# ASSESSING THE USE OF STRANDED PELAGIC *SARGASSUM* BIOMASS AS COMPOST FOR RED MANGROVE SEEDLING GROWTH IN THE CONTEXT OF MANGROVE RESTORATION IN JAMAIACA

A Thesis

Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Natural Resource Management: Marine & Terrestrial Ecosystems

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## ABSTRACT

The use of *Sargassum*seaweed for agricultural purposes is well documented and has proven to be an effective, economically viable and environmentally preferable alternative to the wasteful disposal of *Sargassum*biomass in landfills. The use of *Sargassum* compost (SC) to improve soil conditions for mangrove restoration is theoretically possible but is not a well-documented and researched subject. Research done by Trench et al. (2022) concluded that, under certain conditions, a 75% SC- sand mixture improved the growth, and health of red mangrove *(Rhizophora mangle)* seedlings.This project aimed to further this research by investigating the impacts of different 75% *SC*-sand mixtures on red mangrove seedlings. This investigation utilized unwashed *Sargassum* biomass harvested from the nearshore waters (Golden *Sargassum)*, foreshore (Brown *Sargassum)*, and backshore (Black *Sargassum)* of the Palisadoes/Port Royal coastline. These samples were composted over a three (3) month period then combined with mangrove nursery sand to create the 75% SC- sand mixtures. Forty (40) Red Mangrove were equally planted and grown in the SC mixtures and a control (no SC) soil. The growth and health of these seedlings as well as the soil parameters of each seedling (pH, temperature, and moisture) were recorded over a twenty-one (21) week period. From the results obtained it was observed that seedlings grown inGolden SC were significantly taller and healthierthan those grown in the other treatments but were not significantly different from those grown in the control soil except with regards to the number of leaves. Additionally, the pH and moisture percentage of Golden SC treated soil was significantly different from that of both the control soil and other SC treatments. Overall, the use of 75% Sargassum compost was not shown to significantly improve the growth or health of red mangrove seedlings when compared to seedlings grown in untreated mangrove nursery sand. However, Golden SC showed that it can be used to significantly improve leaf production of red mangrove seedlings most likely through increasing the organic content of treated soil which also improves the water retention ability of treated soil and lowers pH. Further testing is required to verify these results.

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## GLOSSARY

- United Nations Environment Programme- Caribbean Environment Programme (UNEP-CP)
- *Sargassum* Compost (SC)
- University of the West Indies (UWI)
- Port Royal Marine Lab (PRML)
- The National Environment and Planning Agency (NEPA)

## LIST OF FIGURES, TABLES, ILLUSTRATIONS, CHARTS, ETC,

# INTRODUCTION

***Rationale***

Jamaica is home to three (3) species of mangrove trees; these are the Red Mangrove *Rhizophora mangle*, the Black Mangrove *Avicennia germinans* and the White Mangrove *Laguncularia racemosa*. These trees form expansive mangrove forests on the coastlines of the island serving as coastal protection, and as a habitat and nursery for many marine species, many of which are commercially harvested *(Trench 2022)*. They also serve key roles in the mangrove-seagrass-coral coastal dynamic serving to protect the other marine ecosystems from excessive sedimentation and nutrient loading. It is estimated that these mangrove forests cover approximately 9,800 hectares of land across the island *(Ortega et al. 2019).* However much like other countries, Jamaica is experiencing a loss of mangrove habitat due primarily to human activity. These activities include destructive harvesting of mangroves for charcoal, solid waste pollution, clearance of lands and changes in surface water hydrology and drainage. *(Trench 2022; Ellison, Felson, and Friess 2020; Valiela, Bowen, and York 2001*). Majority of this loss can be attributed to the direct loss of Mangrove trees during clearance of large acres of land for housing, resort, agriculture,and commercial developments which has led to a decline in Jamaica's coastline and the ecosystem services these forests provide*(Ortega et al. 2019)*.

The Jamaican coastline has also been threatened by large floating mats of the pelagic Phaeophyta*Sargassum sp.* Since 2011 these mats have been periodically inundating Caribbean shorelines causing a number of negative impacts to both the bio-physical and socio-economic environments of the countries and areas which experience these inundations.The impacts to the coastal community are particularly noteworthy as theymay include severe eutrophication of coastal waters leading to mortality of near-shore coral, seagrass and mangrove communities which may take decades to recoveror may never completely recover*(United Nations Environment Programme- Caribbean Environment Programme 2021; Van Tussenbroek et al. 2017; Resiere et al. 2023).*

The removal and disposal of the seaweed is a costly endeavour; Mexico, one of the Caribbean countries most affected by the influxes estimates that annual harvested volumes of the seaweed ranged from 10,105–40,932 $m^3$ per kilometre, resulting in an annualcleanup cost ofUS$0.3–1.1 million per kilometre*(Rodríguez-Martínez et al. 2023)*. The Inter-American Development Bank (IDB) estimates that *Sargassum* cleanup in 2018 costs the Caribbean US $120 million, a figure expected to increase as theinfluxes appear to be increasing in frequency, duration, and magnitude*(Christie 2019)*. The UNEP-CEP noted that *Sargassum* has also been identified as a potential resource that may be commoditised as it has a variety of possible uses in agriculture, bioenergy, bioplastics, bioremediation, construction, and cosmetics. This utilization of the seaweed for various purposes offsets the annual cleanup costs and stands as a more economically and preferable option to the costly collection and wholesale disposal of the seaweed in landfills. *(United Nations Environment Programme- Caribbean Environment Programme 2021).*

One notable use of *Sargassum* is in the agricultural industry. There is extensive historical use of seaweeds, especially large brown seaweeds like *Sargassum*, to improve crop yield and overall health in agriculture worldwide

*(McHugh 2003)*. Other research into the use of *Sargassum* for agriculture has yielded similar positive results wherein the use of *Sargassum* directly, *Sargassum* compost or other derived products had a positive impact on the growth and health of various agricultural crops as well as sand dune vegetation.*(López-Contreras et al. 2021; CARDI 2015; McHugh 2003; Desrochers et al. 2020; Williams and Feagin 2010).*

Recently, research into mangrove restoration found that soil amelioration using treated sewage effluent *(Erftemeijer et al. 2021)* and *Sargassum c*ompost *(Trench et al. 2022)*was proven to enhance the growth and improve the overall health of mangrove seedlings.The use of *Sargassum* compost in particular was found to, under certain conditions, improve the growth of Red Mangrove (*Rhizophora mangle*) seedlings in Jamaica and may be used to support mangrove restoration attempts without "*proportional contamination of the ecosystem*"*(Trench et al. 2022)*. Given this, the use of *Sargassum* compost may serve as a possible avenue for the removal of the invasive seaweed from shorelines while improving the health of damaged Jamaican mangrove coastlines. However, the use *Sargassum* compost for mangrovegrowthandrestoration is not a particularly well researched area. As such additional research is required to investigate the use and associated costs of *Sargassum* compost for mangrove restoration.

This research seeks to expand on the experiments conducted in 2022 by Trench et al. by exploring the impact of *Sargassum*seaweed harvested under different conditions and composted to an ideal75:25 compost: sand ratio using relatively low-cost methods on Red Mangrove seedlings. This experiment hopes to improve the success of mangrove restoration efforts in Jamaicawhile contributing to the collective body of knowledge regarding the proper utilization and disposal of invasive and rather disruptive*Sargassum*biomass.

***Aim***

The aim of this project is toassess the soil amelioration potential of *Sargassum* compost collected and prepared from different areas of the beach at Port Royal, Kingston, Jamaica.

***Objectives***

This project has the following objectives:

1. To produce compost using *Sargassum* collected from different areas of a beach (Palisadoes/Port Royal). These areas are:
    a. Nearshore Waters
    b. Foreshore
    c. Backshore.
2. To analyse the growth and health of Red Mangrove seedlings grown in the three (3) types of 75% *Sargassum*compost (SC).after twenty-one (21) weeks.
3. To analyse the impact of *Sargassum* Compost on soil parameters (pH, moisture, and temperature).

***Hypothesis***

1. There is no significant difference in the health and growth of mangrove seedlings treated different types of *Sargassum* Compost (SC).
2. There are no significant differences in soil parameters (temperature, moisture, pH)between the SC treatments and the control (no SC)
3. There is no significant difference in plants grown in SC (of varying types) and the control (no SC).

## LITERATURE REVIEW

### ***An Introduction to the SargassumSeaweed***

*Sargassum*is a genus of multicellular marine plants, known as brown algae (Phaeophyta), which are found in temperate and tropical oceans worldwide. Commonly known as seaweed these plants are pelagic, meaning that they are typically found floating in open areas of the ocean far from shore and exist within the water column rather than close to the *seafloor(López-Contreras et al. 2021)*. Some species of *Sargassum* have what is known as a "*benthic phase*" which is a stage of their life where they are attached to the ocean floor. Other species of *Sargassum* are "holopelagic" meaning that they do not have this benthic phase and instead grow, reproduce, and spend the entirety of their lives floating within or at the surface of the water column. *(United Nations Environment Programme- Caribbean Environment Programme 2021).* During this pelagic phase individual *Sargassum* plants can become entangled with one another forming large mats or rafts of *Sargassum*. These mats can travel great distances when propelled by ocean currents and wave actions until they reach existing landmasses where they are deposited on the shoreline. These pelagic mats of *Sargassum* are generally beneficial as they serve as sheltered habitats and a food source for various marine organisms including fish, marine mammals, birds, invertebrates, and sea turtle hatchlings (Webber and Aiken 2018). Additionally, these *Sargassum* mats provide nutrients to benthic communities when they lose their buoyancy and sink into deep water and, when deposited on land, these mats serve a role in shoreline stabilization (López-Contreras, et al. 2021).

However when excessive amounts of the seaweed occur in a particular coastal area its presence and subsequent decomposition can have several negative impacts on human well-being and coastal ecosystems while simultaneously causing impacts to multiple sectors related to the marine environment such as the tourism and fisheries sector *(United Nations Environment Programme- Caribbean Environment Programme 2021).* Starting in 2011 the Caribbean and Tropical Atlantic regions began experiencing *Sargassum* inundation events which led to notable damage and disruptions across coastal sectors and ecosystems. These events led to extensive research into *Sargassum;* its cause, biology, ecology, and mitigation measures either to prevent or reduce the impacts of future *Sargassum* events.

***Sargassum events in The Caribbean and Tropical Atlantic Regions***

In 2011 the Caribbean region, in particular the eastern Caribbean, and Tropical and Sub-Tropical Atlantic region including equatorial Brazil and the coastal West Africa, began experiencing never before seen influxes of *Sargassum* in their coastal waters. This influx of *Sargassum* was depositied on shorelines and caused severe disruptions to coastal industries and notable damage to coastal ecosystems. A similar influx of *Sargassum* occurred in spring of 2014 which continued to 2015. The 2014/2015 was especially noteworthy because by Autumn of 2014 the mean concentration of *Sargassum* was 10 times greater than the 2011 event and 300 times greater than any other autumn over the last two decades *(Schell et. al 2015).* No *Sargassum* events were recorded in 2016 and 2017 however the events resumed in 2018 and have continued into 2021.

