Searching for Nature Stories 2010

Why are mangrove leaves not bitten by herbivores?

Form. 6

Group 1

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Content

1. Abstract

In this project, we aim at looking into how the concentration of salts and tannin in leaves affect its trend to be consumed by worms.

As mangrove leaves normally contain a higher proportion of tannin and salts than common ones, theoretically they will be less possibly eaten.

Our School Camp

In our experiments, we will first analyze the content of different species of mangrove leaves in terms of salts and tannin with a kind of common leaf as control. This will be followed by the study of whether worms will choose common leaves over mangrove leaves.

2. Reasons of the investigation

From the book Forest in the Water (by Lee Shing-yip) it states mangrove leaves contain a kind of tea-coloured chemical, tannin, that gives rise to its red colour and prevents it from herbivore attacks.. We wonder whether mangrove leaves really have a higher concentration of tannins than other common plant leaves, and whether this is one of the reasons for the food choice of herbivores. Moreover, the book claims that mangrove leaves have a very special adaptation to salty water. They tend to drop leaves with accumulated salt, sometimes excreting salt through their leaf surfaces. Therefore we also intend to find out how the concentrations of salts are different between different types of plants and mangroves as well as that between fallen and fresh leaves.

3. Objective

To investigate the relationship of salt and tannin content of leaves with its tendency to be eaten by worms

4. Prediction

- Mangrove leaves should have higher concentration of salt than non-mangrove leaves.
- Mangrove leaves should have higher concentration of tannin.
- Worms should eat non-mangrove leaves but not mangrove leaves.

5. Materials and Method

5.1 Outline of the investigation process

Our school camp is located at Tsam Chuk Wan, Sai Kung. We chose it owing to our familiarity with the site. There are altogether four different species of mangrove in our school camp, *Kandelia obovata*, *Excoecaria agallocha*, *Bruguiera gymnorrhiza* and *Aegiceras corniculatum.* In this investigation, we are going to compare the amount of salt and the presence of tannins in mangrove and non-mangrove plants. An unknown non-mangrove plant and associate mangrove species *Hibiscus tiliaceus* were also used for investigation. Fresh leaves and fallen leaves were both acquired from the school camp during the field trip.

Kandelia obovata(秋茄樹)

Excoecaria agallocha(海漆)

Bruguiera gymnorrhiza(木欖)

Aegiceras corniculatum(桐花樹)

There are 3 experiments to be carried out in this project. First, the presence of tannins is tested by ferric chloride. Upon addition, appearance of black colour indicates the presence of tannin. The concentration of tannin present in the sample can also be determined by observing the intensity of black colour. Second, the chloride ion test was conducted with the aim of finding out the concentration of salt in the sample. The leaf extract samples were titrated against silver nitrate solution, thus obtaining the corresponding concentration of chloride ion. Lastly, to verify which kind of leaves herbivores prefer, a number of *Zophobas morio* are put into a tray containing different kinds of leaves and kept for a few days.

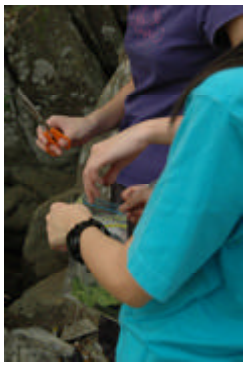
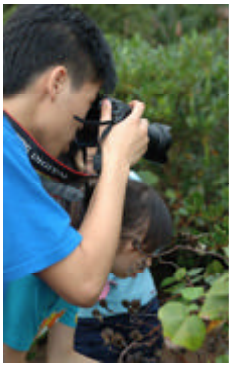
Left:
Unknown non-mangrove plant
Right:
Hibiscus tiliaceus (黃槿)

5.2 Information of the Field Trip

Date	4/3/2010	20/3/2010
Time	5pm-6pm	3:30pm-5pm
Venue	Sai Kung Tsam Chuk Wan Queen Elizabeth School Camp	
Max. Temp. (Sai Kung)	23.7℃	25.4℃
Min. Temp. (Sai Kung)	21.5℃	18.2℃
Relative Humidity	81 - 95 Percent	68 - 92 Percent
Rainfall	0.1 mm	Trace
Tides	0.6-1.0m	0.7-1.2m

