# A Study of microplastics contamination in soil, seawater and seagrass at Sikao District, Trang Province.

Thanwarat Jenwitchumet and Kantaros Asbilly

Patchara Pongmanawut and Neungruthai Chaimanee

Princess Chulabhorn Science High School Trang

Thailand

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**Abstract:**

This research aims to study the amount of microplastics in soil, seawater and sea grass. The study was conducted at Pak Klong Beach and Ao Kham, Sikao District, Trang Province. The study was conducted by collecting samples of water, soil and 3 prominent sea grass species; *Enhalus acoroides*, *Halophila ovalis* and *Cymodocea rotundata*. Analysis of water quality, microplastics in soil, in seawater and in seagrass of all 3 species. The results of the study found that the pH and salinity of the water in both areas were not significantly different, but the DO values were slightly higher in Ao Kham than at Pak Klong Beach. The amount of microplastics in soil and seawater was found to be not significantly different in the two study areas. It was found that the amount of microplastics in the soil was higher than in the seawater. When studying the amount of microplastics in the soil in areas with all 3 species of seagrass, it was found that soil in areas with *Enhalus acoroides* had the highest amount of microplastics (19.00 ± 1.04, Pak Khlong beach and 14.00 ± 0.99 piece/300g, Ao Kham), followed by areas with *Halophila ovalis* and *Cymodocea rotundata* respectively. When studying the amount of microplastics in seagrass, it was found that *Cymodocea rotundata* had the highest amount of microplastics (5.67 ± 1.45 piece/300g, Pak Khlong beach and 5.00 ± 0.89 piece/300g, Ao Kham), followed by *Enhalus. acoroides* and *Halophila ovalis* respectively. The size of microplastics found in each species of seagrass in both areas was not different. Microplastics found ranged in size from 1mm-20 µm. Most were found in the shape of filaments and black color. This suggests that these microplastics may have come from fishing nets or equipment used in fishing in the area.

**Keywords:** Microplastics *Halophila ovalis* *Cymodocea rotundata* *Enhalus acoroides*

**Research Question:**

1. Are there any differences in water quality between Pak Khlong Beach and Ao Kham?

2.Are the amounts of microplastics in the soil and the seawater around Pak Khlong Beach and Ao Kham different?

3. Do the quantities of microplastics in soil in different species of seagrass areas differ?

4. Do the quantities of microplastics in different species of seagrass differ?

5. Do the size, color and shape of microplastics in different species of seagrass differ?

**Research Hypothesis:**

1. The water quality at Pak Khlong Beach and Ao Kham differs.

2. The area around Pak Khlong Beach has a higher quantity of microplastics in both soil and seawater compared to Ao Kham.

3. The quantity of microplastics in the soil varies among different species of seagrass.

4. The quantity of microplastics differs among different species of seagrass.

5. The size of microplastics varies among different species of seagrass.

**Introduction and Review of Literature:**

Coastal blue carbon ecosystems (CBCEs) are widely distributed in the transitional zone between terrestrial and marine environments, from polar to tropical regions. These ecosystems primarily consist of tidal marshes, mangrove forests, and seagrass beds. Seagrass is a crucial ecosystem due to its exceptional capacity as a carbon sink and its significant importance for marine life. It serves as a highly productive food source for marine and endangered species such as dugongs and sea turtles. According to a survey conducted, seagrass beds in Trang province are predominantly in fair to moderate condition. Notable species include *Halophila ovalis, Cymodocea rotundata,* and *Enhalus acoroides* (Department of Marine and Coastal Resources, 2023)

Plastic waste cannot degrade quickly, leading to its accumulation in large quantities. When degraded, it transforms into microplastics, particles smaller than 5 millimeters in diameter. These microplastics can absorb organic pollutants and metals. Consequently, they serve as significant vectors for introducing toxins into ecosystems and causing bioaccumulation within organisms, progressively increasing in concentration along the food chain.

Currently, there has been an increase in microplastic pollution in the Andaman Sea. Accumulation of microplastics has been found in beach sediments along seagrass beds (Krismaat Pathanasin, 2021), as well as in seawater (Kwanthip Pongpan, 2018). Additionally, marine microplastics have been discovered, and it's been suggested that accumulated microplastics in seagrasses may have adverse effects on seagrass (M. Gerstenbacher, 2022).