According to the United Nations Environment Programme- Caribbean Environment Programme (UNEP-CEP) in their 2021 *Sargassum* White Paper the volume of *Sargassum* recorded across the Tropical Atlantic region ranged from 12.7M tonnes in to 27M tonnes for 2018-2020. The scale of these *Sargassum* influxes also led to declaration of emergency conditions in several Caribbean countries e.g. Tobago in 2015, Barbados in 2018, and Mexico in 2019. Trinidad was affected so much by the 2015 *Sargassum* event that the seaweed was labelled as a "*natural disaster*" *(Webber and Aiken 2018)*. The UNEP-CEP described these *Sargassum* influxes as "*a multi-regional transboundary issue, demanding coordination and collaboration within and across impacted regions*". *(United Nations Environment Programme- Caribbean Environment Programme 2021)*. The UNEP-CEP goes on to state that the *Sargassum* issue is "*an emerging hazard for a region that is already subject to numerous hazards*" and is unlikely to resolve itself without significant research and inteverntions. However, the UNEP-CEP also noted that *Sargassum* has also been identified as a potential resource that may be commoditised as it has a variety of possible uses in agriculture, bioenergy, bioplastics biomediation, construction and cosmetics. Through their paper the UNEP-CEP attempts to compile background information on the *Sargassum* events and their impacts across the Atlantic and Caribbean regions in order to promote interest in the topic across a diverse audience of technical and nontechnical readers for future research into potential uses and improvement of mitigative measures.

***Sourceof the Sargassum influx***

According to UNEP-CEP research into the cause of the recent *Sargassum* events is still ongoing however these events are the result of complex interactions of several contributing factors such as land-based nutrient pollution, changes ocean currents and climate change. It is however generally agreed across all literature reviewed for this project that pelagic *Sargassum* in the Atlantic and Caribbean is composed of two species, *Sargassum fluitans* and *Sargassum natans.* These species have several forms differentialed by physical variations in blade length and pneumatocysts (gas-filled bladders which provide bouyancy) (Parr 1939). *Sargassum fluitans* III (*S. fluitans III*) and *Sargassum natans I (S. natans I)* were the most common forms of *Sargassum* found in the North Atlantic, Caribbean Sea and Gulf of Mexico for several decades prior to the 2011 event*(Schell et. al 2015).*Inquiries of the 2014/2015 *Sargassum* event carried out found that *Sargassum natans VIII (S. natans VIII),* a historically rare form of *S. natans* in the Atlantic*,* was the predominant form of *Sargassum* in the inundations. This was significant as this information contrasted previous assumptions that the *Sargassum* influx was from The Sargasso Sea. The Sargasso Sea is a region in Western Central Atlantic Ocean bounded by four ocean currents forming an ocean gyre with large, persistent quantities *Sargassum*(Deacon 1942). Schell concluded, based on their research into the composition and abundance of *Sargassum* found in the Caribbean, that the Sargasso Sea exhited no connection to the Caribbean inundation events as it was predominantly composed of *S. natans I* which could not be linked to the Caribbean events. Later research into the *Sargassum* events identified an area between the Gulf of Guinea and the north coast of Brazil as a new "consolidation region" for *S. natans VIII.* This area is generally agreed to be the source of influxes to the Caribbean and West Africa *(United Nations Environment Programme- Caribbean Environment Programme 2021)* Schell also noted that pelagic mats of *S. natans VIII* were observed to have less resident fauna when compared to other forms of pelagic *Sargassum* leading to lower species richness and abundance. Additionally based on their observations Schell predicted that *S. natans VIII* has a lower value as foraging and nursery habitat for known macro-faunal associates including fish, turtles and seabirds as few of these organisms were present near *S. natans VIII* dominated mats during their research and collection period.

***Sargassum and Jamaica***

According to Webber and Aiken (2018), Jamaica was affected by the 2011 *Sargassum* event, though not as severely as other countries. The 2015 event however saw much greater quantities of the seaweed in Jamaican waters with *Sargassum* identified on at least sixteen (16) beaches across the island. The seaweed caused disruptions to the fishing and tourism industries, negatively affected coastal mangroves, coral reefs, seagrasses, and turtle nesting sites, and caused issues in coastal communities through the production of hydrogen sulphide during the seaweed's decomposition.

In 2018, as part of the country's attempts to mitigate the negative effects of*Sargassum* on Jamaica's beaches and coastal waters, Jamaica's environmental regulation Agency (The National Environment and Planning Agency- NEPA) partnered with the Faculty of Science and Technology (FST) at Jamaica's University of the West indies (Mona) to establish a research group to investigate potential commercial uses of *Sargassum*. Through their investigations the group found that the *Sargassum* mats surrounding Jamaica were *S. natans* I and *VIII*, and *S. fluitans III.* However, unlike the research of Schell et al (2015) the research group found that in the *S. natans*VIII only accounted for around 10% of the sampled *Sargassum* mats around Jamaica while *S. fluitans III* accounted for approximately 70% and *S. natans I* for approximately 20%. *(Webber, et al. 2019)*. The group's analyses of the chemical properties of the sampled *Sargassum* revealed that each species/form had different chemical profiles with varying levels of chemical constituents. *S. natans*VIII contained the highest concentration of Nitrogen followed by S. fluitans III and *S. natans I.* The research group's data also showed that metallic compounds, including harmful lead, mercury and arsenic were present in varying concentrations across all species/forms of *Sargassum* sampled.

The group also noted that research activities into the development of commercial applications of *Sargassum* was ongoing. Notably, this research included the production of *Sargassum* compost for soil improvement under the high volume/low value approach to the utilization of *Sargassum*. Results of this compost research presented in the report showed that height and weight of corn treated with *Sargassum* compost was improved when compared to the untreated control corn.

***Use of Sargassum Compost (SC) for Wetland Restoration in Jamaica***

It is estimated that since 1997 Jamaica has lost over 700 hectares of mangrove forest over the last 2 decades, however approximately 70% of these mangroves may be recovered *(Ortega et al. 2019).* The recovery of lost mangrove forests is underway and has been successful in areas, however the success of mangrove rehabilitation projects may be hindered by disrupted hydrology, nutrient poor sediment conditions and lack of natural recruitment of seedlings from nearby mangrove forests *(Trench et al. 2022; Valiela, Bowen, and York 2001)*.Mangrove restoration may involve, restoration of or improvement of hydrological conditions, the replacement/enrichment of nutrient poor sediment with that of higher organic content as well as the use of hardy nursery grown seedlings to compensate for low natural recruitment. *(Ellison, Felson, and Friess 2020; Kodikara et al. 2017)*. Red Mangrove seedlings in particular are commonly used in restoration efforts as they are an easily propagated mangrove species and notably hardier than seedlings from other species. In 2009 Williams and Feagin, afterpersonally witnessing increased vegetative growth of the dune plants on raked *Sargassum*s wrack, found that collected and unwashed*Sargassum* biomass, administered in various ways,positively increased the growth of sand dune plants*(Williams and Feagin 2010)*.The team concluded that the use of*Sargassum* fertilizer could feasibly be used in the improvement and restoration of sand dunes impacted by both natural and anthropogenic impacts and was"a *positive, natural and efficient methodof dealing with the accumulation of wrack on the beach*"*(Williams and Feagin 2010)*.In 2022, following up on the Williams and Feagin'sexperiments and others regarding the use of *Sargassum*as fertilizerand mangrove restoration*(Thompson, et. al 2020)*, research was conducted by a team at the Discovery Bay Marine Lab (DBML) in St. Ann Jamaica, led by Mr. Camillo Trench, into the impact of *Sargassum* compost(SC) on *R. mangle*seedlings over forty (40) weeks. The team treated the seedlings with varying concentrations of prepared SC (0, 25, 50, 100%) and grew the seedlingsunder "wet"nursery (which simulated natural waterlogged mangrove conditions with diurnal tidal water levels with normal seawater or brackish water salinity)or "dry" nursery (no "tidal" fluctuations and twice daily watering)conditions and concentrations of prepared SC*(Trench et al. 2022)*. The team found that the plants did poorly under "wet" nursery conditions with plants treated with 50% or more concentrations of SC experiencing 90-100% mortality after 6 weeks. All seedlings grown under dry conditions survived to the end of the experiment with those grown in 75% SC treatment showing significantly increased height and number of leaves when compared to the other treatments and the control treatment (0% SC). Under dry conditions the poorest seedling growth was seen in control (0% SC) and the Pure (100% SC). The poor growth of the 100% SC seedlings was attributed to high concentrations of concern elements Sodium (Na) and Arsenic (As), both elements that *Sargassum* is known to have in high concentrations,in the roots of the seedlings which was the highest of any of the treatment methods. Despite this, it was also found through elemental analysis that the plants (roots or leaves)SC had minimal concentrations of the six (6) elements (Na, Mg, K, Ca, As,and Se) which were found in high concentrations in the SC. The roots of the Pure SC seedlings only had 11.9% and 21.5% respectively of the Arsenic and Sodium concentrations found in the SC. These findings showed that the *R. mangle* seedlingswere able to minimize the uptake of these harmful elements. The research concluded that the through the creation of compost and subsequent soil amelioration, there is the potential use, *"without proportional contamination of the ecosystem*," of"*nuisance Sargassum spp. bloomsto support mangrove restoration, leading to increased* *benefits to coastal communities being affected by the inundations,*" without ""(Trench et al. 2022).

***Issues surrounding the use of Sargassum.***

The potential benefits of *Sargassum* as fertilizer have been established in the reviewed literature(McHugh 2003; Thompson, Young, and Baroutian 2020; Addico and deGraft-Johnson 2016)However, there are several issues concerning the harvesting, treatment, storage, and general preparation of *Sargassum* before it can be used as fertilizer or for any other industry. López-Contreras, et al. (2021) explores these issues and has proposed several feasible solutions which can be adjusted for specific locations in the Caribbean. Other researches have outlined issues identified from their own research and have also suggested possible solutions. There is general agreement among the researchers that further investigations are necessary to develop and improve techniques for the handling and treatment of *Sargassum* for its use in any industry. A summary of the issues and proposed solutions are listed below:

1. *The unpredictable supply of Sargassum biomass for use.*

The unpredictability of *Sargassum* events in terms of quantities, locations and appearance time greatly complicates the establishment of sustainable long-term *Sargassum* based industries *(López-Contreras, et al. 2021)*. López-Contreras and other scientists suggest that *Sargassum*should be used not as the primary source of organic biomass for industry but instead should be used to supplement existing industries which use a more stable supply of organic matter *(CARDI 2015; López-Contreras et al. 2021; United Nations Environment Programme- Caribbean Environment Programme 2021).*For example available *Sargassum* could be co-processed at existing composting facilities which use food waste or agricultural waste. This reduces the reliance on *Sargassum* and allows the industry to continue during periods where the *Sargassum* is in short supply.

