Research Title: Correlation between Soil Quality and Biological Activities of Selected Plants in Coastal Zone of Samet, Chonburi, Thailand

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ABSTRACT

Investigating the correlation between soil quality and biological activities of selected plants in coastal zone of Samet, Chonburi, Thailand is the main aim of the current environmental and biological research. The physicochemical factors of the soil were tested using the standard equipment from Extech. The extracts of the experimental plants were used for various screening to discover the capacity of the plant in inhibiting the growth of microorganisms and other plants like *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli*, *Saccharomyces cerevisiae*, and *Rhizopus nigricans*. The results of various experiments were observed, gathered, and compared using one-way ANOVA and Tukey HSD Test. Based on the experimentations, results and gathered data, the researchers concluded that there was a correlation between soil quality and biological activities of the selected plants in coastal zone of Samet, Chonburi, Thailand. The higher amount of soil nutrients enabled the plants to produce substances that yield to various biological activities. Moreover, there are significant differences ($p<0.05$) in soil temperature (5cm and 10cm depth) and relative humidity except for soil pH and air temperature ($p>0.05$). In addition, more research will be conducted to evaluate the other soil parameters and other biological activities of the experimental plants.

Keywords: Correlation, Soil parameter, Biological Activity, and ANOVA

INTRODUCTION

Chonburi, an eastern coastal city, is well known for its rapid economic growth. As a result, various infrastructures have been constructed, including factories, bridges, and buildings. Moreover, living standards have greatly improved, which is advantageous to the locals. In addition, Chonburi boasts over 10,373 acres of mangrove forest, which is among the largest in the country (Department of Marine and Coastal Resources, 2018). Mangrove forests possess abundant natural resources and spectacular life forms. Plants like mangroves, produce various substances like secondary metabolites that protect them from microbial pathogens and abiotic stresses in their environment (Schafer et al., 2009). These compounds are also responsible for plants biological activities that are also beneficial to human beings. The production of these valuable compounds is linked to the nutrients that plant absorb from the soil. Previous research emphasized that reasonable proportion of nutrition (NPK) factors directly promote the absorption and assimilation of plants, thereby affecting their growth and development (Yildirim et al., 2011). However, the intensity of anthropogenic activities may have had an immense impact on the survival of plant especially, in terms of their nutrient absorption which has great impact on their growth and development. Given the rapid changes occurring in the coastal area of Samet, Chonburi, Thailand that could affect the diversity of life particularly the plants, it is imperative to evaluate the soil quality of this place.

The situations above prompted the researchers to conduct environmental research entitled "Correlation between Soil Quality and Biological Activities of Selected Plants in Coastal Zone of Samet, Chonburi, Thailand. This current study aimed to determine if there is correlation between the quality of soil and biological activities of the two selected plants namely Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* that are abundant in the area.

Research Questions:

1. Is there a correlation between the soil quality and biological activities of selected plants in Samet, Chonburi, Thailand?
2. Is there a significant difference in soil parameters measured in coastal zone of Samet, Chonburi, Thailand?

3. What biological activity is possessed by Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)*?

Objectives:

1. To investigate if there is correlation between soil quality and biological activities of selected plants in Samet, Chonburi, Thailand.
2. To determine if there are significant differences in soil parameters measured in coastal zone of Samet, Chonburi, Thailand.
3. To explore the biological activities possessed by Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)*.

Hypotheses:

Alternative: There is a correlation between soil quality and biological activities of selected plants in Samet, Chonburi, Thailand and there is a significant difference in soil parameters measured in coastal zone of Samet, Chonburi, Thailand.

Null: There is no correlation between soil quality and biological activities of selected plants in Samet, Chonburi, Thailand and there is a significant difference in soil parameters measured in coastal zone of Samet, Chonburi, Thailand.

Materials:

Materials and equipment used in this science project.

Petri Dish	Autoclave	Inoculating loops
Soil Survey Instruments	Mueller Hinton Agar	Vernier Caliper
Sabouraud Dextrose Agar	Stirring Rod	Forceps
Thermo Hygrometer	NPK Concentration Sensor	Erlenmeyer flask
Beakers	Alcohol Lamp	Test tubes
Hot plate	Filter Paper	Graduated cylinder

Research Methodology

A. Study Site

The study site is located at in coastal zone of Samet, Chonburi, Thailand with a Latitude 13°20'39"N, Longitude 100°56'56"E.

FIGURE 1. The study site in Samet, Chonburi, Thailand.

B. Survey and Preparation of Materials

The researchers went to the Center of Expertise on Eco-tourism for Mangrove Conservation, Chonburi Province Office and as ked permission to conduct a field study in the coastal area of Samet Municipality to determine the soil quality of the place. After the survey and selection of study site, needed laboratory materials and equipment for soil testing were procured from the science laboratory of Chonradsadornumrung School.

Soil Quality Testing

Various soil parameters were considered in assessing the quality of soil in Samet, Chonburi such as NPK concentration, soil pH, temperature, moisture, soil texture, soil consistency, soil fertility, also the air temperature and relative humidity of the study site was included. To determine the soil pH, the following steps were carried out: 40 g of dried and sieved soil with 40 mL of distilled water (or other amount in a 1:1 soil to water ratio) was mixed in a beaker using the stirring rod, the mixture was allowed to settle until a supernatant (clearer liquid above the settled soil) formed, the pH of the supernatant was measured using the pH meter. Extech standard thermo-hygrometer was used to determine the air temperature and relative humidity of the study site. Moreover, NPK concentration sensors were used to determine the amount of nitrogen (N), phosphorus (P), and potassium (K) present in the soil.

FIGURE 2. *In situ* measurement of soil quality.

Screening for the Allelopathic Potential of Experimental Plants.

Allelopathic activity of the plants were evaluated using these steps: petri dishes were sterilized in the autoclave for 15 minutes at 121° Celsius with 15 psi to ensure that no other contaminants are present in the equipment. Filter papers and 4 treatments were prepared.

FIGURE 3. Allelopathy test

Control (distilled water) is the first treatment, 100% ethanolic extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* for the second and third treatments, and combined extracts for the fourth treatment. The filter papers were soaked in each treatment and then placed in separate petri dishes. Then, 5 Mung beans were added in each petri dish with certain treatment and were incubated in the room temperature and observed for 5 days. The length of the shoot and the root of mung beans in every treatment were measured using the vernier caliper.

Agar Diffusion Test/Kirby-Bauer Antibiotic Testing/ Antibacterial Screening

The pure bacterial cultures of *Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli* were purchased from Thailand Institute of Scientific and Technological Research. The 3 bacterial samples were used to determine the antibacterial property of the plants. Standard disk diffusion agar method from the book of Engelkirk et.al., 2011 was employed for this test. Five treatments with 3 replications were used in this process and the zone of inhibition was measured after 24 hours.

Agar Diffusion Test/Antifungal Screening

Agar diffusion test was used to evaluate the potency of the selected plants in inhibiting the growth of the two experimental fungi- *Saccharomyces cerevisiae* and *Rhizopus nigricans.* There were 5 treatments used in this test with 3 replications. After 24 hours, the zone of inhibition of each treatment for *S. cerevisiae* was observed and then measured with the use of the Vernier Caliper. Whilst the result for *R. nigricans* was observed and measured after 3 days.

FIGURE 4. Antimicrobial testing.

Results and Discussions

The figures below until the next page show the data encoded on Globe web page from December 2023 to January 2024. Figures 5 to 10 shows the Globe data entry for air temperature, relative humidity, soil temperature, surface temperature, soil fertility, and soil pH measured in coastal zone of Samet, Chonburi, Thailand.

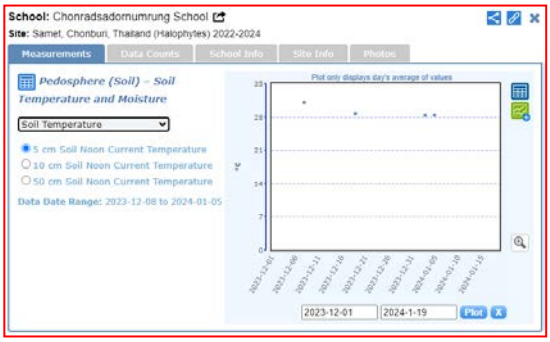


Figure 5. Globe Data Entry for soil temperature (5 cm).

