Research Question: What effect does the distance (in meters) from the ground have on the salinity (in micro siemens) of the leaves on the *Avicennia* in the mangroves of Sedili Besar, Malaysia?

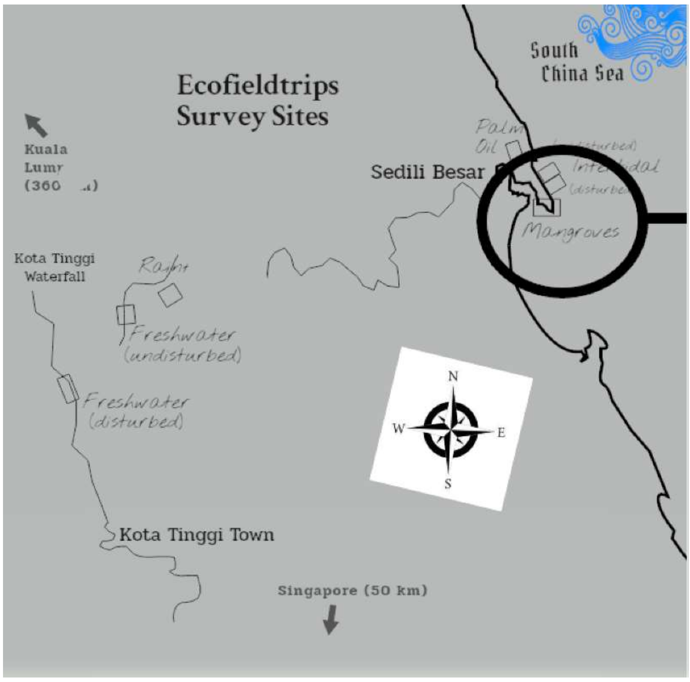
1: Background information

1.1: About Mangroves

The Mangrove experimented on was an ecosystem of plant habitats at a brackish condition (a condition where there are both sea water and natural water is present). There is a high diversity of) mangroves in south east Asia. There is a high abundance of them because places like Malaysia are right

near the equator. This means that there are normally very constant conditions (daylight, temperature, etc.). The minimal change in abiotic pressure is good for the Mangroves because it makes it easier for the species present to survive, as there is lesser adaptation required.

However, abiotic changes are still present. In Sedili the main abiotic pressure that makes it difficult for the mangroves to survive is tidal change. The tide changes twice a day which means the ecosystem has to deal with 2 alternating conditions (temperature, salt level, oxygen level (which leads to bacteria breaking down substances in a lack of oxygen most of the time)), with unstable substrates too. This is why there are many adaptations made by the trees in the mangroves.



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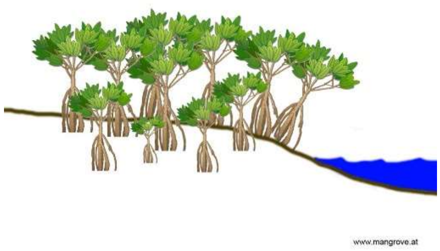
(TTS Survey Booklet March 2019, 2019)

There are 4 main communities for mangroves:

- **Fringing**- These mangroves are the typical types of mangroves. They are directly exposed to tides and sea waves.
- **Riverine**- These mangroves grow along flowing water (like tidal rivers). These provide best for mangrove growth.
- **Basin**- These mangroves are partially impounded depression. There is barely any water present, unless a high tide is present.
- **Overwash**- These are mangroves that are washed over by tides. They are normally quite small and narrow extensions.

The community being experimented were fringing conditions. Also, during the experiment, there was a low tide.



(Mangrove forests, 2019)

1.2: Uses of Mangroves

It is extremely important to protect the mangroves. Here are a few reasons why:

- The mangroves act as a marine nursery- they protect young fish and creatures. It has been proven that reef fish spend their juvenile years in the mangroves.
- They are used for coastal protection- they stop erosion of land and can decrease damage from tsunamis.
- They help improve water quality- they protect litter from enter and spreading through the oceans.
- They act as a carbon sink- they store carbon dioxide (CO_2) in mud and produce oxygen (O_2). They produce much more oxygen than other ecosystems like boreal and temperate.