The researchers aim to investigate the amount of microplastics in both soil and seawater, which may affect the quantity and size of microplastics in three species of sea grass: *Halophila ovalis, Cymodocea rotundata,* and *Enhalus acoroides* at Pak Khlong Beach and Ao Kham in Trang province. The findings will contribute to the conservation of sea grass. The data obtained from this study will be beneficial for individuals seeking to further their understanding and knowledge in this field.

**Research Methods and Materials:**

**Materials**

1. Quadrate size 50×50 cm.
2. Sieve size 5 mm. and 1 mm.
3. Hotplate magnetic stirrer
4. Filter fabric 300 and 20 µm
5. Stereo microscope
6. Hot air oven
7. Vacuum pump
8. Manta net
9. Wet mount slide
10. Microscope
11. pH meter
12. DO meter
13. Reflectometers
14. Ferrous Sulfate ($FeSO_4$) 0.05 M
15. Hydrogen Peroxide ($H_2O_2$) 30%
16. Potassium Hydroxide (KOH) 1%
17. Normal Saline Solution
18. Distilled Water

**Methods**

**1.Study sites**

Study contamination of microplastic at Trang province from two study sites. Point 1, located at Pak Khlong Beach, situated at coordinates (latitude 7°60'91" N longitude 99°27'85"E)and Point 2, located at Ao Kham positioned at coordinates (latitude 7°50'22" N longitude 99°30'10"E),Figure 1.

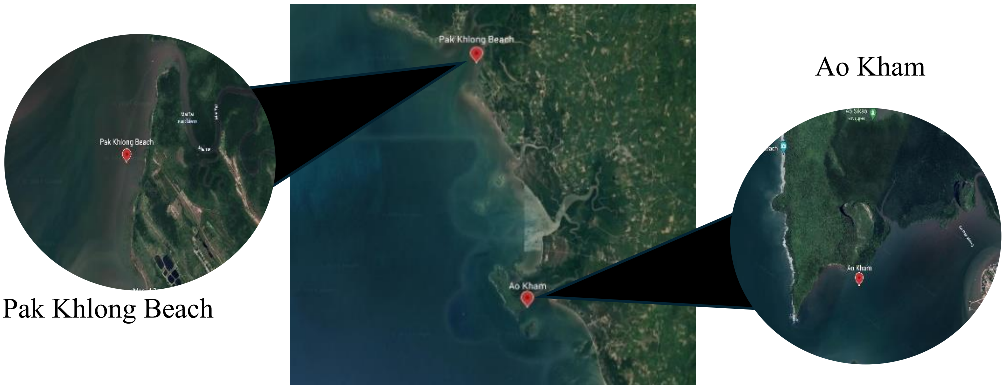


Figure 1: Shows the study sites

**2.Data Collection**

**2.1 Collection of water quality**

1.To measure water quality according to the GLOBE protocol by measure pH, dissolved oxygen, and salinity in the seawater as follows:

2. Determine the sampling points by randomly dividing into 3 points per study site.

3. Study seawater properties using various indices, including pH, dissolved oxygen levels, and salinity. Measure pH using a pH meter, dissolved oxygen using a DO meter, and salinity using reflectometers. Collect water samples for laboratory analysis of microplastics.

4. Send the data into the GLOBE Data Entry.

**2.2 Study of soil microplastic**

1. Determine sampling points by randomly dividing the study sites into 3 points per species of seagrass for each study sites.

2. Collect soil samples by placing a 50×50 cm quadrate and oven-drying the soil at 60 degrees Celsius for 24 hours.

3. Sieve the dried soil samples through 5 mm and 1 mm mesh sieves. Count and record the quantity of soil retained on the sieves, then photograph and record the results.

4. Weigh the soil samples that passed through the 1 mm sieve from each quadrate of the study sites, and analyze in amount of 300 g sample.

5. Place the soil samples in 1000 ml beakers and add saltwater at a 1:1 ratio with the soil volume. Stir with a glass rod and let settle for approximately 5 minutes.