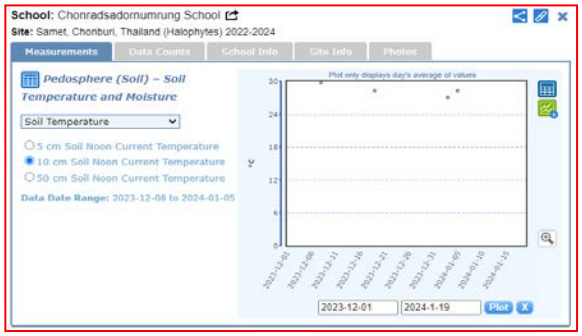


Figure 6. Globe Data Entry for soil temperature (10 cm).

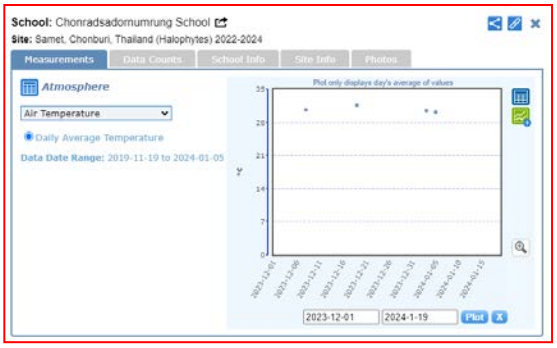


Figure 7. Globe Data Entry for air temperature.

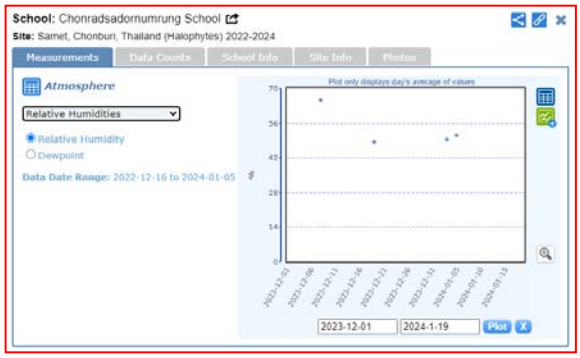


Figure 8. Globe Data Entry for relative humidity.

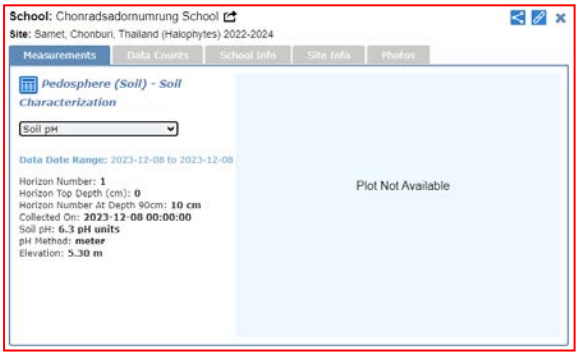


Figure 9. Globe Data Entry for soil pH.

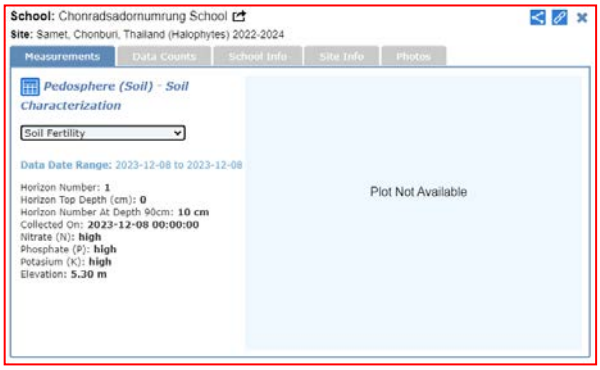


Figure 10. Globe Data Entry for soil fertility

Table 1. Average results of soil characterization measured in coastal zone of Samet, Chonburi, Thailand.

Parameters	8 Dec. 2023 (4:20 PM)	19 Dec. 2023 (9:15 AM)	3 Jan. 2024 (4:30 PM)	5 Jan. 2024 (4:30 PM)
Soil pH	6.33	4.73	5.06	5.6
Soil Temperature (5 cm)	31	28.67	28.33	28.33
Soil Temperature (10 cm)	29.67	28.33	27	28.33
Relative Humidity (%)	65.33	48.33	49.33	51
Air Temperature (°C)	30.57	31.6	30.4	30.07
Soil Color	Gray	Gray	Gray	Gray
Soil Texture	Clay	Clay	Clay	Clay
Soil Consistency	Firm	Firm	Firm	Firm
Soil Moisture	Wet	Wet	Wet	Wet
Nitrogen (N)	High	High	High	High
Phosphorus (P)	High	High	High	High
Potassium (K)	High	High	High	High

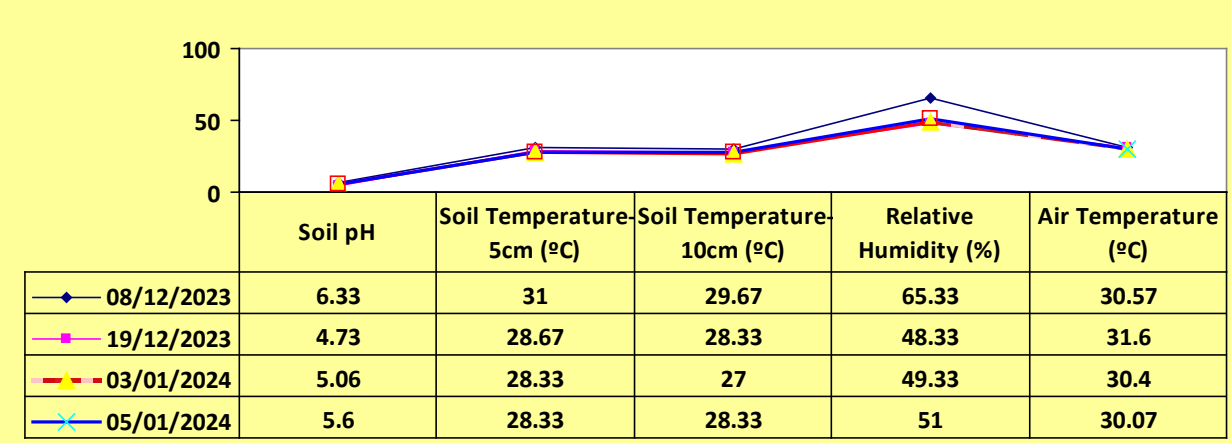
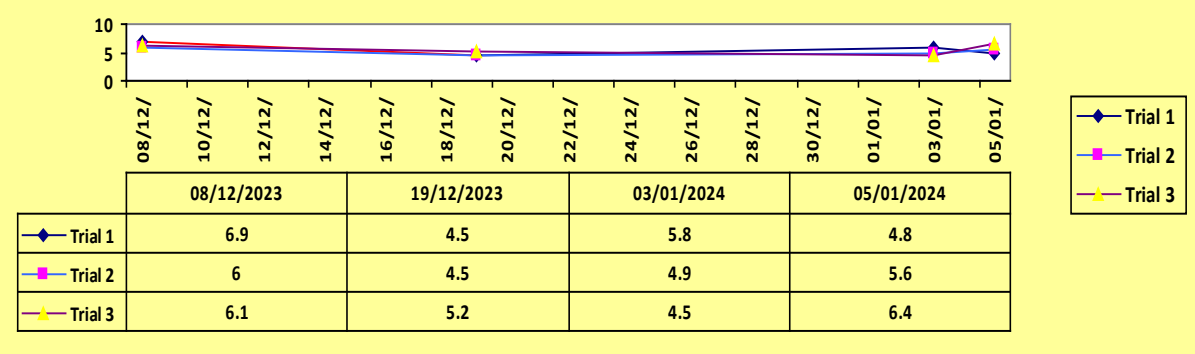


Figure 11. Average results of all soil parameters measured in coastal zone of Samet,

Table 1 and Figure 11 shows the average results of all soil parameters measured in coastal zone of Samet, Chonburi, Thailand. These results were summarized after 4 series of experiments that started from 8 December 2023 to 5 January 2024. The average soil pH ranges from 5.6 – 6.33, soil temperature at 5cm depth ranges from 28.33 - 31°C, soil temperature at 10cm depth ranges from 27 - 29.67°C, relative humidity ranges from 48.33 –

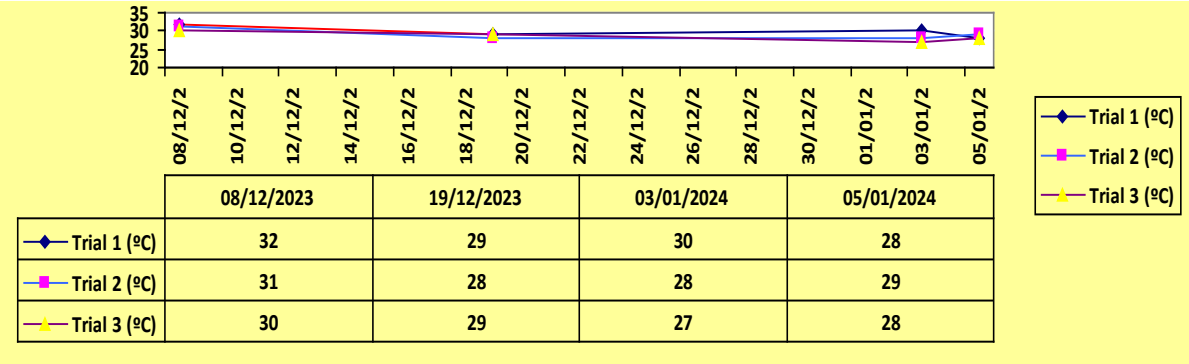
65.33%, and air temperature ranges from 30.07 – 31.6°C. The soil tested from the experimental site also possess the following characteristics: gray color, clay soil structure, clay texture, firm consistency, wet, and has high concentration of Nitrogen (N), Phosphorus (P), and Potassium (K).