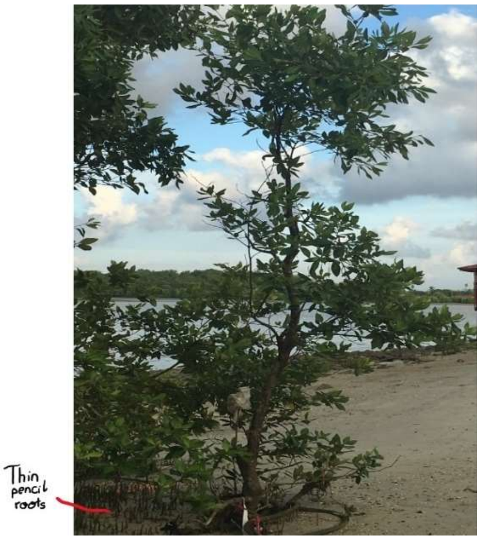
1.3: Protection of Mangroves

Though the mangroves play such an essential role in our community, they under threat by many (manmade) problems, like:

- Construction materials and construction occurring by the coastline.
- Fish/shrimp farms. Especially, considering shrimp farms require brackish conditions, many mangroves like the ones tested in the experiment, are under threat of shrimp farms being built here.
- Extraction of charcoal for things such as barbeques. This is because mangrove charcoal is considered better for cooking compared to regular charcoal.
- Littering, like plastic and cloth.

1.4: About the *Avicennia*

The tree being experimented on, is the *Avicennia* tree. It has thin pencil roots and normally small leaves.



(Avicennia tree taken by me, *2019)*

It is visible in the photo that the *Avicennia* tree is near the sandy part of this Mangrove. This was true for all the *Avicennia* trees. The trees were mostly close together except for 2 which were quite far away. Still, the soil moisture felt around the same (still sandy).

The *Avicennia* adapted to excrete salt because of the different salinity levels from the different tides occur throughout the day in the mangroves. They use the glands present in the tree (specifically the leaf) as a way to excrete any excess salt. If one were to lick the bottom of the leaf, it would taste salty. Through the research on its mechanisms, a hypothesis about how the salt is secreted was created; Each cell's cytoplasm is connected by channels in the cell membrane. A network of pumps and channels work together to move the sodium ions to the glands of the plants to excrete them.

(Top and Bottom of an *Avicennia* leaf taken by me, *2019)*

2.1: Hypothesis

"As the height of the leaf increase, the salinity of the leaf decreases. Hence, the relationship is inversely proportional." This hypothesis is made because of the assumption that it would take lesser energy to transport excess salt to a leaf that is nearer to the roots.

2.2: Null hypothesis

"There is no relationship between the height of the leaf and the salinity of the leaf."

3: Variables

3.1: Independent variable

The independent variable is the distance from the ground to the leaves (in meters). Taking a sample of 3 leaves and then measuring another 30 cm from that point and taking another 3 leaves. Do this 2 more times. This changed so a varies amount of results can be achieved and a conclusion can be made on whether the height effects the salinity of the leaf or not.

3.2: Dependent variable

Variable	How will this be measured?	Why will this measured?
Proximal: The leaves being collected	This will be done by going to the mangroves, measuring the different heights and then taking 3 leaves from that height	The leaves will be used to find the ultimate variable
Ultimate: The salinity of the leaves	This will be done by finding the conductivity (number of free ions) in the leaf.	Once this is found, the amount of NaCl ions can be determined. This is because as there is a higher conductivity, there are more ions present (which can be assumed to be salt ions)

3.3: Control variables

Variable	How will this be done?	Why is this done?
Genus	To identify the *Avicennia*, look for thin pencil roots next to the tree and relatively small leaves. These are 2 indicators that can confirm that the tree is an *Avicennia*. Another factor is that the leaves of the *Avicennia* would excrete salt.	So there is no variation in salinity because of the species. The *Avicennia* is the only species to be tested on.
Day, Time of day, Tide	The leaves were all picked around 6-7 am so the salinity levels were similar throughout the picking of the leaves	To make sure that this doesn't have an effect on the salinity of the tree because the only effect should be height.

Area of mangrove (soil moisture)	The leaves were all picked from the same mangroves and around the same area in the mangroves.	To make sure this is isn't a factor that could affect the salinity of the leaves.
The volume of water added with the small leaf circles	Through the preliminary, a line was drawn on the sampling container to show which point to be filled to. After this, the water was filled with a syringe and the volume came to 10.5 ml.	This is to have a high enough volume that the probe is completely covered and a minimal volume so that the leaves aren't too diluted.
The number and size of the leaf circles	During the preliminary, 10 holes were put in and the result came to 349 µS which was between the range (0-2000 µS).	To make sure that there is an equal amount of leaf in each container so that it can't change the results in any way. This is also to ensure that the range on the conductivity probe is constant

