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Carbon Storage in Rock Garden Seagrass Bed, Rayong Province

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ปีการศึกษา 2563

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Carbon Storage in Rock Garden Seagrass Bed, Rayong Province

Warisara Rotsirisathit

A Senior Project in Partial Fulfillment of the Requirements
for the Degree of Bachelor of Science in Marine Science
Department of Marine Science, Faculty of Science, Chulalongkorn University
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หัวข้อโครงการ	ปริมาณคาร์บอนเก็บกักของพื้นที่หญ้าทะเลบริเวณร็อคการ์เด้นท์ จังหวัดระยอง
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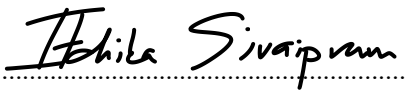

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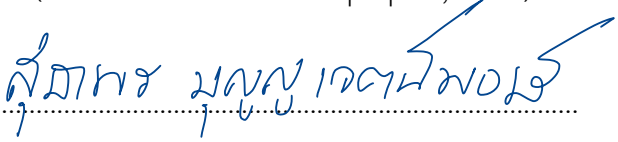

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บทคัดย่อ

ปริมาณคาร์บอนไดออกไซด์ในอากาศที่เพิ่มสูงขึ้นอย่างต่อเนื่องจนให้เกิดปรากฏการณ์สภาพภูมิอากาศเปลี่ยนแปลงนั้นทำให้มนุษย์สนใจหาวิธีการแก้ไข และหนึ่งในวิธีที่จะลดปริมาณคาร์บอนไดออกไซด์ในการอากาศคือการกักเก็บคาร์บอนโดยธรรมชาติ หญ้าทะเลไม่เพียงแต่ให้บริการทางระบบนิเวศหลายประการต่อทะเลและชายฝั่งเท่านั้น แต่ยังเป็นแหล่งกักเก็บคาร์บอนที่สำคัญ (Blue carbon) อีกด้วย ดังนั้นงานวิจัยครั้งนี้จึงมุ่งเน้นการวัดปริมาณคาร์บอนอินทรีย์ในพื้นที่หญ้าทะเล โดยเลือกศึกษาบริเวณหมู่บ้านร็อคการ์เด้นท์ ซึ่งเป็นพื้นที่หญ้าทะเลที่ใหญ่ที่สุดในจังหวัดระยอง ตัวอย่างดินตะกอนและหญ้าทะเลถูกเก็บจาก 19 สถานีด้วยวิธีการเก็บแบบตารางกระจายความน่าจะเป็น (distributed probability-based grid method) ในเดือนพฤศจิกายน และธันวาคม 2563 ในพื้นที่ศึกษาพบหญ้าทะเล 2 ชนิดได้แก่ *Halodule pinifolia* และ *Halodule uninervis* อยู่ในพื้นที่ที่มีขนาดอนุภาคดินตะกอนที่พบส่วนใหญ่เป็นทราย จากการศึกษาปริมาณคาร์บอนอินทรีย์ในดินตะกอนที่วัดโดยเครื่อง elemental analyzer พบว่า ปริมาณคาร์บอนอินทรีย์ในกอหญ้าทะเล (0.97 ± 0.68 mg C/g) สูงกว่านอกกอหญ้าทะเล (0.46 ± 0.11 mg C/g) อย่างมีนัยยะสำคัญ ($p < 0.05$) เมื่อเปรียบเทียบชนิดหญ้าทะเล พบว่า *H. uninervis* สามารถสะสมคาร์บอนอินทรีย์ (0.09 ± 0.05 kg C/m^2) ได้มากกว่าใน *H. pinifolia* (0.06 ± 0.05 kg C/m^2) นอกจากนี้การแยกส่วนของคาร์บอนอินทรีย์หญ้าสองชนิดมีความแตกต่างกัน คือ ใน *H. uninervis* มีแนวโน้มที่จะสะสมคาร์บอนส่วนใหญ่ในส่วนใต้ดิน (ลำต้นใต้ดินและราก) ในขณะที่ *H. pinifolia* มีแนวโน้มที่จะสะสมมากในส่วนเหนือดิน (ลำต้นและแผ่นใบ) การศึกษาในครั้งนี้แสดงให้เห็นถึงความสำคัญของการกักเก็บคาร์บอนอินทรีย์ในหญ้าทะเล และแสดงให้เห็นถึงความสัมพันธ์ของการกักเก็บคาร์บอนอินทรีย์ในหญ้าทะเลแต่ละชนิด และในแต่ละส่วนของหญ้าทะเลซึ่งจะนำไปสู่การวางแผนการพัฒนาและอนุรักษ์พื้นที่ให้มีประสิทธิภาพมากยิ่งขึ้น

คำสำคัญ: Blue carbon, คาร์บอนเก็บกัก, คาร์บอนอินทรีย์, หญ้าทะเล, พื้นที่ชายฝั่ง

Project Title Carbon storage in Seagrass Bed at Rock Garden, Rayong
Name Miss Warisara Rotsirisathit
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Abstract

Seagrass meadows are important coastal ecosystems providing many ecological services including acting as natural sinks for carbon storage and sequestration and thereby offering solutions to mitigate climate change caused by elevated atmospheric carbon dioxide concentration. However, most seagrass beds have been threatened by coastal developments and various other anthropogenic activities. This may in turn reduce the amount of carbon stored in coastal ecosystems (i.e., blue carbon). As a result, this study aims at assessing organic carbon storage in the seagrass beds at Rock Garden Village, which are the largest beds in terms of area in Rayong Province. Sediment and seagrass from 19 sediment cores as predetermined by distributed probability-based grid method were collected in November and December 2020. The organic carbon content was determined by using the elemental analyzer. Two seagrass species present in the study area were *Halodule pinifolia* and *Halodule uninervis*. They were both found in sediments made up mostly of sand. In sediments, carbon concentration inside the seagrass patch was significantly ($p < 0.05$) higher (0.97 ± 0.68 mg C/g) than those outside the patch (0.46 ± 0.11 mg C/g). Overall, the *H. uninervis* stored more organic carbon (0.09 ± 0.05 kg C/m^2) than the *H. pinifolia* (0.06 ± 0.05 kg C/m^2). Furthermore, *H. uninervis* preferentially stores carbon in below-ground parts (i.e., rhizomes and roots), whereas *H. pinifolia* preferentially stores carbon in above-ground parts (i.e., leaf blades and leaf sheaths). This study emphasizes the importance of organic carbon accumulation in seagrass, along with the relationship between organic carbon accumulation in each species of seagrass and in each part of seagrass, which will lead to development of seagrass ecosystem conservation.

Keywords: Blue carbon, carbon storage, organic carbon, seagrass bed, coastal habitat

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Warisara Rotsirisathit

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Chapter 1
Introduction

1.1 Motivation

Climate change from anthropogenic activities is one of the top concerns for countries worldwide. As carbon dioxide (CO_2) concentration in the atmosphere continues to increase, it is important that we find various measures to combat this problem. Natural carbon sink is one of the ways of capturing carbon and may serve as a sustainable solution to mitigate the rising atmospheric CO_2. Various coastal ecosystems, including mangrove swamps and seagrass meadows, are capable of transforming CO_2 into organic carbon in the biomass and storing that organic carbon in the sediments for an extended period of time.

Seagrass ecosystems are widely distributed along the coasts from the tropics to the temperate regions. They have a significant role in coastal and marine ecosystem by providing numerous ecological services (Nordlund *et al.,* 2016) such as being nursery grounds and habitats for fish, invertebrates, and endangered species (e.g., dugongs and sea turtles). Moreover, seagrass meadows are important to coastal protection as they are capable of decreasing wave velocity and thus protecting the coastlines from erosion. Seagrasses are involved in the carbon sequestration process and can accelerate sedimentation in the meadows (Kennedy *et al*., 2010). In addition, while covering only 0.1% of the ocean surface, the complexity of seagrass roots enhance trapping and storing sediments including organic carbon, which accounts for 20% of the global carbon sequestration in marine sediments (Kennedy *et al*., 2010; Duarte *et al.,* 2013).

Rayong is one of the rapidly growing provinces on Thailand's eastern seaboard. Many developments along its coastlines can threaten the existence of coastal ecosystems. The Seagrass bed adjacent to Rock Garden Village is one of the major meadows in this area considering the coverage area. Moreover, Adulyanukosol (2006) reported the total seagrass area of 14945 acres was found in 2006, while only 643 acres remained in 2017 (DMCR, 2017). The main causes of degradation were human activities and nature, such as fishing, waste, sediment, cruising of tourist boats, and seasonal variation (Adulyanukosol, 2006).

Therefore, we selected this particular location to quantify the organic carbon content in the seagrass meadow both in the biomass and in the sediments.

1.2 Project Objectives

1.2.1 To calculate the quantity of organic carbon in the area

1.2.2 To determine the relationship of organic carbon content, seagrass types and sediment characteristics

1.3 Scope of This Study

1.3.1 Study area: Rock Garden Village, Rayong

1.3.2 Seagrass species: *Halodule* spp.

1.3.3 Analysis methods:

- Grain size: wet sieving and gravimetry
- Organic carbon: Pregl-Dumas combustion by using CHN analyzer

1.4 Expected Outcomes

1.4.1 The result on the amount of organic carbon in the study area may serve as the baseline information for conservation and management purposes.

1.4.2 The result on the quantity of organic carbon in the research area might be used for carbon credit calculation which could be sold to large companies needing to reduce carbon emissions and bring revenues to the municipality.

Chapter 2

Literature review

2.1 Seagrass Ecosystems

Seagrasses are marine flowering plants thriving in the coastal intertidal and subtidal zones. There are 72 seagrass species worldwide, divided into 6 families including (1) Cymodoceae (5 genera: *Halodule, Cymodocea, Syringodium, Thalassodendron* and *Amphibolis*), (2) Hydrocharitaceae (3 genera: *Thalassia, Halophila* and *Enhalus*), (3) Posidoniceae (1 genus: *Posidonia*), (4) Zosteraceae (3 genera: *Zostera, Heterozostera* and *Phyllospadix*), (5) Zanichelliaceae (4 genera: *Zannichelia, Althenia, Pseudalthenia* and *Lepilaena*) and (6) Ruppiaceae (1 genus: *Ruppia*) (Den Hartog and Kuo, 2006; Short *et al.,* 2011).