One-way ANOVA and Tukey HSD test were used to determine if there was a significant difference in all soil parameters measured quantitatively in coastal area of Samet, Chonburi. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05 for soil temperature measured at 5 cm and 10 cm depth and relative humidity suggesting that the one or more treatments is/are significantly different. For water soil pH and air temperature, the p-value corresponding to the F-statistic of one-way ANOVA is higher than 0.05, suggesting that the treatments are not significantly different for that level of significance. It means that there were no significant changes in the soil temperature measured for 4 consecutive times from the sampling site.



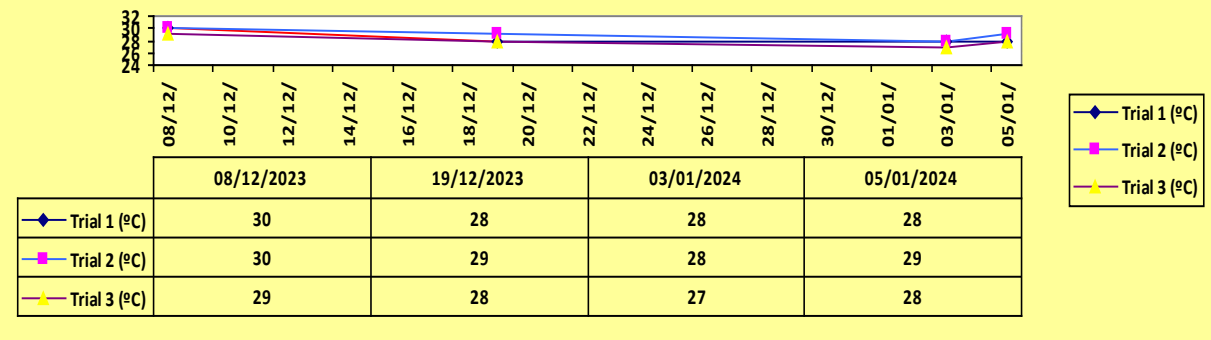
	08/12/2023	19/12/2023	03/01/2024	05/01/2024
Trial 1	6.9	4.5	5.8	4.8
Trial 2	6	4.5	4.9	5.6
Trial 3	6.1	5.2	4.5	6.4

FIGURE 12. Soil pH



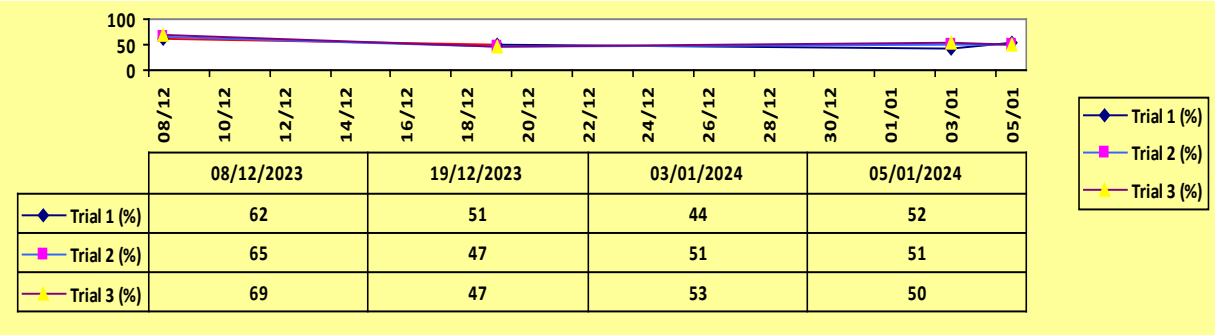
	08/12/2023	19/12/2023	03/01/2024	05/01/2024
Trial 1 (ºC)	32	29	30	28
Trial 2 (ºC)	31	28	28	29
Trial 3 (ºC)	30	29	27	28

FIGURE 13. Soil temperature at 5 cm depth (°C).



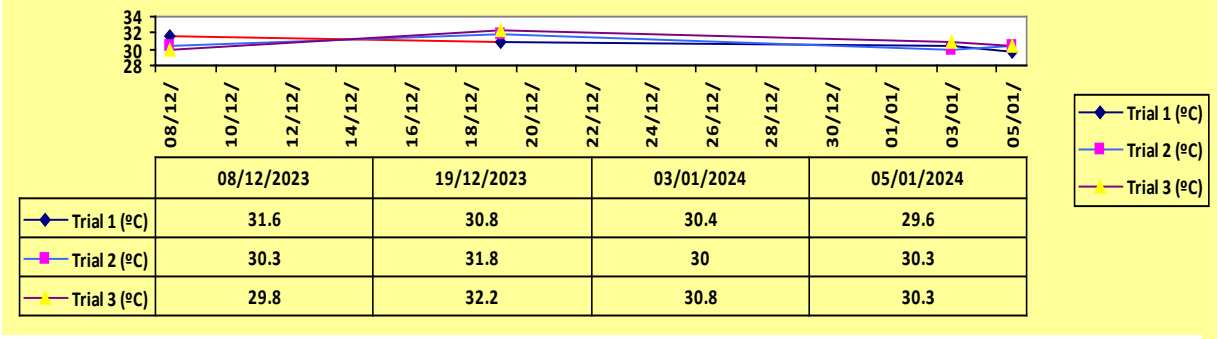
	08/12/2023	19/12/2023	03/01/2024	05/01/2024
Trial 1 (ºC)	30	28	28	28
Trial 2 (ºC)	30	29	28	29
Trial 3 (ºC)	29	28	27	28

FIGURE 14. Soil temperature at 10 cm depth (°C).



	08/12/2023	19/12/2023	03/01/2024	05/01/2024
Trial 1 (%)	62	51	44	52
Trial 2 (%)	65	47	51	51
Trial 3 (%)	69	47	53	50

FIGURE 15. Relative humidity (%).



	08/12/2023	19/12/2023	03/01/2024	05/01/2024
Trial 1 (ºC)	31.6	30.8	30.4	29.6
Trial 2 (ºC)	30.3	31.8	30	30.3
Trial 3 (ºC)	29.8	32.2	30.8	30.3

FIGURE 16. Air Temperature (°C).

FIGURE 12 to 16 shows all the soil parameters (soil pH, soil temperature, relative humidity, and air temperature) measured in coastal zone of Samet, Chonburi, Thailand from 8 December 2023 to 5 January 2024. Each parameter was measured 3 times to get the valid results. All of the graphs above revealed that there are changes in all factors measured for 4 times. Analysis of variance (ANOVA) was the statistical method used to compare these results and to find out if there are significant differences among the collected data.

Table 2. *In vitro* Allelopathic Screening results on the growth of Mung Beans (*Vigna radiata*).