2. *Harvesting and Storage.*

The unique physio-chemical properties of *Sargassum* and the locations where it appears create several difficulties for harvesting and storage. Webber, et al. (2019) stated that one of the greatest challenges for the difficulties of Small Island Developing States (SIDS) like Jamaica is harvesting large quantities of *Sargassum* for use without damaging the environment/species. The use of heavy machinery to harvest*Sargassum* along beaches causes compactions which can kill sand dwelling organisms, crush turtle nests, or remove large quantities of sand from the beach, negatively affecting the overall beach ecosystem *(United Nations Environment Programme- Caribbean Environment Programme 2021; Webber and Aiken 2018)*. Additionally, when out of the water *Sargassum*mats, depending on storage conditions, decay quickly releasing toxic hydrogen-sulphide gas and other heavy metal pollutants. This decay reduces its potential for future use and can introduce harmful chemicals into the immediate environment *(López-Contreras, et al. 2021)*. It has also been argued that pelagic *Sargassum*rafts should not be harvested as they serve as essential habitats for a wide range of organisms and its removal can disrupt recruitment of various commercially and ecologically important marine species *(Webber and Aiken 2018)*. Therefore, it has been suggested by Webber, López-Contreras and UNEP-CEP that the harvesting nearshore *Sargassum (*located less than 1km from shore) is the most suitable option. This option prevents nearshore *Sargassum*, which is most likely to be stranded, from reaching the coast thus minimizing their environmental impact. Furthermore, these nearshore mats would have less associated fauna than their pelagic counterparts due to proximity to shore and high possibility of stranding if not harvested. It is also agreed that additional research is needed to develop appropriate technologies to predict when and where the mats are likely to come ashore and improve existing technologies for the harvesting and storage of *Sargassum.*

3. *Preparation.*

Before it can be used harvested *Sargassum*must be suitably prepared. The level of preparation required depends on the intended use of the harvested *Sargassum*. Generally, preparation involves the removal of plastics and other undesired components in the biomass such as wildlife. As highlighted by McHugh (2003) there are various preparation methods for the use of *Sargassum*as fertilizer ranging from minimal preparation (such as sun drying) to more intensive preparations involving complex chemical treatment. The level and overall effectiveness of these preparations varies by location and requires further investigation. Chemical analyses of the harvested *Sargassum*should play a major role in determining the use of any harvested *Sargassum* and the type and level of treatment required in itspreparation*(Tonon et al. 2022)*. Chemical analyses of *Sargassum*have high shown that the harvested mats can have high concentrations of salt, nutrients, and heavy metal elements. During decay or treatment of harvested *Sargassum* these compounds may leach into the environment where they can cause issues with nearby flora and fauna or contribute to nutrient loading in nearby bodies of water*(Webber and Aiken 2018)*. As such determining the concentration of potential toxic compounds within harvested *Sargassum* is very important to evaluate potential risks and create good practices for treatment *(López-Contreras, et al. 2021).* A comparison of the findings of Webber et al (2019)and Trench et al (2022)to the findings of Addico and deGraft-Johnson shows how varied the levels of heavy metals can be in harvested *Sargassum.* Addico et al.analysed samples of invasive *S. natans and S. fluitans*found off the Western coast of Ghana in 2016 and found that six (6) of the seven (7) heavy metals investigated (Copper, Iron, Lead, Zinc, Cadmium, Mercury, Arsenic and Chloride) were present in concentrations above their established toxic limit and as such would cause life threatening illnesses if introduced to human systems without significant treatment to remove these toxic compounds. Addico attributed these high levels of metals to local marine pollution from dyes, certain fertilizers, metals, pesticides, and industrial wastes from in-land sources and emphasized*Sargassum*'s ability to absorb toxic compounds from the surrounding environment in a relatively short time. By comparison the*Sargassum* sampled by Webber and Trench had concentrations of heavy metals under established toxic limits and would be relatively safe for use with lower levels of treatment required. This difference in results illustrates the importance of determining the chemical composition of harvested *Sargassum* in the selection of suitable treatment measures for its use.

The research conducted by Trench et al (2022) has shown that Red Mangrove seedlings are more resistant to the harmful elements present in

*Sargassum* compost. However even these plants were not immune to the negative effects under higher concentrations of SC. Given the variability*Sargassum*and its possessed concentrations of harmful elements, it must be reiterated that its use should be clearly monitored and researched.

# MATERIALS AND METHODS

## *Equipment List*

The following equipment were used during the course of this project:

- Collection net
- Safety work gloves
- Four (4) 5-gallon buckets
- Mangrove nursery sand
- 0.2L of gravel
- Forty (40) seedling bags
- Red mangrove seedlings (40)
- Two (2) 30-centimeter rulers
- One (1) SnapGate10-inch measuring tape
- Three (3) opaque storage bins (for storage of completed compost)
- 2.0 Gallon watering pail
- One (1) Taylor soil thermometer
- One (1) Kelway Soil Tester
- Four (4) rectangular plastic crates

## *Software Used*

- IBM SBSS Statistics Version 26
- Microsoft Excel for Microsoft 365 MSO (Version 2306 Build 16.0.16529.20100) 32-bit.

- Microsoft Office for Microsoft 365 MSO (Version 2306 Build 16.0.16529.20100) 32-bit.

***Compost Production***

Unwashed *Sargassumsp* biomass was harvested from three (3) different areas of the Palisadoes-Port Royal coastline inKingston, Jamaica in late October 2022. The harvested *Sargassum* was classified as follows:

a. “Golden” *Sargassum*which was collected from nearshore waters, prior to shoreline beaching, using a collection net.
b. “Brown” *Sargassum,*which was beached for between 1-2 days wascollected by hand from the foreshore.
c. “Black”*Sargassum*, beached on a shorelinefor more than 2 weeks, was collected by hand from the backshore.

Each *Sargassum*biomass sample had all non-*Sargassum* plants and debris removed and discardedbefore being placed in separate largeplastic bags. These bags were then stored unsecured, allowing air through but not rainwater, and placed into labelledplastic crates for ease of transport. These crates were stored at the UWI PRML,between the wet lab building and property boundary wall, forthree (3) months. During this time the bags were periodically “turned” and to effect mixing. Each bag was also lightly moistened with unaltered sea water, collected from the lab shoreline, to prevent dehydration of sample. ***(See Plate 1)***

***Plate 1: View of Collected Sargassum at the start of the Experiment October 2022.***
Golden *Sargassum* (Left), Brown *Sargassum* (Middle), Black *Sargassum*(Right),

In February of 2023, approximately three (3) months from the start of composting, each compost was collected and mixed with mangrove nursery sand obtained from the UWI PRML to create three (3) treatment compost soil mixtures with a75:25 ratio of SC to mangrove sand. A fourth control mixture was also created using only mangrove sand.

***Propagule Establishment***

During the composting period, forty (40) identical Red Mangrove seedlings were soaked in fresh water until roots emerged. Following this, ten (10) seedling bags were prepared for each compost treatment soil mixture and left for one (1) week to settle. After the passage of this week any growing vegetation was removed and discarded and one (1) mangrove seedlingwas placed into eachbag ensuring that the roots of the seedlings were adequately covered by the compost soil. The bags were labelled according to the type of compost they contained. Approximately 0.2 L of gravel, also acquired from UWI PRML, was added to top of each bag above the mangrove soil for bag stability.The seedlings were randomly placed in two (2) plastic crates for ease of transport,with twenty (20) seedlings per crate***(See Plate 2)***. These seedling crates were then placed in alow traffic area at UWI PRMLensuring that they were not in direct sunlight for the entirety of the day.The crates were also covered with 50% shade cloth to provide additional sunlight protection and were also elevated using cement blocks to allow for proper seedling bag drainage.All seedlings were watered daily with approximately 0.05gallons of fresh water each.

The seedling crates were moved to a private residence at Harbour View, Kingston at Week 6 (26 March 2023). The crates were placed in a similar low traffic and partially covered conditions.

***Plate 2: Views of Randomly Placed Mangrove Seedlings with Gravel in Plastic Crates.***

***Monitoring***

Weekly measurements of the seedlings, starting on the 15th of February 2023, were made for the first ten (10) weeks after initial planting. After the passage of ten (10) weeks the seedlings were monitored every three (3) weeks. The following parameters were recorded during each recording session:

- Height (cm), measured from the uppermost apical shoot of the seedling down to soil level.
- Number of nodes on the main stem, not including the nodes on the branching stems.
- Number of leaves, including all leaves on branching stems.
- Health (using the following health index)

    0- Plant appears dead - no green areas present on stem; leaves may still be attached but are entirely dried and withered.

    1-Surviving (plant has green material) but has no leaves.

    2-Surviving, plant possesses only few leaves (withered and pale in colour).

    3- Moderate health, leaves are present but are pale and withered.

    4-Good health, leaves are not quite fully green but still succulent (not thin or curled).

    5-Best health possible, leaves are fully green and succulent.

- pH
- Soil temperature (°C)
- Soil Moisture Percent (%)

Dead seedlings,that is those which had a recorded health of 0 for two (2) consecutive recordings, were removed from the analysis.

***Statistical Tests***

The Statistical tests were conducted on the recorded data using SPSS Version 26 to determine if there was a significant difference in plant parameters across the different treatments. A significance (*p*) value less than 0.05 was accepted as significant for all parameters tested. Normality was tested using the Shapiro - Wilks analysis for all parameters. Following this test, it was found that normality was not achieved amongst all the parameters ($p<0.000$ for all parameters) as such the non-parametric Kruskal-Wallis Test was used to assess the significance between the compost treatment methods and the measured parameters (seedling height, number of nodes, number of leaves, health, soil pH, soil temperature, and soil moisture percentage). Pairwise post-hoc tests were also carried out to determine which SC treatment methods were significantly different from each other. The non-parametric Spearman's Ranked Correlation test was also used to determine if the significance and strength of relationships of the measured parameters. The non-parametric Wilcoxon Signed-Rank Test was also used to determine if there was any significant difference between the initial and final soil parameters (Soil temperature, pH, and soil moisture percentage) for all compost treatments.

# RESULTS

***Statistical Tests***

A Shapiro-Wilk Tests of Normality found that all the measured parameters were not normally distributed (*p* = <0.000 in all cases). Given this, the following non-parametric tests were conducted to determine significant differences in the data; the Kruskal-Wallis Test (***Table 1)***,A Spearman's Ranked Correlation (***Table 2)*** and Wilcoxon Signed-Rank Test (***Table 3).*** Details of the tests are as follows.

*Analysis of Variance - Kruskal-Wallis Test*

The Kruskal-Wallis Testconducted with the recorded data found that there were statistically significant differences in seedling height, number of nodes, number of leaves,soil pH,seedling health (*p* = < 0.000 in all cases) and moisture (*p* =0.001), between the different SC compost treatments. It was also found that there were no significant differences in soil temperature between the different SC compost treatments (*p* =0.055), ***See***

***Table*** 1

**Test Statistics**[a,b]

| | Seedling Heights (cm) | Number of nodes | Number of leaves | Soil pH | Moisture Percentage (%) | Soil Temperature (°C) | Health index (0-5) |
|---|---|---|---|---|---|---|---|
| Kruskal-Wallis H | 136.512 | 176.627 | 165.360 | 25.865 | 16.909 | 7.585 | 116.680 |
| df | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Asymp. Sig. | .000 | .000 | .000 | .000 | .001 | .055 | .000 |

a. Kruskal Wallis Test

b. Grouping Variable: Sargassum Compost (SC) Treatment

***Table 1: Kruskal-Wallis Test Results***

Pairwise post-hoc tests were conducted on the parameters which showed significant differences to determine which SC treatment pairs were significantly different from each other. From these teststhe following observations were made with regards to the measured parameters:

***NB**** Please note that the Boxplot diagrams below show the Median value of the measured parameter (bar), the 25-75% interquartile range (box), non-outlier range (whisker) and outlier values (dots)

*Seedling Height*

Golden SC, and Sand (No SC) were not significantly different from each other (p=1.000). All other SC treatments were significantly different from each other (p = < 0.000 in all cases).RedMangrove seedlings treated with the control (no SC sand) had the highest mean height among the treatment methods of 43.178cm. This was followed by the Golden SC seedlings which had a mean height of 42.75cm, Brown SC seedlings with a mean height of 39.327cm and Black SC seedling with a mean of 36.195cm. The Golden SC seedlings however had the tallest individual seedling height of 52.5cm followed by Sand (No SC) seedling with a height of 51.2cm. Black SC seedlings had the shortest recorded height of 29.8cm. (***SeeFigure 1).***