6. Filter the clearer water through a 300 µm filter cloth, then re-filter it with the filter cloth.

Repeat this process 3-5 times until no visible particles are floating on the water's surface. Rinse the soil sample adhering to the filter cloth with distilled water into a 500 ml beaker.

7. Filter the filtered water through a 20 µm filter cloth, then rinse the soil sample adhering to the filter cloth with distilled water into a 500 ml beaker.

8. Place the sample into the process (WPO) by pour 20 ml of 30% hydrogen peroxide and 20ml of Ferrous Sulfate ($FeSO_4$) 0.05 M

9. Place the beakers on a hot plate and heat, adding hydrogen peroxide incrementally by 20 ml each time, repeating until no reaction occurs or the temperature stabilizes.

10. Add sodium chloride and reheat until the salt is dissolved completely.

11. Transfer the solution to a tall container and let stand for 24 hours.

12. Place the soil sample adhering to the 300 µm filter cloth onto a 20 µm filter cloth and Oven dry at 60 degrees Celsius for approximately 3 hours or until dry.

13. The dried samples is examined under a stereo microscope and record observations of shape, color and quantity.

**2.3 Study of water microplastic**

1. Deploy the manta net, which has a known cross-sectional area, and attach a flow meter to the forested area. Drag it across the water surface for 5 minutes, repeating the process 3 times.

1. Filter the samples from the bottle through a 300 µm filter cloth, then transfer the samples adhering to the filter cloth into a 500 ml beaker.

3. Subject the samples to the WPO process, similar to the soil samples.

4. After digestion, add 6 g of salt per 20 ml of solution, then transfer to a tall container and leave it overnight. Use distilled water to rinse the sample solution in the beaker while transferring.

5. After 1 night, filter the water through a 300 µm filter cloth. Filter the solution that passes through the 300 µm filter cloth through a 20 µm filter cloth, then oven dry at 60 degrees Celsius for approximately 3 hours or until dry.

6. The dried samples is examined under a stereo microscope and record observations of shape, color and quantity.

**2.4 Study of seagrass microplastic**

1. Determine the sampling point by randomly selecting an area with three species of seagrass : *Halophila ovalis, Cymodocea rotundata* and *Enhalus acoroides* in each study area.

2. Collect seagrass samples, with a quantity of 50×50 centimeters. Randomly collect 45 seagrass plants and divide them into bergamot leaves. *Halophila ovalis* 15 samples , *Cymodocea rotundata* 15 samples and *Enhalus acoroides* 15 samples.(a total of 45 samples)

3. Record the physical characteristics of seagrass, including the length of roots and leaves for each species of seagrass.

5. Take a seagrass sample, weigh it on foil, and place it in the refrigerator.

6. Rinse the sample with distilled water, then the sample into small pieces with scissors, place them in a 500ml beaker.

7. Place the sample into the process (WPO) by pour 180ml of 30% hydrogen peroxide and 20ml of 1% potassium hydroxide.

8. Place the magnetic stirring rod into the mixer. When making the sample, close the foil beaker mouth. When making the sample, close the foil beaker mouth. After digestion is completed, add 6 g of salt to every 20 milliliters of solution, stir to dissolve, pour into a high cylinder, and let stand for 1 night. When sampling, use distilled water to remove sediment from the beaker.

10. After leaving it overnight, remove the sample with a 20 μm filter cloth.

12. Bake the sample at 60 ° C for approximately 3 hours or until dry.

13. The dried samples is examined under a stereo microscope and record observations of shape, color and quantity.

**2.5 Study of the leaf cross section of seagrass.**

1. Take a seagrass sample, cut it into small pieces, and stain it with toluene blue O for 1 minute.

2. Place the water droplet with the sample on a clean slide. Apply the sample onto the water droplet.

3. Close with sliding glass and gently tilt downwards. Be careful not to produce bubbles.

4. Observe the sample slides under a microscope and take photos.

**3.Statistical Data**

1. Analyze the water quality, microplastics in soil, microplastics in three species of seagrass, and microplastics in water, using mean and standard deviation.

2. Compare water quality using a t-test: Two-Sample Assuming Equal Variances.

3. Compare the quantity, color, and shape of microplastics in soil, and the quantity, size, color, and shape of microplastics in different species of seagrass, using Two-way ANOVA.