Treatments	Replications	Seedlings (Length of the Root and Shoot)						
		1 (cm)	2 (cm)	3 (cm)	4 (cm)	5 (cm)	Total (cm)	Mean (cm)
Control (Distilled Water)	1	6.38	8.96	5.71	4.84	2.81	28.7	**5.74**
	2	6.72	3.57	10.91	3.5	4.20	28.90	**9.63**
	3	5.87	2.94	5.82	3.83	4.5	22.96	**7.65**
100% *Thespesia populnea* Extract	1	0	0	0	0	0	0	**0**
	2	0	0	0	0	0	0	**0**
	3	0	0	0	0	0	0	**0**
100% *Rhizophora apiculata* Extract	1	0	0	0	0	0	0	**0**
	2	0	0	0	0	0	0	**0**
	3	0	0	0	0	0	0	**0**
Combined Extracts	1	0	0	0	0	0	0	**0**
	2	0	0	0	0	0	0	**0**
	3	0	0	0	0	0	0	**0**

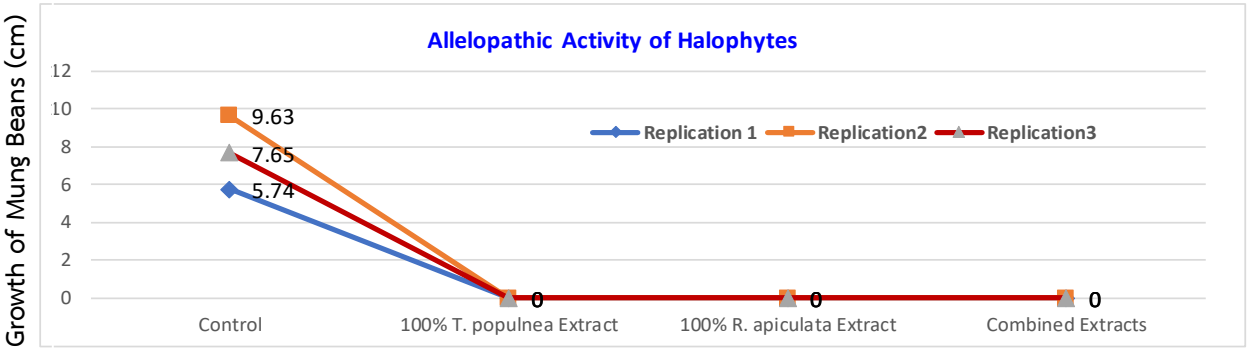


Figure 17. Results of the Allelopathic Screening on the growth Mung Beans (*Vigna radiata*).

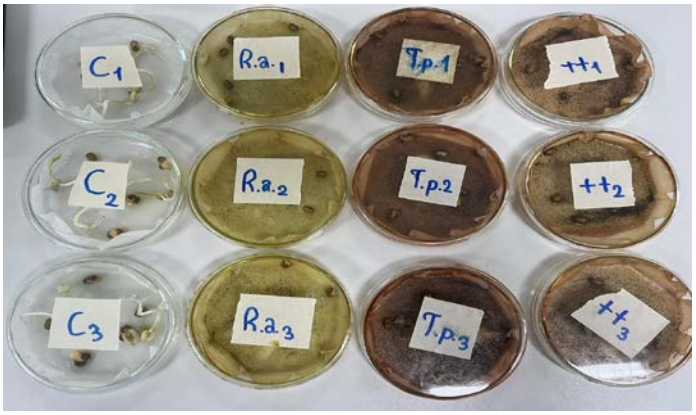


Figure 18. Results of Allelopathic Test after 5 days observation.

TABLE 2 and **FIGURE 17-18** shows the results of the *In vitro* allelopathic screening of the 100% and combined ethanolic extracts of *Thespesia populnea* and *Rhizophora apiculata* against mung beans. It can be seen clearly that among the 4 treatments, the growth of mung beans can only be observed in the control set-up. Hence, the alternative hypothesis has to be accepted because the ethanolic extracts of the two plants are effective allelopathic agent in inhibiting the growth of mung beans after 5 days observation.

One-way ANOVA was used to determine the differences among the treatments used in allelopathic screening using the ethanolic extracts of the experimental plants against Mung Beans (*Vigna radiata)*. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments were significantly different. With this, the alternative hypothesis has to be accepted since the extracts are effective allelopathic agent in inhibiting the growth of mung beans.

Results of the Antibacterial Screening

Table 3. Average zone of inhibitions of the plants against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Saccharomyces cerevisiae,* and *Rhizopus nigricans.*

Treatments	*B. subtilis* (mm)	*S. aureus* (mm)	*E. coli* (mm)	*S. cerevisiae* (mm)	*R. nigricans* (mm)
+ Control	58.5	54.4	61.40	36.10	42
- Control	0	0	0	0	0
T. populnea	10.4	0	6.43	0	0
R. apiculata	7.7	6.20	14.10	0	0
Combined Extracts	17.3	6.30	8.40	0	0

Table 3 shows the average zone of inhibitions of the individual and combined ethanolic extracts of *Thespesia populnea* and *Rhizophora apiculata* against *Bacillus, subtilis, Staphylococcus aureus, Escherichia coli, Saccharomyces cerevisiae,* and *Rhizopus nigricans*. It can be seen clearly that the extracts of selected plants are effective in inhibiting the growth of the bacteria but are not effective antifungal agents. One-way ANOVA was used to determine the differences among the treatments used in antibacterial and antifungal testing.

It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the one or more treatments are significantly different. Hence, the alternative hypothesis has to be accepted because the extracts exhibited an antibacterial property.

Table 4. Growth inhibition of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Bacillus subtilis.*

TREATMENTS	REPLICATIONS				
	1 (mm)	2 (mm)	3 (mm)	Sum (mm)	Mean (mm)
Positive Control (Doxycycline)	54.5	60.8	60.3	175.6	**58.5**
Negative Control (Distilled Water)	0	0	0	0	**0**
100% *T. populnea* Extract	12.9	9.2	9	31.1	**10.4**
100% *R. apiculata* Extract	8	8.7	6.4	23.1	**7.7**
Combined Extracts	7.6	12.2	32.2	52	**17.3**

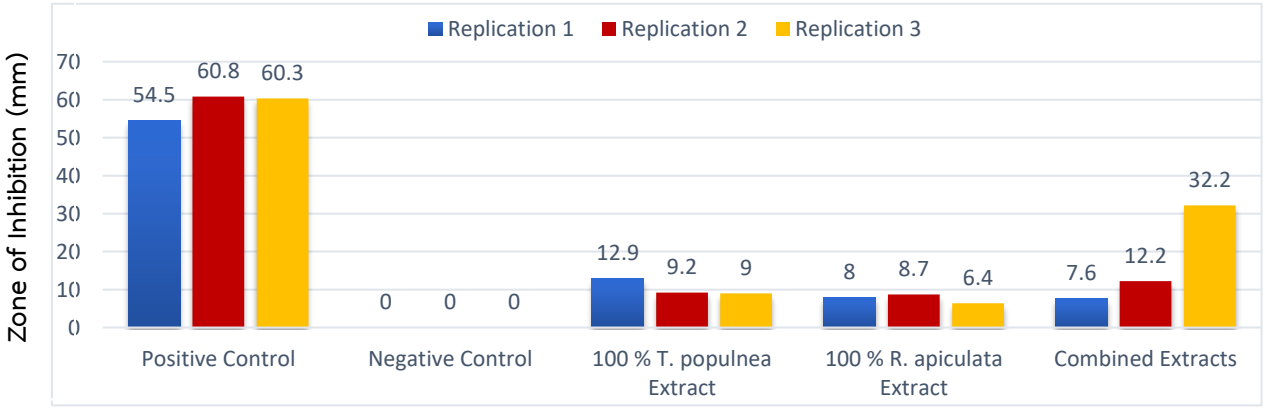


Figure 19. Zone of Inhibition of the ethanolic leaf extracts against *Bacillus subtilis.*

Table 4 and **Figure 19** shows the zone of inhibitions of the individual and combined extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Bacillus subtilis.* It can be seen clearly that the extracts of selected plants can inhibit the growth of the gram-positive bacteria. One-way ANOVA was used to determine the differences among the treatments used in antibacterial testing. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that one or more treatments are significantly different. Hence, the alternative hypothesis has to

be accepted because there was a significant difference between the extracts of halophytes and the control (Doxycycline).