Thirteen seagrass species can be found In Thailand. Twelve seagrass species are present along the Gulf of Thailand coast including *Enhalaus acoroides*, *Thalassia hemprichii*, *Halophila beccarii*, *Halophila decipiens*, *Halophila minor*, *Halophila ovalis, Halodule pinifolia*, *Halodule uninervis*, *Cymodocea serrulata*, *Cymodocea rotundata*, *Syringodium isoetifolium,* and *Ruppia maritima* (DMCR, 2015).

Seagrass meadows are important to coastal and marine ecosystems as they provide various ecosystem services (Figure 1) including supporting the wellbeing of local communities, hosting marine biodiversity, and ensuring food security through commercial and subsistence fishing activity (Cullen-Unsworth *et al*., 2014). Moreover, they can contribute indirect social and economic values to tourism. For instance, tourists are attracted to visiting seagrass meadows in Green Island, Australia for sea turtle watching, snorkeling seagrass trails in Porth Dinllaen, UK or joining educational walk-in around seagrass meadows in Chumbe island, Zanibar (Nordlund *et al*., 2013; Cullen-Unsworth *et al*., 2014). Seagrass ecosystems are also crucial in preventing coastal erosion along the shoreline.

Carbon sequestration and storage within meadows also contribute to climate change mitigation by storing 19.9 Pg of organic carbon globally (Kennedy *et al*., 2010; Fourqurean *et al.*, 2012; Stankovic *et al.,* 2021), and seagrass meadows losses could release 11 to 299 Tg C/year back to the atmosphere (Fourqurean *et al.,* 2012; Pendleton *et al*., 2012; Alongi *et al.,* 2016).

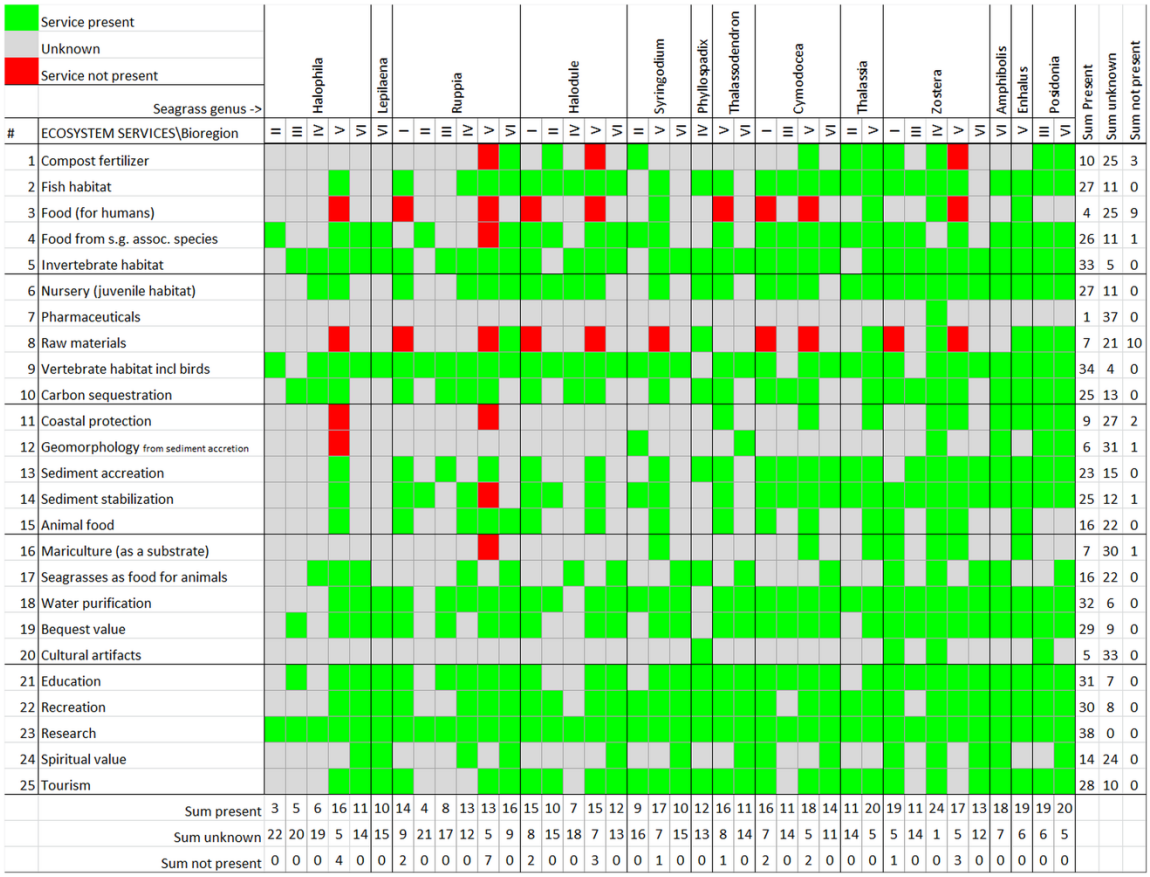

Figure 1. Ecosystem services provided by seagrass meadows. Green boxes denote present services while grey and red boxes represent unknown service and service not present, respectively. Seagrass bioregion is identified according to Short *et al.* (2007): I=Temperate North Atlantic, II=Tropical Atlantic, III= Mediterranean, IV= Temperate North Pacific, V= Tropical Indo-Pacific, VI= Temperate Southern Oceans (Source: Nordlund *et al*., 2016).

2.2 Blue Carbon

Seagrass meadows may account for up to 1% of marine primary production and they are considered one of the most productive amongst biospheres on the planet (Duarte and Cebrián, 1996; Duarte and Chiscano, 1999; Duarte *et al.,* 2013a). Global net primary production of seagrass meadow is in the range of 0.05 to 0.17 Pg C per year (Duarte *et al.,* 2013b), with autotrophic epiphytes accounting for 20 to 60 percent of the total, ranging from 13.3 g dw /m^2 per year to 755 g dw /m^2 per year (Duarte *et al.,* 2013a).

Maximum seagrass production varies significantly by species, with average turnover rates of 2.6 ± 0.3 and 0.77 ± 0.12 percent per day in the aboveground and belowground, respectively (Duarte and Chiscano, 1999). Tropical seagrass meadows have a higher metabolic

rate than temperate areas, but overall net community production (NCP) shows no significant difference (Duarte *et al.,* 2010).

The carbon stored in marine ecosystems is also known as 'blue carbon', and mangrove forests and seagrass meadows are two key blue carbon habitats owing to their capacity to trap and sequester carbon in their ecosystems. Many conservation efforts and ecosystem restoration plans have emerged over the past few years to enhance carbon sequestration (Pendleton *et al*., 2012; Alongi *et al*., 2016). Only 0.2% of the sea surface is occupied by marine vegetated ecosystems, yet they supply approximately half of organic carbon in sediments (Duarte *et al.,* 2013b).

Carbon sequestration has a high potential to mitigate antropogenic greenhouse gas emissions. Photosynthesis, a process that changes inorganic carbon into organic carbon stored within the vegetative biomass, initiates carbon storage in the sediment. Seagrass meadows with an average aboveground biomass exceeding 41 g dw/m^2 are considered as CO_2 sink, with the majority of meadows being autotrophic ecosystems (Duarte *et al*., 2010; Duarte *et al*., 2013a).

Organic carbon in sediments in seagrass beds can be preserved in oxidation-resistant sediments for millennia without being transformed back into CO_2 during remineralization (Fourqurean *et al.,* 2012). Remineralization depletes carbon stocks stored in ecosystems but seagrass beds have a number of carbon preservation mechanisms important for storing organic carbon within sediments for centuries to millennia, including:

(1) the slow microorganism degradation process due to low nutrient content (nitrogen and phosphorus) to support microbial growth (Duarte *et al*., 2013a);

(2) low oxygen concentration in bed sediments leads to inefficient microbial metabolism (Duarte *et al*., 2013a; Duarte *et al*., 2013b; Srinamngeon, 2020);

(3) belowground biomass (i.e., seagrass roots and rhizomes) accounting for up to 50% (Duarte *et al*., 2013a); and

(4) seagrass canopies' ability to reduce wave turbulence and hinder sediment resuspension (Duarte *et al*., 2013a).

Moreover, seagrass meadows have great potential to thicken the seafloor, about 1 mm each year. Particularly in long-term seagrass occupied areas are able to form thick carbon deposits (Kennedy *et al.*, 2010; Duarte *et al.,* 2013a). Up to 11 m of sediment were estimated

to deposit over 6000 years of seagrass growth at Port Lligat in Spain, making it the thickest sedimentary deposition found in a seagrass meadow (Lo Iacono *et al*., 2008).

Despite their significant ecosystem roles, seagrass ecosystems are estimated to decline at an alarming rate of 7% since 1990 (Waycott *et al.*, 2009; Nordlund *et al.,* 2018) and 14% of all seagrass species are at extinction risk (Short *et al*., 2011) and thereby reducing carbon sink (Meleod *et al.*, 2011).

2.3 Rock Garden Seagrass Beds

Approximately 14,945 acres of the total seagrass area was found along the coastlines of Rayong according to survey in 2006 (Adulyanukosol and Poovachiranon, 2006) while only 643 acres were reported in 2017 meadows in the eastern part of Thailand, with the largest beds spanning an area of over 327.3 acres around Rock Garden Village (DMCR, 2015). This number may vary at different times of the year by 25-50% (DMCR, 2015).

At Rock Garden, *Halodule* spp. were identified as the dominant species (DMCR, 2015; Potisarn *et al*, 2017; Wanna and Phongpha, 2018; Srinamngeon, 2020). Here, two species were reported, namely: *Halodule pinifolia* and *Halodule uninervis*. They can be distinguished by the morphology of their leaf tips. *H. pinifolia* has a round leaf tip while *H. uninervis* has a trident leaf tip. Moreover, *H. uninervis* are usually larger than *H. pinifolia* which has thin and delicate leave blades (Waycott *et al.,* 2004).

2.4 Blue carbon quantification

There are mainly 3 methods to analyzed carbon content.

