arsenic levels, the concentration in Sargassum is still high enough to pose potential risks to public health. Risk assessments, particularly for children, suggest that dermal absorption and adherence of Sargassum to the skin are key factors influencing arsenic exposure. A study by [Brittany's Paper] showed a slight but elevated cancer risk due to dermal exposure to arsenic from Sargassum, underscoring the need for further investigation into the potential risks of beachgoers interacting with stranded Sargassum.

In addition to the volatilization of arsenic from the Sargassum itself, our results show that a small amount of arsenic was transferred from the Sargassum to the sand beneath it. The initial arsenic concentration in the sand within the SS and SO mesocosms was between 4.1 and 4.2 mg/kg, while the final concentrations were 4.3 mg/kg in the SS mesocosms and 4.1 mg/kg in the SO mesocosms. Although these concentrations remain above the residential risk threshold of 2.1 mg/kg, the increase in sand arsenic levels in the SO mesocosms is noteworthy, as it suggests that arsenic may become more bioavailable in the sand due to rainwater leaching. While the transfer of arsenic to sand is relatively small, further studies are needed to evaluate the potential health risks associated with arsenic exposure from beach sand, particularly in areas where Sargassum blooms are frequent. Mc Intyre et al. (in review) suggested that the risks posed by arsenic in sand are lower compared to those from the Sargassum itself, but the potential for bioavailability and dermal exposure warrants further investigation.

The results observed in this study are limited. Beach sand, known to have very low organic content, is does not readily absorb contaminants including arsenic. The slight decrease of arsenic in the SO mesocosm was likely due to the washing out of arsenic by rainwater. The slight increase of arsenic in the SS mesocosm was due to the release of arsenic from the overlying Sargassum layer. In either case, arsenic was not readily sorbed by the sand. If an alternate soil type were to underly the Sargassum layer in the SS mesocosm it is likely that more arsenic could be absorbed with a lower fraction transferred to leachate. The stronger sorption of arsenic to a different soil type may have an impact on the fraction that is volatilized. Of interest would be to investigate the conditions under which arsenic can be trapped by an underlying soil layer and its impact on volatilization. Such a study would require an understanding of the microbial community, as the arsenic transformations that facilitate volatilization are likely microbially mediated. Enhancing the volatilization or capture of arsenic by an underlying soil layer could potentially mediate impacts to leachate.

#### III.4.5 Arsenic Speciation

Throughout the study, inorganic As(V) was the dominant species across all compartments, although its proportion decreased in Sargassum over time. By Day 70, DMA(V) had become the predominant form in the Sargassum, indicating transformation consistent with the mechanisms of Sargassum detoxification. Datta et al. (2025) evaluated the biotransformation of arsenic in Sargassum thunbergia throughout its growth stages. Their conceptual model illustrates arsenate (as As(V)) entering the cell through phosphate transporters in the cell membrane due to the similarity in the structure of arsenate (as As(V)) and phosphate. Once within the cell, As(V) is converted to As(III) which then binds with phytochelatins and glutathione to sequester arsenic into vacuoles. Once in the vacuoles methylation occurs converting the As(III) to MMA which quickly transforms to DMA, with excretion in the form of DMA. The dominance of As(V) and DMA during all measurements within the current study, in Sargassum, sand, and leachate, is consistent with the conceptual model established by Datta et al. 2025. The appearance of As(III) in the last measurement of the Sargassum, and in the SS leachates can be due to the last phase of Sargassum degradation where the arsenic internal to the cells may be getting released. The presence of As(III) in the SO sand may be due to microbial mediated processes or from historic releases of arsenic from Sargassum prior to the time the sand samples were collected.

The dominance of As(V) in Sargassum is consistent with the findings from other studies (Peng et al. 2023). Studies of *Sargassum fulvellum*, a species that is consumed in Asia, found that 87% (García-Salgado et al. 2012) and 98% (Khan et al. 2015) of the arsenic was present as As(V) with trace levels of As(III) and AsB (Khan et al. 2015) or DMA and arsenosugars (García-Salgado et al. 2012). Studies of *Sargassum aquifolium* and *Sargassum echinocarpum* found a dominance of As(V) (over 67%) but with considerable proportions of the remaining arsenic as arsenosugars (Kim et al. 2024).

The change in speciation is significant due to differences in toxicity. As(V) and As(III) are considered highly toxic, with As(III) being more acutely toxic than As(V). DMA(V) is less toxic than inorganic species but still poses health concerns, particularly due to its potential carcinogenicity. The shift in species composition from primarily inorganic to partially methylated forms indicate active biotransformation processes and may affect both the environmental persistence, bioavailability of arsenic and toxicity to humans.

#### III.4.6 Implications for Management and Risk

The results of this study have direct implications for the management of stranded Sargassum. Because Sargassum facilitates arsenic mobilization through leaching and volatilization, beach management practices that involve onsite stockpiling or composting may inadvertently contribute to localized contamination or atmospheric release. Mitigation strategies should consider offsite removal or containment methods that minimize environmental exposure pathways. Additionally, the persistence of arsenic in both Sargassum and sand above regulatory thresholds for residential soil suggests a need for risk assessment, particularly in recreational beach settings. Children are especially vulnerable to arsenic exposure due to higher rates of dermal contact and hand-to-mouth behavior. Risk assessments should incorporate both leachate exposure and volatilization pathways.

#### III.4.7 Future Directions

Several areas warrant further investigation. First, the microbial communities associated with Sargassum should be characterized to identify key organisms involved in arsenic methylation and volatilization. Second, experiments should be extended beyond 70 days to determine the full degradation timeline of Sargassum and whether arsenic levels eventually decline below regulatory thresholds. Third, studies should assess how different substrate types (e.g., organic-rich soils vs. beach sand) influence arsenic retention or transformation. Finally, atmospheric sampling techniques should be deployed in future mesocosm or field studies to directly measure volatile arsenic species and assess their environmental and health implications. Collectively, these findings highlight the multifaceted role of Sargassum in coastal arsenic cycling and emphasize the need for integrated strategies that address both environmental fate and public health risks associated with beach-cast seaweed.

#### **III.4 Conclusions**

This study underscores the intricate biogeochemical dynamics of arsenic cycling in coastal systems impacted by Sargassum strandings. Our mesocosm experiments demonstrate that Sargassum not only alters shoreline hydrology by retaining surface moisture and modifying evaporation dynamics, but also facilitate the transformation and loss of arsenic through volatilization. The volatilization of arsenic observed in the Sargassum-covered systems suggests active microbial mediation, with a substantial proportion of the initial arsenic content being released into the atmosphere in potentially volatile forms. This pathway may represent an unrecognized vector for arsenic dispersion in nearshore environments, with implications for local air quality and ecological exposure.

Despite notable reductions in arsenic concentration in the Sargassum over the 70-day experimental period, final concentrations in the Sargassum tissue remained above the Florida Department of Environmental Protection's (FDEP) residential soil cleanup target levels. This residual contamination raises concerns regarding potential public health risks, particularly from dermal contact and incidental ingestion during recreational beach activities. Previous risk assessments have highlighted that dermal contact with Sargassum and potential arsenic dermal absorption are key variables governing human exposure, and our findings reinforce the need for detailed toxicological studies to quantify these exposure pathways under real-world conditions.

Additionally, the downward leaching of arsenic into underlying sand layers, although limited, suggests that Sargassum can contribute to localized contamination of beach substrates. While the magnitude of this transfer was relatively small, the persistence of arsenic in sand above residential screening levels points to a potential chronic exposure source, especially in areas where Sargassum strandings are frequent or long-lasting. The altered moisture dynamics and associated changes in microbial habitat conditions further complicate the ecological implications of stranded Sargassum, as microbial community shifts may affect nutrient cycling, contaminant transformation, and pathogen persistence in coastal zones.

Collectively, these findings emphasize the need for integrated management approaches to Sargassum accumulation that account for its role in environmental persistence, contaminant dynamics, and public health exposure. Future research should aim to elucidate the microbial mechanisms underpinning arsenic volatilization, assess the atmospheric fate and transport of volatilized species, and evaluate mitigation strategies that minimize human and ecological exposure to arsenic in Sargassum-impacted coastal settings.

Overall, the results indicate that Sargassum plays a critical role in both arsenic volatilization and release to leachate, affecting not only its own biomass but also influencing the surrounding sand and water that infiltrates (i.e., leachates). These interactions highlight the complex environmental processes at play and suggest that Sargassum may serve as a significant vector for arsenic movement in coastal ecosystems.

# CHAPTER IV SUMMARY AND CONCLUSIONS

# **CHAPTER IV**

# SUMMARY AND CONCLUSIONS

# **IV.1 Summary and Conclusions**

This study confirms that Sargassum is a significant source of arsenic in the beach environment, unlike seagrasses, which commonly accumulate alongshore but do not contain elevated arsenic levels. Results from the mesocosm experiments show that as Sargassum decomposes onshore, approximately half of its arsenic content is released through volatilization and another 40% is lost through leaching. The leachate contained elevated arsenic concentrations (>300  $\mu$ g/L), most of which passed through the underlying sand.

To address key regulatory questions:

- What are the background levels of arsenic at the beach? Average arsenic concentrations in beach sand (2.2 mg/kg) and seagrass (1.9 mg/kg) were close to the Florida Soil Cleanup Target Level (SCTL) of 2.1 mg/kg. In contrast, Sargassum had much higher average concentrations (36.4 mg/kg).
- Does Sargassum contribute to excessive arsenic levels at beaches?
   Yes. Sargassum bioaccumulates arsenic and, even during decomposition, retains levels more than ten times higher than the Florida SCTL. A detailed risk assessment is recommended to evaluate exposure risks to beachgoers.
- Will Sargassum compost increase arsenic levels if applied near the beach?
   Yes. Mesocosm results show that Sargassum retains about 20% of its original arsenic content after decomposition (from 34 mg/kg to 7.7 mg/kg), which still exceeds the SCTL.
   Leachate arsenic concentrations remained high (>300 μg/L), but the potential health risks from leachate and volatilized arsenic require further investigation.

These findings provide critical insight for beach managers and solid waste operators. Arsenic remains a key concern when recycling Sargassum, particularly in systems lacking containment or treatment measures for leachate or emissions.

#### **IV.2 Recommendations**

Results show that Sargassum does contribute to elevated arsenic concentrations in the beach environment. Risk assessments are needed to evaluate the impact to beach goers, especially children due to their play behaviors. Risks should also be evaluated to beach shore ecosystems which may be impacted directly from the arsenic in the leachate. The impacts from the volatilization of arsenic should also be evaluated.

Given that Sargassum releases considerable arsenic as it decomposes, studies are needed to develop technologies for the capture of the arsenic. We envision a system designed to encourage Sargassum decomposition naturally. This system would be underlaid by a passive capture system designed to absorb arsenic from leachate. More work is recommended to evaluate arsenic capture technologies in the context of Sargassum recycling.

#### **IV.3 Practical Benefits for End Users**

Currently, FDEP is working to include Sargassum as its own category of waste to be composted. Their assessment uses results from our previously funded Hinkley Center research. As it stands now under the Florida Administrative Code (FAC), chapter 62-709, Sargassum can be composted under the Source-Separated Organics Processing Facility (SOPF) registration program as yard waste. The status of Sargassum as yard waste was used to establish the Sargassum composting facility in Fort Lauderdale. With climate change and other factors, the Sargassum making its way onshore is increasing. As such, Sargassum inundations will impact more coastal communities and the need will grow to develop solutions for handling the influx. Recognizing this growing need the FDEP is in the process of developing regulations specific to Sargassum (and seaweed strandings as a whole). We have been keeping in communication with representatives at the Division of Waste Management at FDEP on this rule change. They have provided the research team with feedback and questions they are interested in answering. They specifically mentioned the need to understand the background levels of arsenic at the beach before further evaluating its use in the nearshore environment, for dune stabilization, and/or as a substrate for vegetation within adjacent beach park areas. Also, we have served on compost and Sargassum panels with FDEP representatives at the Recycle Florida Today (RFT) Conference, twice, along with representatives from the U.S. Composting Council. As we update our research since the last RFT Conference, we have continued to meet with the representatives from the FDEP Division of Waste Management to inform them of our findings and to provide feedback on the wording of future FDEP regulations, specifically whether it should be specific to Sargassum or inclusive of seaweed as a whole. Identifying the background levels of arsenic at the beach and in Sargassum provides information useful for FDEP managers as they develop rules for the recycling of Sargassum. It is now known that Sargassum retains some of its arsenic (about 20%) upon decomposition and the levels after decomposition continue to exceed Florida SCTLs. It is also now known that, of the arsenic lost by the Sargassum, about half is volatilized while the other half is lost to leachate, with leachate showing high levels of arsenic ( $>300 \mu g/L$ ). This new knowledge will allow for more informed rule-making for the handling, management, recycling, and disposal of Sargassum.