Table 5. Growth inhibition of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Staphylococcus aureus.*

TREATMENTS	REPLICATIONS				
	1 (mm)	2 (mm)	3 (mm)	Sum (mm)	Mean (mm)
Positive Control (Doxycycline)	50.4	58.1	54.6	163.1	**54.4**
Negative Control (Distilled Water)	0	0	0	0	**0**
100% *T. populnea* Extract	0	0	0	0	**0**
100% *R. apiculata* Extract	7.2	6.2	6.1	19.8	**6.6**
Combined Extracts	6.5	6.6	6.8	19.9	**6.63**

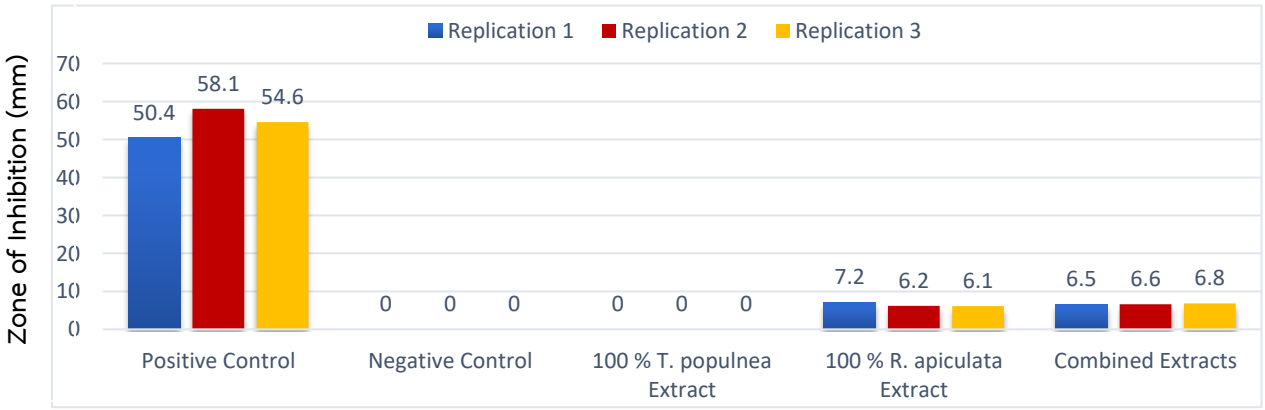


Figure 20. Zone of Inhibition of the ethanolic leaf extracts against *Staphylococcus aureus.*

Table 5 and **Figure 20** shows the zone of inhibitions of the individual and combined extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Staphylococcus aureus.* It can be seen clearly that the extracts of selected plants can inhibit the growth of the gram-positive bacteria except for *T. populnea* extract. One-way ANOVA was used to determine the differences among the treatments used in antibacterial testing. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that one or more treatments are significantly different. Hence, the alternative hypothesis has to be accepted because there was a significant difference between the extracts of halophytes and the control (Doxycycline).

Table 6. Growth inhibition of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Escherichia coli.*

TREATMENTS	REPLICATIONS				
	1 (mm)	2 (mm)	3 (mm)	Sum (mm)	Mean (mm)
Positive Control (Doxycycline)	66.4	53.6	64.3	184.3	**61.40**
Negative Control (Distilled Water)	0	0	0	0	**0**
100% *T. populnea* Extract	6.5	6.5	6.3	19.3	**6.43**
100% *R. apiculata* Extract	18.7	10.6	13.1	42.4	**14.10**
Combined Extracts	8.2	7.5	9.5	25.2	**8.40**

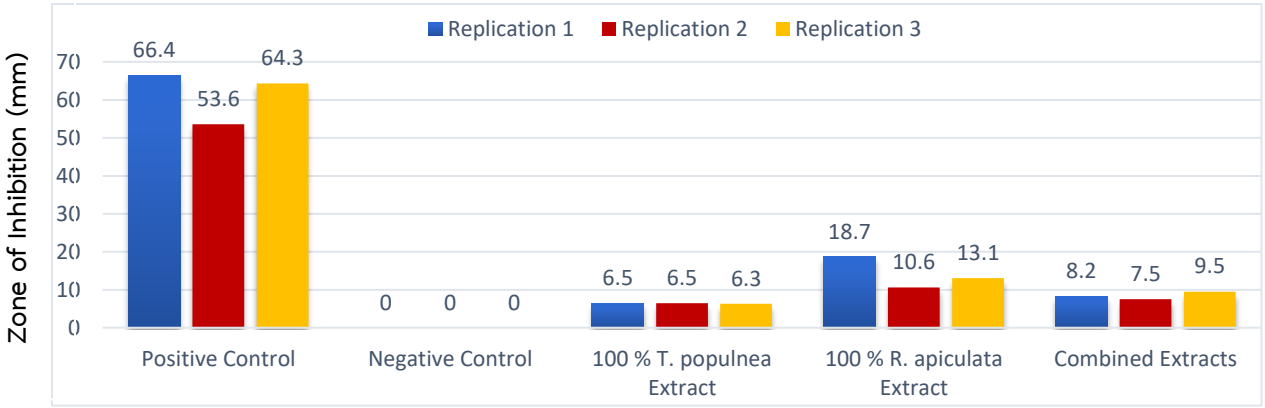


Figure 21. Zone of Inhibition of the ethanolic leaf extracts against *Escherichia coli.*

Table 6 and **Figure 21** shows the zone of inhibitions of the individual and combined extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Escherichia coli.* It can be seen clearly that the extracts of selected plants can inhibit the growth of the gram-negative bacteria. One-way ANOVA was used to determine the differences among the treatments used in antibacterial testing. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that one or more treatments are significantly different. Hence, the alternative hypothesis has to be accepted because there was a significant difference between the extracts of halophytes and the control (Doxycycline).

Results of the Antifungal Screening

Table 7. Growth inhibition of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Saccharomyces cerivisiae.*

TREATMENTS	REPLICATIONS				
	1 (mm)	2 (mm)	3 (mm)	Sum (mm)	Mean (mm)
Positive Control (Fluconazole)	34.3	36.6	37.4	108.3	**36.10**
Negative Control (Distilled Water)	0	0	0	0	**0**
100% *T. populnea* Extract	0	0	0	0	**0**
100% *R. apiculata* Extract	0	0	0	0	**0**
Combined Extracts	0	0	0	0	**0**

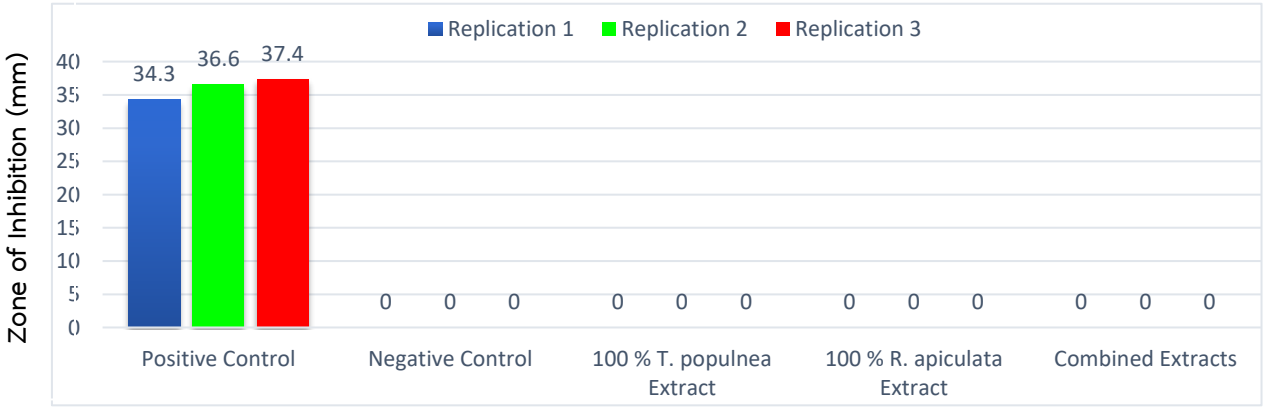


Figure 22. Zone of Inhibition of the ethanolic leaf extracts against *Saccharomyces cerevisiae.*

Table 7 and **Figure 22** shows the zone of inhibitions of the individual and combined extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Saccharomyces cerevisiae.* It was found out that the extracts of selected plants cannot inhibit the growth of unicellular fungi. One-way ANOVA was used to determine the differences among the treatments used in antifungal testing. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that one or more treatments are significantly different. Hence, the alternative hypothesis has to be accepted because there was a significant difference between the extracts of halophytes and the control (Fluconazole). This result is in favor of the control because the plants are not effective antifungal agents.