5.3 Equipment

Scissors
Labels
Plastic bags
Mangrove guide book


We were taking photos and getting the samples

5.4 Preparation of aqueous leaf extract

Apparatus

Blender
$100cm^3$ Beakers
Scissors
Bunsen Burner
Wire gauze
Tripod
spatula
Insulating mat
Muslin cloth
Glass rod
Pipettes
Pipette filler
Electronic balance

Procedures

1. The leaves were made into powder using a blender.
2. 4g of the powdered leaf were weighed and transferred into a $100cm^3$ beaker containing 25 cm^3 of distilled water.
3. The beaker was heated using a Bunsen Burner for 5 minutes.

4. The solution was squeezed out using muslin cloth.
5. Another 25 cm^3 of distilled water was added and squeezed out again in order to wash the sample adhered to the beaker wall.

5.5 Tannin test

Apparatus

Spotting Tile	Droppers
100cm^3 Beakers	Measuring Cylinder
250cm^3 Beaker	Electronic Balance
Spatula	

Chemical

Ferric chloride

Procedures

1. 0.5g of ferric chloride was measured into a 250cm^3 beaker.
2. 200cm^3 of distilled water was added, a 0.25% ferric chloride solution was prepared.
3. 0.5cm^3 of samples was added into each well of the tile.
4. 1 drop of ferric chloride solution was added to each sample.
5. The colour change of the sample was compared before and after addition of ferric chloride solution.

**Appearance of black colour indicates the presence of tannin.*

5.6 Test for Chloride ion

Apparatus

Beakers	Burette
Burette	Pipette
Conical flask	Pipette filler
Dropper	Clamp & stand
White tile	

Chemical

0.01M silver nitrate	potassium chromate

Procedures

1. 10cm^3 of sample was pipetted to a conical flask
2. 2.5cm^3 of potassium chromate was added as an indicator.
3. The solution was diluted to 100cm^3.
4. The sample was titrated against 0.01M silver nitrate until the end point was

reached, i.e. when the red precipitate of potassium nitrate had started to appear.

5. The burette was refilled when necessary.
6. The volume of silver nitrate used was recorded.

5.7 Effect of different kinds of leaves on *Zophobas*

Experimental animals

Zophobas morio was used for the experiment. It is a species of darkling beetle, whose larvae are known by the common name **superworm** or **zophobas**. Superworms are often chosen as an educational project. They are large in size and easy to be taken care of. They eat almost everything, for instance, fruits, vegetables and peels.

Apparatus

Plastic box Leaf samples (one per kind)