From a more global perspective, this study, supported by the Hinkley Center, has continued to contribute towards the overall understanding of possible reuse options for Sargassum compost. In Caribbean countries which have been hit hard by Sargassum inundations, Sargassum compost may be the only resource available for growing food crops on remote Caribbean islands. The soil on many Caribbean islands is made of calcium carbonate minerals which are not rich in organics. Sargassum is a resource that is useful for adding nutrients needed for plants to grow. However, before we encourage the use of Sargassum compost for growing food crops, the impacts of the arsenic found in it needs to be better understood. This study addresses this need by emphasizing that the arsenic is released from the Sargassum. It is possible that the arsenic released can be sorbed by underlying soils, especially soils high in organic content. This sorbed arsenic can then be potentially transferred to food crops. More work is needed to confirm by how much the Sargassum compost increases the arsenic levels in food crops, especially in organic rich soils.

# REFERENCES AND PERTINENT LITERATURE

- Abdool-Ghany, A. A., Blare, T., Solo-Gabriele, H. M. (2023a). Assessment of *Sargassum* spp. management strategies in southeast Florida. *Resources, Conservation & Recycling Advances*, 19, 200175. <a href="https://doi.org/10.1016/j.reradv.2023.200175">https://doi.org/10.1016/j.reradv.2023.200175</a>
- Abdool-Ghany, A. A., Pollier, C. G., Oehlert, A. M., Swart, P. K., Blare, T., Moore, K., Solo-Gabriele, H. M. (2023b). Assessing quality and beneficial uses of *Sargassum* compost. *Waste Management*, 171, 545–556. <a href="https://doi.org/10.1016/j.wasman.2023.09.030">https://doi.org/10.1016/j.wasman.2023.09.030</a>
- Abdool-Ghany, A. A., Sahwell, P. J., Klaus, J., Gidley, M. L., Sinigalliano, C. D., Solo-Gabriele, H. M. (2022). Fecal indicator bacteria levels at a marine beach before, during, and after the COVID-19 shutdown period and associations with decomposing seaweed and human presence. *Science of the Total Environment*, 851, 158349. https://doi.org/10.1016/j.scitotenv.2022.158349
- Alleyne, K., Neat, F., & Oxenford, H. A. (2023). An analysis of arsenic concentrations associated with *Sargassum* influx events in Barbados. *Marine Pollution Bulletin*, 192, 115064. https://doi.org/10.1016/j.marpolbul.2023.115064
- Anderson, S. A., Turner, S. J., & Lewis, G. D. (1997). Enterococci in the New Zealand environment: implications for water quality monitoring. *Water Science & Technology*, 35(11–12), 325–331. https://doi.org/10.2166/wst.1997.0754
- Badgley, B. D., Ferguson, J., Vanden Heuvel, A., Kleinheinz, G. T., McDermott, C. M., Sandrin, T. R., Kinzelman, J., Junion, E. A., Byappanahalli, M. N., Whitman, R. L., & Sadowsky, M. J. (2011). Multi-scale temporal and spatial variation in genotypic composition of Cladophoraborne Escherichia coli populations in Lake Michigan. *Water Research*, 45(2), 721–731. <a href="https://doi.org/10.1016/j.watres.2010.08.041">https://doi.org/10.1016/j.watres.2010.08.041</a>
- Banks, K. W., Riegl, B. M., Richards, V. P., Walker, B. K., Helmle, K. P., Jordan, L. K. B., Phipps, J., Shivji, M. S., Spieler, R. E., & Dodge, R. E. (2008). The Reef Tract of Continental Southeast Florida (Miami-Dade, Broward and Palm Beach Counties, USA). *Coral Reefs of the USA*, 175–220. https://doi.org/10.1007/978-1-4020-6847-8 5
- Beckinghausen, A., Martinez, A., Blersch, D., & Haznedaroglu, B. Z. (2014). Association of nuisance filamentous algae Cladophora spp. with E. coli and Salmonella in public beach waters: impacts of UV protection on bacterial survival. *Environmental Science Processes & Impacts*, 16(6), 1267–1274. https://doi.org/10.1039/c3em00659j
- Block, C.N., Shibata, T., Solo-Gabriele, H.M., Townsend, T.G., (2007). Use of handheld X-ray fluorescence spectrometry units for identification of arsenic in treated wood. Environ Pollut 148(2), 627-633. <a href="https://doi.org/10.1016/j.envpol.2006.11.013">https://doi.org/10.1016/j.envpol.2006.11.013</a>
- Boehm, A., Griffith, J., McGee, C., Edge, T., Solo-Gabriele, H., Whitman, R., Cao, Y., Getrich, M., Jay, J., Ferguson, D., Goodwin, K., Lee, C., Madison, M., & Weisberg, S. (2009). Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *Journal of Applied Microbiology*, *107*(5), 1740–1750. <a href="https://doi.org/10.1111/j.1365-2672.2009.04440.x">https://doi.org/10.1111/j.1365-2672.2009.04440.x</a>
- Bousso, N.C., Brehmer, P., Ndiaye, W., Stiger-Pouvreau, V., Kane, C., Gautier, M., Faye, M., Fricke, A., Diadhiou, H.D., Aroui Boukbida, H., Weinberger, F., Ramasamy, B., Diedhiou, F., Diop, M.S., Balde, B.S., Simon, G., Quack, B., (2024). Unusual holopelagic Sargassum mass beaching in Northwest Africa: Morphotypes, chemical composition, and potential valorisation. Sci Total Environ 955, 177018. https://doi.org/10.1016/j.scitotenv.2024.177018
- Breuninger, E.S., Tolu, J., Aemisegger, F., Thurnherr, I., Bouchet, S., Mestrot, A., Ossola, R., McNeill, K., Tukhmetova, D., Vogl, J., Meermann, B., Sonke, J.E., Winkel, L.H.E., (2024).

- Marine and terrestrial contributions to atmospheric deposition fluxes of methylated arsenic species. Nat Commun 15(1), 9623. https://doi.org/10.1038/s41467-024-53974-z.
- Chávez, V., Uribe-Martínez, A., Cuevas, E., Rodríguez-Martínez, R. E., Van Tussenbroek, B. I., Francisco, V., Estévez, M., Celis, L. B., Monroy-Velázquez, L. V., Leal-Bautista, R., Álvarez-Filip, L., García-Sánchez, M., Masia, L., & Silva, R. (2020). Massive Influx of Pelagic *Sargassum* spp. on the Coasts of the Mexican Caribbean 2014–2020: Challenges and Opportunities. *Water*, *12*(10), 2908. <a href="https://doi.org/10.3390/w12102908">https://doi.org/10.3390/w12102908</a>
- Chow, D. (2023, March 11). A giant seaweed bloom that can be seen from space threatens beaches in Florida and Mexico. *NBC News*. Retrieved March 14, 2023, from <a href="https://www.nbcnews.com/science/environment/sargassum-seaweed-threatens-beaches-florida-mexico-rcna73862">https://www.nbcnews.com/science/environment/sargassum-seaweed-threatens-beaches-florida-mexico-rcna73862</a>
- Cipolloni, O. A., Gigault, J., Dassié, M. P., Baudrimont, M., Gourves, P. Y., Amaral-Zettler, L., & Pascal, P. Y. (2022). Metals and metalloids concentrations in three genotypes of pelagic *Sargassum* from the Atlantic Ocean Basin-scale. *Marine Pollution Bulletin*, *178*, 113564. <a href="https://doi.org/10.1016/j.marpolbul.2022.113564">https://doi.org/10.1016/j.marpolbul.2022.113564</a>
- Dassié, E. P., Gourves, P. Y., Cipolloni, O., Pascal, P. Y., & Baudrimont, M. (2021). First assessment of Atlantic open ocean Sargassum spp. metal and metalloid concentrations. *Environmental Science and Pollution Research*, *29*(12), 17606–17616. https://doi.org/10.1007/s11356-021-17047-8
- Datta, R.R., Papry, R.I., Asakura, Y., Kagaya, R., Wong, K.H., Mashio, A.S., Hasegawa, H., (2025). Arsenic biotransformation by macroalgae Srgassum thunbergii: Influence of growth stages and phosphate availability on uptake and reductive methylation. Chemosphere 377, 144333. https://doi.org/10.1016/j.chemosphere.2025.144333
- Datta, R.R., Papry, R.I., Asakura, Y., Kato, Y., Hong, W.K., Mashio, A.S., Hasegawa, H., (2024). Effect of salinity on arsenic uptake, biotransformation, and time-dependent speciation pattern by Sargassum species. Chemosphere 362, 142712. https://doi.org/10.1016/j.chemosphere.2024.142712
- Davis, D., Simister, R., Campbell, S., Marston, M., Bose, S., McQueen-Mason, S.J., Gomez, L.D., Gallimore, W.A., Tonon, T., (2021). Biomass composition of the golden tide pelagic seaweeds Sargassum fluitans and S. natans (morphotypes I and VIII) to inform valorisation pathways. Sci Total Environ 762, 143134. https://doi.org/10.1016/j.scitotenv.2020.143134.
- Davis, T. A., Ramirez, M., Mucci, A., & Larsen, B. (2004). Extraction, isolation and cadmium binding of alginate from *Sargassum* spp. *Journal of Applied Phycology*, *16*(4), 275–284. <a href="https://doi.org/10.1023/b:japh.0000047779.31105.ec">https://doi.org/10.1023/b:japh.0000047779.31105.ec</a>
- Devault, D. A., Massat, F., Baylet, A., Dolique, F., & Lopez, P. J. (2021). Arsenic and chlordecone contamination and decontamination toxicokinetics in Sargassum sp. *Environmental Science and Pollution Research*, 29(1), 6–16. <a href="https://doi.org/10.1007/s11356-020-12127-7">https://doi.org/10.1007/s11356-020-12127-7</a>
- Devault, D. A., Pierre, R., Marfaing, H., Dolique, F., & Lopez, P. J. (2020). Sargassum contamination and consequences for downstream uses: a review. *Journal of Applied Phycology*, *33*(1), 567–602. <a href="https://doi.org/10.1007/s10811-020-02250-w">https://doi.org/10.1007/s10811-020-02250-w</a>
- Devault, D.A., Massat, F., Baylet, A., Dolique, F., Lopez, P.J., (2022). Arsenic and chlordecone contamination and decontamination toxicokinetics in Sargassum sp. Environ Sci Pollut Res Int 29(1), 6-16. https://doi.org/10.1007/s11356-020-12127-7