Table 8. Growth inhibition of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Rhizopus nigricans.*

TREATMENTS	REPLICATIONS				
	1 (mm)	2 (mm)	3 (mm)	Sum (mm)	Mean (mm)
Positive Control (Fluconazole)	61.2	19.5	45.3	126	**42**
Negative Control (Distilled Water)	0	0	0	0	**0**
100% *T. populnea* Extract	0	0	0	0	**0**
100% *R. apiculata* Extract	0	0	0	0	**0**
Combined Extracts	0	0	0	0	**0**

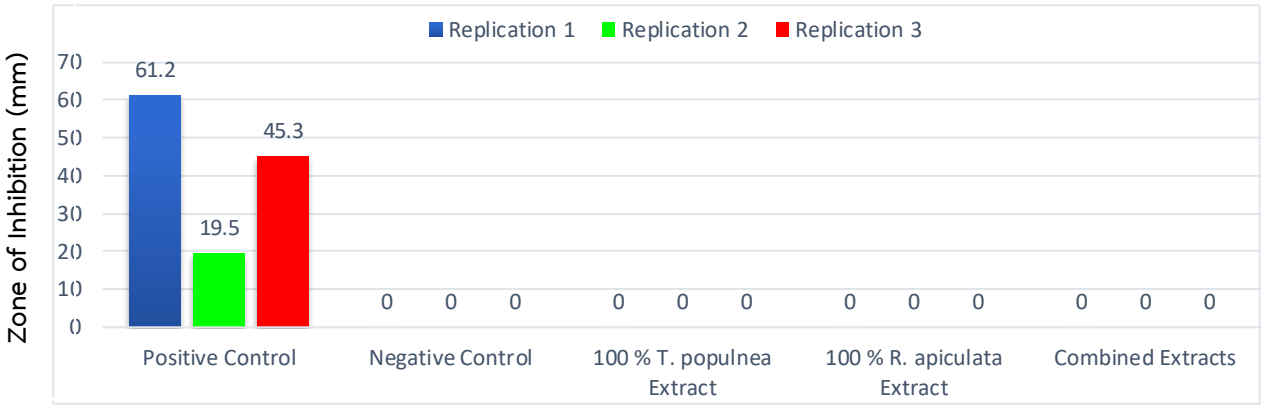


Figure 23. Zone of Inhibition of the ethanolic leaf extracts against *Rhizopus nigricans.*

Table 8 and **Figure 23** shows the zone of inhibitions of the individual and combined extracts of Portia Tree (*Thespesia populnea)* and Tall-Stilt Mangrove (*Rhizophora apiculata)* against *Rhizopus nigricans.* It was found out that the extracts of selected plants cannot inhibit the growth of unicellular fungi. One-way ANOVA was used to determine the differences among the treatments used in antifungal testing. It was found out that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that one or more treatments are significantly different. Hence, the alternative hypothesis has to be accepted because there was a significant difference between the extracts of halophytes and the control (Fluconazole). This result is in favor of the control because the plants are not effective antifungal agents.

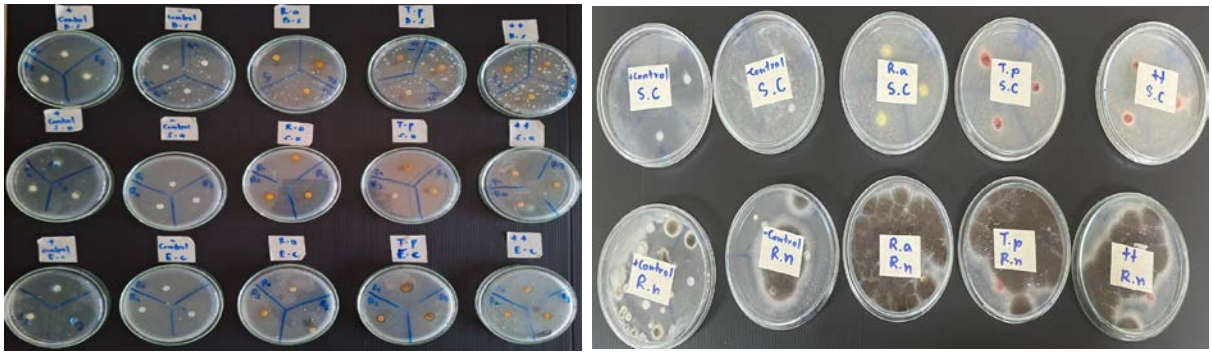


FIGURE 24. Results of the antibacterial and antifungal testing using the ethanolic extracts of selected plants.

Table 9. Soil Quality and Biological Activities of Selected Plants

Soil Quality and Biological Activities	*Thespesia populnea*	*Rhizophora apiculata*	Combined Extracts
Nitrogen (N)	High	High	High
Phosphorus (P)	High	High	High
Potassium (K)	High	High	High
Antibacterial Activity against *Staphylococcus aureus*	+	-	+
Antibacterial Activity against *Bacillus subtilis*	+	+	+
Antibacterial Activity against *Escherichia coli*	+	+	+
Antifungal Activity against *Saccharomyces cerevisiae*	-	-	-
Antifungal Activity against *Rhizopus nigricans*	-	-	-
Allelopathic Potential	+	+	+
(+) : possessed by the plant (positive); (-) : is not possessed by the plant (negative)			

Discussion

The results of field measurement, ANOVA, and post-hoc Tukey HSD test showed that there was a significant difference in some soil parameters measured in coastal zone of Samet, Chonburi, Thailand such as soil temperature measured at 5 cm and 10 cm depth, and relative humidity but there was no significant difference in soil pH and air temperature. It shows that there were no significant changes in the soil quality of the study site despite of

the changes taking place and dry season. The acquired data is pertinent to the study of Milosevic et al., 2020 that claims that the physical characteristics of soil are largely fixed and won't change over time. The main factors that can contribute to changes in soil health are soil organisms, including the abundance and diversity of bacteria, fungi, and nematodes, as they respond sensitively to anthropogenic disturbance (Lynch, 2015).

The soil in coastal zone of Samet, Chonburi is so rich in nutrients due to high concentration of organic matters and minerals such as nitrogen, phosphorus, and potassium as shown in Table 1. The richness of the soil is noticeable due to the presence of abundant and various plants in the area. The nutrients absorbed by the plants like phosphorus is involved in energy metabolism and photosynthesis during plant growth (Yan et al., 2021). Whereas, potassium plays an important role in carbohydrate and protein metabolism (Hassanein et al., 2021), indicating that a reasonable proportion of nutrition (NPK) factors directly promote the absorption and assimilation of plants, thereby affecting their growth and development (Yildirim et al., 2011). Since plant's biological and physiological needs are satisfied, they were able to produce various compounds both primary and secondary compounds that are necessary for their growth and development as well as survival in their natural habitat with biotic and abiotic stressors (Briskin, 2000).

Research done by many researchers revealed that plants produce natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes and some of which also involved in defense against abiotic stress and also important for the communication of the plants with other organisms (Schafer et.al., 2009). The existence of biologically active phytochemicals such as alkaloids, flavonoids, steroids, saponins, and terpenoids in the plants makes them medicinally important. The experimental plants exhibited different types of biological activities such as antibacterial and allelopathy because of the phytonutrients that they possess.

Conclusion

Based on the experimentations, results and gathered data, the researchers concluded that there was a correlation between soil quality and biological activities of the selected plants in coastal zone of Samet, Chonburi, Thailand. Moreover, there are significant

differences ($p<0.05$) in soil temperature (5cm and 10cm depth) and relative humidity except for soil pH and air temperature ($p>0.05$).

Recommendations

For the improvement of the study, further research will be conducted to evaluate the other soil parameters in Samet, Chonburi, Thailand also more biological activities of the plants will be investigated. Moreover, the phytochemical compounds of the plants will be evaluated to find out the specific substances present in the plants that are responsible for their biological activities.

GLOBE Badges

I am a Collaborator

The effective completion of this biological and environmental research was made possible because of the collective efforts of various individuals. The management of the Center of Expertise on Eco-tourism for Mangrove Conservation, Chonburi Province Office, granted permission to the researchers to carry out a study in Samet Municipality's coastal zone, which is home to a variety of halophytes that are flourishing. The team had received sufficient thoughts about the elements that require further investigation in the area from the survey conducted in the aforementioned office. During the conduct of the study, the researchers were thoroughly guided and given knowledge by their teachers namely Ms. Rawadee Meesuk and Mr. Marvin Servallos. Furthermore, the soil quality testing was carried out properly because of some of the materials provided by the home economics department of the school such as shovel and hand forks. Thorough guidance and invaluable ideas from the above names were very significant to completely understand all the scopes of this research. Finally, the researchers of this science project have cooperated to finish the work entirely from the planning stage, experiments, analyzing of data, and packaging of the final research paper.

I Make an Impact

The success of this experiment would greatly benefit the public, the administration of the Center of Expertise on Eco-tourism for Mangrove Conservation, Chonburi Province Office, and government officials because the results of soil quality measurement in the natural habitat of halophytes would give them valuable information about the current

condition of Samet soil where various halophytes are thriving and other flora are thriving. Moreover, the result of antimicrobial screening is very helpful because the community in Thailand can use the aqueous extracts of halophytes as an alternative method in fighting the adverse effects caused by bacteria. This research will significantly impact including the agriculture sector because the result of allelopathic test will propel them to use organic materials like halophyte extracts in inhibiting the growth of unwanted plants instead of using commercial herbicides that are expensive and harmful to the body. Most importantly, the methods and results gathered in this study have great impact to the community of Chonradsadornumrung School, especially to the students because it serves as an eye opener for them that young learners like the researchers can have a valuable contribution in studying their surroundings like soil quality, also in using the natural resources as alternative agents in inhibiting the growth of pathogenic microbes.