Procedures

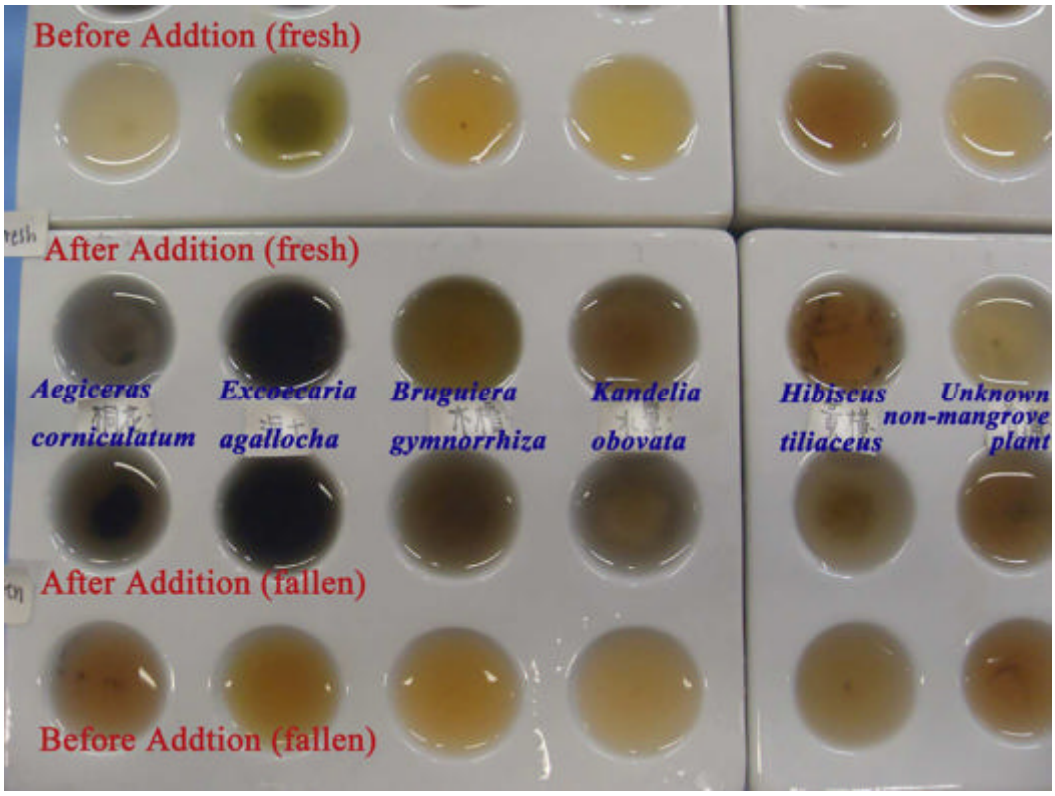
1. One sample from each kind of leaves was placed inside the big plastic box.
2. Photos were taken to record the original appearance of the leaves.
3. Superworms were introduced into the plastic box.
4. The set-up was left for a few days.
5. Photos were taken to record the final appearance of the leaves.
6. The final photos of the leaves were compared with the original one.

5.8 Assumptions of all the tests

- Assume that when the red colour starts to appear, the end point of the titration of chloride ion has been reached, i.e. all chloride ions have reacted with the silver nitrate.
- Assume all chloride ions present in the sample comes from sodium chloride.
- Assume all the sodium chloride and tannin in the leaves samples had been extracted.
- Assume *Zophobas* would eat the leaves that most insects would prefer to eat.
- Assume *Zophobas* would bite the leaves that they prefer to eat the most.
- Assume only tannin in the sample would react with ferric chloride and gives the precipitate.
- Assume the black colour intensity in the test result of tannin is directly proportional to the concentration of tannin inside the sample.

6. Results

Result of Tannins test



Species	Colour intensity	Species	Colour intensity
Fresh unknown non-mangrove plant	-	Fallen unknown non-mangrove plant	+
Fresh *Hibiscus tiliaceus*	+	Fallen *Hibiscus tiliaceus*	+
Fresh *Kandelia obovata*	++	Fallen *Kandelia obovata*	++
Fresh *Bruguiera gymnorrhiza*	+++	Fallen *Bruguiera gymnorrhiza*	+++
Fresh *Excoecaria agallocha*	+++++	Fallen *Excoecaria agallocha*	+++++
Fresh *Aegiceras corniculatum*	+++	Fallen *Aegiceras corniculatum*	++++

‘ – ‘ indicates the absence of black colour

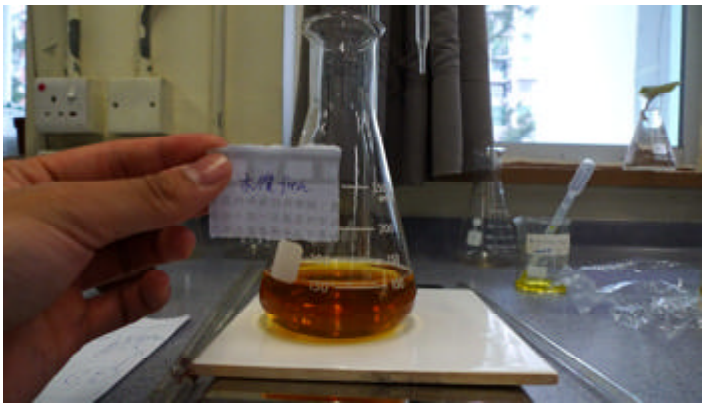
‘ + ‘ indicates the degree of colour intensity