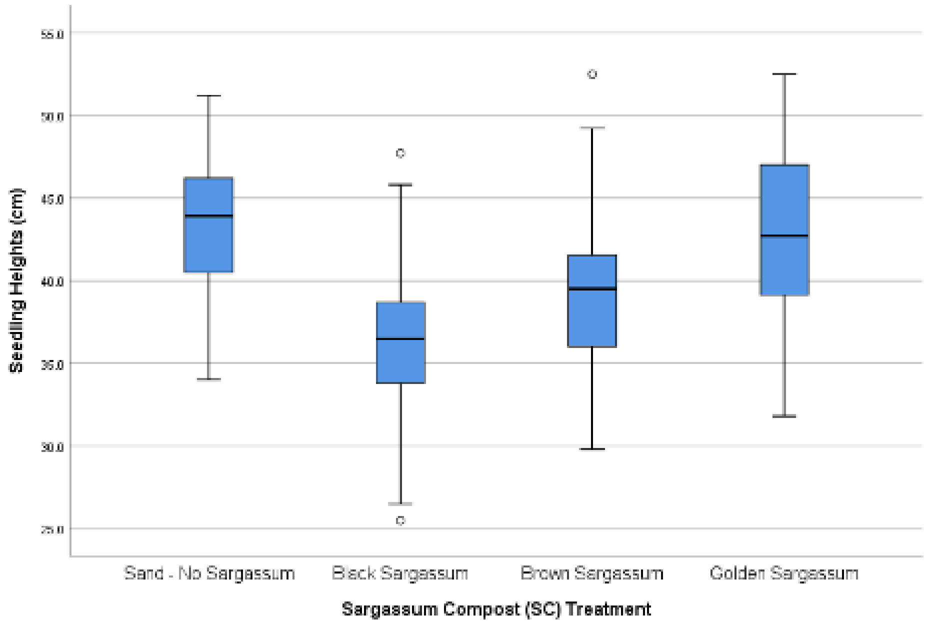


***Figure 1: Box Plot Diagram of Seedling Heights (cm) across SC Treatments***

*Number of Nodes*

Golden SC and Sand treated seedlings were the only pairs which were not significantly different from each other (*p* =0.392). All other SC treatments showed significant differences (*p = <0.000 to 0.002).* Golden SC seedlings were found to have the highest mean number of nodes of 3.39 nodes. Black SC seedlings had the lowest mean number of nodes at 2.21 nodes. Golden SC seedlings also had the highest number of individual nodes of eight (8) nodes. Black SC seedlings had the lowest number of nodes at one (1) node. (***See* Error! Reference source not found.*)***

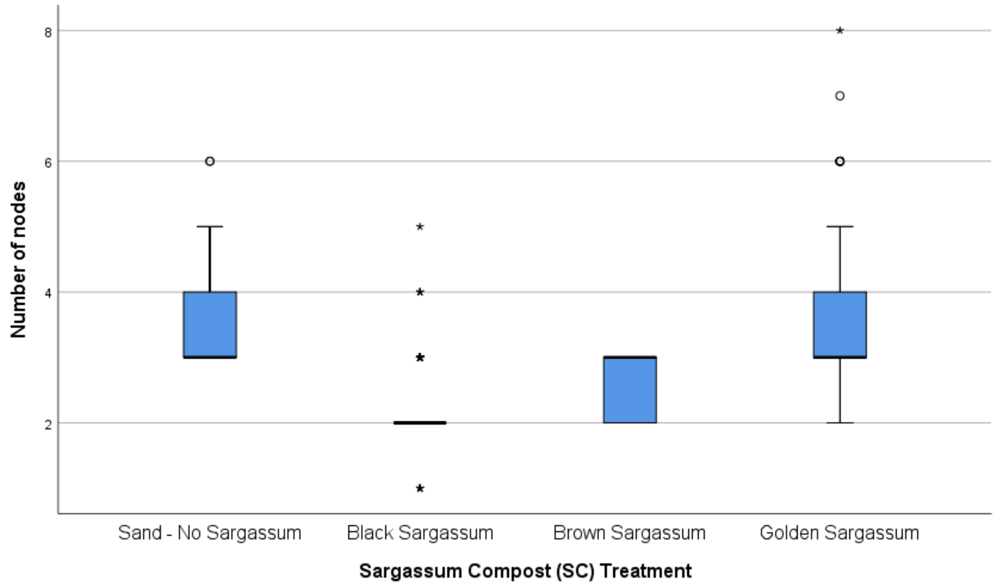


***Figure 2 Box Plot Diagram of Number of Nodes across SC Treatments***

*Number of Leaves*

Brown SC and Black SC were not significantly different from each other (p=1.000). All other SC treatments were significantly different from each other (p = 0.009 for Gold SC to Sand and p = < 0.000 in all other cases). Sand seedlings were found to have the highest mean number of leaves of 3.65 leaves, followed by the Golden SC seedlings which had a mean of 2.94 leaves. Brown SC seedlings had the lowest mean number of leaves of 0.74 leaves followed by Black SC seedlings with 0.85 leaves. Golden SC seedlings had the highest number of leaves on any individual plant of eleven (11) leaves, while all treatments had at least one (1) plant with zero (0) leaves. (***See Figure 3)***

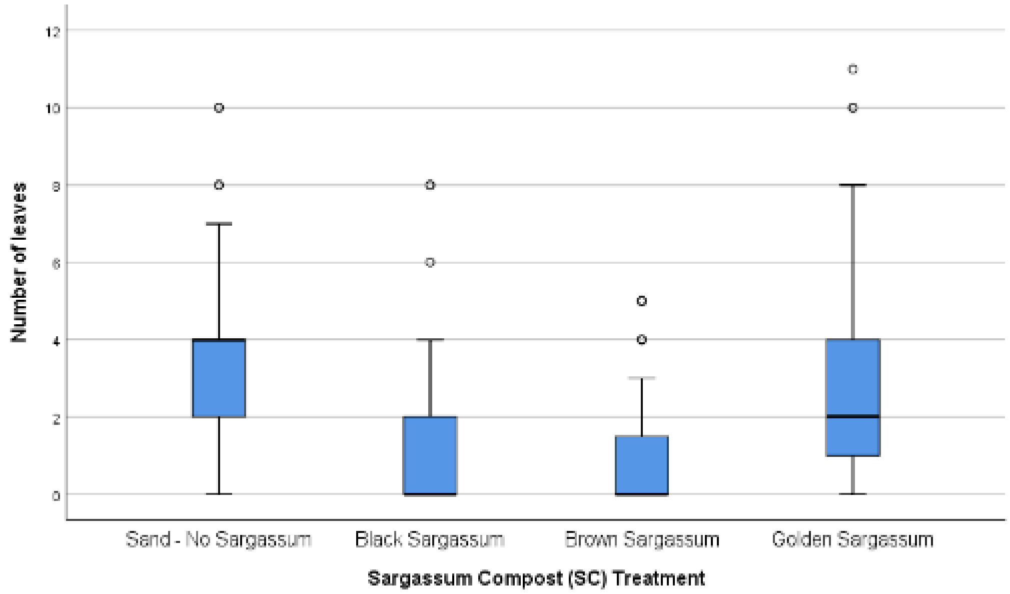


***Figure 3:Box Plot Diagram of Number of leavesacrossSCTreatments***

*Soil pH*

Golden SC and Brown SC as well as Sand (No SC) and Black SC were not significantly different from each other (p=1.000 in both cases). All other SC treatments were significantly different from each other (p = 0.003 to 0.028 in all other cases.***Figure 4*** below shows that none of the SC treatments had a pH exceeding 7 following planting of the red mangrove seedlings. Black SC soil had the highest average pH of 6.53 while Brown SC had the lowest average pH of 6.26. Brown SC soil also had the lowest recorded pH of 4.1.

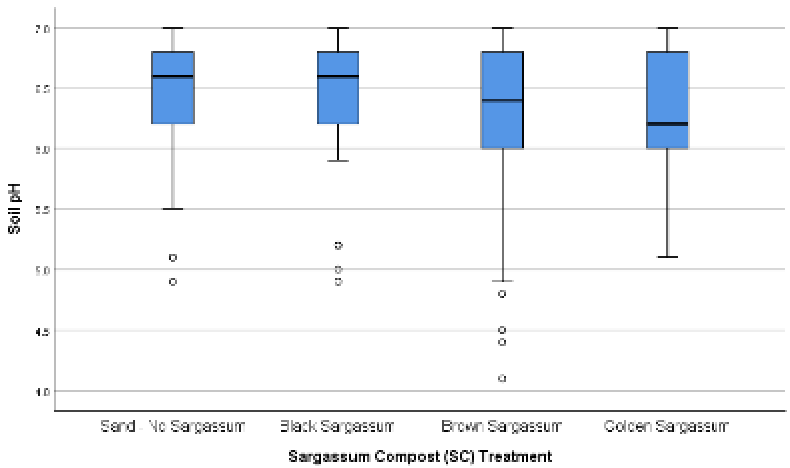


***Figure 4: Box Plot Diagram of Soil pH across SCTreatments***

*Health Index*

Brown SC and Black SC as well as Golden SC and Sand (No SC) were not significantly different from each other (p=0.802 and 1.000 respectively). All other SC treatments were significantly different from each other (p = < 0.000 in all other cases). Golden SC seedlings had the highest mean health of 3.51, followed by the Sand Seedlings with a mean health of 3.40. Both of these values fall within the "Moderate Health" category of the defined health Index. Black and Brown SC seedlings have mean health of 1.5 and 1.14 respectively which fall into the "Surviving but poor health" category of the index. All plants observed had at least one (1) individual seedling which fell into the "Best Health" health index (Category 5) and at least one (1) individual seedling which fell into "Appears Dead" health index (Category 0) at some point during the recording period. ***(See Figure 5).*** Of the plants observed, no plant which received a health score of 0 for two (2) consecutive weeks recovered and survived to the end of the monitoring period.

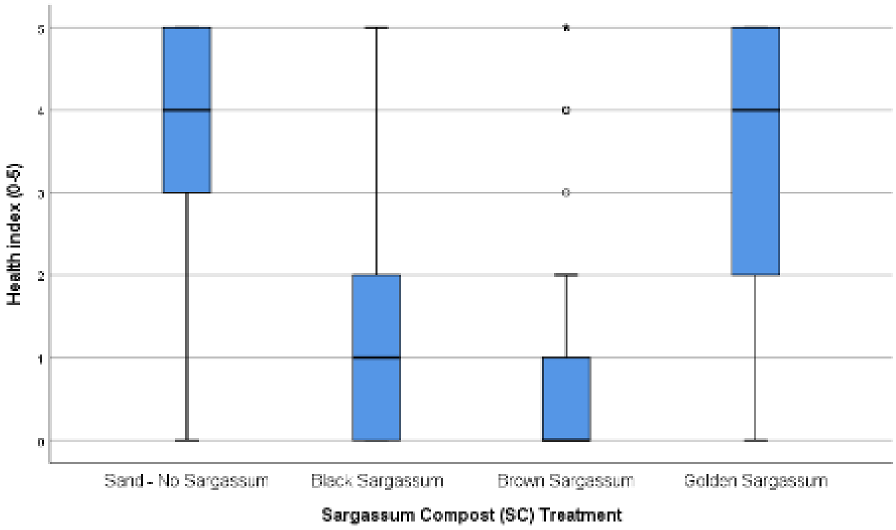


***Figure 5:Box Plot Diagram of Seedling Health Index across SCTreatments***

When the frequency of health index recordings was analysed, it was found that both the Golden SC and Sand Seedlings had the most recorded instances of "Best Health" with forty (40) recorded instances, while the Golden SC seedlings had the most instances of "Good Health" (Category 4) with fifty (50) recorded instances. Sand Seedlings also had the highest recorded instances of "Moderate Health" (Category 3) with eleven (11) instances ***(Figure 6)***. Black SC Seedlings had the most instances of "Surviving" health and Brown SC seedlings had the most "Appears Dead" instances with forty (48) instances.