**Results:**

**1.Study of seawater quality**

The study on water quality in the Pak Khlong Beach and Ao Kham revealed that there was no statistically significant difference in pH and salinity between the two study sites. However, the dissolved oxygen (DO) levels in Ao Kham were slightly higher compared to Pak Khlong Beach. (figure 2-4)

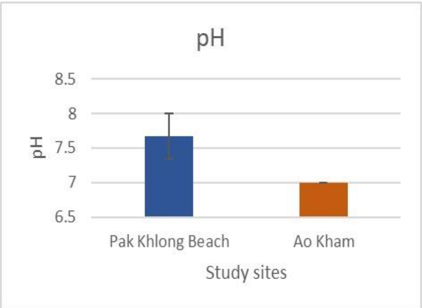


Figure 2: The chart show pH

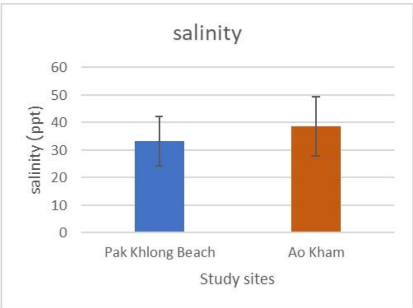


Figure 3 : The chart show salinity

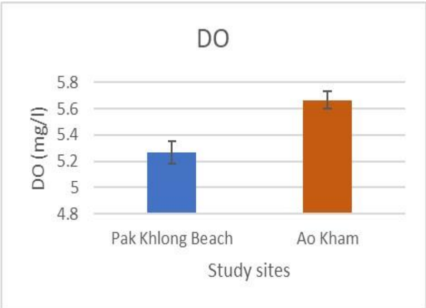


Figure 4 : The chart show DO

**2.Study of microplastics in soil**

A study on microplastics in soil revealed that the quantity of microplastics in Pak Khlong Beach and in Ao Kham had no statistically significant difference. However, in various species of seagrass sources, there were statistically significant differences. The highest quantity of microplastics in soil was found in *Enhalus acoroides*, followed by *Halophila ovalis* and *Cymodocea rotundata* in both areas, as shown in Table 1.

Table 1 shows the quantities of microplastics in soil.

| Study sites | Species | Microplastic numbers | | | | |
|---|---|---|---|---|---|---|
| | | >5mm | 5mm-1mm | 1mm-300µm | 300-20µm | Total |
| **Pak Khlong Beach** | *H. ovalis* | 2.00 ± 0.58 | 2.00 ± 1.53 | 9.33 ± 0.88 | 8.67 ± 0.33 | 15.00 ± 1.26 |
| | *C. rotundata* | 1.33 ± 0.67 | 1.00 ± 0.58 | 2.33 ± 1.20 | 2.00 ± 1.00 | 6.67 ± 0.30 |
| | *E. acoroides* | 2.00 ± 1.15 | 4.33 ± 0.88 | 7.67 ± 1.53 | 13.67 ± 2.33 | 19.00 ± 1.04 |
| **Ao Kham** | *H. ovalis* | 1.33 ± 0.67 | 2.33 ± 0.33 | 3.33 ± 0.88 | 3.00 ± 1.53 | 10.00 ± 0.44 |
| | *C. rotundata* | 0.33 ± 0.33 | 1.67 ± 0.88 | 3.67 ±0.67 | 2.33 ± 1.20 | 8.00 ± 0.69 |
| | *E. acoroides* | 0.67 ± 0.33 | 3.67 ± 0.67 | 4.67 ± 0.67 | 5.00 ± 1.53 | 14.00 ± 0.99 |

The study of the shapes and colors of microplastics found two distinct shapes: fiber and fragment. It was found that the fiber shape is more prevalent than the fragment shape in study sites. Regarding the colors of microplastics, it was observed that black microplastics are the most abundant in both study sites (Figure 5-6).