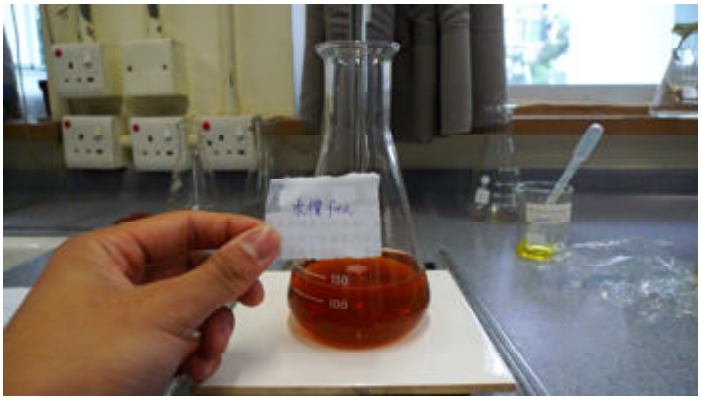
Result of test for chloride ion

	Fresh ***Aegiceras corniculatum***		**Fallen** ***Aegiceras corniculatum***		**Fresh** ***Excoecaria agallocha***		**Fallen** ***Excoecaria agallocha***	
Final reading/cm^3	21.60	23.50	59.40	63.90	45.00	44.30	36.70	37.10
Initial reading/cm^3	1.70	3.50	0.10	5.10	1.30	2.00	1.80	3.20
Vol. used/cm^3	19.90	20.00	59.30	58.80	43.70	42.30	34.90	33.90
Average vol. used/cm^3	19.5		59.05		43.00		34.40	

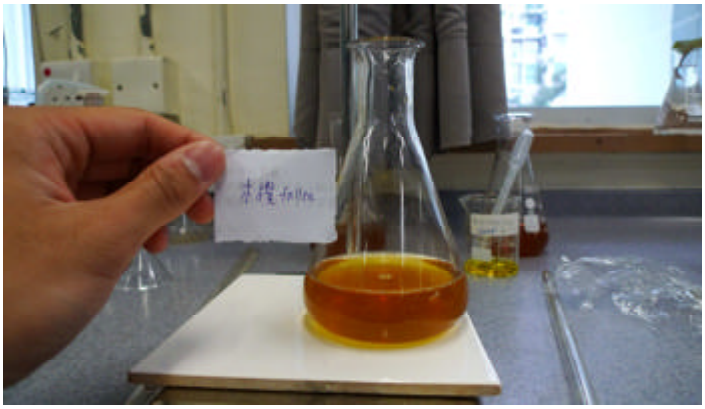
	Fresh ***Bruguiera gymnorrhiza***		**Fallen** ***Bruguiera gymnorrhiza***		**Fresh** ***Kandelia obovata***		**Fallen** ***Kandelia obovata***	
Final reading/cm^3	25.00	26.20	63.30	57.45	24.70	22.30	48.50	49.00
Initial reading/cm^3	0.60	2.30	11.40	7.25	2.70	0.80	5.15	4.95
Vol. used/cm^3	24.40	23.90	51.90	50.20	22.00	21.50	43.35	44.05
Average vol. used/cm^3	24.15		51.05		21.75		43.70	

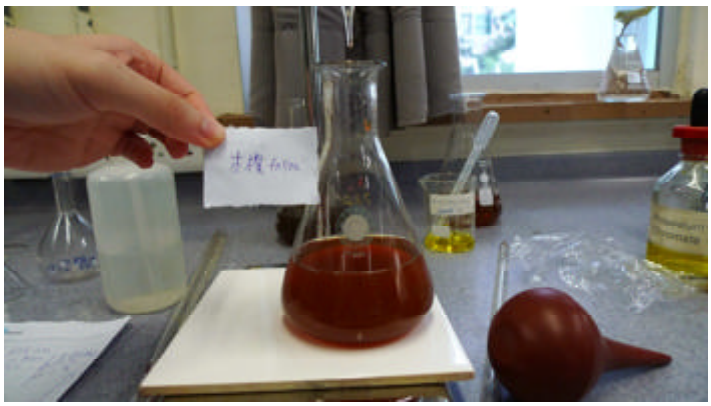
	Fresh ***Hibiscus tiliaceus***		**Fallen** ***Hibiscus tiliaceus***		**Fresh unknown non-mangrove plant**		**Fallen unknown non-mangrove plant**	
Final reading/cm^3	6.50	14.40	43.55	53.10	12.00	20.10	6.70	12.90
Initial reading/cm^3	0.95	9.90	4.05	14.00	3.65	12.00	0.20	6.70
Vol. used/cm^3	5.55	4.50	39.50	39.10	8.35	8.10	6.50	6.20
Average vol. used/cm^3	5.025		39.30		8.23		6.35	