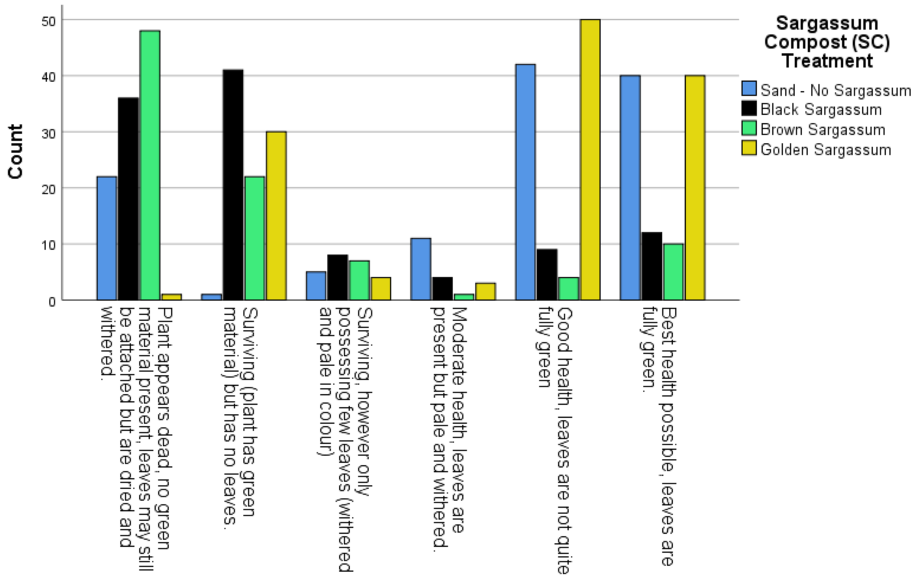


***Figure 6: Frequency Histogram of Seedling Health Index across SCTreatments***

*Moisture*

Black SC and Golden SC, as well as Sand and Golden SC were significantly different from each other (p = 0.004 and 0.005). All other compost treatments were not significantly different from each other (p= 0.175 to 1.000).As shown in ***Figure 7***, all treatment soils recorded at least one instance of 100%moisture however only soil treated with Black SC had a recorded instance of 0% moisture. Golden SC soil had the highest mean moisture of 86.22% while Sand soil had the lowest mean moisture of 77.79%

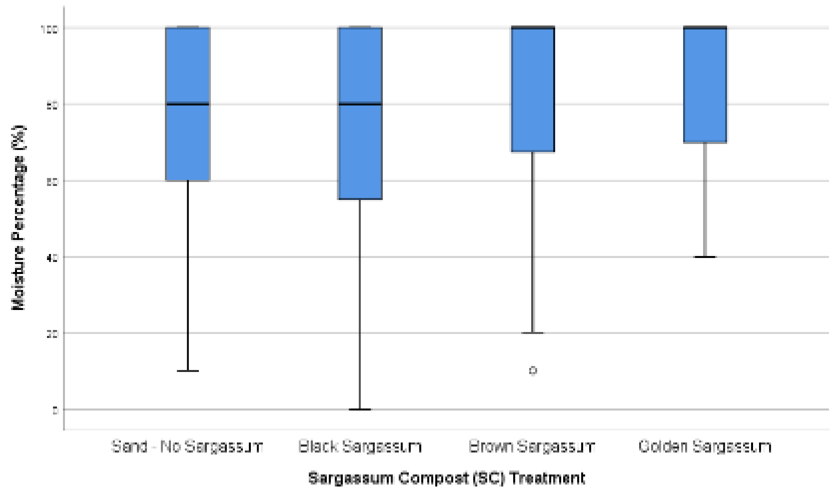


***Figure 7:Box Plot Diagram of Soil Moisture Percentage (%) across SCTreatments***

*<u>Test for Correlation</u>*

A Spearman's Ranked Correlation test was undertaken. The correlation coefficient ($r_s$) was used to determine the strength and direction of any significant correlations between parameters ***See Table 2***. The following results were obtained:

- SC treatment showed significant negative correlation with the number of leaves (*p=0.003,* $r_s$*=-0.025),* soil pH (*p=0.000,* $r_s$= -0.205) and significant positive correlation with moisture percentage (*p=<0.000,* $r_s$= 0.180). There was no significant correlation with all other parameters.

- Seedlings heights showed significant positive correlation with the number of nodes, number of leaves, health index, moisture percentage and soil temperature (*p=<0.000 to 0.008,* $r_s$= 0.116 to 0.628). Seedling height showed significant negative correlation with soil pH (*p=0.000,* $r_s$= -0.157). Seedlings heights did not significantly correlate to the SC treatment methods.
- Seedling Health Index showed significant positive correlation with the number of nodes, number of leaves, moisture percentage (*p=0.000 to 0.016,* $r_s$= 0.113 to 0.521) and significant negative correlation with soil pH (*p=<0.000,* $r_s$= -0.188)

**Correlations**

| | | | Sargassum Compost (SC) Treatment | Seedling Heights (cm) | Number of nodes | Number of leaves | Health index (0-5) | Soil pH | Moisture Percentage (%) | Soil Temperature (°C) |
|---|---|---|---|---|---|---|---|---|---|---|
| Spearman's rho | Sargassum Compost (SC) Treatment | Correlation Coefficient | 1.000 | .022 | -.025 | -.138** | .031 | -.205** | .180** | -.011 |
| | | Sig. (2-tailed) | . | .636 | .596 | .003 | .513 | .000 | .000 | .812 |
| | | N | 520 | 451 | 450 | 450 | 451 | 451 | 450 | 451 |
| | Seedling Heights (cm) | Correlation Coefficient | .022 | 1.000 | .628** | .600** | .521** | -.157** | .125** | .116* |
| | | Sig. (2-tailed) | .636 | . | .000 | .000 | .000 | .001 | .008 | .014 |
| | | N | 451 | 451 | 450 | 450 | 451 | 451 | 450 | 451 |
| | Number of nodes | Correlation Coefficient | -.025 | .628** | 1.000 | .715** | .519** | -.017 | -.022 | .252** |
| | | Sig. (2-tailed) | .596 | .000 | . | .000 | .000 | .711 | .648 | .000 |
| | | N | 450 | 450 | 450 | 450 | 450 | 450 | 449 | 450 |
| | Number of leaves | Correlation Coefficient | -.138** | .600** | .715** | 1.000 | .704** | -.125** | .078 | .185** |
| | | Sig. (2-tailed) | .003 | .000 | .000 | . | .000 | .008 | .101 | .000 |
| | | N | 450 | 450 | 450 | 450 | 450 | 450 | 449 | 450 |
| | Health index (0-5) | Correlation Coefficient | .031 | .521** | .519** | .704** | 1.000 | -.188** | .113* | .016 |
| | | Sig. (2-tailed) | .513 | .000 | .000 | .000 | . | .000 | .016 | .728 |
| | | N | 451 | 451 | 450 | 450 | 451 | 451 | 450 | 451 |
| | Soil pH | Correlation Coefficient | -.205** | -.157** | -.017 | -.125** | -.188** | 1.000 | -.529** | .425** |
| | | Sig. (2-tailed) | .000 | .001 | .711 | .008 | .000 | . | .000 | .000 |
| | | N | 451 | 451 | 450 | 450 | 451 | 451 | 450 | 451 |
| | Moisture Percentage (%) | Correlation Coefficient | .180** | .125** | -.022 | .078 | .113* | -.529** | 1.000 | -.274** |
| | | Sig. (2-tailed) | .000 | .008 | .648 | .101 | .016 | .000 | . | .000 |
| | | N | 450 | 450 | 449 | 449 | 450 | 450 | 450 | 450 |
| | Soil Temperature (°C) | Correlation Coefficient | -.011 | .116* | .252** | .185** | .016 | .425** | -.274** | 1.000 |
| | | Sig. (2-tailed) | .812 | .014 | .000 | .000 | .728 | .000 | .000 | . |
| | | N | 451 | 451 | 450 | 450 | 451 | 451 | 450 | 451 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

***Table 2: Spearman's Ranked Correlation Test***

*Wilcoxon Signed-Rank Test for Soil Parameters*

A Wilcoxon Signed-Rank Test (***Table 3)*** was used to determine if there were any significant differences between the initial and final soil parameters (Soil temperature, pH, and soil moisture percentage) for all compost treatments***(See Table 4).*** The test showed that overall, there was a significant difference only in soil pH for all SC treatments. ($p$= 0.002). Further testing showed that the significance was due to the difference between the final and initial pH of Golden SC.

**Test Statistics[a]**

| | Final Soil pH - Initial Soil pH | Final Moisture Percentage (%) - Initial Moisture Percentage (%) | Final Soil Temperature (°C) - Initial Soil Temperature (°C) |
|---|---|---|---|
| Z | -3.064[b] | -1.203[c] | -.473[c] |
| Asymp. Sig. (2-tailed) | .002 | .229 | .636 |

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

c. Based on positive ranks.

***Table 3:Wilcoxon Signed-Rank Test for Initial and Final Soil Parameters***

| Parameter | INITIAL AVERAGES | | | | FINAL AVERAGES | | | |
|---|---|---|---|---|---|---|---|---|
| | Sand | Black | Brown | Gold | Sand | Black | Brown | Gold |
| Temperature | 28.00 | 28.67 | 27.67 | 27.33 | 26.30 | 26.20 | 25.80 | 33.40 |
| pH | 5.73 | 6.00 | 5.27 | 3.87 | 6.40 | 6.70 | 6.58 | 6.47 |
| % moisture | 46.00 | 58.33 | 100.00 | 100.00 | 76.50 | 54.50 | 76.00 | 84.50 |

***Table 4: Average Physicochemical soil parameters prior to Seedling Placement (February 2023) and at end of Monitoring Period (July 2023)***

As is seen ***Table 4***above, at the start of the experiment, Black SC soil had highest average temperature (28.7°C) as well as the highest (most basic) average pH (6.0) when compared to the other soil treatments. Browm SC and Golden SC soils had the highest moisture percentage.

At the end of the experiment, Golden SC soil had the highest average moisture (84.5%), and temperature (33.4°C) while Black SC soil remained the most basic soil with a pH (6.7) when compared to the other soil treatments. Browm SC soil had the lowest temperature, Sand soil had the lowest pH and Black SC had the lowest moisture percentage.

### *Survival Rate*

At the end of the monitoring period Golden SC seedlings had the highest survival rate of 80%, followed by the control seedlings with a survival rate of 70%. Brown SC seedlings had the lowest survival rate of 0% and Black seedlings had a survival rate of 20% by the end of the monitoring period. (***SeeTable 5)***

| SC Treatments | Number of Mangrove Seedlings remaining alive in each treatment per week | | | | | | | | | | | | | % Survival |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 14 | Week 17 | Week 21 | |
| Sand- No Sargassum | 10 | 10 | 9 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | **70%** |
| Black Sargassum | 10 | 10 | 10 | 8 | 6 | 5 | 4 | 4 | 5 | 4 | 3 | 3 | 2 | **20%** |
| Brown Sargassum | 10 | 10 | 7 | 6 | 3 | 2 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | **0%** |
| Golden Sargassum | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 8 | **80%** |

***Table 5: Mangrove Seedling Survival Rate (%) across Monitoring Period.***

***Other Observations***

<u>*Compost Production*</u>

For the purpose of this project the collected *Sargassum* was classified as “Compost” when it had decomposed to the point where it was moist, malleable crumbling and had lost most of its vegetative fibrousness,resemblingdark brown soil or organic humus.Approximately one (1) month after the start of composting, the harvested Golden *Sargassum*had reached an advanced state of decomposition, losing most of its vegetativefibrousness and greatly resembled dark brown soil***(See Plate 3***). At the end of the composting periodapproximately three (3) months after collection, the following was observed ***(See Plate 4)***:

- Golden*Sargassum*was noticeably darker in colour and was no longer fibrous.
- Brown *Sargassum* had reached the desired “compost” stage of decomposition similar to that of Golden *Sargassum*.
- Black *Sargassum*was noticeably less decomposed that the other samples and retained much of itsoriginal fibrousness.This *Sargassum* achieved decomposition similar to the other samples in July of 2023 approximately nine (9) months after initial harvesting.