(1) Wet oxidation commonly known as Walkley-Black method. This method rely on chemical oxidation with chromate in the presence of sulfuric acid which can be easily done at low cost but may only work on oxidizable fraction of the samples and produce hazardous waste.

(2) Loss on Ignition (LOI). This relies on combusting the samples and considering the mass loss as the combustible component (including organic carbon). This may not be the most direct method for organic carbon quantification but can be done easily in labs having muffle furnaces.

(3) Elemental analysis. This method can turn organic carbon in the samples into CO_2 via combustion at high temperature and capable to quantify all carbon content in the sample (Howard *et al*., 2014).

2.5 Previous studies on carbon storage in Thailand

Prathep (2012) studied carbon stock in seagrass and sediment in Trang and Ranong by using an elemental analyzer. Sediment in both sites was made up of sand. At Trang sites, sediments underlying *Halodule uninervis* had 4.66% and 1.34% carbon content for those of grainsize <63 μm and >63 μm, respectively. In Ranong, on the other hand, sediments found inside *H. uninervis* beds were of 3.63% and 2.48% carbon content for sediment of grainsize <63 μm and >63 μm, respectively. Average above- and belowground carbon biomass of *H. uninervis* reported in Trang was 36.04% and 33.03%, respectively and in Ranong was 36.90% and 35.25%, respectively.

Srinamngeon *et al.* (2016) analyzed carbon accumulation in seagrass at Khungkraben bay by Walkley and Black titration method. *Halodule pinifolia* did not exhibit any difference between the aboveground (38.93%) and belowground (42.53%) biomass.

Srinamngoen *et al.* (2018) estimated carbon accumulation at Khungkraben Bay by using the Walkley and Black titration method. *H. pinifolia* did not seem to be selective in terms of where they stored carbon: 44.23% aboveground vs 43.98% belowground. In addition, the organic carbon content in sediment underlying *H. pinifolia* was 1.049 ± 0.31%.

Srinamngoen *et al.* (2020) investigated blue carbon in seagrass beds from 4 areas in eastern part of Thailand: Sattahip, Chonburi; Rock Garden - Nernkho, Rayong; Khungkraben, Chanthaburi and Ko Kradat, Trat. The Walkley and Black titration technique was used to determine the quantity of carbon in various seagrass species. There was no significant difference in carbon biomass in *H. pinifolia* across the four study locations: 88.28% in Sattahip, 85.00% in Khungkraben, 95.91% in Rock Garden-Nernkho, and in *H. uninervis* 71.31% in Ko Kradat. Furthermore, *H. uninervis* reported in this study was the second-largest carbon biomass storage compared to other seagrass species found across the study sites at 2883.10 g C/m^2, which is 71.31% at Ko Kradat. Meanwhile, the organic carbon in the sediments were 0.22% in Sattahip, 1.06% in Khung Kraben, 0.22% in Rock Garden-Nernkho and 0.24% in Ko Kradat.

Chapter 3

Methodology

3.1 Study site

Rock Garden Village seagrass bed is located around 12°39'46.2" N and 101°39'28.2" E (Figure 2). It is one of the largest seagrass beds in Rayong Province with the total seagrass area of about 643 acres (DMCR, 2017). *Halodule uninervis* and *Halodule pinifolia* were reported as the dominant seagrass species in this area (DMCR, 2015; Potisarn *et al.,* 2017; Wanna and Phongpha, 2018).

3.2 Site selection and sampling

Seagrass and sediment core samples were collected at low tide during the dry season at Rock Garden Village (Figure 2). In November 2020, three sediment cores (R1, R2, and R5) were collected outside the seagrass patch and two (R3 and R4) were collected inside the seagrass patch using acrylic sediment core liners with a 10-cm diameter and a 50-cm length. In addition, 10 additional short sediment cores (D1 - D10) and long cores (A1 – A4) were randomly sampled in December 2020. Immediately after sampling, the cores were subsampled by cutting into sections. Sediments were sectioned evenly for those taken with longer cores. Then, the sediment samples were kept inside clean plastic zip-lock bags. As summarized in Table 1, different cores may contain varying sediment depths and sectioning intervals. All samples were carefully stored in an icebox while transporting to Chulalongkorn University where samples were kept frozen prior to further analysis.

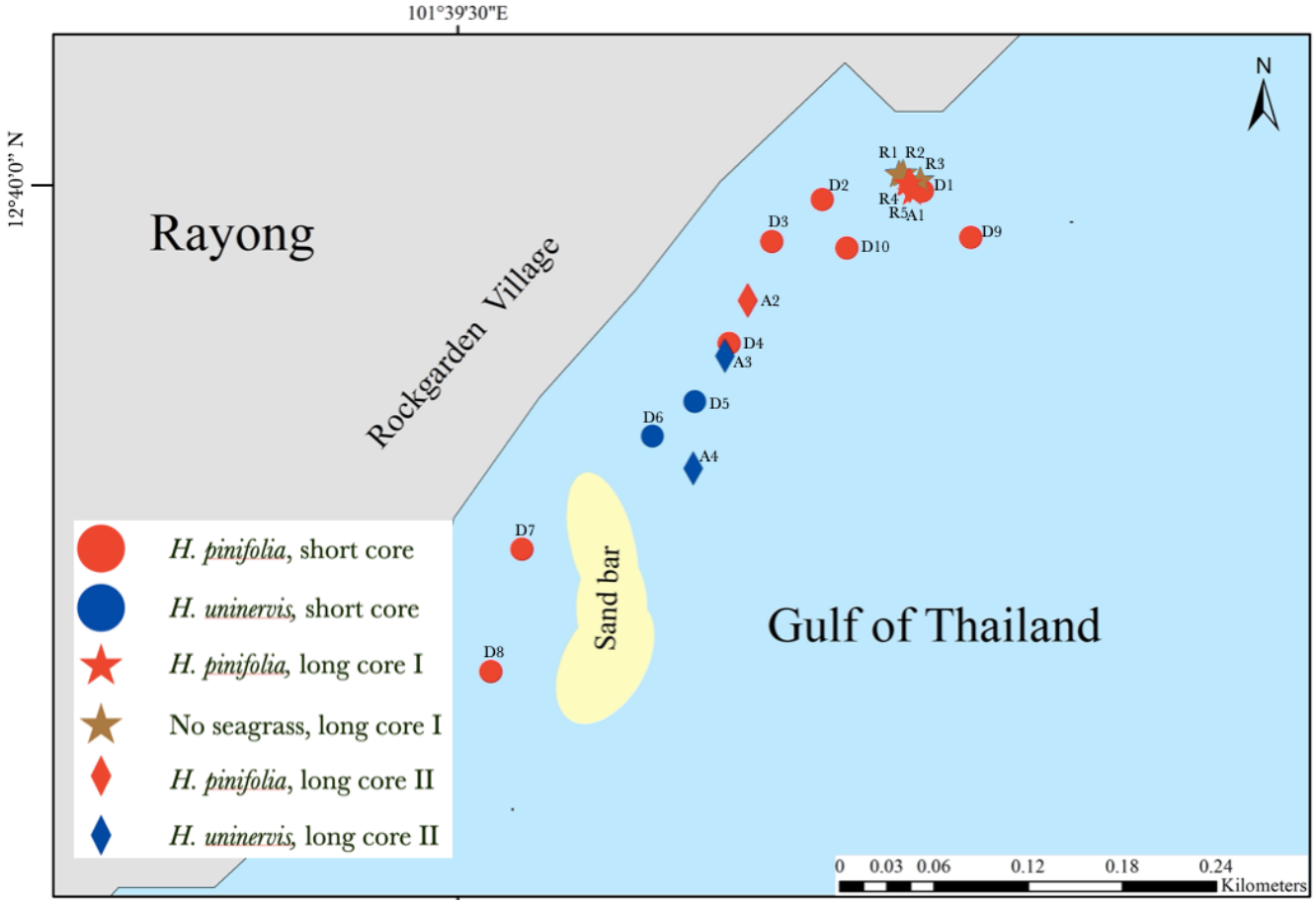

Figure 2. The study location and stations. Red symbols represent *Halodule pinifolia* while blue symbols represent *Halodule uninervis*. Filled circles, stars and diamonds denote short cores, long cores I and long cores II, respectively. The brown stars denote stations with no seagrass (i.e., outside the patch). Long cores I were samples with maximum depth at 20 cm and subsampled at 5-cm intervals while long core II were samples with maximum depth at 16 cm and sub-sampled at 2-cm intervals.

Table 1. Detailed descriptions of each sediment core taken during this study.

Station	Collecting month	Seagrass Species	Latitude	Longitude	Maximum depth (cm)	Subsamples per core
R1	November	no	-	-	20	4
R2	November	no	-	-	15	3
R3	November	*H. pinifolia*	-	-	15	3
R4	November	*H. pinifolia*	-	-	20	4
R5	November	no	-	-	20	4
A1	December	*H. pinifolia*	12.665688°N	101.660924°E	22	11
A2	December	*H. pinifolia*	12.665028°N	101.659996°E	16	8
A3	December	*H. uninervis*	12.664711°N	101.659865°E	16	8
A4	December	*H. uninervis*	12.664067°N	101.659683°E	16	8
D1	December	*H. pinifolia*	12.665654°N	101.660998°E	3	1
D2	December	*H. pinifolia*	12.665606°N	101.660426°E	3	1
D3	December	*H. pinifolia*	12.665365°N	101.660135°E	3	1
D4	December	*H. pinifolia*	12.664781°N	101.659891°E	3	1
D5	December	*H. uninervis*	12.664450°N	101.659693°E	6	2
D6	December	*H. uninervis*	12.664252°N	101.659450°E	6	2
D7	December	*H. pinifolia*	12.663604°N	101.658703°E	6	2
D8	December	*H. pinifolia*	12.662902°N	101.658524°E	6	2
D9	December	*H. pinifolia*	12.665389°N	101.661276°E	3	1
D10	December	*H. pinifolia*	12.665328°N	101.660566°E	3	1

3.3 Chemical and equipment

3.2.1 Chemical and equipment for grain size analysis

(a) Chemical

I. 50% (v/v) Hydrogen peroxide

II. 50% (v/v) Hydrochloric

III. 10% (w/v) Sodium hexametaphosphate ($Na_6[(PO_3)_6]$)

(b) Equipment

I. Beakers
II. Hot plate
III. Syringe
IV. Rubber tube
V. Oven
VI. 63 microns sieve
VII. 5 ml pipette
VIII. 1 liter cylinder

3.2.2 Chemical and equipment for organic carbon analysis in sediment

(a) Chemical

I. 10% (v/v) Hydrochloric

(b) Equipment

I. Ultra microbalances: Perkin Elmer AD-6 Autobalance
II. Elemental analyzer: Perkin Elmer series II 2400
III. Syringe
IV. Rubber tube

3.2.3 Chemical and equipment for organic carbon analysis in seagrasses

(a) Equipment

I. Agate mortar and pestle
II. Ultra microbalances: Perkin Elmer AD-6 Autobalance
III. Elemental analyzer: Perkin Elmer series II 2400

3.4 Sample preparation

3.4.1 Sediment samples

After the seagrass biomass was taken out of the sediment samples, the drying process was done in a freeze-dryer (Heto, Lyopro 6000). Then, the dry weights were obtained by using a precision balance.