I am a Data Scientist

The researchers have studied systematically the current condition of soil sample in coastal zone of Samet, Chonburi, Thailand. The results were collected, recorded, and analyzed properly. The soil quality was correlated to the biological activities of the plants. In addition, botany and microbiology experiments were integrated to this environmental research. All of the data gathered from the field measurement were analyzed using some statistical models like ANOVA (Analysis of Variance) with post-hoc Tukey HSD (Honestly Significant Difference) Test. The results of the analysis were discussed and presented properly. Moreover, the results of the experiment were linked to the research done by other researchers.

I am a STEM Professional

This environmental research is not possible without the invaluable insights from various STEM Professionals. The researchers were able to formulate research topic and questions by asking their Biology teacher pertaining to possible studies that they can pursue that is relevant to Globe mission and vision. During data gathering and interpretation, the statistician of the school was consulted about the accurate statistical method that can be used in interpreting the data about soil quality in Samet, Chonburi, Thailand as well as the results of biological testing. From the consultation with the school's statistician, the researchers learned that ANOVA (Analysis of Variance) and post-hoc Tukey HSD (Honestly

Significant Difference) Test are the most appropriate tool to analyze the collected data. Moreover, the researchers also asked help from the statistician of the school to calculate and interpret the results of the study.

Acknowledgment

The researchers of the study would like to acknowledge the following for making this environmental research possible. First, they would like to convey their genuine thanks to the Head of Chonradsadornumrung School English Program, Ms. Rawadee Meesuk for her utmost support, suggestions, and encouragement as well as for providing all the Laboratory equipment and chemicals that they need in their study. Second, heartfelt thanks are also conveyed by the researchers to their Science teacher- Mr. Marvin Servallos for his thorough guidance towards the completion of the study. Third, sincere gratitude is given by the researchers to the administration of the Center of Expertise on Eco-tourism for Mangrove Conservation, Chonburi Province Office for allowing them to conduct a study in the coastal area of Samet Municipality. Finally, the researchers would like to give their special thanks to the committee of IPST and Globe Student Research Competition for conducting this prestigious event that enabled young scientists to share their scientific discoveries.

References

Books

Burton, Gwendolyn R. W. et al. (2004). *Microbiology for the Health Sciences. USA,* Lippincott: Williams and Wilkins.

Engelkirk, Paul G. et.al. (2011). *Burton's Microbiology for the Health Sciences*. Philadelphia, Lippincott: Williams & Wilkins.

Guevara, Beatrice Q. et al. (2005). *A Guidebook to Plant Screening: Phytochemical and Biological*. Manila, Philippines: UST Publishing House.

Internet

Ballhorn DJ, Kautz S, Heil M, Hegeman AD, 2009. Cyanogenesis of wild lima bean (Phaseolus lunatus L.) is an efficient direct defense in nature. Plant Signaling and Behavior, 4(8): 735-745.

Retrieved from https://www.omicsonline.org/open-access/role-of-secondary-metabolites-in-defense-mechanisms-of-plants-0974-8369-3-128.pdf

Bacon RT, Sofos JN (2003) Characteristics of Biological Hazards in Foods, In: Schmidt RH, Rodrick GE, Editors, Food Safety Handbook, New Jersey: John Wiley & Sons, Inc., 157–195.Retrieved from: https://www.aimspress.com/fileOther/PDF/microbiology/microbiol-03-00529.pdf- February 28, 2020.

Rajkaran, A., Adams, J. & van der Colff, D. 2016. Rhizophora mucronata Lam. National Assessment: Red List of South African Plants version 2017.1. Accessed on 2017/12/07. Retrieved from: http://pza.sanbi.org/rhizophora-mucronata-

Rhoades J, Kandiah A and Mashali A 1992 The use of saline waters for crop production (Rome: FAO United Nations). Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/118/1/012019/pdf

Sawyer C, McCarty P and Parkin G 1994 Chemistry for Environmental Engineering (Singapore: McGraw-Hill, Inc). Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/118/1/012019/pdf

Schafer H, Wink M, 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnology Journal, 4(12): 1684- 1703. Retrieved from https://www.omicsonline.org/open-access/role-of-secondary-metabolites-in-defense-mechanisms-of-plants-0974-8369-3-128.pdf

T. Nakashima, Y. Sakagami, M. Matsuo. 2001. Antibacterial efficacy of cotton fabrics chemically modified by metal salt Biocontrol. Retrieved from https://www.sciencedirect.com/science/article/pii/S1687428513000824

Todd D K and Mays L W 2005 Groundwater Hydrology ed B Zobrist (New Jersey: John Wiley & Sons, Inc.). Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/118/1/012019/pdf

WHO 2011 WHO guidelines for drinking-water quality (Geneva: World Health Organization). Retrieved from https://iopscience.iop.org/article/10.1088/1755-1315/118/1/012019/pdf

https://www.globe.gov/documents/11865/ae27466a-9a69-4fd6-8244-ae1f7e22d844; December 8, 2023

https://www.globe.gov/documents/11865/0fd40183-f2ea-480f-82b6-8d62180d9291; December 8, 2023

https://www.globe.gov/documents/11865/3464b426-6d54-4ba2-9cca-8d398fb38ef8; December 8, 2023

https://www.globe.gov/documents/11865/920675f5-56c0-46a3-97b5-74f9953b2ae4; December 8, 2023

Appendix 1

ANOVA (Analysis of Variance) for soil pH measured for 4 consecutive times in the coastal zone of Samet, Chonburi, Thailand.

Treatment ⟶	A	B	C	D
Input Data ⟶	6.9	4.5	5.8	4.8
	6.0	4.5	4.9	5.6
	6.1	5.2	4.5	6.4

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	4.3867	3	1.4622	3.9254	0.0541
error	2.9800	8	0.3725		
total	7.3667	11			

Conclusion from ANOVA:

The p-value corresponding to the F-statistic of one-way ANOVA is **higher** than 0.05, suggesting that the treatments are not significantly different for that level of significance. The Tukey HSD test multiple comparison tests follow. This post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

Tukey HSD results for soil pH.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	4.5406	0.0494378	* p<0.05
A vs C	3.5947	0.1267775	insignificant
A vs D	2.0811	0.4949983	insignificant
B vs C	0.9460	0.8999947	insignificant
B vs D	2.4595	0.3662722	insignificant
C vs D	1.5135	0.7002289	insignificant

Appendix 2

ANOVA (Analysis of Variance) for soil temperature (5 cm depth) measured for 4 consecutive times in the coastal zone of Samet, Chonburi, Thailand.

Treatment ⟶	A	B	C	D
Input Data ⟶	32.0	29.0	30.0	28.0
	31.0	28.0	28.0	29.0
	30.0	29.0	27.0	28.0

Source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	14.9167	3	4.9722	4.9722	0.0310
error	8.0000	8	1.0000		
total	22.9167	11			

Conclusion from ANOVA:

The p-value corresponding to the F-statistic of one-way ANOVA is **lower** than 0.05, suggesting that one or more treatments are significantly different for that level of significance. The Tukey HSD test multiple comparison tests follow. This post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

Tukey HSD results for soil temperature (5 cm depth).

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	4.0415	0.0813777	insignificant
A vs C	4.6188	0.0457399	* p<0.05
A vs D	4.6188	0.0457399	* p<0.05
B vs C	0.5774	0.8999947	insignificant
B vs D	0.5774	0.8999947	insignificant
C vs D	0.0000	0.8999947	insignificant

Appendix 3

ANOVA (Analysis of Variance) for soil temperature (10 cm depth) measured for 4 consecutive times in the coastal zone of Samet, Chonburi, Thailand.

Treatment ⟶	A	B	C	D
Input Data ⟶	30.0	28.0	27.0	28.0
	30.0	29.0	28.0	29.0
	29.0	28.0	26.0	28.0

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	10.6667	3	3.5556	7.1111	0.0120
error	4.0000	8	0.5000		
total	14.6667	11			

Conclusion from ANOVA:

The p-value corresponding to the F-statistic of one-way ANOVA is **lower** than 0.05, suggesting that one or more treatments are significantly different for that level of significance. The Tukey HSD test multiple comparison tests follow. This post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

Tukey HSD results for soil temperature (10 cm depth).

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	3.2660	0.1749731	insignificant
A vs C	6.5320	0.0074287	** p<0.01
A vs D	3.2660	0.1749731	insignificant
B vs C	3.2660	0.1749731	insignificant
B vs D	0.0000	0.8999947	insignificant
C vs D	3.2660	0.1749731	insignificant

Appendix 4

ANOVA (Analysis of Variance) for relative humidity measured for 4 consecutive times in the coastal zone of Samet, Chonburi, Thailand.