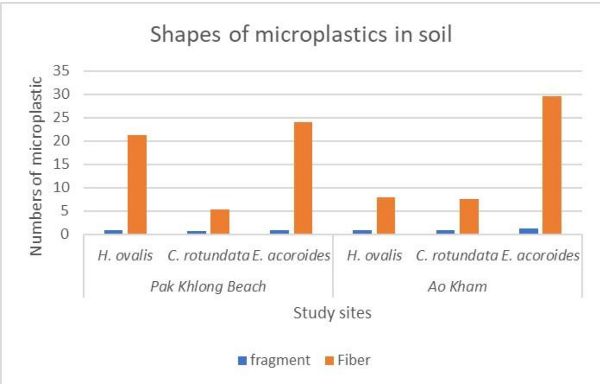


Figure 5: shows the shape of microplastics

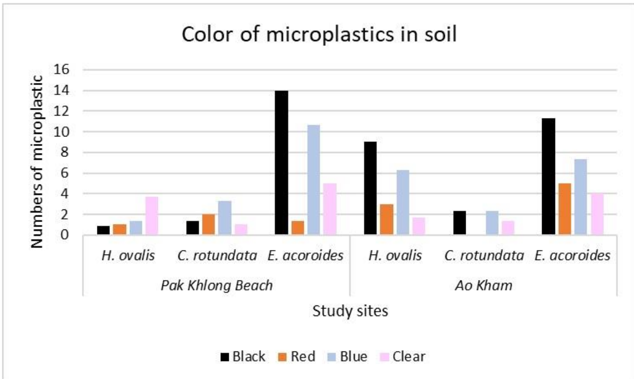


Figure 6: shows the color of microplastics

**3.Study of microplastics in seawater**

The study of microplastics in water revealed that the quantity of microplastics in the water at Pak Khlong Beach and Ao Kham did not differ significantly statistically, as shown in Table 2.

Table 2 shows the quantity of microplastics in seawater.

| Study sites | Microplastic numbers | | |
|---|---|---|---|
| | 1mm-300µm | 300-20 µm | Total |
| **Pak khlong beach** | 1.67 ± 0.67 | 1.33 ± 0.33 | 3.00 ± 0.17 |
| **Ao Kham** | 2.00 ± 1.00 | 2.67 ± 0.88 | 4.67 ± 0.33 |

The study of the shapes and colors of microplastics in seawater revealed two distinct shapes: fiber and fragment. Fiber shapes were more prevalent than fragment in both study sites, and black microplastics were found to be the most abundant color in both study sites. (Figure 7-8).

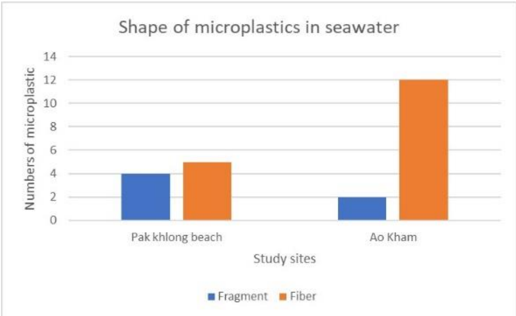


Figure 7: shows the shape of microplastics

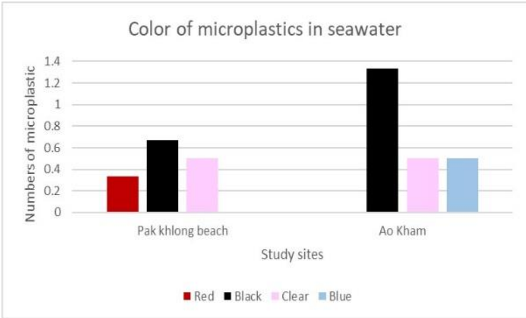


Figure 8: shows the color of microplastics

**4. Study of microplastics in seagrass**

A study on microplastics in various species of seagrass found that the quantity of microplastics in seagrass in the both study sites of Pak Khlong Beach and Ao Kham did not significantly differ statistically. Additionally, it was found that *Cymodocea rotundata* had the highest average amount of microplastics found per seagrass, at 30 g, followed by *Enhalus acoroides* and *Halophila ovalis*, respectively. Furthermore, the size of microplastics in each type of seaweed did not differ between the two study sites, as shown in Table 3.