- Di, X., Beesley, L., Zhang, Z., Zhi, S., Jia, Y., Ding, Y., (2019). Microbial Arsenic Methylation in Soil and Uptake and Metabolism of Methylated Arsenic in Plants: A Review. Int J Environ Res Public Health 16(24). https://doi.org/10.3390/ijerph16245012
- Dubey, B., Townsend, T.G., and Solo-Gabriele, H.M. (2007). Quantities of Arsenic-Treated Wood in Demolition Debris Generated by Hurricane Katrina. Environmental Science & Technology, 41(5),1533-1536. http://doi.org/10.1021/es0622812
- Fauser, P., Sanderson, H., Hedegaard, R. V., Sloth, J. J., Larsen, M. M., Krongaard, T., Bossi, R., & Larsen, J. B. (2012). Occurrence and sorption properties of arsenicals in marine sediments. *Environmental Monitoring and Assessment*, 185(6), 4679–4691. https://doi.org/10.1007/s10661-012-2896-2
- Florida Department of Environmental Protection (FDEP), (2005). Florida Administrative Code, Chapter 62-777, Referenced Guidelines. Groundwater and Surface Water Cleanup Target Levels. FDEP, Tallahassee, FL. <a href="https://floridadep.gov/sites/default/files/2-GroundwaterandSurfaceWaterCleanupTargetLevels-3.pdf">https://floridadep.gov/sites/default/files/2-GroundwaterandSurfaceWaterCleanupTargetLevels-3.pdf</a>
- Florida Department of Environmental Protection (FDEP) (2013). *Soil Cleanup Target Levels*. <a href="https://floridadep.gov/waste/district-business-support/documents/table-ii-soil-cleanup-target-levels">https://floridadep.gov/waste/district-business-support/documents/table-ii-soil-cleanup-target-levels</a>
- Fourest, E., & Volesky, B. (1997). Alginate properties and heavy metal biosorption by marine algae. *Applied Biochemistry and Biotechnology*, 67(3), 215–226. https://doi.org/10.1007/bf02788799
- Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. Á., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., McGlathery, K. J., & Serrano, Ó. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, *5*(7), 505–509. https://doi.org/10.1038/ngeo1477
- Garcia-Salgado, S., Quijano, M.A., Bonilla, M.M., (2012). Arsenic speciation in edible alga samples by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry. Anal Chim Acta 714, 38-46. https://doi.org/10.1016/j.aca.2011.12.001
- Gobert, T., Gautier, A., Connan, S., Rouget, M.L., Thibaut, T., Stiger-Pouvreau, V., Waeles, M., (2022). Trace metal content from holopelagic Sargassum spp. sampled in the tropical North Atlantic Ocean: Emphasis on spatial variation of arsenic and phosphorus. Chemosphere 308(Pt 1), 136186. https://doi.org/10.1016/j.chemosphere.2022.136186
- Graca, B., Jędruch, A., Bełdowska, M., Bełdowski, J., Kotwicki, L., Siedlewicz, G., Korejwo, E., Popińska, W., & Łukawska-Matuszewska, K. (2022). Effects of beach wrack on the fate of mercury at the land-sea interface A preliminary study. *Environmental Pollution*, *315*, 120394. https://doi.org/10.1016/j.envpol.2022.120394
- Hatt, D. C., Bally, N. K., Iporac, L. A. R., Olszak, S., Campbell, J. E., & Collado-Vides, L. (2024). Comprehensive Analysis of Biomass, Nutrient, and Heavy Metal Contributions of Pelagic *Sargassum* Species (Phaeophyceae) Inundations in South Florida. *Phycology*, 4(2), 235-255. <a href="https://doi.org/10.3390/phycology4020013">https://doi.org/10.3390/phycology4020013</a>
- Iporac, L. a. R., Hatt, D. C., Bally, N. K., Castro, A., Cardet, E., Mesidor, R., Olszak, S., Duran, A., Burkholder, D. A., & Collado-Vides, L. (2022). Community-based monitoring reveals spatiotemporal variation of sargasso inundation levels and morphotype dominance across the Caribbean and South Florida. *Aquatic Botany*, *182*, 103546. <a href="https://doi.org/10.1016/j.aquabot.2022.103546">https://doi.org/10.1016/j.aquabot.2022.103546</a>

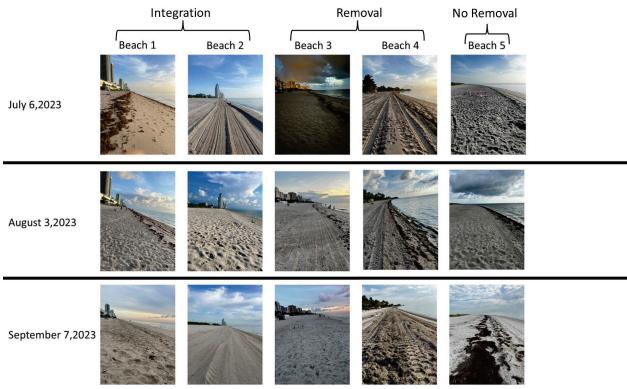
- Jones, A. S., Marini, J., Solo-Gabriele, H. M., Robey, N. M., Townsend, T. G., 2019. Arsenic, copper, and chromium from treated wood products in the U.S. disposal sector. *Waste Management*, 87, 731-740. <a href="https://doi.org/10.1016/j.wasman.2019.03.004">https://doi.org/10.1016/j.wasman.2019.03.004</a>
- Kelly, E. A., Feng, Z., Gidley, M. L., Sinigalliano, C. D., Kumar, N., Donahue, A. G., Reniers, A. J., & Solo-Gabriele, H. M. (2018). Effect of beach management policies on recreational water quality. *Journal of Environmental Management*, 212, 266–277. https://doi.org/10.1016/j.jenvman.2018.02.012
- Khan, N., Ryu, K.Y., Choi, J.Y., Nho, E.Y., Habte, G., Choi, H., Kim, M.H., Park, K.S., Kim, K.S., (2015). Determination of toxic heavy metals and speciation of arsenic in seaweeds from South Korea. Food Chem 169, 464-470. https://doi.org/10.1016/j.foodchem.2014.08.020
- Kim, S.T., Conklin, S.D., Redan, B.W., Ho, K., (2024). Determination of the Nutrient and Toxic Element Content of Wild-Collected and Cultivated Seaweeds from Hawai'i. ACS Food Sci Technol 4(3), 595-605. https://doi.org/10.1021/acsfoodscitech.3c00476
- Kinzelman, J. L., Pond, K. R., Longmaid, K. D., & Bagley, R. C. (2004). The effect of two mechanical beach grooming strategies on Escherichia coli density in beach sand at a southwestern Lake Michigan beach. Aquatic Ecosystem Health & Management, 7(3), 425–432. <a href="https://doi.org/10.1080/14634980490483953">https://doi.org/10.1080/14634980490483953</a>
- Liranzo-Gomez, R.E., Gomez, A.M., Gomez, B., Gonzalez-Hernandez, Y., Jauregui-Haza, U.J., (2023). Characterization of sargassum accumulated on Dominican beaches in 2021: Analysis of heavy, alkaline and alkaline-earth metals, proteins and fats. Mar Pollut Bull 193, 115120. <a href="https://doi.org/10.1016/j.marpolbul.2023.115120">https://doi.org/10.1016/j.marpolbul.2023.115120</a>
- Maurer, A.S., Gross, K., Stapleton, S.P., (2022). Beached Sargassum alters sand thermal environments: Implications for incubating sea turtle eggs. Journal of Experimental Marine Biology and Ecology,546, 151650. <a href="https://doi.org/10.1016/j.jembe.2021.151650">https://doi.org/10.1016/j.jembe.2021.151650</a>. McGillicuddy, D. J., Morton, P. L., Brewton, R. A., Hu, C., Kelly, T. B., Solow, A. R., & Lapointe, B. E. (2023). Nutrient and arsenic biogeochemistry of *Sargassum* in the western Atlantic. *Nature Communications*, 14(1). <a href="https://doi.org/10.1038/s41467-023-41904-4">https://doi.org/10.1038/s41467-023-41904-4</a>
- Mohan, P., Strobl, E., (2024). Tourism and marine crises: The impact of Sargassum invasion on Caribbean small island developing states. Ocean & Coastal Management, 251, 107091. https://doi.org/10.1016/j.ocecoaman.2024.107091
- Neff, J. M. (1997). Ecotoxicology of arsenic in the marine environment. *Environmental Toxicology and Chemistry*, 16(5), 917–927. <a href="https://doi.org/10.1002/etc.5620160511">https://doi.org/10.1002/etc.5620160511</a>
- Nordlund, L. M., Jackson, E. L., Nakaoka, M., Samper-Villarreal, J., Beca-Carretero, P., & Creed, J. C. (2018). Seagrass ecosystem services What's next? *Marine Pollution Bulletin*, 134, 145–151. <a href="https://doi.org/10.1016/j.marpolbul.2017.09.014">https://doi.org/10.1016/j.marpolbul.2017.09.014</a>
- Ofori, R. O., & Rouleau, M. D. (2021). Modeling the impacts of floating seaweeds on fisheries sustainability in Ghana. *Marine Policy*, *127*, 104427. https://doi.org/10.1016/j.marpol.2021.104427
- Olguin-Maciel, E., Leal-Bautista, R.M., Alzate-Gaviria, L., Dominguez-Maldonado, J., Tapia-Tussell, R., (2022). Environmental impact of Sargassum spp. landings: an evaluation of leachate released from natural decomposition at Mexican Caribbean coast. Environ Sci Pollut Res Int 29(60), 91071-91080. <a href="https://doi.org/10.1007/s11356-022-22123-8">https://doi.org/10.1007/s11356-022-22123-8</a>
- Ortega-Flores, P. A., Servière-Zaragoza, E., De Anda-Montañez, J. A., Freile-Pelegrín, Y., Robledo, D., & Méndez-Rodríguez, L. C. (2022). Trace elements in pelagic *Sargassum* species in the Mexican Caribbean: Identification of key variables affecting arsenic

- accumulation in S. fluitans. *Science of the Total Environment*, 806, 150657. https://doi.org/10.1016/j.scitotenv.2021.150657
- Oxenford, H. A., Cox, S.-A., van Tussenbroek, B. I., Desrochers, A. (2021). Challenges of Turning the Sargassum Crisis into Gold: Current Constraints and Implications for the Caribbean. Phycology, 1(1), 27-48. <a href="https://doi.org/10.3390/phycology1010003">https://doi.org/10.3390/phycology1010003</a>
- Percival, E., & McDOWELL, R. H. (1990). Algal polysaccharides. In *Methods in plant biochemistry* (pp. 523–547). <a href="https://doi.org/10.1016/b978-0-12-461012-5.50021-5">https://doi.org/10.1016/b978-0-12-461012-5.50021-5</a>
- Pfeifer, L., & Classen, B. (2020). The Cell Wall of Seagrasses: Fascinating, Peculiar and a Blank Canvas for Future Research. Frontiers in Plant Science, 11:588754. <a href="https://doi.org/10.3389/fpls.2020.588754">https://doi.org/10.3389/fpls.2020.588754</a>
- Planer-Friedrich, B., Lehr, C., Matschullat, J., Merkel, B.J., Nordstrom, D.K., Sandstrom, M.W., (2006). Speciation of volatile arsenic at geothermal features in Yellowstone National Park, Geochimica et Cosmochimica Acta, 70(10), 2480-2491. https://doi.org/10.1016/j.gca.2006.02.019
- Raize, O., Argaman, Y., & Yannai, S. (2004). Mechanisms of biosorption of different heavy metals by brown marine macroalgae. *Biotechnology and Bioengineering*, 87(4), 451–458. <a href="https://doi.org/10.1002/bit.20136">https://doi.org/10.1002/bit.20136</a>
- Resiere, D., Mehdaoui, H., Florentin, J., Gueye, P., Lebrun, T., Blateau, A., Viguier, J., Valentino, R., Brouste, Y., Kallel, H., Megarbane, B., Cabie, A., Banydeen, R., Neviere, R., (2021). Sargassum seaweed health menace in the Caribbean: clinical characteristics of a population exposed to hydrogen sulfide during the 2018 massive stranding. Clin Toxicol (Phila) 59(3), 215-223. <a href="https://doi.org/10.1080/15563650.2020.1789162">https://doi.org/10.1080/15563650.2020.1789162</a>
- Rivero, N. (2023, March 14). Coming soon to Florida beaches: Massive, messy and maybe record mounds of seaweed. *AOL*. Retrieved March 14, 2023, from <a href="https://www.aol.com/news/coming-soon-florida-beaches-massive-093000127.html">https://www.aol.com/news/coming-soon-florida-beaches-massive-093000127.html</a>
- Robledo, D., Vázquez-Delfín, E., Freile-Pelegrín, Y., Vásquez-Elizondo, R. M., Qui-Minet, Z. N., & Salazar-Garibay, A. (2021). Challenges and opportunities in relation to *Sargassum* events along the Caribbean Sea. *Frontiers in Marine Science*, 8. <a href="https://doi.org/10.3389/fmars.2021.699664">https://doi.org/10.3389/fmars.2021.699664</a>
- Rodriguez-Martinez, R.E., Gomez Reali, M.A., Torres-Conde, E.G., Bates, M.N., (2024). Temporal and spatial variation in hydrogen sulfide (H(2)S) emissions during holopelagic Sargassum spp. decomposition on beaches. Environ Res 247, 118235. <a href="https://doi.org/10.1016/j.envres.2024.118235">https://doi.org/10.1016/j.envres.2024.118235</a>
- Rodriguez-Martinez, R.E., Roy, P.D., Torrescano-Valle, N., Cabanillas-Teran, N., Carrillo-Dominguez, S., Collado-Vides, L., Garcia-Sanchez, M., van Tussenbroek, B.I., (2020). Element concentrations in pelagic Sargassum along the Mexican Caribbean coast in 2018-2019. PeerJ 8, e8667. <a href="https://doi.org/10.7717/peerj.8667">https://doi.org/10.7717/peerj.8667</a>
- Rodríguez-Martínez, R.e., Torres-Conde, E.G., Jordán-Dahlgren, E., (2023). Pelagic Sargassum cleanup cost in Mexico, Ocean & Coastal Management, 237, 106542. https://doi.org/10.1016/j.ocecoaman.2023.106542
- Savage, L., Carey, M., Hossain, M., Islam, M.R., de Silva, P., Williams, P.N., Meharg, A.A., (2017). Elevated Trimethylarsine Oxide and Inorganic Arsenic in Northern Hemisphere Summer Monsoonal Wet Deposition. Environ Sci Technol 51(21), 12210-12218. <a href="https://doi.org/10.1021/acs.est.7b04356">https://doi.org/10.1021/acs.est.7b04356</a>
- Shibata, T., Solo-Gabriele, H.M., Fleming, L., Cai, Y., and Townsend, T.G., 2007. A Mass Balance Approach for Evaluating Leachable Arsenic and Chromium from an In-Service CCA-