***Plate 3: Views of Golden Sargassum One (1) Month into Composting (November 2022)***

***Plate 4: Views of Sargassum at the end of Three (3) Month Composting Period, February 2023***
*Golden Sargassum (Left), BrownSargassum (Middle), BlackSargassum(Right),*

<u>*Non-Target Seedlings*</u>

Three (3) types of seedlings were found in the potting soil during the monitoring period. These were named Seedlings 1-3 (S1- S3). S1 was identified as a Seagrape seedling (*Coccolobauvifera*). S2 plants were identified as a succulent seedling spread from a nearby parent plant. S3 plants could not be identified.***(See Plate 5 -Plate 7)***

*Plate 5: S1 Seedlings (Sea grape)*

*Plate 6: Unidentified S2 Seedlings*

*Plate 7:Unidenfied S3 Seedlings and Seedling Parent Plant (Right)*

*Predation*

During the first two (2) weeks of the experiment, three (3) plants, BlackSCSeedlings #4 and #5 (BLK4 and BLK5) and Golden SC Seedling #3 (GLD 3),showed herbivory damage at the apex of BLK 4 and GLD 3 and along the stem of BLK 5) ***(See Plate 8).*** This damage occurred while the plants were at the PRML. Given the presence of crab burrows adjacent to the location of the seedlings, it is suspected that the plants were predated upon byopportunistic land crabs, namely the Black Land Crab *Gecarcinusruricola*or the Blue Land Crab*Cardisomaguanhumi*. The offending crab(s) were however unable to be captured and identified.

BLK 5 seedling did not recover from this damage and died in the weeks following the predation. BLK 4 seedling partially recovered and survived till week 17 however the plant did not produce any additional leaves or undergo any significant regrowth. GLD 3 seedling however recovered from the predation, undergoing re-growth at its damaged apex, and producing two (2) branching stems with eleven (11) leaves by the end of the monitoring period.***(See Plate 9)***

***Plate 8: Herbivory Damage at seedlings BLK 4 (left) and BLK 5 (right)***

***Plate 9: GLD 3 Seedling One Week Following Predation Incident (left) and at the end of Monitoring Period (right)***

## DISCUSSION

### *Limitations*

As previously stated, the use of *Sargassum* compost (SC) for mangrove restoration is not heavily researched; the research of Trench et al. 2022 currently stands as one of the few publisheddocuments regarding the topic. The use of *Sargassum*in various forms for agricultural purposes is however a well-researched and well-documented study. *Sargassum*, or other similar brown algae, haveproven effective in the improving the growth rate of tomatoes *(McHugh 2003)*, rice(*Sunarpi et al. 1970)*, wheat*(Beckett and Van Staden 1989)*, corn*(Webber et al. 2019)* and a number of other agricultural crops *(CARDI 2015; Thompson et al. 2020; McHugh 2003)*.The lack of direct research into the use of *Sargassum* into mangrove restoration is a notable limitation of this project. As such much of this research was influenced by research conducted by Trench et. al. Additionally, due to time constraints this research did not include an elemental analysis of the compost itself, nor the mangrove seedlings treated by the various composts. As such any theories regarding the decline in the health of seedlings treated by the various SC do not have element concentrations or other conclusive evidence to support/refute them.

This project attempted to replicate the ideal compost conditions of Trench et al which produced the most satisfactory results. However, it must be noted the differences in methodology,location, climate,*Sargassum*, and Red Mangrove seedlings used are highly likely to have affected the results. *Sargassum* in particular has been proven to have varying chemical composition depending on the location, season and oceanic conditions *(Tonon et al. 2022)*. It is also possible that the varieties of*Sargassum* harvestedfor this project in Port Royal, Kingstonare from different influx events and origin locations and as such experienced different oceanic conditions which led to differing characteristics.

***Compost Production***

It was observed during the course of this project that the rate of controlled decomposition of *Sargassum*biomass into useable compostrelates directly to the area it was harvested from and most likely corresponds to the moisture and level of decomposition of the vegetation itself. Moisture of the chosen compost material is a critical part of the composting process and significantly effects the rate of compost decomposition *(Cooperband 2002)*. The Golden *Sargassum*sample decomposed fastest into useable compost while the Black *Sargassum*which had been beached for at least 2 weeks and most likely had the lowest internal moisture of the collected samples decomposed slowest into compost of similar consistency to the other two (2) samples.

Major issues in the use of *Sargassum* as compost include the expense and potential impacts of harvesting the seaweed, especially in cases where inundation events are extensiveand may require heavy machinery, the excessive salt and metal content of the seaweed  and the risk of the release of harmful hydrogen sulphide gas should the seaweed be left to decay on beaches. *(Abdool-Ghany et al. 2022; Tonon et al. 2022; Addico and deGraft-Johnson 2016)*. The methodology used for this project may be used to mitigate some of these issues in low magnitude influx cases. This experiment showed that viable compost was able to be produced from *Sargassum* in varying states of decomposition without the use of heavy machinery or significant treatment of the compost. Additionally, it should be noted that while the salinity of the compost was not measured for this project, other research*(Abdool-Ghany et al. 2022)* has, without any washing or significantchemical treatment of the harvested seaweed, produced effective *Sargassum* compostwith conductivity (saltiness) levels within the acceptablecompost standards outlined by the U.S Composting Council (USCC).Abdool-Ghany et al (2022) was also able to produce compost with arsenic and other metalswithin acceptable USCC and United States Environmental Protection Agency (US EPA) standards without washing or chemicaltreatment. Furthermore, Abdool-Ghany et al was able to reduce the arsenic content of the compost by mixing it with grass clippings and other vegetative mulch.Other research was also able to produce viable compost comparable to commercially available inorganic fertilizer without any washing or chemical treatment of compost *(Walsh et al. 2020)*and with as little as 20% waterper volume of harvested compost*(Muarif et al. 2022)*. The compost produced by Muarif et al. (2022) was able to be used to substitute up to 75% of commercially available inorganic fertilizer producingthe same growth and yield of shallot onions as 100% inorganic fertilizer.

These experiments show that the viable and effective SC can be produced without significant chemical treatment to reduce heavy metals or the use of large volumes of fresh water for washing to reduce salt content.Furthermore, the research of Walsh (2020) shows that the chemical composition of SC can be modified with the use of common composting materials such as wood chips, leaf litter and food waste. It should however be noted that the level of treatment required for harvested *Sargassum* depends on the intended use of the seaweed and the chemical composition of the *Sargassum* itself. For example,the concentration of arsenic (As) found and other elements in the experiment ofAbdool-Ghany et al (2022) meet industry standards for fertilizers but fall into toxic ranges for mammals*(Addico and deGraft-Johnson 2016)* and *Sargassum*used by  the would not be suitable for use as livestock feedAbdool-Ghany et al in its current form would have limited usage.

The use of Golden *Sargassum*, that is *Sargassum* harvested prior to beaching, for composting can, under the right conditions, produce viable compost within one (1) month of harvesting as was seen in this experiment. The use of recently beached Sargassum, though slower, can still be used to create viable compost in a relatively short time period. Black Sargassum, which took the longest time to become compost could reasonably still be used as composting material nine (9) months after it was harvested as it showed no obvious signs of fungal growth, insect infestation or other physical issues which would prevent its use as compost. This shows that at, least in the case of Black SC in this experiment, the long-term storage of viable SC is feasible. Further testing is still required to determine the efficacy of this compost and determine the exact length of time that the produced SC remains viable.

Given the above statements,some of the common issues typically associated with the production of viable compost using Sargassum, that is collection, preparation, and storage,can theoretically be circumvented without significant financial investment. Large magnitude influxes of the seaweed will however still require the use of heavy machinery or large amounts of manpower to collect and process. The cost of such an endeavour can be offset by the income generated from the direct sale or usage of the compost produced.The unpredictable nature of Sargassum influxes can be circumvented by the use of the seaweed as a secondary component of already established composting systems*(CARDI 2015)*. As such when the influxes do occur, they can be easily integrated into these already established systems without issue.

***Impact of SC onthe Growth of Red Mangrove Seedlings***

For this experiment the mangrove seedlings were grown in “dry” nursery conditions with a 75% SC mixture as this percentage was found by Trench et al (2022) to produce the most positive seedling growth. Golden SC treated seedlings showed the best performance of the SC treatments with the tallest, healthiest, and longest surviving plants.However, it was found that seedlings grown in the control (No SC) medium had the highest mean growth rate, and the second highest survival rate when compared to theother SC mixtures. Statistical tests showed that the heights, health, and number of nodes of seedlings treated with Golden SC and Sand (No SC) were not significantly different from each other. The number of leaves produced by the seedlings were however statistically different from in the Golden SC and Sand treatments. All other SC treatments were significantly different from each other with the Golden SC seedlings showing the highest survival rate and growth rate when compared to the other treatments. Additionally, seedling height was found to have no significant correlation with the SC treatments.

These results indicate that while Golden SC had the most positive impact on the seedlings compared to the other treatments, it was no more effective than growing the seedlings in untreated nursery sand. The Black SC and Brown SC treatments in particular worsened the health of the seedlings leading to the total mortality of the Brown SC seedlings by Week 14 and 80% mortality of the Black SC by Week 21. The Brown SC seedlings were expected to perform better than the Black SC seedlings given that Black *Sargassum* given its advanced beaching age would have already lost majority of its nutrients to natural decomposition and leaching. These results contradict those obtained by Trench et al (2022) which found that under "dry" nursery conditions, allmangrove seedlings treated with SC compost showed improved growth and height over the control seedlings; those treated with 75% SC performed best when compared to the other treatments. The difference in results is difficult to explain but may be attributed to several factors including differences in methodology, location and climate, the nature and chemical composition of the harvested *Sargassum*as well as the initial health and age of the Red Mangrove seedlings used for the experiment.

One possible explanation of the results is the elemental composition of the *Sargassum* used for compost in this experiment. *Sargassum* is known to contain harmful metalelements (Copper, Iron, Lead, Zinc, Cadmium, Mercury, Arsenic and Chloride) in concentrations varying from negligible to fatal to humans*(Addico and deGraft-Johnson 2016; Webber et al. 2019; Abdool-Ghany et al. 2022; Muarif et al. 2022)*.It has also been shown that Red Mangrove seedlings have adaptations which allow them to grow in limited concentrations of harmful metals and other chemicals while minimizing sequestration of these elements however at high enough concentrations these elements may still cause root damage leading to the poor health or death of the plants. *(Trench et al. 2022)*. Higher concentrations of arsenic or other heavy metals in the recently beached Brown *Sargassum* used for the experiment may have contributed to the observed mortality. The Black *Sargassum* used in this experiment may have lost some of these harmful chemicals through leaching or natural decomposition during the extended period that it was beached leading to a lower observed mortality in the Brown SC seedlings.

Additionally, the concentration of beneficial compounds in Sargassum, namely carbon, (C), nitrogen (N), phosphorus (P), potassium (K), sodium (Na), sulfur (S)and various plant hormones also vary based on the season, climate and oceanic conditions which the seaweed experiences prior to beaching*(Abdool-Ghany et al. 2022)*.The Sargassum used for this experiment may not have been from the same influx cohort and source location and as such may have had differing levels of the beneficial compounds.