Table 3 shows the quantities of microplastics in Seagrass.

| **Study sites** | **species** | **Microplastic numbers** | | |
|---|---|---|---|---|
| | | **1mm-300μm** | **300-20μm** | **Total** |
| **Pak Khlong Beach** | *H. ovalis* | 2.00 ± 0.58 | 1.00 ± 0.58 | 1.50 ± 043 |
| | *C. rotundata* | 6.67 ± 2.33 | 4.67 ± 2.03 | 5.67 ± 1.45 |
| | *E. acoroides* | 4.00 ± 0.58 | 3.33 ± 1.33 | 3..67 ± 0.94 |
| **Ao Kham** | *H. ovalis* | 2.33 ± 0.88 | 3.00 ± 1.53 | 2.67 ± 0.80 |
| | *C. rotundata* | 5.67 ± 1.45 | 4.33 ± 1.20 | 5.00 ± 0.89 |
| | *E. acoroides* | 3.67 ± 0.33 | 2.00 ± 0.58 | 2.83 ±0.48 |

The study of the shapes and colors of microplastics found two shapes in three species of seagrass: fiber and fragment. Fiber shapes were more prevalent than fragmented ones in both study sites, and black microplastics were found to be the most abundant color in all three species of seagrass (Figure 9-10).

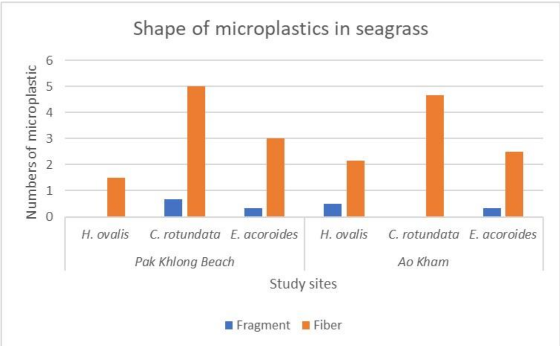


Figure 9: shows the shape of microplastics

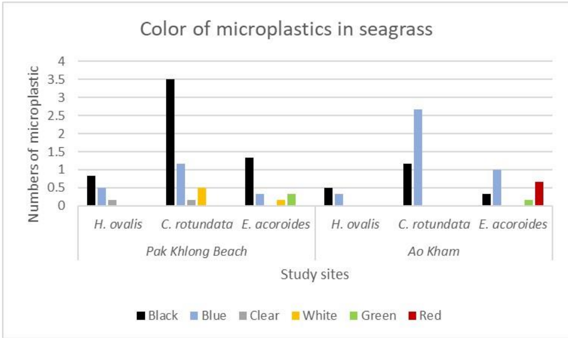


Figure 10: shows the color of microplastics

**5. Study of the leaf cross section of seagrass.**

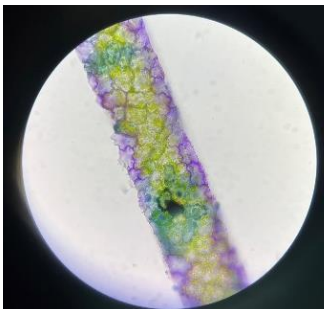

Figure 11: *Halophila ovalis*

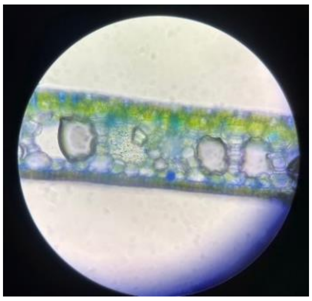

Figure 12: *Cymodocea rotundata*

Figure 13:*Enhalus acoroides*

From studying the leaf cross section of seagrasses, it is evident that the surface structure varies significantly between different species. *Cymodocea rotundata* has thin leaves with extensive internal gaps, allowing microplastics to accumulate easily. In contrast, *Halophila ovalis* has fewer internal gaps within its leaves, while *Enhalus acoroides* has thicker leaf surfaces, making it difficult for microplastics to accumulate within the leaves (Figure 11-13).