3.4.2 Seagrass samples

Seagrasses were separated from the soil matrix and rinsed with water. Then they were separated into two groups: aboveground and belowground. After that, the drying process was done in a freeze-dryer (Heto, Lyopro 6000). Then, the dry weights of aboveground and belowground were obtained as biomass of seagrass by using a precision balance.

3.5 Laboratory analysis

3.5.1 Grain size analysis

Dry sediments were passed through a 2-cm sieve to remove larger items. Then, any organic matter that might create clumping in the sediments was removed by using 50% (v/v) hydrogen peroxide. After that, 50% (v/v) hydrochloric acid was added to the sample slurry to remove any carbonates. Once all the bubbling was gone, neutralization was done by adding distilled water until the pH was higher than 6. At this point, wet samples were dried inside an oven set at 110°C for at least 12 hours or until it was completely dry. Then the dry weights of the samples were taken.

Samples were then analyzed by the wet sieving/pipette method as described in Sompongchaiyakul (1989). Briefly, previously weighed dry sediments were wet-sieved using a 63-micron mesh. sediment bigger than 63 microns, sand component, was dried in a 110°C oven at least 12 hours or until it was completely dried, then weighed the sample with precision balance.

A sediment smaller than 63 microns was loaded into a 1000-ml cylinder. Ten ml of 10% (w/v) sodium hexametaphosphate ($Na_6[(PO_3)_6]$) was added then adjusted volume to 1000 ml. The slurry was mixed thoroughly and left to sit at 23 °C for 3 hours 52 minutes. Five ml of the slurry from the 5-cm depth was pipetted out to a container and dried in a 110°C oven. This constitutes the clay component, then obtain the weight by precision balance. The amount of silt in the sample was calculated from subtracting the sand and clay from the initial gross dry weight.

Sediment characteristic was classified by percentage of each sediment type, then plot in Shepard (1954) ternary diagram based on Wentworth (1922) grain size classification as shown in Figure 3.

3.5.2 Organic carbon in sediment

The dry sediment samples were first homogenized then sieve dry content with 200 μm mesh size to separate non-sediment part out. Any carbonates in the sample that passed through the sieve were removed by adding 10% (v/v) hydrochloric acid. After acidification, rinsing out the samples by using distilled water until pH was close to neutral. Another round of freeze-drying was conducted before packing samples in tin capsules with accurate weight recorded. Finally, carbon content was analyzed by elemental analyzer (Perkin Elmer series II 2400).

To report organic carbon content in sediment in unit of mg C/g dw, organic carbon percentage obtain from elemental analyzer would be multiply by ten (Howard *et al.,* 2014).

3.5.3 Organic carbon in seagrasses

Each dry vegetative sample was pounded in a mortar, homogenized and then packed in a tin cup. The exact weight of each sample was determined by an ultra-microbalance (Perkin Elmer AD-6 Autobalance). The organic carbon contents in seagrass samples were analyzed by an elemental analyzer (Perkin Elmer series II 2400).

Carbon biomass reported in kg C/m^2 were calculated by percentage of organic carbon determined by elemental analyzer multiply the biomass of seagrass in each sample, then divided by area of each core (Howard *et al.,* 2014).

3.6 Data analysis

All statistical analyses were performed by IBM SPSS® statistical software 26.0. Comparison between the carbon content in the sediments inside and outside the seagrass patches were done by using a student's t-test while correlation coefficients between the organic carbon content and different sediment characteristics were carried out by using Pearson's correlation analysis. Statistical significance was set either at 95% ($p \leq 0.05$) and 99% ($p \leq 0.01$) confidence levels.

Chapter 4

Results and Discussions

4.1 Sediment characterization

Sand made up the majority of the sediment grain in all samples (Figure 3 and Table 2). Approximately 90% sand was found in samples from the outside the seagrass patch and 80% was found in samples inside the seagrass patch. A large sand bar was found nearby the seagrass bed area. The presence of this sand bar was a recent occurrence according to the local residents and this may suggest a higher sediment deposition in the area. It should also be noted that our sediment samples have a higher sand composition than previously reported in Potisarn *et al*. (2017) which conducted a study in the same area in 2017 and reported sediment type in the area as sandy clay.

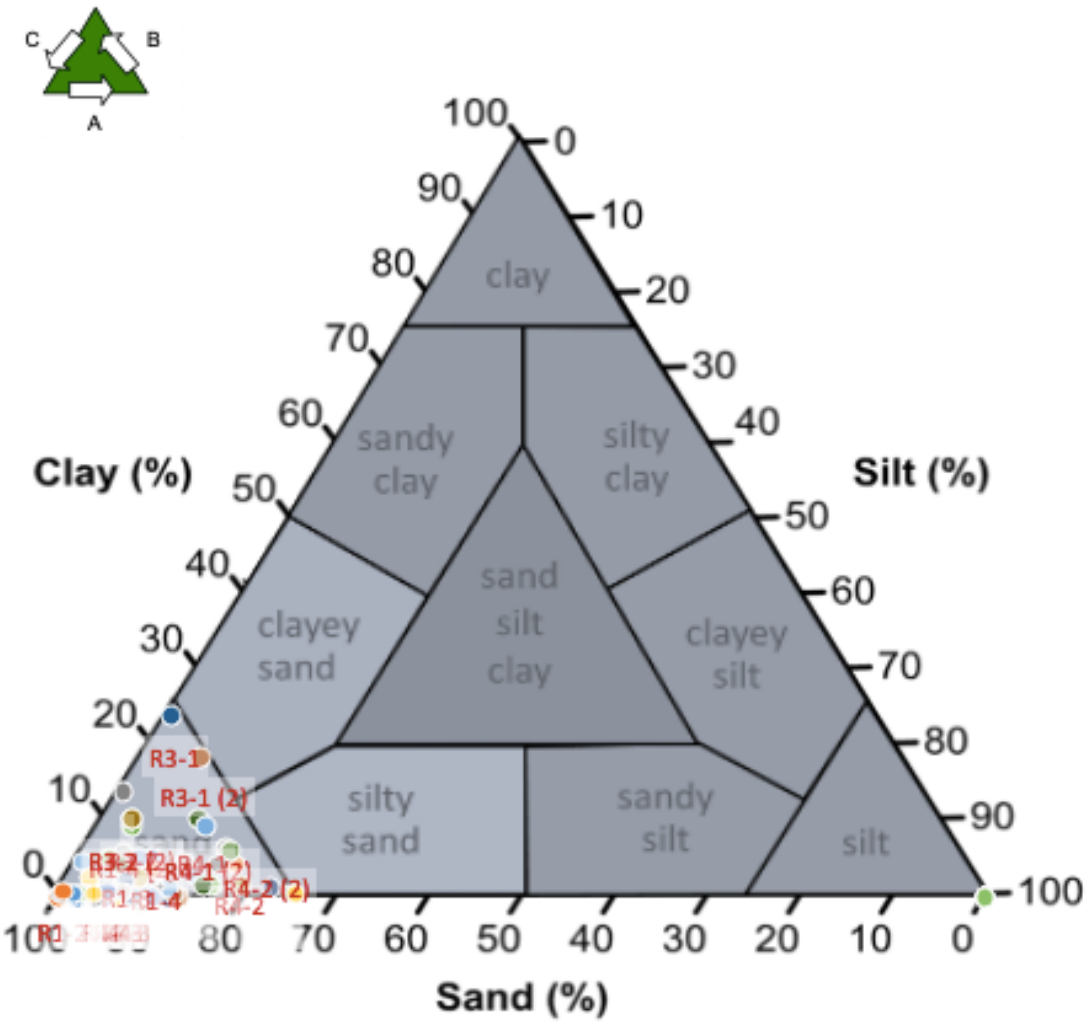

Figure 3. Sediment characteristics of samples taken from Rock Garden Village.

Table 2. Sediment composition in each core from Rock Garden Village.

Core	Species	Sedimentary fraction			Sediment type
		%sand	%clay	%silt	
R1-1	-	93.3	0.0	6.7	Sand
R1-2	-	98.8	0.0	1.2	Sand
R1-3	-	88.8	6.2	5.0	Sand
R1-4	-	86.0	10.5	3.5	Sand
R3-1	*H. pinifolia*	72.9	21.5	5.6	Sand
R3-2	*H. pinifolia*	85.4	11.8	2.8	Sand
R3-3	*H. pinifolia*	92.1	0.0	7.9	Sand
R4-1	*H. pinifolia*	78.0	10.4	11.6	Sand
R4-2	*H. pinifolia*	77.4	7.6	14.9	Sand
R4-3	*H. pinifolia*	92.9	0.0	7.1	Sand
R4-4	*H. pinifolia*	94.0	0.0	6.0	Sand
R5-1	-	95.3	0.0	4.7	Sand
R5-2	-	97.2	0.0	2.8	Sand
R5-3	-	84.0	16.0	0.1	Sand
R5-4	-	89.9	0.0	10.1	Sand
A1-1	*H. pinifolia*	77.1	6.7	16.2	Sand
A1-2	*H. pinifolia*	93.7	5.2	1.1	Sand
A1-3	*H. pinifolia*	91.8	3.9	4.2	Sand
A1-4	*H. pinifolia*	92.6	3.0	4.3	Sand
A1-5	*H. pinifolia*	-	-	-	n/a
A1-6	*H. pinifolia*	95.2	0.0	4.84	Sand
A1-7	*H. pinifolia*	97.1	0.4	2.6	Sand
A1-8	*H. pinifolia*	98.4	0.0	1.6	Sand
A1-9	*H. pinifolia*	-	-	-	n/a
A1-10	*H. pinifolia*	94.8	5.8	-0.7	Sand
A1-11	*H. pinifolia*	87.7	0.5	11.8	Sand
A2-1	*H. pinifolia*	95.2	0.3	4.5	Sand
A2-2	*H. pinifolia*	91.1	0.2	8.7	Sand
A2-3	*H. pinifolia*	89.2	0.4	10.4	Sand

Table 2. (cont.).