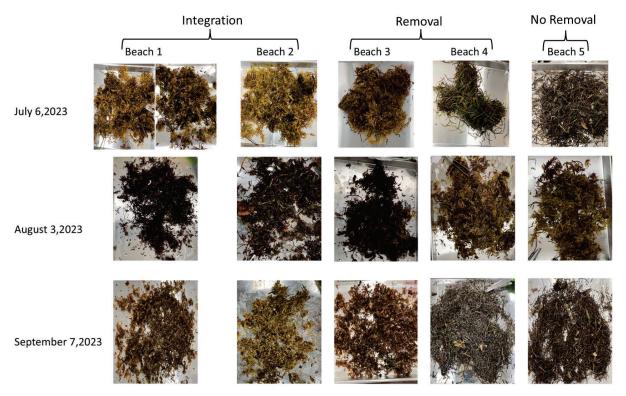
- Treated Wood Structure. *The Science of the Total Environment*, *372*, 624-635. <a href="http://doi.org/10.1016/j.scitotenv.2006.10.037">http://doi.org/10.1016/j.scitotenv.2006.10.037</a>.
- Theirlynck, T., Mendonça, I. R. W., Engelen, A. H., Bolhuis, H., Collado-Vides, L., Van Tussenbroek, B. I., García-Sánchez, M., Zettler, E., Muyzer, G., & Amaral-Zettler, L. (2023). Diversity of the holopelagic *Sargassum* microbiome from the Great Atlantic *Sargassum* Belt to coastal stranding locations. *Harmful Algae*, *122*, 102369. https://doi.org/10.1016/j.hal.2022.102369
- Tonon, T., Machado, C. B., Webber, M., Webber, D., Smith, J., Pilsbury, A., Cicéron, F., Herrera-Rodriguez, L., Jimenez, E. M., Suarez, J. V., Ahearn, M., Gonzalez, F., Allen, M. J. (2022). Biochemical and Elemental Composition of Pelagic Sargassum Biomass Harvested across the Caribbean. Phycology, 2(1), 204-215. https://doi.org/10.3390/phycology2010011
- Tucker, R. (2023, March 13). Giant seaweed blob twice the width of the US takes aim at Florida. *The Hill*. Retrieved March 14, 2023, from <a href="https://thehill.com/homenews/3896298-giant-seaweed-blob-twice-the-width-of-the-us-takes-aim-at-florida/">https://thehill.com/homenews/3896298-giant-seaweed-blob-twice-the-width-of-the-us-takes-aim-at-florida/</a>
- US Environmental Protection Agency (USEPA), (1980). Resource Conservation and Recovery Act (RCRA), specifically Chapter 42 of the United States Code Parts 260-279.
- US Environmental Protection Agency (US EPA). (1992). *Method 3010A Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by FLAA or ICP Spectroscopy*. https://www.epa.gov/sites/default/files/2015-12/documents/3010a.pdf
- US Environmental Protection Agency (US EPA). (1994). *Method 1312: Synthetic Precipitation Leaching Procedure* (SW-846). <a href="https://www.epa.gov/sites/default/files/2015-12/documents/1312.pdf">https://www.epa.gov/sites/default/files/2015-12/documents/1312.pdf</a>
- US Environmental Protection Agency (US EPA), (1994). SW-846 Test Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry. <a href="https://www.epa.gov/sites/default/files/2015-06/documents/epa-200.7.pdf">https://www.epa.gov/sites/default/files/2015-06/documents/epa-200.7.pdf</a>
- US Environmental Protection Agency (US EPA). (1996). *Method 3050B Acid Digestion of Sediments, Sludges, and Soils*. <a href="https://www.epa.gov/sites/default/files/2015-06/documents/epa-3050b.pdf">https://www.epa.gov/sites/default/files/2015-06/documents/epa-3050b.pdf</a>
- US Environmental Protection Agency (US EPA). (2014). Enterococci in water by membrane filtration using membrane-Enterococcus indoxyl-β-D-glucoside Agar (mEI). EPA-821-R-14-011, United States Environmental Protection Agency, Washington, DC, USA.
- US Environmental Protection Agency (US EPA). (2018). *Method 6010D Inductively Coupled Plasma—Optical Emission Spectroscopy*. <a href="https://www.epa.gov/sites/default/files/2015-12/documents/6010d.pdf">https://www.epa.gov/sites/default/files/2015-12/documents/6010d.pdf</a>
- Vazquez-Delfin, E., Freile-Pelegrin, Y., Salazar-Garibay, A., Serviere-Zaragoza, E., Mendez-Rodriguez, L.C., Robledo, D., (2021). Species composition and chemical characterization of Sargassum influx at six different locations along the Mexican Caribbean coast. Sci Total Environ 795, 148852. <a href="https://doi.org/10.1016/j.scitotenv.2021.148852">https://doi.org/10.1016/j.scitotenv.2021.148852</a>
- Verhougstraete, M. P., Byappanahalli, M. N., Rose, J. B., & Whitman, R. L. (2010). Cladophora in the Great Lakes: impacts on beach water quality and human health. *Water Science & Technology*, 62(1), 68–76. <a href="https://doi.org/10.2166/wst.2010.230">https://doi.org/10.2166/wst.2010.230</a>
- Volesky, B., & Holan, Z. R. (1995). Biosorption of heavy metals. *Biotechnology Progress*, 11(3), 235–250. <a href="https://doi.org/10.1021/bp00033a001">https://doi.org/10.1021/bp00033a001</a>
- Wang, M., Hu, C., Barnes, B. B., Mitchum, G., Lapointe, B., & Montoya, J. P. (2019). The great Atlantic *Sargassum* belt. *Science*, 365(6448), 83–87. https://doi.org/10.1126/science.aaw7912

# Appendix A

Field Photos and Data Tables for Environmental Study



**Figure A.1**. Photos of five beach sites taken during sampling, depicting the environmental conditions at the time of sample collection. The images illustrate the physical state of the beaches under different management practices—Integration of beach wrack, Removal of beach wrack, and No Removal—highlighting the variability in wrack presence, sand conditions, and beach management approaches. Two sampling teams collected samples starting at their designated beaches at sunrise. Sampling commenced at Beach 1 at sunrise and then continued to Beach 2. Similarly sampling commenced at Beach 3 at sunrise and commenced to 4 and 5. Beach grooming usually started at beaches 1 through 4 shortly after sunrise and for this reason, beach grooming activities are more obvious for the second beaches (Beaches 2 and 4) visited by each team.



**Figure A.2**. Photos of wrack collected from three sampling periods at beaches practicing Integration, Removal, and No Removal management styles.

**Table A.1**. Raw data from the study, presenting the concentrations of bacteria and arsenic across various management styles—Integration, Removal, and No Removal. All water samples were collected in ankle deep water and are identified as "Ankle Water". Sand identified as "Sand Under", "Supratidal" and "Bladed". Wrack identified as "Sargassum" and "Seagrass".

Officer, Su	prandar ar	ia biauca . Wi	ack identified as S	argassum and	Scagrass.	
Beach	Sample		Bacteria Concentration	Arsenic	Management	Moisture
Identifier	Date Date	Sample Type	(CFU/100 mL or	Concentration		
Identifier	Date		`	(mg/kg)	style	content
1	7/6/2023	Ankle Water	dry g) <sup>a</sup> 80.0	ND <sup>b</sup>	Intoquation	NA <sup>c</sup>
1				3.60	Integration	
	7/6/2023	Sand Under	36.1		Integration	0.0923
1	7/6/2023	Sargassum	875	33.25	Integration	0.7153
1	7/6/2023	Supratidal	344	4.13	Integration	0.0031
1	8/3/2023	Ankle Water	91.0	ND	Integration	NA
1	8/3/2023	Sand Under	353	4.12	Integration	0.0392
1	8/3/2023	Sargassum	5,292	9.99	Integration	0.5269
1	8/3/2023	Supratidal	72.0	4.41	Integration	0.0164
1	9/7/2023	Ankle Water	74.0	ND	Integration	NA
1	9/7/2023	Sand Under	27.0	4.92	Integration	0.0208
1	9/7/2023	Sargassum	57.0	61.70	Integration	0.2292
1	9/7/2023	Supratidal	248	4.68	Integration	0.0067
2	7/6/2023	Ankle Water	16.0	ND	Integration	NA
2	7/6/2023	Bladed Sand	196	2.02	Integration	0.0264
2	7/6/2023	Sand Under	2.1	1.65	Integration	0.0436
2	7/6/2023	Sargassum	51.6	23.00	Integration	0.6998
2	7/6/2023	Supratidal	69.0	1.30	Integration	0.0010
2	8/3/2023	Ankle Water	244	ND	Integration	NA
2	8/3/2023	Bladed Sand	625	1.73	Integration	0.0337
2	8/3/2023	Sand Under	230	1.89	Integration	0.0311
2	8/3/2023	Sargassum	9,642	10.70	Integration	0.6667
2	8/3/2023	Supratidal	304	1.65	Integration	0.0023
2	9/7/2023	Ankle Water	118	ND	Integration	NA
2	9/7/2023	Bladed Sand	8.8	1.54	Integration	0.0349
2	9/7/2023	Sand Under	0.6	1.77	Integration	0.0203
2	9/7/2023	Sargassum	13.9	64.30	Integration	0.4485
2	9/7/2023	Supratidal	100	1.85	Integration	0.0012
3	7/6/2023	Ankle Water	50.0	ND	Removed	NA
3	7/6/2023	Sand Under	16.6	1.70	Removed	0.0337
3	7/6/2023	Sargassum	15.9	43.70	Removed	0.6845
3	7/6/2023	Supratidal	38.0	1.55	Removed	0.0019
3	8/3/2023	Ankle Water	29.0	ND	Removed	NA
3	8/3/2023	Sand Under	648	1.08	Removed	0.0341
3	8/3/2023	Sargassum	8,594	10.90	Removed	0.6891
3	8/3/2023	Supratidal	267	1.18	Removed	0.0025
	01514045	Duprandar	201	1.10	1Como v Cu	0.0023

Table A.1. continued.