**Diccussion:**

The water quality and the quantity of microplastics in both study sites do not statistically differ. This is because both areas have similar geographical features. The soil in *Enhalus acoroides* area has the highest amount of microplastics. This is because the physical characteristics of it, such as the length and density of bristle fibers, make it a trap for microplastics, leading to more accumulation in the soil compared to other areas. Regarding the accumulation of microplastics in seagrass, *Cymodocea rotundata* has the highest quantity, followed by *Enhalus acoroides* and *Halophila ovalis*, respectively. This is because *Cymodocea rotundata* has thin leaves with extensive internal gaps, allowing microplastics to accumulate easily. In contrast, *Halophila ovalis* has fewer internal gaps within its leaves, while *Enhalus acoroides* has thicker leaf surfaces, making it difficult for microplastics to accumulate within the leaves. The size of microplastics found in each type of seagrass in both areas is similar in terms of shape and color, indicating a possible origin from fishing gear and equipment used in fishing activities in the area, which are mostly made of nylon and fiber.( Supakorn Thepwilai,2021)

**Conclusion:**

The results revealed that the pH and salinity values did not differ between the two study sites. However, the dissolved oxygen (DO) level in Ao Kham was slightly higher than in Pak Khlong Beach. The amount of microplastics in the soil and the seawater at Pak Klong Beach and Ao Kham was not significantly different. It was found that *Enhalus acoroides* area in the soil had the highest levels of microplastics followed by *Halophila ovalis* area and *Cymodocea rotundata* area respectively. Moreover, *Cymodocea rotundata* had more microplastics than both *Enhalus acoroides* and *Halophila ovalis* respectively. Additionally, the size of the microplastics which were found in each seagrass from both study sites were not different, their size being from 1 mm – 20 µm Most of the microplastics found are linear and black. According to the size, shape and color found, these microplastics may come from annular or annular ligaments used in fishing activities in this area, and the components of these devices come from ropes and fibers.

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**Citations:**

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**Send the data into the GLOBE Data Entry**

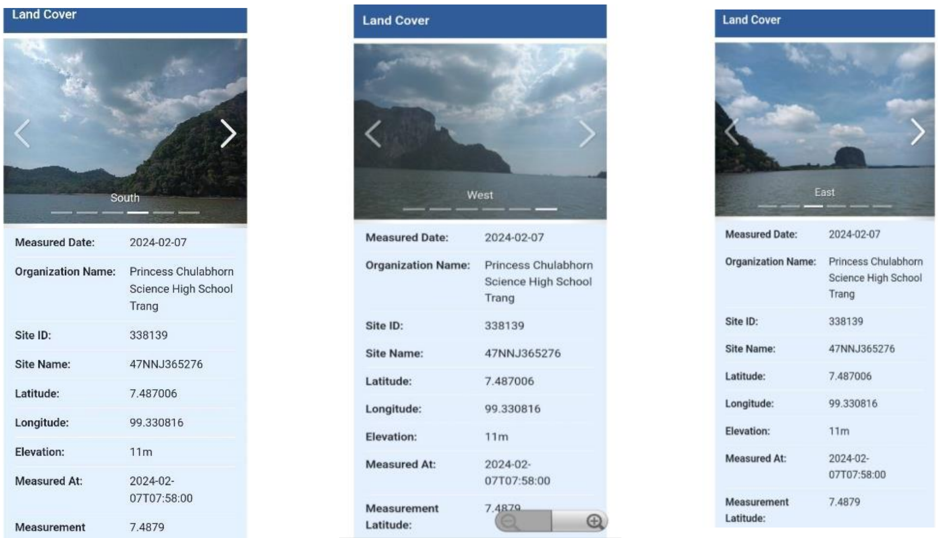


Figure 14: Send Land Cover information

| Salinity | |
|---|---|
| Measured Date: | 2024-02-07 |
| Organization Name: | Princess Chulabhorn Science High School Trang |
| Site ID: | 337330 |
| Site Name: | หาดปากคลอง |
| Latitude: | 7.600253 |
| Longitude: | 99.278839 |
| Elevation: | 20.2m |
| Measured At: | 2024-02-07T14:30:00 |
| Before Salinity Measurement Tide At: | 2024-02-07T15:00:00 |
| After Salinity Measurement Tide At: | 2024-02-07T18:00:00 |
| Before Salinity Measurement Tide Type: | low |
| After Salinity Measurement Tide Type: | high |
| Water Body State: | normal |
| Salinity via Hydrometer: | 33.3 ppt |
| Salinity Kit Mfg: | other |
| GLOBE Teams: | GLOBE PCSHSTrang |