4.1: Equipment

- **Measuring tape *(±0.01)***- Used to measure the height from the ground to the leaf
- **Plastic bags *(x20)***- To store the leaves when going back for testing
- **Conductivity probe *(±3%)***- To measure conductivity of the leaf (hence the salinity of the leaf)
- **Data logger**- Used to show the conductivity (in micro siemens) of the leaf
- **Distilled water**- To clean the conductivity probe and use as solvent for the salt in the leaves
- **Pipette**- To measure the amount of distilled water being put in
- **Sampling containers**- To store the water and leaves in (and shake them in)
- **Hole punch**- To make holes in the leaves

4.2: Method

1. Identify an *Avicennia* tree
2. Use measuring tape to find the height from the ground for the lowest leaf of the tree
3. Take 3 leaves that appear to be the same size from that height
4. Move up in 30 cm intervals and take 3 leaves until 12 leaves from one tree (4 distances) are collected
5. Repeat this with 5 trees in total
6. Calibrate the conductivity probe (set at 0-2000 micro Siemen) with the standardized NaCl that is at 1000 micro Siemen/cm
7. Wash the probe in distilled water and zero it
8. Hole punch 10 small, equal sized circles from a leaf
9. Put the 10 circles in 10.5 ml of water in a sampling container
10. Shake the container for 2 minutes

11. Place the probe in the beaker and wait for the measurement to remain constant and record that measurement
12. Repeat this for all 60 leaves

4.3: Rationale

The conductivity probe helps show salinity because when testing for salinity, NaCl is trying to be found. By making a solution with adding the leaf and water together, a sodium chloride solution is formed. This creates free moving ions that were present in the leaf. From this, conductivity can be tested. So, as conductivity increase, it is assumed that there is more sodium chloride (salt), hence the leaf is more saline. 4 heights were being measured per tree, with 3 leaves being collected for every height. With 5 different tree, that made 20 different heights with 3 repeats each. This gave the data enough variation and repeats without taking up too much time to measure conductivity levels.

4.4: Preliminary findings

- 10 holes made in the leaf (leaf couldn't have any more full holes made in it)
- When putting distilled water in with syringe, made sure to have enough water that the probe was fully covered and keep the volume minimal to prevent excess dilution (10.5 ml of water decided as constant)
- Twisted the container up and down with cap on for 2 minutes and then put the probe in. Measurement took less than 10 seconds to be constant. So, 2 minutes of shaking time decided as constant
- Result came to 349 µS (which means the 0-2000 µS range was good)

5: Risk Assessment

5.1: Environmental Risks

- There are a lot of species, like crabs and shells, in the mangroves. Make sure not to step on them or disrupt their habitats.
- Do not take any excess leaves than necessary, because it is important to conserve the plants that remain in the mangroves.
- Don't step on any of the pencil roots of the *Avicennia*, as they are thin and can easily break.

5.2: Personal Risks

- Experimenting will be done in the morning (around 7 am) so wear sunscreen and have a hat on.
- Have water to keep hydrated.
- Wear insect repellant because there may be flies or other insects that could be dangerous.
- Wear water shoes/boots so it is easier to walk in the mud.

6: Findings

6.1: Raw Data

Distance from ground (m) (±0.01) (x)	Conductivity of leaf (μS) *(±3%)* 1st Repeat	2nd Repeat	3rd Repeat
0.60	293	290	298
0.73	306	300	301
0.90	309	320	315
1.03	318	320	318
1.10	478	308	315
1.14	330	315	322
1.20	345	350	348
1.33	342	340	342
1.40	353	350	351
1.44	372	365	315
1.50	368	310	369
1.63	368	350	351
1.70	310	372	369
1.74	356	345	348
1.85	412	389	395
2.00	352	380	391
2.04	378	369	370
2.15	368	453	450
2.45	412	480	475
2.75	453	400	397

6.2: Processed Data

Distance from ground (m) (±0.01) (x)	Mean Conductivity of leaf (μS) *(±9%)* *(y)*
0.60	294
0.73	302
0.90	315
1.03	319
1.10	367
1.14	322
1.20	348
1.33	341
1.40	351
1.44	351
1.50	349
1.63	356
1.70	350
1.74	350
1.85	399
2.00	374
2.04	372
2.15	424
2.45	456
2.75	417

6.3: Data Analysis

The Pearson's correlation coefficient can be used to find the confidence of these results. This test was chosen as my data was not normally distributed meaning Simpson's correlation coefficient couldn't be used.