Core	Species	Sedimentary fraction			Sediment type
		%sand	%clay	%silt	
A2-4	*H. pinifolia*	86.6	4.2	9.3	Sand
A2-5	*H. pinifolia*	82.9	5.3	11.8	Sand
A2-6	*H. pinifolia*	88.6	4.4	7.0	Sand
A2-7	*H. pinifolia*	87.8	5.6	6.6	Sand
A2-8	*H. pinifolia*	90.9	5.7	3.4	Sand
A3-1	*H. uninervis*	90.9	0.0	9.1	Sand
A3-2	*H. uninervis*	85.9	0.1	14.0	Sand
A3-3	*H. uninervis*	98.1	1.9	0.0	Sand
A3-4	*H. uninervis*	89.2	2.9	8.0	Sand
A3-5	*H. uninervis*	90.7	2.0	7.3	Sand
A3-6	*H. uninervis*	89.9	0.0	11.1	Sand
A3-7	*H. uninervis*	82.6	3.1	14.3	Sand
A3-8	*H. uninervis*	92.1	0.5	7.4	Sand
A4-1	*H. uninervis*	91.9	0.5	7.6	Sand
A4-2	*H. uninervis*	93.9	2.9	3.2	Sand
A4-3	*H. uninervis*	95.9	0.2	3.9	Sand
A4-4	*H. uninervis*	96.2	0.4	3.4	Sand
A4-5	*H. uninervis*	97.5	0.1	2.4	Sand
A4-6	*H. uninervis*	97.9	0.8	1.3	Sand
A4-7	*H. uninervis*	73.8	0.2	26.0	Sand
A4-8	*H. uninervis*	73.5	0.2	26.3	Sand
D1	*H. pinifolia*	93.6	0.7	5.6	Sand
D2	*H. pinifolia*	82.0	1.3	16.7	Sand
D3	*H. pinifolia*	87.3	0.1	12.7	Sand
D4	*H. pinifolia*	86.4	0.4	13.2	Sand
D5-1	*H. uninervis*	79.7	4.7	15.6	Sand
D5-2	*H. uninervis*	78.8	1.4	19.8	Sand
D6-1	*H. uninervis*	75.7	1.0	23.4	Sand
D6-2	*H. uninervis*	82.6	1.5	15.9	Sand

Table 2. (cont.).

Core	Species	Sedimentary fraction			Sediment type
		%sand	%clay	%silt	
D7-1	*H. pinifolia*	86.7	0.9	12.4	Sand
D7-2	*H. pinifolia*	-	-	-	n/a
D8-1	*H. pinifolia*	-	-	-	n/a
D8-2	*H. pinifolia*	95.0	0.5	4.5	Sand
D9	*H. pinifolia*	88.7	0.1	11.2	Sand
D10	*H. pinifolia*	-	-	-	n/a

Remark: Samples with no sediment type information available are marked as n/a.

4.2 Organic carbon in the sediment

The organic carbon concentration in sediment samples measured in this study ranged from 0.3 to 4.50 mg C/g dw (0.03 to 0.45 percent) with an average (± S.E.) of 0.88 ± 0.65 mg C/g dw (0.09 ± 0.06 percent). The higher carbon concentrations in sediment were observed within large seagrass patches as shown in Figure 4. In addition, carbon concentration storing in sediment in other areas in Thailand was shown in Table 3.

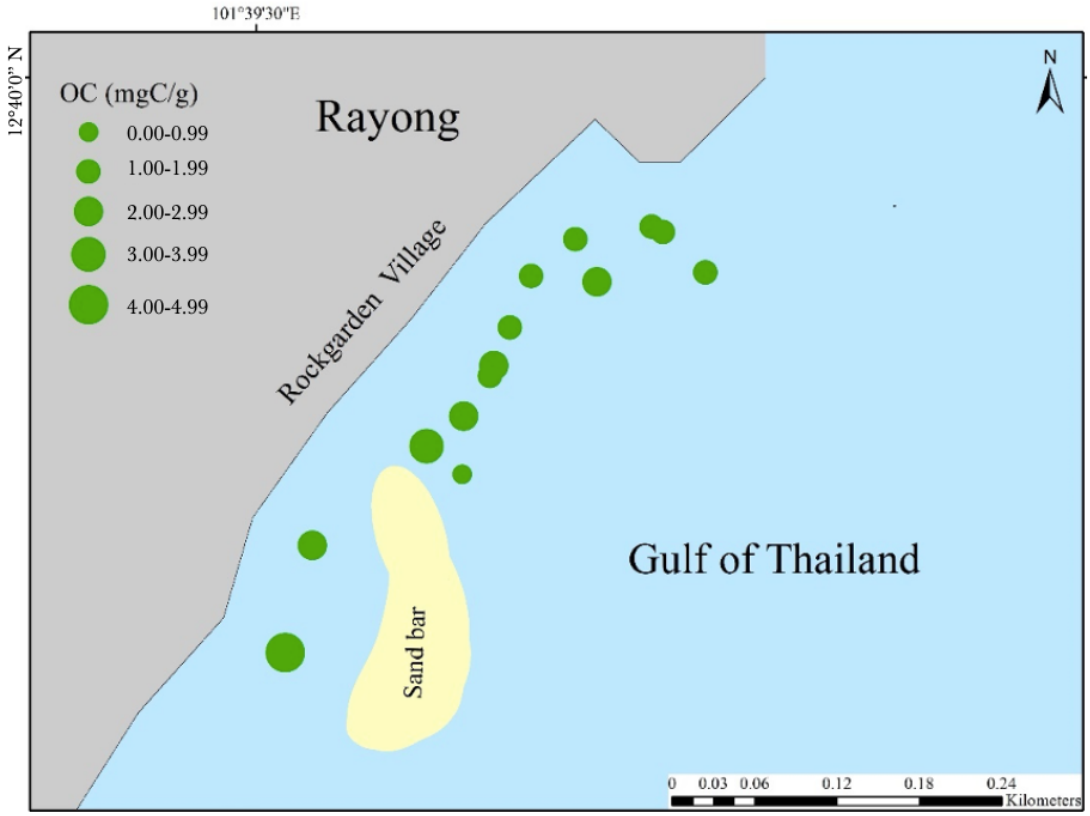

Figure 4. Organic carbon concentration in sediment at Rock Garden.

Table 3. Organic carbon in seagrass bed sediment in Thailand (mean ± S.E.).

Year	Seagrass	Method	Study area	Organic carbon (%)	Reference
2012	*H. uninervis*	Elemental analyzer	Hadchaomai, Trang	4.66	Prathep (2012)
2012	*H. uninervis*	Elemental analyzer	Ranong	3.63	Prathep (2012)
2016	*H. pinifolia*	Walkley and Black titration	Khungkraben	1.05 ± 0.31	Srinamngoen (2018)
2017	*H. pinifolia*	Walkley and Black titration	Sattahip	0.22	Srinamngoen (2020)
	H. minor				
2017	*H. pinifolia*	Walkley and Black titration	Rock Garden-Nernkho	0.22	Srinamngoen (2020)
2017	*E. acoroides*	Walkley and Black titration	Khungkraben	1.06	Srinamngoen (2020)
	H. pinifolia				
2017	*E. acoroides*	Walkley and Black titration	Ko Kradat, Trat	0.24	Srinamngoen (2020)
	C. serrulate				
	H. ovalis				
	H. uninervis				
	T. hemprichii				
2020	*H. pinifolia*	Elemental analyzer	Rock garden	0.07 ± 0.10	This study
2020	*H. uninervis*	Elemental analyzer	Rock garden	0.06 ± 0.08	This study

In this study, organic carbon in sediments stored inside and outside of the seagrass bed were significantly different ($p < 0.05$) with a range of 0.3 to 4.5 mg C/g dw (0.03 to 0.45 percent) for samples inside the patch and 0.3 to 0.8 mg C/g dw (0.03 to 0.08 percent) for samples outside the patch (Figure 5).

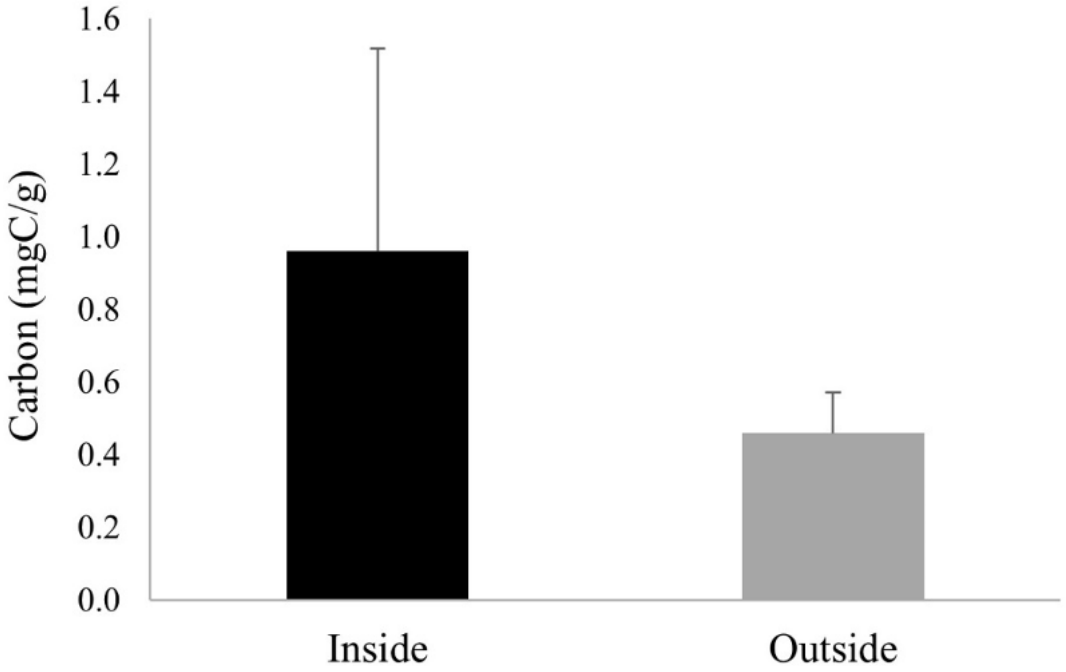

Figure 5. Comparison of organic carbon concentration in sediment inside and outside seagrass patch at Rock Garden. Error bars represent the standard deviations of the data sets.