Show on Map

| pH | |
|---|---|
| Measured Date: | 2024-02-07 |
| Organization Name: | Princess Chulabhorn Science High School Trang |
| Site ID: | 338918 |
| Site Name: | อ่าวมะขาม |
| Latitude: | 7.497402 |
| Longitude: | 99.299012 |
| Elevation: | 0m |
| Measured At: | 2024-02-07T15:45:00 |
| Water Body pH: | 7 pH units |
| Water Body State: | normal |
| pH Method: | paper |
| pH Buffer 7: | true |
| GLOBE Teams: | GLOBE PCSHSTrang |

Show on Map

| Dissolved Oxygen | |
|---|---|
| Measured Date: | 2024-02-07 |
| Organization Name: | Princess Chulabhorn Science High School Trang |
| Site ID: | 337330 |
| Site Name: | หาดปากคลอง |
| Latitude: | 7.600253 |
| Longitude: | 99.278839 |
| Elevation: | 20.2m |
| Measured At: | 2024-02-07T14:30:00 |
| Water Body State: | normal |
| Dissolved Oxygen Salinity: | 33.3 ppt |
| Dissolved Oxygen via Kit: | 5.3 mg/L |
| GLOBE Teams: | GLOBE PCSHSTrang |

Show on Map

| Dissolved Oxygen | |
|---|---|
| Measured Date: | 2024-02-07 |
| Organization Name: | Princess Chulabhorn Science High |

Figure 15: send water quality information into the data entry

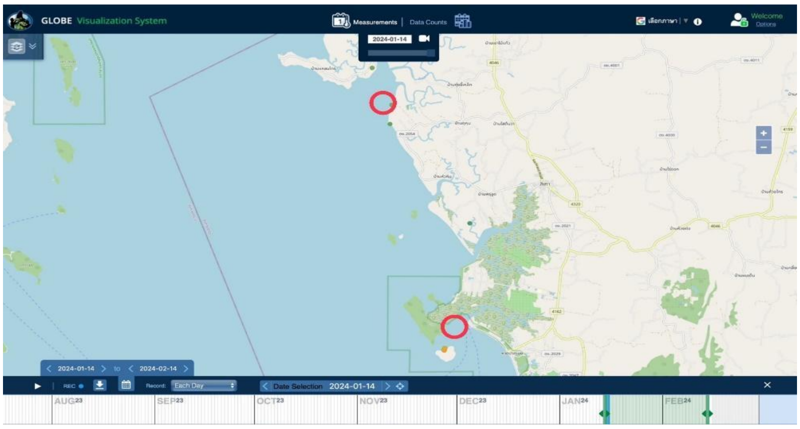


Figure 16: shows the study sites of Pak Khlong Beach and Ao Kham .

**Badge Descriptions:**

**I make an impact**

Understanding the interactions between seagrass and microplastic accumulation reveals the extent of ecosystem damage that individual seagrass species cause from plastic pollution. Seagrass beds, which are important marine habitats It serves as both a breeding ground for marine life and a carbon sink. This makes them indispensable to the health of the ecosystem. It is a guideline for being careful of the effects of microplastic accumulation in each type of seagrass. Including the risk trend of each species of fish species accumulating high levels of microplastics that will affect the survival of seagrass in the future.

**I am a collaborator**

This study has received cooperation from many departments, including friends, teachers, consultants, scientists, and villagers. Collaboration has many benefits in brainstorming, collaborative solutions, and better solutions. Collaboration with school friends makes research collection more detailed. Collaborate with teachers and scientists. Let's learn more detailed information about research work, whether it's suggestions for experimental design methods or research methods. With the cooperation of villagers, it is easier to obtain research seagrass. Multiple efforts were made to help make this study more comprehensive.

**I am a stem storyteller**

Demonstrating effective communication skills to disseminate research findings to both scientific communities and the general public, raising awareness about the critical importance of preserving seagrass ecosystems and mitigating microplastic pollution.