Beach Identifier	Sample Date	Sample Type	Bacteria Concentration (CFU/100 mL or dry g)	Arsenic Concentration (mg/kg)	Management style	Moisture content
3	9/7/2023	Ankle Water	15.0	ND	Removed	NA
3	9/7/2023	Sand Under	32.5	0.58	Removed	0.0068
3	9/7/2023	Sargassum	1662	35.10	Removed	0.2859
3	9/7/2023	Supratidal	121	2.26	Removed	0.0030
4	7/6/2023	Ankle Water	42.0	ND	Removed	NA
4	7/6/2023	Sand Under	259	2.03	Removed	0.0597
4	7/6/2023	Seagrass	2,435	1.55	Removed	0.8112
4	7/6/2023	Supratidal	94.0	2.19	Removed	0.0038
4	8/3/2023	Ankle Water	145	ND	Removed	NA
4	8/3/2023	Sand Under	476	2.45	Removed	0.0670
4	8/3/2023	Sargassum	2,742	62.50	Removed	0.5816
4	8/3/2023	Supratidal	38.8	1.94	Removed	0.0088
4	9/7/2023	Ankle Water	28.0	ND	Removed	NA
4	9/7/2023	Sand Under	1,158	1.92	Removed	0.0445
4	9/7/2023	Seagrass	2966	2.03	Removed	0.5083
4	9/7/2023	Supratidal	586	2.33	Removed	0.002
5	7/6/2023	Ankle Water	7.0	ND	No Removal	NA
5	7/6/2023	Sand Under	54.2	1.29	No Removal	0.0428
5	7/6/2023	Seagrass	2,973	1.77	No Removal	0.5878
5	7/6/2023	Supratidal	429	1.42	No Removal	0.0053
5	8/3/2023	Ankle Water	21.0	ND	No Removal	NA
5	8/3/2023	Sand Under	10.2	1.35	No Removal	0.1313
5	8/3/2023	Sargassum	68.7	48.20	No Removal	0.7613
5	8/3/2023	Supratidal	96.0	1.61	No Removal	0.0046
5	9/7/2023	Ankle Water	79.0	ND	No Removal	NA
5	9/7/2023	Sand Under	18.3	1.06	No Removal	0.0452
5	9/7/2023	Seagrass	3,554	2.18	No Removal	0.5399
5	9/7/2023	Supratidal	168	1.62	No Removal	0.0019

a"Ankle water" has units of CFU/100 mL. All other sample types have units of CFU/dry\_gram, bND=Not Detected. Detection limit of 30 μg/L cNA=Not applicable

# Appendix B Data Tables for Mesocosm Study

**Table B.1**. Arsenic concentrations measured in Sargassum samples collected between March 13, 2024, and May 22, 2024. The table includes the measured arsenic result (mg/kg dry), the dilution factor (Dil), and the Method Detection Limit (MDL) for each sampling date. Dilutions were adjusted as needed to ensure values fell within the quantifiable range.

Date	Sargassum Concentration	Dil	MDL
Date	(mg/kg dry)	DII	WIDL
3/13/2024	31.5	1	0.18
3/16/2024	53.8	1	0.10
3/17/2024	56.9	2	0.17
3/19/2024	66.7	2	0.18
3/23/2024	13.3	1	0.21
3/24/2024	15.3	1	0.13
3/26/2024	17.9	1	0.09
3/30/2024	17.8	1	0.09
4/2/2024	20.8	1	0.09
4/4/2024	16.1	1	0.14
4/6/2024	19.8	1	0.09
4/9/2024	16.7	1	0.08
4/13/2024	16.6	1	0.09
4/20/2024	16.3	1	0.09
4/23/2024	9.74	1	0.12
4/27/2024	12.2	1	0.08
4/30/2024	13.7	0.91	0.12
5/2/2024	13.2	1	0.09
5/4/2024	11.9	0.94	0.09
5/22/2024	7.21	1	0.11

**Table B.2**. Arsenic concentrations in sand samples (mg/kg dry weight) from control and experimental conditions collected between March 13, 2024, and May 22, 2024. Control samples contained sand without Sargassum, while experimental samples included sand beneath decomposing Sargassum. The data reflect changes in arsenic levels over time potentially due to leaching from Sargassum into underlying sand.

	Sand Control	Sand Experimental
Date	Concentrations	Concentrations
	(mg/kg dry)	(mg/kg dry)
3/13/2024	4.79	4.55
3/16/2024	4.83	5.19
3/17/2024	4.28	5.00
3/19/2024	4.59	5.08
3/23/2024	4.75	4.68
3/24/2024	4.50	4.84
3/26/2024	4.90	4.70
3/30/2024	4.53	4.36
4/2/2024	4.95	4.64
4/4/2024	4.30	4.41
4/6/2024	4.67	5.04
4/9/2024	4.53	5.10
4/13/2024	4.57	5.24
4/20/2024	4.77	5.12
4/23/2024	4.48	4.77
4/27/2024	4.24	4.96
4/30/2024	4.52	4.80
5/2/2024	4.89	4.50
5/4/2024	4.53	5.05
5/22/2024	4.46	4.82

**Table B.3**..Elemental concentrations (ppm) in sand and Sargassum samples measured via X-ray fluorescence (XRF). Notably, arsenic (As) was detected in Sargassum (22.33 ppm) but not in sand (ND). Additionally, titanium (Ti), antimony (Sb), and lead (Pb) were only detected in Sargassum, highlighting potential accumulation of certain elements in the biomass.

	Sand	Sargassum
	Concentration	Concentration
	(ppm)	(ppm)
Fe	625	202
Sr	956	469
Zr	53.0	47.1
Mo	39.8	57.4
Ag	248	ND
Zn	148	28.0
Rb	8.00	20.6
As	ND	22.3
Ti	ND	9805
Sb	ND	239
Pb	ND	16.0

# Appendix C Project Administration

**PROJECT TITLE**: Does Sargassum spp. Compost Impact the Arsenic and Bacteria Levels within the Beach Environment

PRINCIPAL INVESTIGATOR: Dr. Helena Solo-Gabriele

AFFILIATION: University of Miami, Dept. of Chemical, Environmental, and Materials

Engineering

CONTACT INFORMATION: hmsolo@miami.edu, 305-284-3467

**LEAD POST-DOCTORAL ASSOCIATE:** Afeefa Abdool-Ghany

AFFILIATION: University of Miami, Dept. of Chemical, Environmental, and Materials

Engineering and Brizaga Inc.

CONTACT INFORMATION: aaa625@miami.edu, 954-298-4073

PROJECT WEB-SITE: <a href="https://eel.miami.edu/projects/sargassum-research/sargassum-re

recycling/index.html

**PROJECT DURATION:** August 1, 2021 to July 31, 2025

ABSTRACT: Sargassum, floating macroalgae species known by scientific names of Sargassum natans and Sargassum fluitans, has been inundating the beaches across Florida in recent years during spring and summer months. These inundations are considered the "new normal" and their volumes are expected to increase in the future due to global climatologic factors which has been contributing to Sargassum blooms in the Atlantic Ocean. Typically, during large strandings events, municipalities hire third party contractors to haul away the Sargassum to a landfill, which is very costly. Once in the landfill, the Sargassum begins to rot and can release hydrogen sulfide. There is a need for municipalities to address these inundations in a sustainable way. Prior research has shown that limitations to the reuse of Sargassum include arsenic concentrations above some regulatory guidelines and bacteria which also occasionally fail standards depending upon how the Sargassum is processed. For example, recent studies have documented that when Sargassum is composted the arsenic levels (6.64 to 26.5 mg/kg), exceed Florida Soil Cleanup Target Level guidelines, which limits the end use of the Sargassum compost. Similarly, Sargassum compost made using tumbler systems exceeded regulatory levels for fecal indicator bacteria, enterococci, and fecal coliform.

One potentially viable option for the reuse of Sargassum is composting locally by setting up staging areas within the beach property, without the need to haul the Sargassum large distances. This compost can then be given away or used locally for dune or mangrove restoration. But excessive levels of arsenic and bacteria continue to be a concern for this potential reuse option. Regulators now raise questions about the background levels of arsenic at the beach and if Sargassum contributes towards excessive arsenic levels at beaches. Another question that arises is, "Will the Sargassum compost increase the arsenic burden at the beach if composted near or on the beach?" To answer these questions (which impact the reuse options for Sargassum) we aim to evaluate the background levels of arsenic and bacteria at beaches with various levels of Sargassum impacts coupled with laboratory experiments to simulate the fate of the arsenic and bacteria from Sargassum compost at the beach. This study is split into two phases. The first phase (Environmental Study with results in Chapter II) focuses on evaluating natural beach environments that are known for little to no accumulation of Sargassum, moderate accumulations, and hotspots for massive Sargassum inundations. Samples from the beach sites

(Sargassum, sand below Sargassum, and beach water) were be analyzed for arsenic and the fecal indicator bacteria (FIB), enterococci. The second phase of the study (Mesocosm Study with results in Chapter III) focuses on controlled laboratory experiments that examined the arsenic levels in sand and Sargassum as it decomposed and was exposed to simulated rain. Assessing the background levels of arsenic in the beach environment allows for a better assessment of its reuse potential. The results of this study will contribute towards sustainable solutions that avoid the need for landfilling the potentially valuable Sargassum resource.

Key Words: Sargassum, seaweed, compost, beach, arsenic

#### **METRICS REPORTING**

This page will be omitted from the report when it is published.

Post-Doctoral Researcher (previously supported by Hinkley funding as a PhD student):

Full Name: Afeefa A. Abdool-Ghany

Email: aaa625@miami.edu

Anticipated Degree: Ph.D. in Chemical, Environmental, and Materials Engineering

(Environmental Emphasis)

Department: Department of Chemical, Environmental, and Materials Engineering, University of

Miami, Coral Gables, FL

#### **Metrics:**

1. Research publications from THIS Hinkley Center Project. JOURNAL ARTICLES

- McIntyre, B., Cerna, M., Ferguson, A., Li, J., & Solo-Gabriele, H. M. (2024). Does Sargassum on Beaches Pose Health Risks to Children Through Arsenic Exposure During Recreational Play? (In Review).
- Abdool-Ghany, A.A., Amirali, A., Reiner, R., Hoffman, S., Tavarez, I., Roca, M., Li, J., Solo-Gabriele, H., 202X. Impacts of Stranded Wrack on Enterococci and Arsenic Levels in the Beach Environment. Environmental Science and Pollution Research (In Review).
- Abdool-Ghany, A.A., Babler, K.M., Bogumil, D., Pollock, S., Li, J., Manning, S.R., Solo-Gabriele, H.M., 202X. Deep Sequencing Results for Samples of Stranded Sargassum. International Journal of Microbiology (In Review).
- Abdool-Ghany, A. A., Blare, T., & Solo-Gabriele, H. M. (2023). Assessment of Sargassum spp. management strategies in southeast Florida. *Resources, Conservation & Recycling Advances*, 19, 200175. <a href="https://doi.org/10.1016/j.rcradv.2023.200175">https://doi.org/10.1016/j.rcradv.2023.200175</a>
- Abdool-Ghany, A. A., Pollier, C., Oehlert, A. M., Swart, P. K., Blare, T., Moore, K. K., & Solo-Gabriele, H. M. (2023). Assessing Quality and Beneficial Uses of Sargassum Compost. *Waste Management*, 171, 545-556. https://doi.org/10.1016/j.wasman.2023.09.030

#### PUBLISHED OUTREACH DOCUMENTS

• Blare, T., Abdool-Ghany, A. A., Solo-Gabriele, H. M. 2022. Cost Estimates for Producing *Sargassum* spp. Compost- English. *University of Florida Institute of Food and Agricultural Sciences EDIS*. (https://hmsolo.miami.edu/wp-content/uploads/2023/03/Blare et al 2023.pdf)

Blare, T., Abdool-Ghany, A. A., Solo-Gabriele, H. M., Gonzalez, E. 2022. Costos
 Estimados de la Producción de Sargazo Compostaje. *University of Florida Institute of* Food and Agricultural Sciences EDIS. (Spanish version) (<a href="https://hmsolo.miami.edu/wp-content/uploads/2023/03/Blare\_et\_al\_2023\_Spanish.pdf">https://hmsolo.miami.edu/wp-content/uploads/2023/03/Blare\_et\_al\_2023\_Spanish.pdf</a>)