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

(Statistics How To, 2019)

x is the distance from the ground, y is the mean conductivity, n is the number of results (20)

$\sum xy$ = 11374.66

$\sum x$ = 30.7

$(\sum x)^2$ = 941.3

$\sum y$ = 7156

$\sum x^2$ = 53.112

$\sum y^2$ = 2592783.7

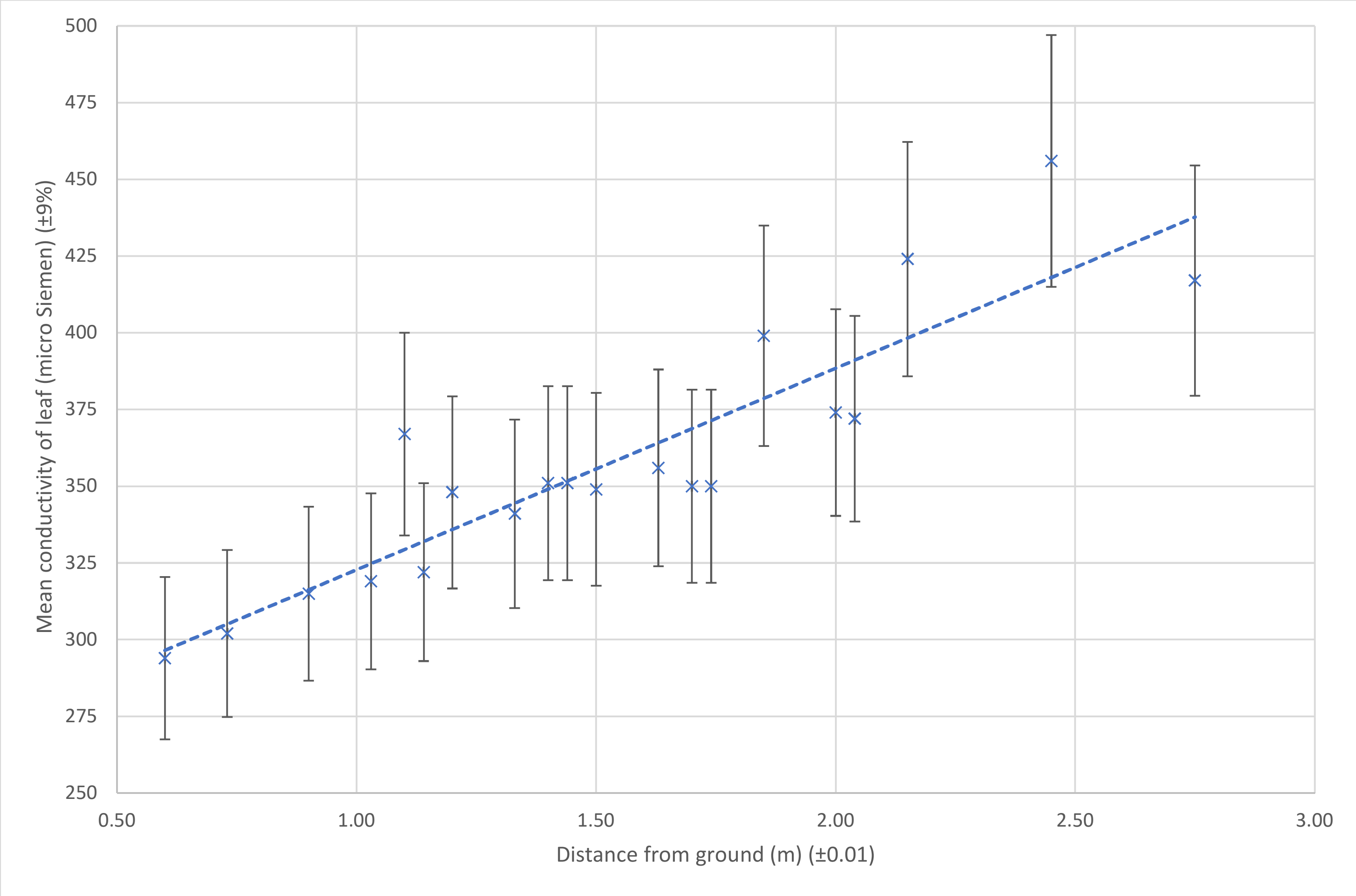
$(\sum y)^2$ = 51208336

$$r = \frac{20(11374.66) - (30.68 \cdot 7156)}{\sqrt{[(20 \cdot 53.112) - 941.2624] \cdot [(20 \cdot 2592783.667) - 51208336]}}$$

r= 0.9002

Some points on the table may look anomalous but when looking at the graph, it seemed unnecessary to ignore them, as there were no major differences made. The Pearson's correlation coefficient was much higher than the minimal confidence value (which is 0.4437 for p>0.95) (Zaionts, 2019).