The average percentage of organic carbon in sediment stored in seagrass beds (*H. pinifolia* 0.07 ± 0.10 percent and *H. uninervis* 0.06 ± 0.08 percent) at Rock Garden Village was lower than the carbon content in *Halodule* spp. in the prior study. In 2012, sediment underlay *H. uninervis* around Hadchaomai, Trang and Ranong reported to store organic carbon 4.66 and 3.63 percent, respectively (Prathep, 2012). In 2016, sediment underlay *H. pinifolia* in Khungkraben was reported to store organic carbon 1.05 ± 0.31 percent (Srinamngoen, 2018). Then in 2017, sediment underlay *H. pinifolia* in Rock Garden-Nernkho was reported to store 0.22 percent of organic carbon. Moreover, sediment underlay multi-species seagrass patch which *H. pinifolia* was included reported to store carbon 0.22 and 1.06 percent in Sattahip and Khungkraben, respectively. Furthermore, in multi-species seagrass patch which *H. uninervis* was included reported to store carbon 0.24 percent at Ko Kradat (Srinamngoen, 2020).

The lower carbon content in sediment when compared to prior study possibly due to sediment type. According to Table 4, carbon content is related to sediment type; sand has an inversion correlation with carbon content, while silt and clay have a positive correlation with carbon content. Correspondingly to Kelleway study reported sediment characteristics as a key physical parameter of carbon storage. Sediment type could control carbon density by carbon density significantly higher in fine sediments and lower in sand (Kelleway *et al.,* 2016).

Table 4. Correlation analysis of sediment composition and carbon in sediment cores.

	Sand	**Silt**	**Clay**	**Carbon**
Sand	1			
Silt	**-0.94****	1		
Clay	-0.30	-0.03	1	
Carbon	-0.16	0.11	0.19	1

4.3 Organic carbon in sediment core at different depth

The amount of organic carbon in the sediment cores within the seagrass patch (Figure 6) did not show any apparent trend from the sediment surface down to the deeper layer. This could be explained by the fluctuating supply of organic carbon into the sediment over time. However, we observed an increasing value downcore for the organic carbon from the cores outside the patch (Figure 7), which could indicate that this area was previously colonized by seagrasses.

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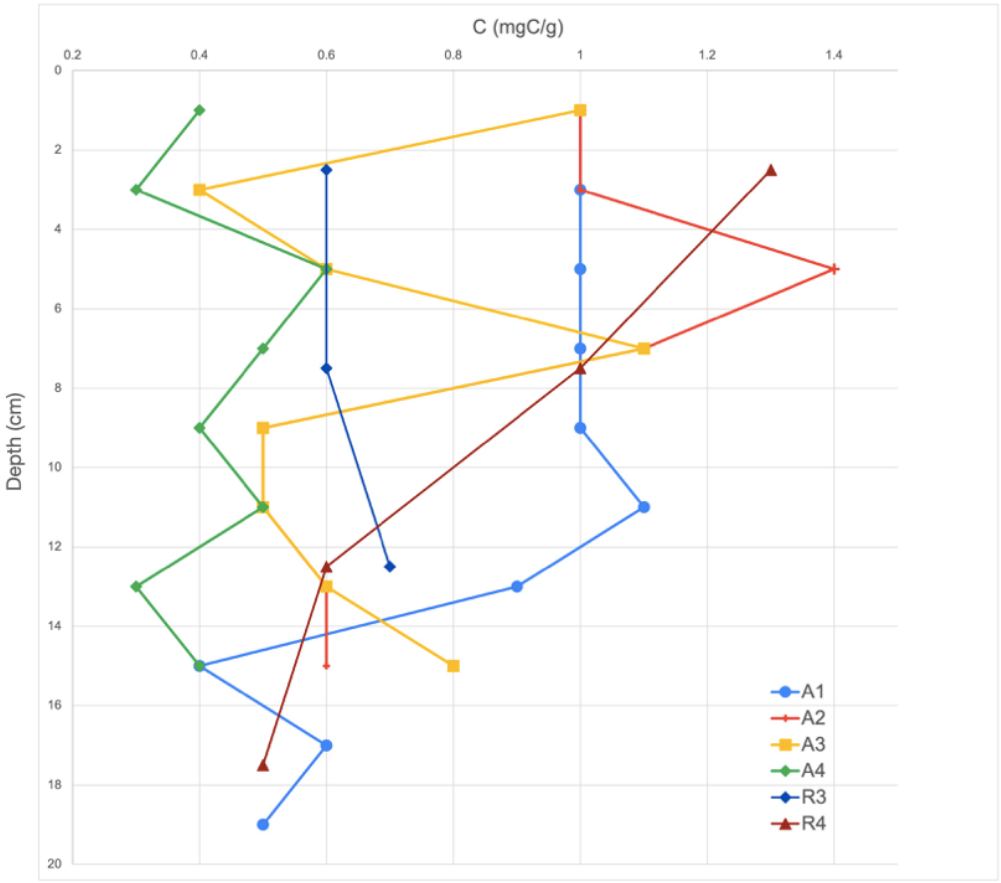

Figure 6. Carbon concentration in sediment at different depths inside seagrass patches.

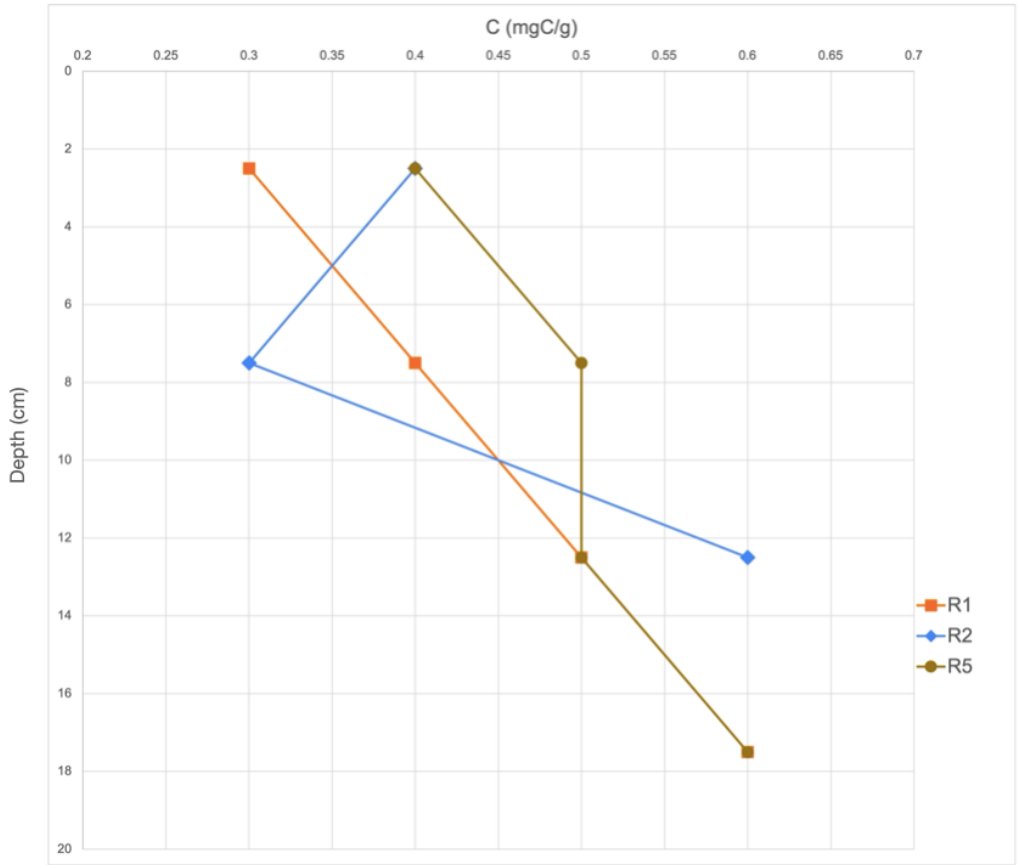

Figure 7. Carbon concentration in sediment at different depths outside seagrass patches.

4.4 Carbon biomass of seagrass

Carbon storage in the seagrass biomass for each species was slightly different (Figure 8). *H. pinifolia* from study site was in range of 0.01 to 0.19 kg C/m^2 or 19.26 to 34.50%, and in *H. uninervis* was in range of 0.03 to 0.19 kg C/m^2 or 23.85 to 30.1%. *H. uninervis* stored more carbon than *H. pinifolia* could: average of *H. uninervis* was 0.09 ± 0.05 kg C/m^2 or 27.88 ± 2.21% and *H. pinifolia* was 0.06 ± 0.05 kg C/m^2 or 27.88 ± 5.00%, respectively. For *H. uninervis*, a larger amount of carbon was in the belowground portion (i.e., 0.07 ± 0.06 kg C/m^2 or 26.75 ± 2.58% aboveground vs 0.11 ± 0.05 kg C/m^2 or 29.00 ± 2.58% belowground). On the other hand, *H. pinifolia* had more carbon stored within aboveground (i.e., 0.08 ± 0.06 kg C/m^2 or 26.80 ± 5.03% aboveground vs 0.04 ± 0.02 kg C/m^2 or 28.53 ± 5.03% belowground).