#### **ABSTRACTS**

- 2025 Association for the Science of Limnology and Oceanography (ASLO) Conference
  - o Title: Health Risks from Stranded Sargassum
  - o Abstract: Potential health risks from Sargassum strandings include exposures to arsenic, infectious microbes, and hydrogen sulfide. This study explores environmental levels of arsenic, pathogenic microbes, and sulfur releases from stranded Sargassum. The elevated levels of arsenic are due to the bioaccumulation, pathogenic microbes especially Vibrio species vary depending upon stranding duration, and sulfur species are volatilized from Sargassum when waterlogged. This poster will introduce the health risks and focus on assessing arsenic. Arsenic levels were documented in Sargassum, sand, and water at five beaches. Mesocosm studies were conducted to evaluate the transport of arsenic from Sargassum, to sand, to water, and to the atmosphere. Results from mesocosm studies indicate that the majority of the arsenic volatilizes. The arsenic that remains in the stranded Sargassum can be a source of exposure. Using levels of arsenic found in Sargassum, sand, and water, along with human exposure factors indicates that cancer risks are on the order of 10<sup>-4</sup>, which is considered low increased risk. This risk to beach goers is driven by the dermal absorption factor.
- 2024 Phycological Society of America Conference
  - o **Title**: Assessing The Agricultural Viability of *Sargassum* Compost: Quality Analysis and Pathogen Investigation
  - o **Abstract**: With fertilizer costs on the rise, Sargassum compost could offer a costeffective alternative for farmers and gardeners. Prior work that included interviews with stakeholders and a comprehensive cost analysis emphasized the potential economic viability and benefits of composting Sargassum from inundations in southeastern Florida, suggesting that beach managers can potentially recoup costs through the sale of compost. The focus of the current study was to evaluate the quality of Sargassum compost against 11 guidelines (nutrients, bacteria) and to evaluate the potential for Sargassum to harbor pathogens. Despite nutrient ratios occasionally falling short of standards, the compost sustained radish growth, indicating its potential agricultural value. Most trace metal levels aligned with regulatory guidelines, although arsenic levels exceeded residential use standards, limiting the use of the compost. Bacteria levels met regulatory standards in large-scale experiments, though not consistently in small-scale trials. Further, preliminary metagenomic sequencing data on short term (STS) and long term (LTS) stranded Sargassum indicated the biomass can be a host to diverse and potentially, pathogenic bacteria (Vibrio and Staphylococcus). Overall, these findings support that stranded Sargassum can be composted for beneficial applications, including fill and farming of non-edible plants. However, quality concerns should be addressed to assess the presence of

microbial contaminants and the concentration of heavy metals to ensure its safe utilization.

- 2022 Goldschmidt Conference Abstract, International Conference on Geochemistry and Related Subjects, organized by the Geochemical Society and the European Association of Geochemistry
  - Title: Is composting a feasible disposal option for beach-stranded Sargassum in South Florida?
  - o **Abstract:** Over the last decade there has been increased proliferation of Sargassum in the north Atlantic Ocean, with massive strandings occurring on near annual frequency in the Caribbean, western Africa, and United States since 2011. Such events have environmental, health, and economic impacts, because Sargassum is known to have a high capacity to absorb metals from the environment [1]. A common disposal method is mechanical collection of the stranded Sargassum and subsequent landfill disposal. Thus, leachates of degrading Sargassum can contribute to contamination in soils and groundwater near landfills. Compost can be a potential solution and can present a sustainable management method if concentrations of potentially toxic metals are below EPA guidelines. The objective of this project is to determine whether composting is a feasible management solution for Sargassum strandings. We assessed compositional characteristics of the compost [nutrient ratios (C:N, P), elemental concentrations, abundance of indicator bacteria] in both small-scale and largescale settings. The first phase (small-scale) of study involved experiments using tumbler composters, which independently evaluated the impacts of washing the Sargassum prior to composting, as well as the impact of mixing with other vegetative wastes (grass, mulch, etc). The second phase (large-scale) involved two 4 yd<sup>3</sup> compost piles with different additives (a control pile and vegetative waste) in a municipal setting. In the first phase, the mixture of Sargassum and grass clippings produced compost with the best C:N ratios and lowest concentrations of toxic metals. Bacteria levels did exceed EPA regulatory limits in this treatment. Preliminary radish bioassay experiments also suggested best growth in the compost treatment mixed with grass clippings. Unwashed Sargassum produced compost with moderate C:N but the highest concentrations of toxic metals. Within the larger scale experiments conducted in the second phase, the Sargassum treatment produced the best C:N ratios and lowest bacteria levels compared to the *Sargassum* and vegetive waste treatment.
    - [1] Rodríguez-Martínez, R. E., et al., (2020). *PeerJ*, 8, e8667.
- 2021 Florida Shore and Beach Preservation Association (FSBPA) Annual Conference
  - o **Title:** Sargassum Invasion: Composting as a Solution
  - o **Abstract:** Sargassum spp. is one of the dominant forms of marine macroalgae (seaweed) found on beaches throughout Florida. Excess Sargassum is washing up on the shores of Florida beaches and originates from the Sargasso Sea in the Northern Atlantic Ocean near Bermuda. Recently there have been large quantities of Sargassum reported in the central Atlantic Ocean and the Caribbean Sea. During the summer of 2018 and 2019, record amounts of Sargassum spp. were documented along beach coastlines resulting in local authorities hauling this

seaweed to the nearest landfill. Hauling and landfill disposal of seaweed can cost the cities and municipalities hundreds of thousands of dollars per year.

The influx of Sargassum onto the shores is important to maintain the ecological balance. The difficulty has been when the amounts of seaweed stranding onshore are excessive. When excessive, the local ecology suffers and the aesthetics of the beach decline. In extreme conditions, the seaweed is so thick on the water surface that turtles are unable to surface for air, thus drowning in embayments where the Sargassum accumulates. When excessive amounts of Sargassum are found on the sand, it also contributes to a decline in the aesthetic quality of Florida beaches and ultimately impacts on the tourism industry. When left on the shore to decompose, the *Sargassum* will release unpleasant odors (hydrogen sulfide) into the environment. It also attracts insects, e.g. sand flies, as it decomposes. Bacteria levels in the seaweed also tend to increase. When the decomposing Sargassum is washed back to the water it results in the issuance of beach swim advisories due to elevated bacteria levels further impacting the economy of the area by limiting access to safe recreational waters along the coast. Thus, coastal communities are looking for alternative ways to handle the material once removed from the beach.

Alternative methods are needed for handling excessive amounts of Sargassum that are found on Florida's coastlines. In order to combat this problem, local government agencies are exploring how to remove the seaweed and are looking for beneficial uses. Composting offers one potential and beneficial alternative. Instead of leaving the seaweed to decompose on shore, or hauling it off to landfills via trucks, Sargassum can be potentially composted. Compost consists of decomposed organic matter. This natural process of recycling organic matter can be used to produce a rich soil amendment. Compost maintains moisture more effectively and provides a rich environment for plants to grow. Seaweed is rich in nutrients that are absorbed from the sea and from the energy from the sun, making it a potentially rich soil amendment. In addition to its use as a soil amendment, it should be ensured that the composting of seaweed is within the standards of heavy metals and bacteria levels so that the constituents are within satisfactory health-based levels. The objective of this project is to evaluate the suitability of producing compost from seaweed in tumbler composters. Four experiments were conducted to evaluate the need for pre-washing and suitable mixes. The treatments included: no washing of Sargassum, washing Sargassum with freshwater, grass clippings mixed with Sargassum, and mulch mixed with Sargassum. These treatments were sampled biweekly and measured for bulk physical-chemical parameters, nutrients, metals, and bacteria. Once the compost was cured, radish bioassays were setup to evaluate the plant growth in each of the treatments. Results indicate that electrical conductivity (saltiness) is not an issue when composting the seaweed (values are well below the U.S Composting Council standards). Preliminary carbon to nitrogen results show that the compost can be used to grow plants. Results from the radish bioassays indicate that the compost can support growth of plants.

2. Research presentations resulting from THIS Hinkley Center Project. The interim results from this study have been presented during the following meetings:

- "Sources of Enterococci to a Coastal Beach Experiencing Elevated Background Levels" Webinar organized by SOP Technologies, Miami FL. July 2020. (Speaker presentation by H. Solo-Gabriele and A. Abdool-Ghany). [This webinar was attended by over 70 individuals.]
- "Sources of Enterococci to a Coastal Beach Experiencing Elevated Background Levels"
   Webinar organized by the City of Hallandale Beach, Hallandale Beach, FL. August 2020. (Speaker presentation by A. Abdool-Ghany).
- "Sargassum Seaweed Management in the State of Florida" Webinar organized by Recycle Florida Today. March 18, 2021. (Speaker presentation by A. Abdool-Ghany and H. Solo-Gabriele).
- "Sargassum Composting- A Solution" Presentation organized by Ana Zangroniz of Florida Sea Grant for Miami Dade County Parks and Recreation. June 24<sup>th</sup>, 2021. (Speaker presentation by A. Abdool-Ghany and H. Solo-Gabriele).
- "Sargassum Composting" Annual Conference organized by Recycle Florida Today. September 8<sup>th</sup>, 2021. (Speaker presentation by A. Abdool-Ghany).
- "Sargassum Invasion: Composting as a solution" Annual conference organized by Florida Shore and Beach Preservation Association. September 17<sup>th</sup>, 2021. (Speaker presentation by A. Abdool-Ghany).
- "Sargassum Composting-A Potential Management Solution" Annual Conference organized by Recycle Florida Today. June 27th, 2022. (Speaker presentation by A. Abdool-Ghany and H. Solo-Gabriele).
- "Is composting a feasible disposal option for beach-stranded Sargassum in South Florida?" Annual Goldschmidt conference. July 10-15, 2022. (Speaker presentation by A. Abdool-Ghany).
- "The Challenge of Managing Seaweed (Sargassum) Deposited on Florida's Beaches." 2023 Solid Waste Association of North American (SWANA) Florida Chapter Conference, Daytona Beach, FL, July 2023 (speaker presentation by H. Solo-Gabriele).
- "Managing Seaweed (Sargassum) Deposited on Florida's Beaches." Hinkley Center Advisory Board Meeting, held at Orange County Public Works, Orlando, FL, May 2024 (speaker presentation by H. Solo-Gabriele)
- "Estimating Children's Health Risks to Arsenic following Recreational Play on Beaches with Sargassum." Poster presentation at the REU program, University of Miami, FL. August 2024. (Speaker presentation by Melanie Cerna).
- "Health Risks from Stranded Sargassum." Association for the Science of Limnology and Oceanography (ASLO) 2025, Aquatic Sciences Meeting, Charlotte, NC (poster presentation by Helena Solo-Gabriele. Poster authors included Afeefa Abdool-Ghany, Brittany Mc Intyre, Melanie Cerna, Isabela Puente, Matthew Roca, Ayaaz Amirali, Jiayu Li, Shahar Tsmaret, Nohhyeon Kwak, Alejandro Iriate, Noah Ross, Oscar Sosa, and Koa Wong).
- 3. List who has referenced or cited your publications from this project (data from Scopus).
  - Almela, V. D., Tompkins, E. L., Dash, J., & Tonon, T. (2023). Brown algae invasions and bloom events need routine monitoring for effective adaptation. Environmental Research Letters, 19(1), 013003.