***Impact of SC on the Soil Parameters***

SC treatments were found to have significantly affected all the tested soil parameters excluding temperature which showed no significant differences between samples. Notably as it relates to soil pH, pairwise comparisons of the samples found that the pH of soil treated with Golden SC was not significantly different from Brown SC treated soil. However, Golden SC treated soil was significantly different from Sand and Black SC treated soils. Additionally, Golden SC soil also had the lowest (most acidic) pH of 3.87 at the start of the monitoring period prior to placement of the seedlings which was found to be significantly different from its pH at the end of the monitoring period of 6.58.These differences in soil pH are most likely due to the high organic content of Golden SC. Brown SC used in the experiment most likely has organic content comparable to that of Golden SC as it also displayed significant difference when compared to Black SC and Sand treated soils. The pH of soil is known to be inversely proportional to its organic content *(Zhou et al. 2019),* that is the higher the amount of organic content present in the soil, in particular nitrates and carbon, the lower and more acidic the pH. Pelagic *Sargassum* is known to have high concentrations of macro and micro nutrients *(Tonon et al. 2022)*as such compost produced using *Sargassum*will have high organic content and be similarly rich in macro and micro nutrients *(K. T. Walsh 2019)*. The Carbon-Nitrogen ratioof produced SC is also comparable to inorganic fertilizers; said SC may be used as an organic replacement to inorganic fertilizers*(Muarif et al. 2022).*

As it relates to soil moisture content, Golden SC treated soil showed significantly different moisture when compared with Black SC soil and Sand. Golden SC and Brown SC overall showed higher and less varied soil moisture values through the experiment. During the experiment it was also found that treatment of the soil with Gold and Brown SC improved the overall water retention capabilities of the soil.

The correlation tests reinforce these findings as the SC treatments showed significant negative correlation with the number of leaves ($p=0.003$, $rs = -0.025$), soil pH ($p=0.000$, $rs = -0.205$) and significant positive correlation with moisture percentage ($p=< 0.000$, $rs = 0.180$). pH showed the strongest correlations while number of leaves showed the weakest correlation.

**CONCLUSION**

The objectives of the project were partially met. A75% *Sargassum*compost – Sand mixture was successfully created using *Sargassum* harvested from different areas (Nearshore waters, foreshore, and backshore) of the Palisadoes/Port Royal Shoreline and tested on Red Mangrove seedlings which were observed over a twenty-one (21) week period. However due to time constraints, the impact of the compost on the soil parameters (pH, moisture, and temperature) were only partially analysed as a chemical analysis of the soils was not undertaken.

Using the recorded data and subsequent statistical analyses, the following conclusions cam be made:

1. There are significant differences in the health and growth of mangrove seedlings treated with different types of *Sargassum* Compost (SC).Mangrove seedlingheight ($p = < 0.000$), number of leaves ($p = < 0.000$), and seedling health ($p = < 0.000$)weresignificantly impacted by the different types of SC. Golden SC had overall positive impacts on the growth of seedlings and the number of leaves while Black and Brown SC negatively impacted the seedlings leading to high mortality of seedling samples.Further testing showed that there were no significant differences in the seedling height, number of nodes, and health index between the Golden SC treatment, the treatment with the most positive results and highest survival rate, and the Control treatment (No SC). However, there is a significant difference in the number of leaves between the Golden SC and the control treatment which may

be attributed to the additional macronutrients provided by the compost.

2. As it relates to soil parameters(temperature, moisture, pH), there were no significant differences in soil temperature between the different SC compost treatments ($p$ =0.055), however soil pH ($p$ = < 0.000), soil moisture ($p$ =0.001) differed significantly between the treatments. Pairwise comparisons of the results showed that the differences were due to the pH of soil treated with Golden SC which was significantly different from other treated soils and significantly different from its initial and final soil pH. The low initial soil pH seen in Golden SC is most likely due to its high organic content which is inversely proportional to pH.Soils treated with Golden SC and Brown SC showed significantly improved water content than the other treatments and the control showing that these treatments may improve water retention in soil.
3. Seedling heights did not significantly correlate to the SC treatment methods showing that a weak relationship between the treatments and seedling growth.

In conclusion,the use of 75% *Sargassum* compost was not shown to significantly improve the growth or health of red mangrove seedlings when compared to seedlings grown in untreated mangrove nursery sand. However,Golden SC in particularshowed that it can be used to significantly improve leaf production of red mangrove seedlings most likely through increasing the organic content of treated soil which also improves the water retention of soil and lowers pH. Further testing is required to verify these results.

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# APPENDICES

## *Appendix A – Sample Data Table*

**Donavan Sankey Sargassum Compost Mangrove Data Sheet**

| Date: 2023 | Location: |
|---|---|
| Time: ____ to ____ | Species: Red Mangrove |
| Data collected by : D.Sankey & M. McLeod | |

| Compost Type | Plant # | Height (cm) | No. of nodes | No. of leaves | Health index (1-5) | pH | % Moisture | Soil Temperature (°C) | Other Observations (leaf, colour, animals, etc) |
|---|---|---|---|---|---|---|---|---|---|
| Sand (no Sargassum) | 1 | | | | | | | | |
| | 2 | | | | | | | | |
| | 3 | | | | | | | | |
| | 4 | | | | | | | | |
| | 5 | | | | | | | | |
| | 6 | | | | | | | | |
| | 7 | | | | | | | | |
| | 8 | | | | | | | | |
| | 9 | | | | | | | | |
| | 10 | | | | | | | | |
| Black (Beached more than 2 weeks) | 1 | | | | | | | | |
| | 2 | | | | | | | | |
| | 3 | | | | | | | | |
| | 4 | | | | | | | | |
| | 5 | | | | | | | | |
| | 6 | | | | | | | | |
| | 7 | | | | | | | | |
| | 8 | | | | | | | | |
| | 9 | | | | | | | | |
| | 10 | | | | | | | | |
| Brown (Beached 1-2 days | 1 | | | | | | | | |
| | 2 | | | | | | | | |
| | 3 | | | | | | | | |
| | 4 | | | | | | | | |
| | 5 | | | | | | | | |
| | 6 | | | | | | | | |
| | 7 | | | | | | | | |
| | 8 | | | | | | | | |
| | 9 | | | | | | | | |
| | 10 | | | | | | | | |
| Golden (In-water harvest) | 1 | | | | | | | | |
| | 2 | | | | | | | | |
| | 3 | | | | | | | | |
| | 4 | | | | | | | | |
| | 5 | | | | | | | | |
| | 6 | | | | | | | | |
| | 7 | | | | | | | | |
| | 8 | | | | | | | | |
| | 9 | | | | | | | | |
| | 10 | | | | | | | | |

**Health index**

0- Plant appears dead, no green material present, leaves may still be attached but are dried and withered.
1-Surviving (plant has green material) but has no leaves.
2-Surviving, however only possessing few leaves (withered and pale in colour).
3- Moderate health, leaves are present but pale and withered.
4- Good health, leaves are not quite fully green.
5- Best health possible, leaves are fully green.

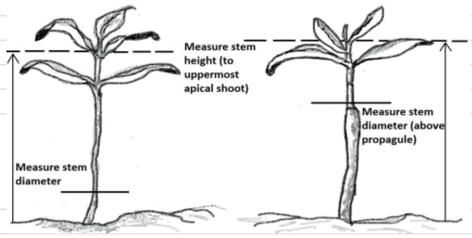


***Plate 10: – Mangrove Seedling Recording Sheet Template***

## *Appendix B–Tests for Normality and Frequency Histograms*

*Normality*

**Tests of Normality**

| | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Corrected Heights (cm) | .035 | 449 | .200* | .959 | 449 | .000 |
| No. of leaves | .209 | 449 | .000 | .849 | 449 | .000 |
| No. of nodes | .298 | 449 | .000 | .809 | 449 | .000 |
| Health index | .234 | 449 | .000 | .828 | 449 | .000 |
| % Moisture | .288 | 449 | .000 | .802 | 449 | .000 |
| Soil pH | .142 | 449 | .000 | .902 | 449 | .000 |
| Soil Temperature (°C) | .178 | 449 | .000 | .926 | 449 | .000 |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