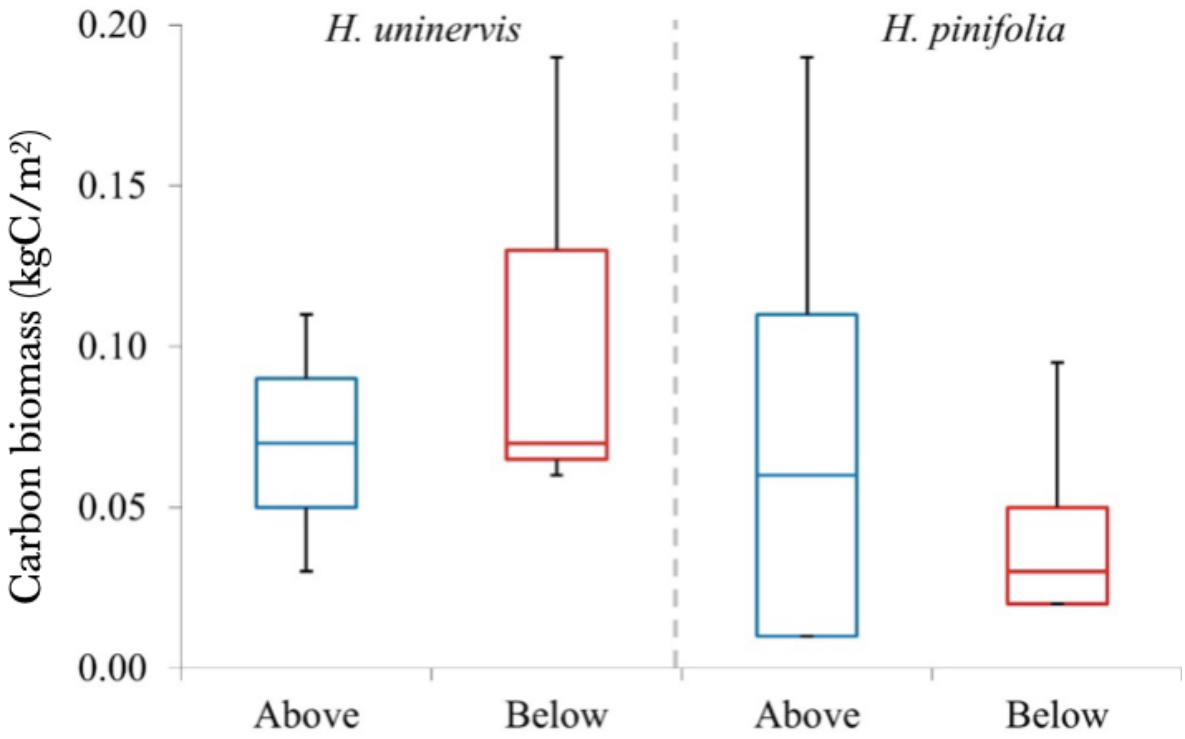

Figure 8. Carbon storage in biomass of the *Halodule* two species at Rock Garden.

The results from this study were lower than other areas reported in Thailand as shown in Table 5. For example, carbon biomass of *H. pinifolia* in Khungkraben in 2016 was 33.93 and 42.53 percent, respectively in aboveground and belowground (Srinamngeon, 2016) and in 2018 reported 44.23 and 43.98 percent, respectively in aboveground and belowground (Srinamngeon, 2018). Likewise, carbon biomass of *H. uninervis* observed in Trang was 36.04

and 33.03 percent, respectively in aboveground and belowground and in Ranong was 36.90 and 35.25 percent, respectively in aboveground and belowground (Prathep, 2012).

Such differences in carbon content might be caused by the conditions of the seagrass meadows (Prathep, 2012). Previous studies may have be conducted at healthier and more pristine sites compared with Rock Garden Village. Moreover, different methods for organic carbon content analysis may introduce variabilities in the obtained values as well.

Table 5. Organic carbon in different species of seagrass in Thailand (mean ± S.E.).

Year	Seagrass	Method	Study area	Organic carbon (%)		Reference
				Aboveground	Belowground	
2016	*H. pinifolia*	Walkley and Black titration	Khungkraben	38.93	42.53	Srinamngoen *et al*. (2016)
2016	*H. pinifolia*	Walkley and Black titration	Khungkraben	44.23	43.98	Srinamngoen *et al*. (2018)
2017	*H. pinifolia*	Walkley and Black titration	Sattahip	88.28		Srinamngoen (2020)
2017	*H. pinifolia*	Walkley and Black titration	Khungkraben	85.00		Srinamngoen (2020)
2017	*H. pinifolia*	Walkley and Black titration	Rock Garden-Nernkho	95.91		Srinamngoen (2020)
2020	*H. pinifolia*	Elemental analyzer	Rock garden	26.80	28.53	This study
2012	*H. uninervis*	Elemental analyzer	Had Chaomai, Trang	36.04	33.03	Prathep (2012)
2012	*H. uninervis*	Elemental analyzer	Ranong	36.90	35.25	Prathep (2012)
2017	*H. uninervis*	Walkley and Black	Ko Kradat, Trat	71.31		Srinamngoen (2020)
2020	*H. uninervis*	Elemental analyzer	Rock garden	26.75	29.00	This study

Chapter 5
Conclusion and Recommendations

5.1 Conclusion

Across 19 cores, two types of seagrass were found: *Halodule pinifoila* and *Halodule uninervis*. Sediment cores collected were of the length of 3 to 22 cm as shown in Table 1. The sediment underlying the seagrass beds at Rock Garden Village was mainly made up of sand with organic carbon content ranging from 0.3 to 4.5 mg C/g dw with an average 0.88 ± 0.65 mg C/g dw, based on 65 subsamples analyzed. The average organic carbon concentration inside the patch (0.97 ± 0.68 mg C/g dw) was significantly higher than that from outside the patch (0.46 ± 0.11 mg C/g dw) ($p < 0.05$).

There was no discernible trend in organic carbon concentrations by depth in sediment cores within the seagrass bed. However, sediment in seagrass patch stores larger amounts of carbon at upper depth. Contrarily to sediment outside the seagrass patch, storing larger amount of carbon at lower depth. The average carbon store underlay *H. pinifoila* is 1.05 ± 0.71 mg C/g dw and in *H. uninervis* is 0.82 ± 0.57 mg C/g dw.

This study also found a difference in average organic carbon content between the two seagrass species. The average biomass in *H. uninervis* was 0.09 ± 0.05 kg C/m^2, and had 0.07 ± 0.06 kg C/m^2 in their aboveground biomass and 0.11 ± 0.06 kg C/m^2 in their belowground biomass. Meanwhile, *H. pinifoila* biomass was 0.06 ± 0.05 kg C/m^2, and stored 0.08 ± 0.06 kg C/m^2 and 0.04 ± 0.02 kg C/m^2 in their aboveground and belowground biomass respectively. Overall, *H. uninervis* had a higher carbon content than *H. pinifoila.*

5.2 Recommendations

1. The leaf tip of seagrass is a confirmation key to identify *Halodule* spp. to a species level. Since this study did not find any complete leaf tips, we used other morphology including leaf width and leaf length for species identification. The author suggests carefully collecting seagrass samples for further work.

2. Samples from this work were collected either at dusk or at night during the low tide. While this provided easy access to the seagrass patches, it was hard to locate the sampling sites. The author suggests collecting samples during the time of the year when low tide was during the daytime and focus on the day of the spring tide. However, if nighttime sampling has to be done to accommodate seasonal sampling, different plans need to be thought out to accommodate the visibility issue.

3. For further work, satellite images can be used to estimate the area size and seagrass and combine with the groundwork data done in this work.

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Appendices

Appendix A: Precision and accuracy of organic carbon analysis

Table a1. Certified value from analyzing carbon: acetanilide.

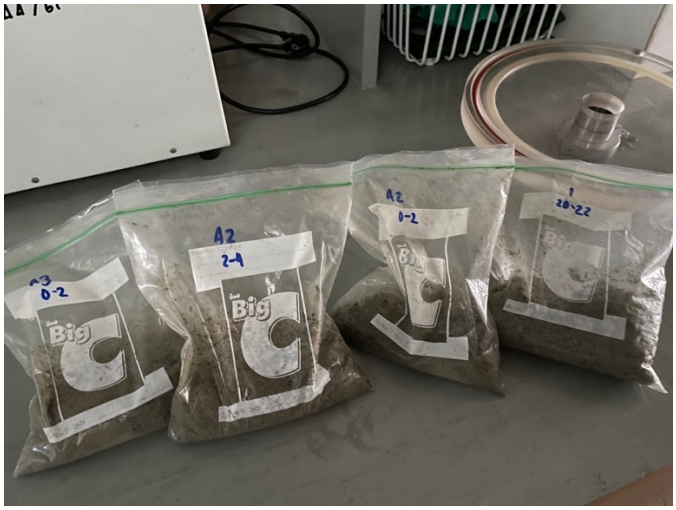
	C in N241-0324 REV-A					Mean±SD	Certified Value
	1	2	3	4	5		
Value	16.9	17.339	15.83	15.92	16.8	16.56±0.59	16.5±1.5

Table a2. %RPD from analyzing organic carbon.

	%RPD
%OC	2.78±2.62

Appendix B: Laboratory process

Sediment preparation



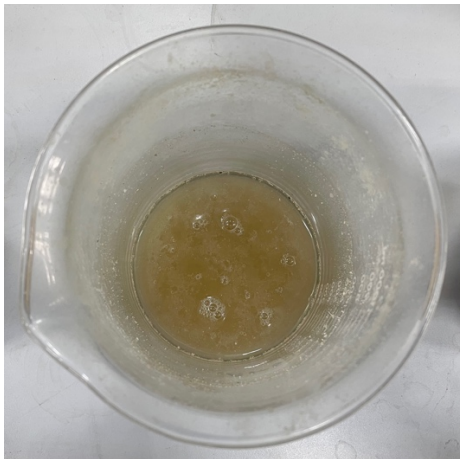
1. Freeze the sediment samples at -20 °C.