- Gabriel, D., Maridakis, C., Fredericq, S. (2024). Gone with the wind: An unexpected Sargassum inundation in the mid-Atlantic Azores archipelago. Marine Pollution Bulletin, 204, art. no. 116522.
- Leal-Bautista, R.M., Rodriguez-Garcia, J.C., Acosta-González, G., Chablé-Villacis, R., Tapia-Tussell, R., Bautista-García, J.E., Olguin-Maciel, E., Alzate-Gaviria, L., González-López, G. (2024). Assessment of Leachate Generated by Sargassum spp. in the Mexican Caribe: Part 1 Spatial Variations, Water (Switzerland), 16 (9), art. no. 1251.
- Elizalde-Mata, A., Trejo-Caballero, M.E., Yánez-Jiménez, F., Bahena, D., Esparza, R., López-Miranda, J.L., Estevez, M. (2024). Assessment of Caribbean Sargassum species for nanocellulose foams production: An effective and environmentally friendly material to water-emerging pollutants removal. Separation and Purification Technology, 341, art. no. 126627
- Oueld Lhaj, M., Moussadek, R., Mouhir, L., Mdarhri Alaoui, M., Sanad, H., Iben Halima, O., Zouahri, A. (2024). Assessing the Evolution of Stability and Maturity in Co-Composting Sheep Manure with Green Waste Using Physico-Chemical and Biological Properties and Statistical Analyses: A Case Study of Botanique Garden in Rabat, Morocco. Agronomy, 14 (7), art. no. 1573.
- Machado, C.B., Marsh, R., Hargreaves, J.K., Oxenford, H.A., Maddix, G.-M., Webber, D.F., Webber, M., Tonon, T. (2024). Changes in holopelagic Sargassum spp. biomass composition across an unusual year. Proceedings of the National Academy of Sciences of the United States of America, 121 (23), art. no. e2312173121.
- Correa-Bustos, A., Berti, F., Salas-Sanjuán, M.D.C., Segura-Pérez, M.L. (2024). Characterization of Mixtures of Rugulopteryx okamurae Compost and Plant Residues to Determine the Most Effective Composition as a Substrate and Source of Nutrients. Horticulturae, 10 (6), art. no. 567.
- Timshina, A.S., Robey, N.M., Oldnettle, A., Barron, S., Mehdi, Q., Cerlanek, A., Townsend, T.G., Bowden, J.A. (2024). Investigating the sources and fate of per- and polyfluoroalkyl substances (PFAS) in food waste compost. Waste Management, 180, pp. 125-134.
- Thomas, C., Filella, M., Ionescu, D., Sorieul, S., Pollier, C. G. L., Oehlert, A. M., Zahajska P, Gedulter N, Agnon A, Ferreira Sanchez D, Ariztegui, D. (2024). Combined genomic and imaging techniques show intense arsenic enrichment caused by detoxification in a microbial mat of the Dead Sea shore. Geochemistry, Geophysics, Geosystems, 25(3), e2023GC011239.
- Chávez-Vergara B., Solleiro-Rebolledo E., López-Martínez R., Beltrán-Paz O., Ceniceros-Gómez Á., Yañez-Mendoza G., The release of arsenic is a hidden risk during the in-situ decomposition of landed sargassum litter (2025) Aquatic Botany, 199, art. no. 103884. <a href="https://doi.org/10.1016/j.aquabot.2025.103884">https://doi.org/10.1016/j.aquabot.2025.103884</a>
- Zeng H., Wu J., Yu C. Biotransformation of agar extraction waste into cultivation matrix using an adaptively evolved Paenibacillus mucilaginosus strain (2025) World Journal of Microbiology and Biotechnology, 41 (4), art. no. 108 https://doi.org/10.1007/s11274-025-04332-8
- 4. How have the research results from THIS Hinkley Center project been leveraged to secure additional research funding?
  - We submitted a pre-proposal to EREF, but it was not awarded.

- We have also submitted a proposal to Commissioner Raquel Regalado of Miami-Dade County. It was intended to evaluate a composting operation located in Crandon Beach. The objective of the proposal was to evaluate the suitability of producing compost from seaweed on a large scale.
- An NSF-RAPID proposal was submitted by Dr. Jiayu Li, PI, and Dr. Helena Solo-Gabriele, coPI. The purpose of the proposal was to evaluate sulfur emissions from Sargassum and to evaluate the microbial communities. This proposal has since been funded.
- A proposal was submitted to the Google challenge to evaluate sequestration of carbon dioxide via Sargassum efforestration of the ocean. This proposal was not funded.
- A proposal was submitted to Miami-Dade Innovation Authority in collaboration with a local company to identify sustainable solutions for repurposing Sargassum seaweed. This proposal was not funded.
- A proposal was submitted to the Conservation, Food & Health Foundation to identify beneficial uses for Sargassum compost for farming in the Caribbean. This proposal was funded.
- A proposal was submitted to the US Environmental Protection Agency South Florida Program to evaluate Biochar made from Sargassum for the reduction of CyanoHABs and toxins. This proposal was not funded.
- We have been submitting concept papers to NSF to evaluate the chemistry of arsenic in Sargassum. Both the Division of Chemical Oceanography and Division of Physical Oceanography have exhibited an interest and have requested that we submit a full proposal.
- A proposal was submitted to the Global Outreach Initiative of the Association for the Science of Limnology and Oceanography (ASLO) for funding towards an outreach initiative to develop best practices for Sargassum composting.
- Additional proposals are pending.
- 5. What new collaborations were initiated based on THIS Hinkley Center project?
  - Collaboration with BiocharNow, led by James Gaspard, focused on creating biochar from Sargassum collected by the University of Miami research team. James Gaspard provided expertise and support in the pyrolysis process, transforming the Sargassum into biochar to assess its potential benefits for environmental management and soil improvement.
  - Legena Henry UWI and Rum&Sargassum. In this collaboration, the research team will
    be supplied with biodigestate and biogas samples from a rum distillery, which were
    tested for arsenic levels. This initiative aims to understand the safety and potential
    applications of byproducts from the rum production process in environmental and
    agricultural contexts.
  - Caribbean Agricultural Research and Development Institute (CARDI). CARDI's
    extensive farmer network facilitated the collection of crop and soil samples to evaluate
    the use of Sargassum-derived products, such as fertilizer and compost. Additionally,
    CARDI offers opportunities to collaborate on future training programs for farmers in the
    Caribbean, enhancing their knowledge of sustainable practices involving sargassumbased agricultural inputs.

- Upon initiation of this project, we have been in contact with the City of Fort Lauderdale. Mark Almy and his team have been gracious enough to show us their composting operations.
- One of our TAG members (Chip Jones) has allowed us to tour his facilities and see the machines that are used in operation. We met with him and took a tour of his operations on December 11, 2020.
- Another TAG member (Mark Richards) offered for us to tour Crandon Beach to get an idea of the influx of seaweed that plagues the unique area. We toured Crandon Beach with Mark Richards on December 29, 2020.
- We are in contact with Dr. Kimberly Moore, from the University of Florida, IFAS. She has provided guidance on the quality of compost and helped to design the radish bioassay experiments. We are working with her to establish a set of standards that can exclusively be used for sargassum compost.
- Afeefa worked in Dr. Amanda Oehlert's lab to analyze the metals and phosphorous found in the tumbler composters as well as the compost piles.
- Dr. Peter Swart invited us to be a part of the proposals submitted to Commissioner Regalado. We also analyze for nutrients in his lab.
- Through Dr. Blare, we have collaborated with individuals in the agricultural community who are helping to set up interviews with growers that work with Sargassum compost.
- Recycle Florida Today and the Organic Compost Council have been big supporters of our research by promoting our work through meetings they organize.
- We have met with the CEO/founder of Sustainscape Inc, Dennis de Zeeuw. His company produces fertilizer from Sargassum. He has two products that he uses throughout his jobs in Broward County. Dr. Blare and Afeefa met with him on September 20, 2021.
- The CEO/founder of Algas Organics, Johanan Dujon, reached out to us to hear more about our research. We will also hear more about the operation he is running and how he deals with Sargassum. We plan on meeting him on September 22, 2021.
- Ana Zangroniz who is a Florida Sea Grant Extension Agent at the UF/IFAS Extension Miami-Dade County, reached out to us requesting that we present our research to Miami-Dade County. From this presentation we also were in contact with Tom Morgan, who is the Chief of Operations for Miami-Dade County Parks, Recreation and Open Spaces Dept.
- Rebecca Wakefield who is the Chief of Staff in the office of Commissioner Raquel Regalado, reached out to us to find out more about our research. She has indicated an interest in developing a coalition to address the seaweed disposal issue.
- Ultima, a startup sequencing company, process Sargassum samples we provided for microbial communities.
- We are currently working with UMiami faculty, Dr. Cynthia Silveira, in evaluating the results from the microbial community analyses.
- We have since teamed up with UMiami faculty, Dr. Jiayu Li, with whom we are collaborating on an NSF funded project focused on Sargassum emissions and microbial communities.
- We have been in communication with Dr. Schonna Manning of FIU who serves as Dr. Afeefa Abdool-Ghany's post-doctoral advisor. We have discussed research ideas that integrate micro- and macro-algae.

- We have been participating in the FORCE Team initiatives to promote composting in Florida. This group is led by Miriam Zimms of Kessler and Associates and the group serves as a forum to promote communication and collaboration.
- 6. How have the results from THIS Hinkley Center funded project been used by the FDEP or other stakeholders?
  - Members of the FDEP have participated in our TAG meetings and in meetings organized by our collaborators. They include Karen Moore, Lauren O'Connor, and Chris Perry. The FDEP has provided us with guidance in the process for obtaining permits for on-beach composting projects. They have also provided us with guidance in terms of applicable regulations. Currently they are considering classifying sargassum compost as yard trash. The regulations for yard trash do not include arsenic and as a result seaweed compost would pass FDEP regulatory thresholds. The FDEP is interested in our work because it will help guide the agency in terms of classifying Sargassum compost. They appear to want to encourage recycling and have been keeping up with our work on this project.
  - Representatives from the FDEP indicated during our TAG meeting on March 17, 2022 that they plan to develop regulatory guidelines specific for Sargassum compost. A key component of their decision making will be the results reported from the Hinkley Seaweed projects.
  - Miami-Dade County DERM has since initiated their own Sargassum compost study to confirm levels of arsenic in the compost and its runoff. These results will be used to establish potential permitting requirements for Sargassum compost in the county.
  - Dr. Solo Gabriele met with Ana Zagroniz of Miami-Dade SeaGrant and Miami-Dade County Parks and Recreation to discuss needs in Miami-Dade County on June 27<sup>th</sup> 2024.
  - Dr Solo Gabriele met with Karen Moore and Lauren O'Connor to discuss FDEP initiatives in developing standards for Sargassum compost on July 11<sup>th</sup> 2024.
  - During the March 26, 2025, TAG meetings members of FDEP indicated that they are working on recycling regulations specific to Sargassum. A separate meeting was offered to discuss with the FDEP further.

#### **ACKNOWLEDGEMENTS**

- This project was funded by the Hinkley Center for Solid and Hazardous Waste Management.
- We thank the City of Fort Lauderdale for their support in this project and for allowing us to conduct part of our prior projects onsite.
- We thank the beach managers, organic growers, and business owners who provided feedback concerning composting operations through the interview process set up as part of this study.
- We are thankful to all beach managers who provided us with access to the beach sites and especially to the park rangers at Crandon Park Beach who facilitated access to the preserve area of the beach.
- We are grateful to all the Technical Awareness Group (TAG) members listed in the following table, plus the individuals who took part in the TAG meetings who are listed in the table that follows for participating in meetings and for their input and feedback.

## RESEARCH TEAM MEMBERS

Name	Affiliation and Address
Helena Solo-Gabriele	Professor, Principal Investigator
(Year 1 through 3)	University of Miami, 1251 Memorial Drive McArthur Bldg
	R 518, Coral Gables, FL 33146
	Graduate Ph.D. Student
	University of Miami, Dept. of Chemical, Environmental,
Afeefa Abdool-Ghany	and Materials Engineering, Coral Gables, FL 33146
(Year 1 through 3)	Postdoctoral Associate
(Teal Tullough 5)	Florida International University, Institute of Environment,
	11200 SW 8th Street, OE 148, Miami, FL 33199
	Brizaga Inc., Department of Planning and Analytics, 2101
	W Commercial Blvd Ste 4600, Fort Lauderdale, FL 33309
	Assistant Professor, Co-Principal Investigator
Trent Blare	University of Florida Institute of Food and Agricultural
(Year 2)	Sciences, Tropical Research and Education Center,
	Homestead, FL, 33031
	Assistant Professor, Co-Principal Investigator
Amanda Oehlert	University of Miami, Dept. of Marine Geosciences -
(Year 1)	Rosenstiel School of Marine, Atmospheric, and Earth
	Science, Miami, FL, 33149
	Professor, Co-Principal Investigator
Peter Swart	University of Miami, Dept. of Marine Geosciences -
(Year 1)	Rosenstiel School of Marine, Atmospheric, and Earth
	Science, Miami, FL, 33149
Brittany Mc Intyre	Graduate Student University of Miami
Melanie Cerna	Undergraduate Student University of Florida

### HINKLEY CENTER

Name	Affiliation and Address
John Schert	Director (through December 2022)
	University of Florida, Gainesville, FL
Dr. Timothy Townsend	Director (Starting January 2023)
	University of Florida, Gainesville, FL
Steve Laux	Administrator
Hannah Sackles	Administrator

**TECHNICAL AWARENESS GROUP (TAG) MEMBERS.** Note: Participation in the TAG group does not imply an endorsement of the research. The TAG group are individuals who are interested in the research and are capable and willing to provide input. This input is considered by the research team as the research project progresses.