Treatment ⟶	A	B	C	D
Input Data ⟶	62.0	51.0	44.0	52.0
	65.0	47.0	51.0	51.0
	69.0	47.0	53.0	50.0

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	571.0000	3	190.3333	18.5691	0.0006
error	82.0000	8	10.2500		
total	653.0000	11			

Conclusion from ANOVA:

The p-value corresponding to the F-statistic of one-way ANOVA is **lower** than 0.05, suggesting that one or more treatments are significantly different for that level of significance. The Tukey HSD test multiple comparison tests follow. This post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

Tukey HSD results for relative humidity.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	9.1970	0.0010053	** p<0.01
A vs C	8.6560	0.0012761	** p<0.01
A vs D	7.7544	0.0026084	** p<0.01
B vs C	0.5410	0.8999947	insignificant
B vs D	1.4427	0.7258676	insignificant
C vs D	0.9017	0.8999947	insignificant

Appendix 5

ANOVA (Analysis of Variance) for air temperature measured for 4 consecutive times in the coastal zone of Samet, Chonburi, Thailand.

Treatment ⟶	A	B	C	D
Input Data ⟶	31.6	30.8	30.4	29.6
	30.3	31.8	30.0	30.3
	29.8	32.2	30.8	30.3

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	3.9358	3	1.3119	3.0749	0.0906
error	3.4133	8	0.4267		
total	7.3492	11			

Conclusion from ANOVA:

The p-value corresponding to the F-statistic of one-way ANOVA is **higher** than 0.05, suggesting that the treatments are not significantly different for that level of significance. The Tukey HSD test multiple comparison tests follow. This post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

Tukey HSD results for air temperature.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	2.7400	0.2862712	insignificant
A vs C	0.4419	0.8999947	insignificant
A vs D	1.3258	0.7681372	insignificant
B vs C	3.1820	0.1895859	insignificant
B vs D	4.0659	0.0794159	insignificant
C vs D	0.8839	0.8999947	insignificant

Appendix 6

ANOVA (Analysis of Variance) for growth inhibition against *Bacillus subtilis.*

Treatment ⟶	A	B	C	D	E
Input Data ⟶	54.5	0.0	12.9	8.0	7.6
	60.8	0.0	9.2	8.7	12.2
	60.3	0.0	9.0	6.4	32.2

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	6,385.9773	4	1,596.4943	42.1172	3.1592e-06
error	379.0600	10	37.9060		
total	6,765.0373	14			

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	16.4668	0.0010053	** p<0.01
A vs C	13.5504	0.0010053	** p<0.01
A vs D	14.3006	0.0010053	** p<0.01
A vs E	11.5905	0.0010053	** p<0.01

Conclusion from ANOVA:

Above table shows the (ANOVA) analysis of variance and Tukey HSD results of the antibacterial activity of oyster and ethanolic extracts of halophytes against *Bacillus subtilis*. It revealed that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments are significantly different. The Tukey HSD test multiple comparison tests followed and it also showed that there was a significant difference between the control (Doxycycline) and the experimental set-ups. It shows that both control and experimental groups are potent antibacterial agent against gram-positive bacteria. Moreover, the null hypothesis of no significant difference in inhibitory effect of the halophyte extracts compared to the doxycycline has to be rejected in favor of the alternative hypothesis.

Appendix 7

ANOVA (Analysis of Variance) for growth inhibition against *Staphylococcus aureus.*

Treatment ⟶	A	B	C	D	E
Input Data ⟶	50.4	0.0	0.0	7.2	6.5
	58.1	0.0	0.0	6.2	6.6
	54.6	0.0	0.0	6.1	6.8

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	6,392.2067	4	1,598.0517	523.7224	1.4464e-11
error	30.5133	10	3.0513		
total	6,422.7200	14			

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	53.9074	0.0010053	** p<0.01
A vs C	53.9074	0.0010053	** p<0.01
A vs D	47.4623	0.0010053	** p<0.01
A vs E	47.3301	0.0010053	** p<0.01

Conclusion from ANOVA:

Above table shows the (ANOVA) analysis of variance and Tukey HSD results of the antibacterial activity of oyster and ethanolic extracts of halophytes against *Staphylococcus aureus*. It revealed that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments are significantly different. The Tukey HSD test multiple comparison tests followed and it also showed that there was a significant difference between the control (Doxycycline) and the experimental set-ups. It shows that both control and experimental groups are potent antibacterial agent against gram-positive bacteria. Moreover, the null hypothesis of no significant difference in inhibitory effect of the halophyte extracts compared to the doxycycline has to be rejected in favor of the alternative hypothesis.

Appendix 8

ANOVA (Analysis of Variance) for growth inhibition against *Escherichia coli.*

Treatment ⟶	A	B	C	D	E
Input Data ⟶	66.4	0.0	6.5	18.7	8.2
	53.6	0.0	6.5	10.6	7.5
	64.3	0.0	6.3	13.1	9.5

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	7,353.9640	4	1,838.4910	140.6219	9.6151e-09
error	130.7400	10	13.0740		
total	7,484.7040	14			

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	29.4280	0.0010053	** p<0.01
A vs C	26.3463	0.0010053	** p<0.01
A vs D	22.6578	0.0010053	** p<0.01
A vs E	25.4042	0.0010053	** p<0.01

Conclusion from ANOVA:

Above table shows the (ANOVA) analysis of variance and Tukey HSD results of the antibacterial activity of oyster and ethanolic extracts of halophytes against *Escherichia coli*. It revealed that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments are significantly different. The Tukey HSD test multiple

comparison tests followed and it also showed that there was a significant difference between the control (Doxycycline) and the experimental set-ups. It shows that both control and experimental groups are potent antibacterial agent against gram-negative bacteria. Moreover, the null hypothesis of no significant difference in inhibitory effect of the halophyte extracts compared to the doxycycline has to be rejected in favor of the alternative hypothesis.

Appendix 9

ANOVA (Analysis of Variance) for growth inhibition against *Saccharomyces cerevisiae.*

Treatment ⟶	A	B	C	D	E
Input Data ⟶	34.3	0.0	0.0	0.0	0.0
	36.6	0.0	0.0	0.0	0.0
	37.4	0.0	0.0	0.0	0.0

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	3,127.7040	4	781.9260	1,509.5097	7.4052e-14
error	5.1800	10	0.5180		
total	3,132.8840	14			

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	86.8766	0.0010053	** p<0.01
A vs C	86.8766	0.0010053	** p<0.01
A vs D	86.8766	0.0010053	** p<0.01
A vs E	86.8766	0.0010053	** p<0.01

Conclusion from ANOVA:

Above table shows the (ANOVA) analysis of variance and Tukey HSD results of the antifungal activity of oyster and ethanolic extracts of halophytes against *Saccharomyces cerevisiae.* It revealed that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments are significantly different. The Tukey HSD test multiple comparison tests followed and it also showed that there was a significant difference between the control (Fluconazole) and the experimental set-ups. It shows that experimental groups are not effective antifungal agents because they did not exhibit any zone of inhibitions.

Appendix 10

ANOVA (Analysis of Variance) for growth inhibition against *Rhizopus nigricans.*

Treatment ⟶	A	B	C	D	E
Input Data ⟶	61.2	0.0	0.0	0.0	0.0
	19.5	0.0	0.0	0.0	0.0
	45.3	0.0	0.0	0.0	0.0

source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
treatment	4,233.6000	4	1,058.4000	11.9488	0.0008
error	885.7800	10	88.5780		
total	5,119.3800	14			

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	7.7294	0.0019845	** p<0.01
A vs C	7.7294	0.0019845	** p<0.01
A vs D	7.7294	0.0019845	** p<0.01
A vs E	7.7294	0.0019845	** p<0.01

Conclusion from ANOVA:

Above table shows the (ANOVA) analysis of variance and Tukey HSD results of the antifungal activity of oyster and ethanolic extracts of halophytes against *Rhizopus nigricans.* It revealed that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the treatments are significantly different. The Tukey HSD test multiple comparison tests followed and it also showed that there was a significant difference between the control (Fluconazole) and the experimental set-ups. It shows that experimental groups are not effective antifungal agents because they did not exhibit any zone of inhibitions.

Appendix 11

Pictorials (*In situ* Measurement of Soil Quality)

Measuring Soil Temperature

Measuring Soil Fertility

Measuring Soil pH

Measuring the NPK content of Soil

Measuring Soil Moisture

Measuring the NPK content of Soil