6.4: Graph comparing the relationship between the height and salinity of the leaf



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Graph made by me using Excel, 2019)

Vertical bars are the percentage uncertainty of the mean conductivity of the leaf (±9%)

6.5: Conclusion

Through the graph, it is visible that as the height of the *Avicennia* leaf increases, the salinity of the leaf increases. This suggests a directly proportional relationship. This was also proven by my positive correlation coefficient and positive gradient from the graph. As my hypothesis suggested the correlation should have been negative, my both my null hypothesis and hypothesis have been rejected. This may be because of transpiration. Transpiration is the uptake of water (minerals, ions and nutrients) through a plant's xylem and the water's evaporation from the leaf. The factor of transpiration to be focused on is light intensity. As light intensity increases, the rate of transpiration increases. Also, presumably, as the height of the leaf increases, there is more sunlight present for that leaf. Hence, there is more transpiration (and evaporation of water) through leaves that are higher up in the tree. Assuming there would be some excess salt in the water of the tree, this would prove why there would be more salt excreted by leaves that are taller in the tree.

By Pearson's correlation coefficient, it is proven that the results have a strong confidence. This means that the null hypothesis is rejected. But, the hypothesis stated is also rejected. This is because the hypothesis states an inverse relationship (when height increases, salinity decreases), but the observations show a direct relationship. However, looking at my graph, a lot of the error bars are overlapping, with some of the bars not going through the line of best fit. This suggests that the relationship cannot be proven, and that no real correlation should be found. This is because the error bars suggest the data could have been anywhere in that area.

6.6: Evaluation

6.6.1: Strengths

The leaves picked out were around the same size to decrease the possibility of an error where the size of the leaf may have affected the amount of salt on the leaf. The conductivity probe was washed with distilled water and zeroed it after every recording. This way there was systematic error. The Pearson's correlation coefficient was 0.9002. There were 20 different points (n-2= 18). This means the minimum 95% confidence coefficient for this experiment is 0.4438. This means the results for this experiment showed a strong positive correlation. The bottom of the leaf was never touched, to prevent any salt from rubbing off.

6.6.2: Weaknesses/Limitations

	The effects they have on the experiments	**Improvements that could be made**
It is correlation, not causation	There is no way to be sure that the height of the leaf does have an effect on the salinity of the leaf. A reason is the fact that the results showed conductivity and not direct salinity. There is no way to be sure that conductivity directly links to the amount of sodium ions in the leaf. Any other ions that may have been	One could look at the structure of an *Avicennia* tree to find whether water is more likely to flow to the higher leaves first or the lower leaves first (through the xylem)

	on the leaf would have affected the results.	
The leaf was left in a Ziploc bag for at least 15-20 minutes	The salt could have been rubbed off and left in the bag	Do the experiment right after removing the leaf
The distance was measured to a branch and the leaves were then picked from that branch	The leaves may have differed in distance compared to the branches, meaning the	Measure each leaf at the distance needed, not the branch.
Only 10 small circles were picked from the leaf	There may have been more salt on other areas of the leaves, which means the salinity level may have been lower than the actual level.	One way this could be improve is by using the entire leaf in a bigger container.
The trees the leaves were taken from weren't the same size	This may change the amount of nutrients and ions taken up by the tree. This means the leaves at different distances would not be comparable. This could have been a large source of error made in the data.	Use a smaller distance, and use similarly sized trees to get the leaves.

6.6.3: Extensions

To improve on this experiment as a whole, another species' salinity levels could have been experimented on (like *Rhizophora,* another tree present in the mangroves) to show how high the salinity of the *Avicennia* was. This would also emphasize the fact that the *Avicennia* uses a different way to excrete salt (from the leaves). Another way is to look at a different area. This allows the comparison between the 2 sets of data to improve on the reliability of the data. Another way to improve on the experiment is to use electrophoresis. This can be used to separate charged molecules in the leaf. It is then possible to test for the amount of salt ions present in the solution. To show that there is actually a difference made by the mangroves, it is possible to grow an *Avicennia* tree in normal conditions (without the salinity levels changing) and test the leaf. This will give a control to the experiment that will prove a significance in having the *Avicennia* from the mangroves being tested.

7: Bibliography

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Avicennia tree: 27th March 2019, Sedili Besar, Malaysia

Top and Bottom of an Avicennia leaf: 27th March 2019, Sedili Besar, Malaysia

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