Name	Affiliation
Kimberly Moore	Professor in Environmental Horticulture, Distinguished Teaching Scholar University of Florida, IFAS Fort Lauderdale Research and Education Center
Ligia Collado-Vides	Senior Lecturer: Associate Chair
Dan Meeroff	Professor and Associate Chair Department of Civil, Environmental & Geomatics Engineering, Florida Atlantic University
Ashley Smyth	Assistant Professor, Biogeochemistry, Tropical Research and Education Center, University of Florida
Kimberly Moore	Professor in Environmental Horticulture, Distinguished Teaching Scholar, University of Florida, IFAS: Fort Lauderdale Research and Education Center
Randall Penn	UF IFAS Extension Agent - Sarasota County
Armando Ubeda	UF/IFAS Extension Sarasota County
Michelle Mularz	Extension Services-Environmental Horticulture Agent
Ana Zangroniz	Florida Sea Grant Extension Agent UF/IFAS Extension Miami-Dade County
Shelly Krueger	Florida Sea Grant Agent II University of Florida IFAS Extension, Monroe County
Vincent Encomio	Sea Grant- Martin and St. Lucie Counties Extension Agent
Emilio Lopez	CEO of SOP Technologies
Alejandro Quintás	NEAT Sand
Chip Jones	President of Beach Raker
Chris Snow	Vice President of Corporate Affairs, Consolidated Resource Recovery, Inc.
David Hill	Co-Chair Organics Recycling Committee Recycle Florida Today
Nandra Weeks	GeoSyntec Consultants
Cathie Schanz	Director of Park, Recreation, and Open Spaces
Mary Beth Morrison	Director of Environmental Programs, Solid Waste Authority of Palm Beach County
Enrique Sanchez	Deputy Director, Parks and Recreation of the City of Fort Lauderdale
Mark Almy	Park Operations Superintendent Parks and Rec. Admin.

# TECHNICAL AWARENESS GROUP (TAG) MEMBERS (Cont'd)

## Name Affiliation

Roland Samimy	Chief Resilience and Sustainability Officer
Christopher Bumpus	Chief of Conservation, Miami Dade Parks and Rec
Paul Vitro	Division Chief at Miami-Dade County Parks, Recreation and Open Spaces Department
Heather Tedlow	Interpretive Nature Coordinator, Miami Dade Parks and Rec
Samir Elmir	Director of Environmental Health & Engineering Service Florida Department of Health in Miami-Dade County
Karen Moore	Environmental Administrator-FDEP Division of Waste Management
Lauren O'Connor	Government Operations Consultant-FDEP Division of Waste Management
Chris Perry	FDEP Division of Waste Management

TAG MEETING PARTICIPAN	TS. Note: Participation in the TAG meetings does not imply an
endorsement of the research.	
MEETING 1 (Online)	March 17, 2023
Name	Affiliation
Afeefa Abdool-Ghany	University of Miami
Aliza Karim	Miami WaterKeeper
Amanda Oehlert	University of Miami-RSMAS
Amede Dimonnay	Broward Engineering and Permitting Division
Ana Zangroniz	Florida Sea Grant Extension Agent for Miami Dade-County
Chip Jones	Beach Raker
Chris Snow	Consolidated Resource Recovery, Inc.
Christopher Perry	Florida Department of Environmental Protection
Danielle Jimenez	Division of Environmental Resource Management (DERM)
Daniel Meeroff	Florida Atlantic University
David Hill	Co-Chair Organics Recycling Committee
Elizabeth Kelly	Martin County
Emilio Lopez	SOP Technologies
Evan Blanchard	Brizaga
Fanny Navarro	Miami- Dade County Parks, Recreation and Open Spaces; Sea Turtle Conservation Program
Helena Solo-Gabriele	University of Miami
Isaac Bearg	Organic Waste Management Consultant
Jairo Gonzalez	
Karen Moore	Florida Department of Environmental Protection
Kimberly Moore	University of Florida, IFAS
Lauren O'Connor	Florida Department of Environmental Protection
Libbie	Farmer in the British Virgin Islands
Mark Richards	Miami-Dade County
Mary Beth Morrison	Solid Waste Authority of Palm Beach County
Nandra Weeks	Geosyntec Consultants
Peter Swart	University of Miami-RSMAS
Rachel Harris	Loxahatchee River District
Roland Samimy	The Village of Key Biscayne
Shelly Krueger	University of Florida, Florida Sea Grant Agent for Monroe County
Steve Laux	Hinkley Center for Solid and Hazardous Waste Management
Susan Noel	Loxahatchee River District
Tom Morgan	Miami-Dade County Parks
Trent Blare	University of Florida-IFAS-Homestead
Vincent Encomio	Florida Sea Grant Agent for Martin and St. Lucie County

	<b>S.</b> Note: Participation in the TAG meetings does not imply an
endorsement of the research.  MEETING 2 (Online)	September 19, 2023
Name	Affiliation
Afeefa Abdool-Ghany	University of Miami, now at FIU
Alejandro Quintas	NEAT Sand
Alexandra Stiffler	University of Miami
Aliza Karim	Miami Waterkeeper
Amanda Oehlert	University of Miami-RSMAES
Angela Delaney	Broward County Marine Resources
Brittany Mc Intyre	University of Miami
Clara Sidan, Assistant Director at City of Miami	Resilience and Public Works Department
Craig Ash	Waste Management
Cynthia Silveria	University of Miami
Dan McChesney	Shapiro Enterprises
Doug Farrington	ADAR Technologies
Eli Rosa Estevez	City of Miami
Elizabeth Kelly	Martin County
Emilio Lopez	SOP Technologies
Griffin Alexander	Biscayne Bay Aquatic Reserve
Griselle Correa	City of Miami (NPDES and Stormwater Department)
Helena Solo-Gabriele	University of Miami
Jared Jacobs	Fertile Earthworm Farms
Jiayu Li	University of Miami
Julia Poliadis	Fertile Earthworm Farms
Kimberly Moore	University of Florida, IFAS
Lanette Sobel	Fertile Earthworm Farm
Libbie	Farmer in the British Virgin Islands
Ligia Collado-Vides	Florida International University
Mary Beth Morrison	Solid Waste Authority of Palm Beach County
Melanie Cerna	Florida International University
Nohhyeon Kwak	University of Miami
Peter Klaich	Shapiro Enterprises
Peter Swar	University of Miami-RSMAES
Roland Samimy	The Village of Key Biscayne
Ron Portell	ADAR Technologies
Samantha Tiffany, Environmental Resource Manager	City of Miami Beach
Schonna Manning	Florida International University

TAG MEETING PARTICIPANTS. Continued from prior page		
MEETING 2 (Online)	September 19, 2023	
Name	Affiliation	
Shahar Tsameret	University of Miami	
Sonia Brubaker, Chief Resilience Officer & Director	City of Miami	
Steve Laux	Hinkley Center for Solid and Hazardous Waste Management	
Tom Morgan	Miami-Dade County Parks and Recreation	
Tracy Mincer	Florida Atlantic University	
Valentina Caccia	Division of Environmental Resource Management (DERM)	
Victoria Lewis	University of Miami	
Vincent Encomio	Florida Sea Grant for Martin and St. Lucie County	
Xavier DeRoos	Renewable Composting	

TAG MEETING PARTICIPANTS. Note: Participation in the TAG meetings does not imply an		
endorsement of the research.	1 21 2024	
MEETING 3 (Online).	June 21, 2024	
Name	Affiliation	
Afeefa Abdool-Ghany	University of Miami, now at FIU	
Alejandro Prado Iriarte	University of Miami	
Alejandro Quintas	NEAT Sand	
Alina Ruta	Miami-Dade Innovation Authority	
Angela Delaney	Broward County Marine Resources	
Bethany Tober	Biscayne Bay Aquatic Reserve	
Brittany Mc Intyre	University of Miami	
Caroline Irvin	Division of Environmental Resource Management (DERM)	
Chadeene Beckles	Caribbean Agricultural Research and Development Institute (CARDI)	
Chrissy Hudson	ADAR Technologies	
Craig Ash	Waste Management	
Dan McChesney	Shapiro Enterprises	
Dan Meeroff	Florida Atlantic University	
Danielle Jimenez	Division of Environmental Resource Management (DERM)	
Elizabeth Kelly	Martin County	
Gloria Antia	City of Miami	
Guangliang Liu	Florida International University	
Hannah Sackles	University of Florida	
Helena Solo-Gabriele	University of Miami	
Isabela Puente	University of Miami	
James Gaspard	BioChar Now	
Jessica Lorenzo	City of Miami Beach	
Jiayu Li	University of Miami	
Josefina Olascoaga	University of Miami	
Kimberly Moore	University of Florida, IFAS	
Koa Wong	University of Miami	
Lanette Sobel	Fertile Earthworm Farm	
Legena Henry	Rum and Sargassum	
Ligia Collado-Vides	Florida International University	
Lisa James	Caribbean Agricultural Research and Development Institute (CARDI)	
Louis DiVita	Hinkley Center for Solid and Hazardous Waste Management	
Mark Almay	City of Fort Lauderdale	
Mary Beth Morrison	Solid Waste Authority of Palm Beach County	

TAG MEETING PARTICIPANTS. Continued from prior page.		
MEETING 3 (Online).	June 21, 2024	
Name	Affiliation	
Melanie Cerna	Florida International University	
Pamela Sweeney	Division of Environmental Resource Management (DERM)	
Rivka Reiner	University of Miami,	
Roland Samimy	The Village of Key Biscayne	
Samir Elmir	Department of Health, Miami Dade-County	
Schonna Manning	Florida International University	
Shahar Tsameret	University of Miami	
Shelly Krueger	Florida Sea Grant for Monroe County	
Stephanie Roche	Broward County's Resiliency Department	
Steve Sternick	Beach Raker	
Susan Noel	Loxahatchee River District	
Thierry Tonon	York University, UK	
Timothy Kirby	City of Miami	
Tristan Alvarez	Caribbean Agricultural Research and Development Institute (CARDI)	
Vincent Encomio	Florida Sea Grant Agent for Martin and St. Lucie Counties	
Wilbur Mayorga	Division of Environmental Resource Management (DERM)	
Yong Cai	Florida International University	

TAG MEETING PARTICIPANTS. Note: Participation in the TAG meetings does not imply an	
endorsement of the research.  MEETING 4 (Online).	March 26, 2025 (Open only to TAG members)
Name	Affiliation
Afeefa Abdool-Ghany	University of Miami, now at Brizaga
Ana Zangroniz	Florida Sea Grant for Miami Dade-County
Ashley Smyth	Tropical Research and Education Center, UF/IFAS
Bill Cooper	University Professor
Brittany Mc Intyre	University of Miami
Chip Jones	Beach Raker
Chris Bumpus	Miami Dade Parks and Recreation
Chris Perry	Florida Department of Environmental Protection
Cristina Fayad Martinez	University of Miami
Crystal	
Dustin Dubois	Hinkley Center for Solid and Hazardous Waste Management
Guangliang Liu	Florida International University
Helena Solo-Gabriele	University of Miami
Hilda	
Isabela Puente	University of Miami
Jake	
Jiayu Li	University of Miami
Joe Ullo	Hinkley Center for Solid and Hazardous Waste Management
Karen Moore	Florida Department of Environmental Protection
Kimberly Moore	University of Florida, IFAS
Koa Wong	University of Miami
Lauren O'Connor	Florida Department of Environmental Protection
Louis DiVita	Hinkley Center for Solid and Hazardous Waste Management
Nohhyeon Kwak	University of Miami
Rebecca Dickman	CETCO
Roland Samimy	The Village of Key Biscayne
Sam Levin	Hinkley Center for Solid and Hazardous Waste Management
Samantha Tiffany	City of Miami Beach
Samir Elmir	Department of Health, Miami Dade-County
Santiago Stebelski De Alba	University of Miami
Shelly Krueger	Florida Sea Grant for Monroe County
Sherry Carpenter	Hinkley Center for Solid and Hazardous Waste Management
Tessa Brown	University of Miami
Tim Townsend	Hinkley Center for Solid and Hazardous Waste Management

TAG MEETING PARTICIPANTS. Continued from prior page.	
MEETING 4 (Online).	March 26, 2025 (Open only to TAG members)
Name	Affiliation
Vincent Encomio	Florida Sea Grant for Martin and St. Lucie Counties
Yang Wong	University of Miami
Yong Cai	Florida International University