***Table 6: Kolmogorov-Smirnov and Shapiro-Wilk Tests of Normality***

*Frequency Histograms*

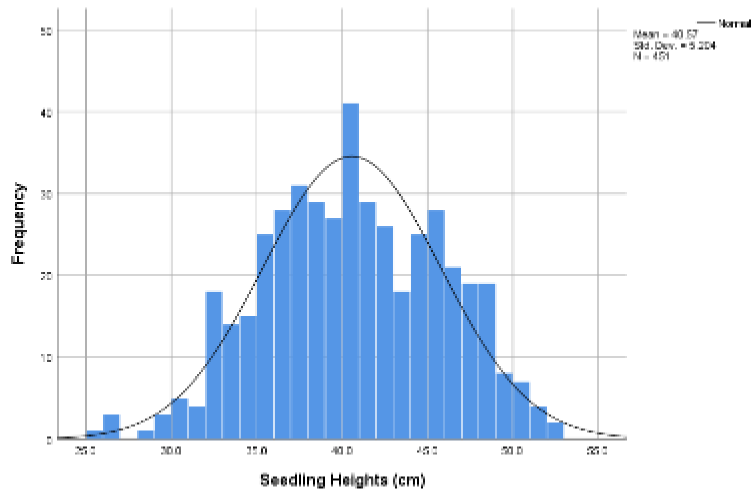


***Figure 8: Seedling Heights Frequency Histogram with Normal Distribution Curve***

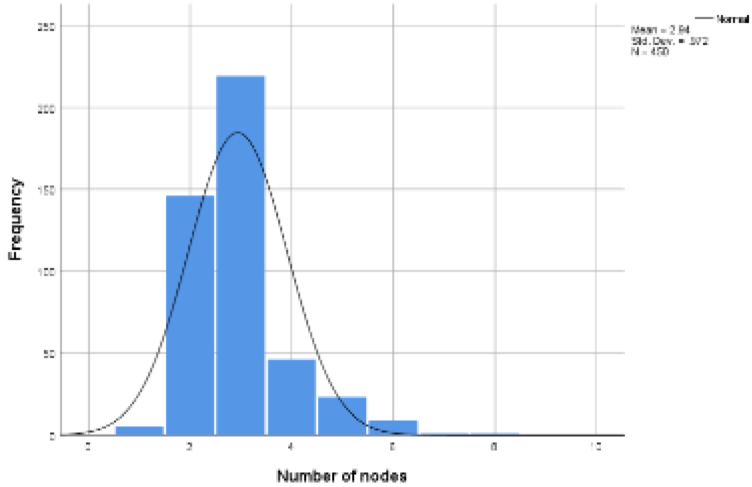


***Figure 9: Seedling Number of Nodes Frequency Histogram with Normal Distribution Curve***

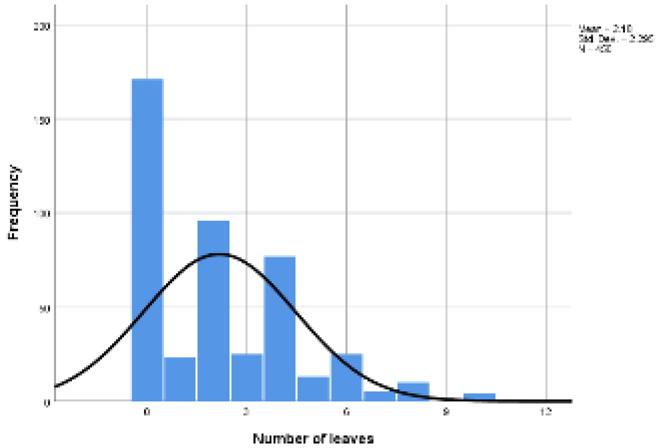


***Figure 10: Seedling Number of Leaves Frequency Histogram with Normal Distribution Curve***

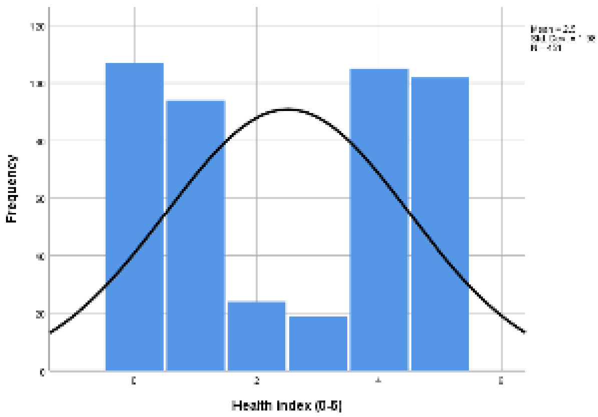


***Figure 11: Seedling Health Index Frequency Histogram with Normal Distribution Curve***

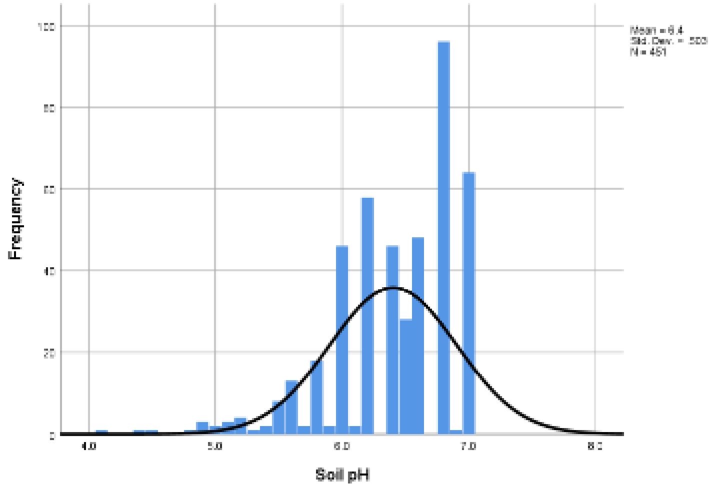


*Figure 12: Soil pH Frequency Histogram with Normal Distribution Curve*

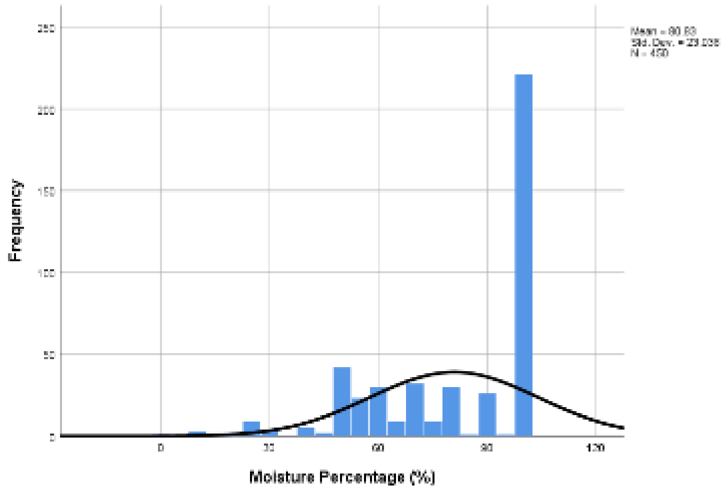


*Figure 13: Moisture Percentage (%) Frequency Histogram with Normal Distribution Curve*

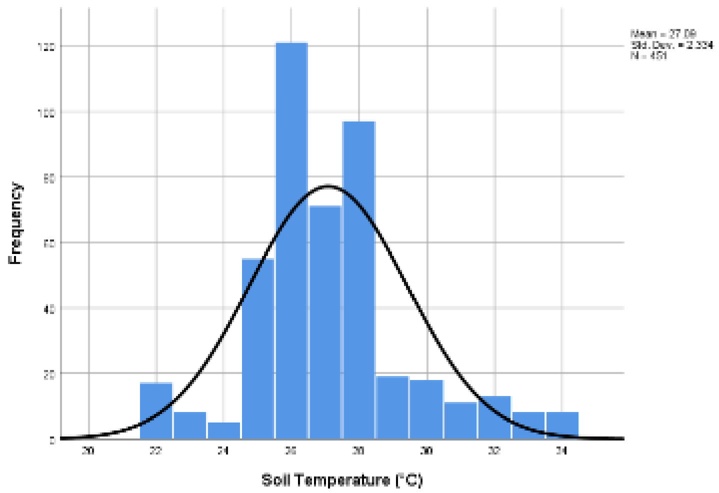


*Figure 14: Soil Temperature Frequency Histogram with Normal Distribution Curve*

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Black Sargassum-Brown Sargassum | -73.923 | 18.413 | -4.015 | .000 | .000 |
| Black Sargassum-Golden Sargassum | -161.298 | 16.945 | -9.519 | .000 | .000 |
| Black Sargassum-Sand - No Sargassum | 176.355 | 17.170 | 10.271 | .000 | .000 |
| Brown Sargassum-Golden Sargassum | -87.375 | 17.814 | -4.905 | .000 | .000 |
| Brown Sargassum-Sand - No Sargassum | 102.432 | 18.028 | 5.682 | .000 | .000 |
| Golden Sargassum-Sand - No Sargassum | 15.057 | 16.525 | .911 | .362 | 1.000 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 7: Kruskal-Wallis Pairwise Comparison of Seedling Heights***

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Black Sargassum-Brown Sargassum | -61.309 | 16.933 | -3.621 | .000 | .002 |
| Black Sargassum-Golden Sargassum | -157.976 | 15.611 | -10.120 | .000 | .000 |
| Black Sargassum-Sand - No Sargassum | 186.033 | 15.790 | 11.782 | .000 | .000 |
| Brown Sargassum-Golden Sargassum | -96.666 | 16.409 | -5.891 | .000 | .000 |
| Brown Sargassum-Sand - No Sargassum | 124.724 | 16.579 | 7.523 | .000 | .000 |
| Golden Sargassum-Sand - No Sargassum | 28.058 | 15.226 | 1.843 | .065 | .392 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 8: Kruskal-Wallis Pairwise Comparison of Seedling Number of Nodes***

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Brown Sargassum-Black Sargassum | 9.061 | 17.718 | .511 | .609 | 1.000 |
| Brown Sargassum-Golden Sargassum | -128.373 | 17.169 | -7.477 | .000 | .000 |
| Brown Sargassum-Sand - No Sargassum | 178.759 | 17.347 | 10.305 | .000 | .000 |
| Black Sargassum-Golden Sargassum | -119.312 | 16.334 | -7.304 | .000 | .000 |
| Black Sargassum-Sand - No Sargassum | 169.698 | 16.521 | 10.272 | .000 | .000 |
| Golden Sargassum-Sand - No Sargassum | 50.386 | 15.931 | 3.163 | .002 | .009 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 9: Kruskal-Wallis Pairwise Comparison of Seedling Number of Leaves***

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Brown Sargassum-Black Sargassum | 26.959 | 17.978 | 1.500 | .134 | .802 |
| Brown Sargassum-Sand - No Sargassum | 136.404 | 17.602 | 7.749 | .000 | .000 |
| Brown Sargassum-Golden Sargassum | -149.620 | 17.393 | -8.602 | .000 | .000 |
| Black Sargassum-Sand - No Sargassum | 109.446 | 16.764 | 6.529 | .000 | .000 |
| Black Sargassum-Golden Sargassum | -122.662 | 16.544 | -7.414 | .000 | .000 |
| Sand - No Sargassum-Golden Sargassum | -13.216 | 16.135 | -.819 | .413 | 1.000 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 10:Kruskal-Wallis Pairwise Comparison of Seedling Health***

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Golden Sargassum-Brown Sargassum | 6.802 | 17.651 | .385 | .700 | 1.000 |
| Golden Sargassum-Sand - No Sargassum | 57.439 | 16.374 | 3.508 | .000 | .003 |
| Golden Sargassum-Black Sargassum | 70.785 | 16.790 | 4.216 | .000 | .000 |
| Brown Sargassum-Sand - No Sargassum | 50.637 | 17.863 | 2.835 | .005 | .028 |
| Brown Sargassum-Black Sargassum | 63.984 | 18.245 | 3.507 | .000 | .003 |
| Sand - No Sargassum-Black Sargassum | -13.347 | 17.013 | -.785 | .433 | 1.000 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 11: Kruskal-Wallis Pairwise Comparison of Soil pH***

| Sample1-Sample2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj.Sig. |
|---|---|---|---|---|---|
| Black Sargassum-Sand - No Sargassum | 2.321 | 16.098 | .144 | .885 | 1.000 |
| Black Sargassum-Brown Sargassum | -37.596 | 17.230 | -2.182 | .029 | .175 |
| Black Sargassum-Golden Sargassum | -53.947 | 15.856 | -3.402 | .001 | .004 |
| Sand - No Sargassum-Brown Sargassum | -35.275 | 16.900 | -2.087 | .037 | .221 |
| Sand - No Sargassum-Golden Sargassum | -51.626 | 15.497 | -3.331 | .001 | .005 |
| Brown Sargassum-Golden Sargassum | -16.351 | 16.669 | -.981 | .327 | 1.000 |

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

***Table 12: Kruskal-Wallis Pairwise Comparison of Soil Moisture***

***Appendix C - Pictorials***

*<u>Compost Production</u>*

***Plate 11:View of BlackSargassum at the End of Composting Period (February 2023)***

*Plate 12: Black Sargassum at the End of Seedling Monitoring Period (July 2023)*

*Mangrove Seedlings*

***Plate 13: Views of Seedlings at Location 1, PRML, Port Royal, Kingston***

***Plate 14: View of Seedlings at Location 2 Harbour View, Kingston***

| Concrete Wall | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Crate 1 - Yellow** | | | | | **Crate 1 - Green** | | | | |
| BLK 6 | SND 8 | BLK 8 | BRN 1 | BLK 7 | GLD 7 | BRN 3 | BRN 5 | BLK 9 | SND 1 |
| BLK 3 | GLD 2 | BRN 7 | SND 10 | BLK 2 | BRN 8 | GLD 6 | BRN 4 | SND 6 | BLK1 |
| SND 5 | BRN 10 | GLD 8 | GLD4 | GLD 9 | BRN 2 | BLK 10 | BRN 6 | GLD 10 | SND 7 |
| SND 2 | BLK 4 | GLD 5 | BRN 9 | SND 9 | GLD 3 | BLK 5 | SND 3 | GLD 1 | SND 4 |
| **Walkway** | | | | | | | | | |

***Table 13: Table Showing Randomized Seedling Locations in Plastic Crates.***

Sand (No SC) = SND, Black SC =BLK, Golden SC = GLD, Brown SC = BRN

***Plate 15: Example of Health Index Category 0 Seedling***

***Plate 16: Examples of Health Index Category 1 Seedlings***

***Plate 17: Example of Health Index Category 2 Seedling***

***Plate 18: Example of Health Index Category 3 Seedling***

***Plate 19: Example of Health Index Category 4 Seedling***

***Plate 20: Examples of Health Index Category 5 Seedlings***