2. Freeze-dry the samples.

3. Select sediment smaller than 2mm by sieve.

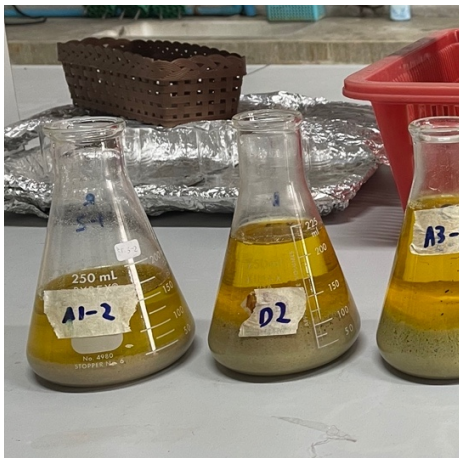
Grainsize analysis

1. Add hydrogen peroxide to remove organic compounds in the sediments.

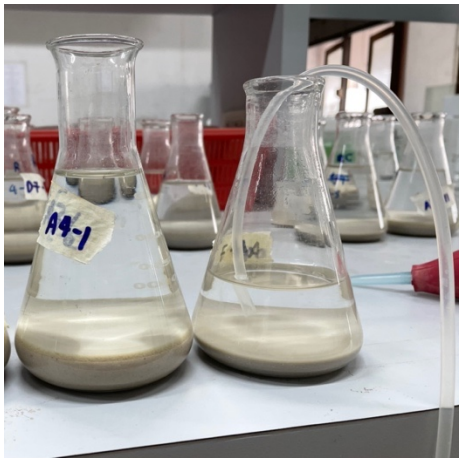


2. Add 50% (v/v) HCl to digest inorganic portions of the samples.

3. Transfer the content from beakers to flasks.



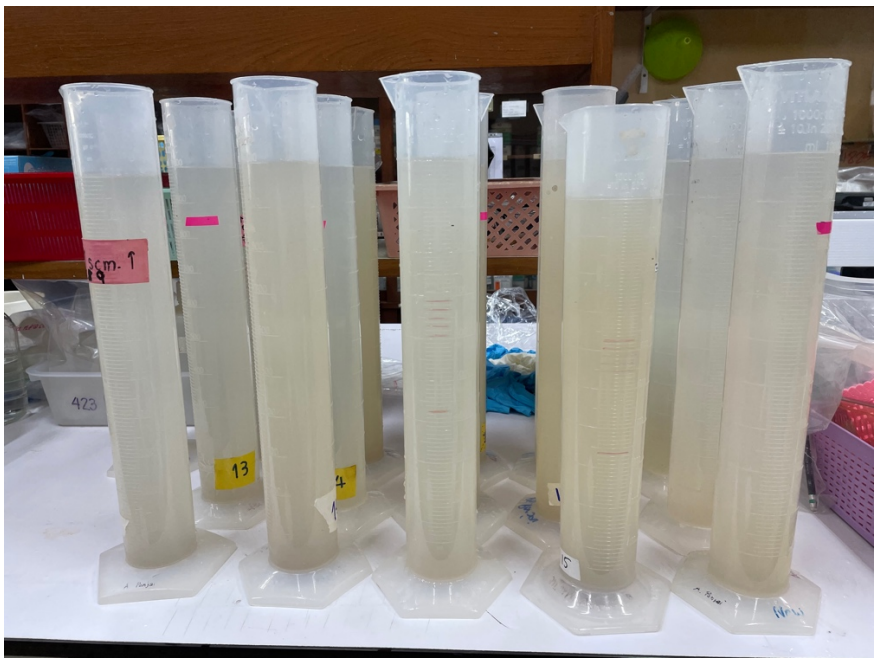
4. Use distilled water to neutralize the samples.



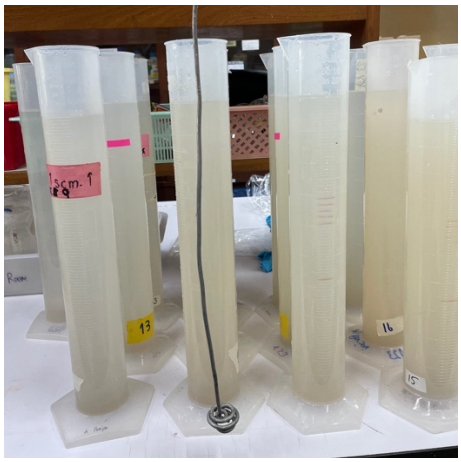
5. Carefully drain out the supernatant.

6. Sieve out sediments with >63 μm diameter (i.e., sand).

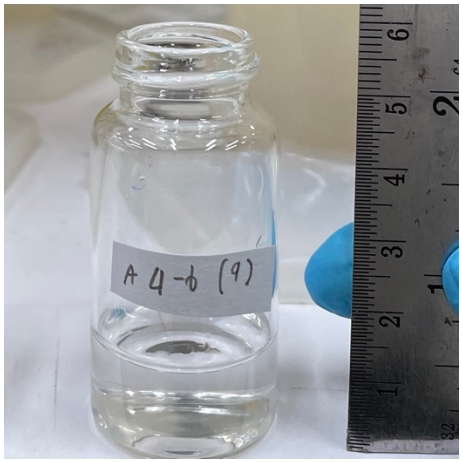
7. Dry the samples at 110°C.



8. Transfer the remaining silt and clay into 1-liter measuring cylinders. Add 10 ml. of 10% (w/v) sodium hexametaphosphate, ($Na_6[(PO_3)_6]$) and filled up with distilled water up to a 1-L mark.

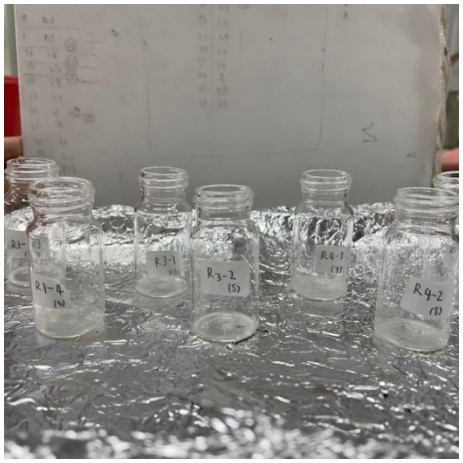


7. Mix the slurry and let it silt for 3 hours and 52 minutes.

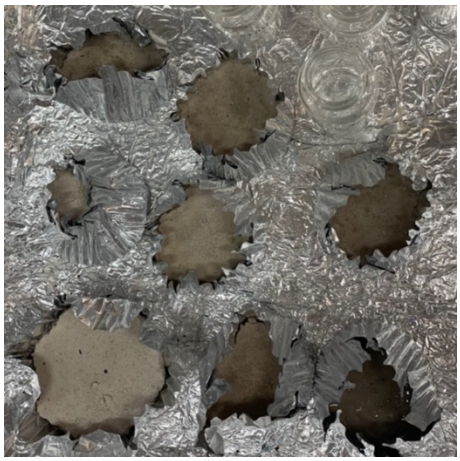


8. Pipet out the solutions containing clay.

9. Dry the clay at 110°C.



10. These are dry clay contained in the vials.



11. Weigh out the dry sand portion.

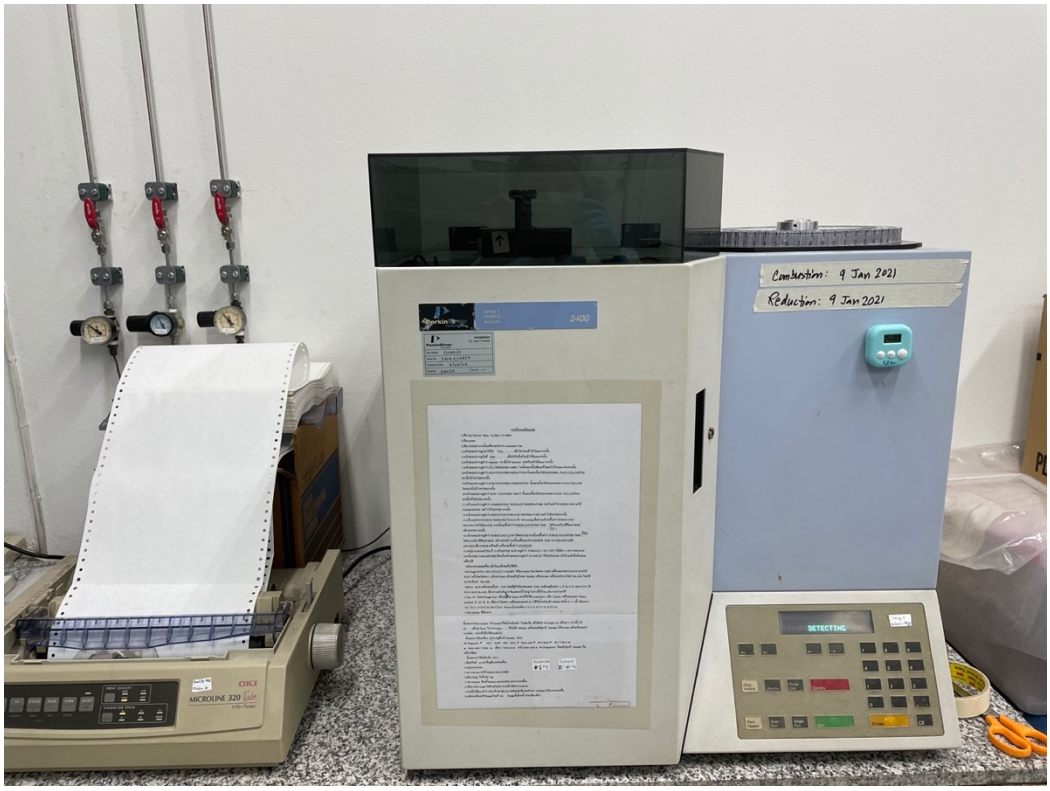
12. Weigh out the clay portion.

Carbon analysis

1. Pack sample in tin cup and weight by ultra-microbalance.

2. Fold tin cup like a ball and make sure each is fully sealed.

3. Store each tin cup in identifiable vial.



4. Place each sample in elemental analyzer with exact weight.

5. Record data.