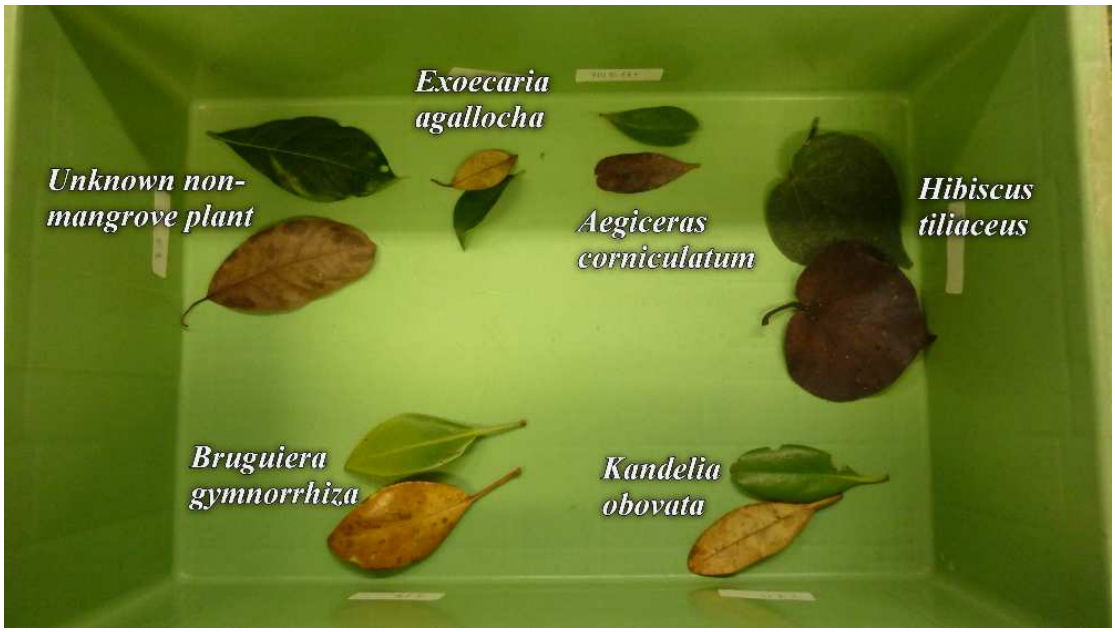
Result of fresh Bruguiera gymnorrhiza before and after end point is reached





Result of fallen Bruguiera gymnorrhiza before and after end point is reached

Result of *Zophobas* Test



Before any Zophobas is put into the box

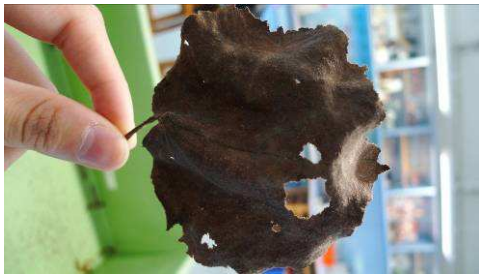
One week later

	a	***b***
1	Fresh *Aegiceras corniculatum*	Fallen *Aegiceras corniculatum*
2	Fresh *Excoecaria agallocha*	Fallen *Excoecaria agallocha*
3	Fresh *Bruguiera gymnorrhiza*	Fallen *Bruguiera gymnorrhiza*
4	Fresh *Kandelia obovata*	Fallen *Kandelia obovata*
5	Fresh *Hibiscus tiliaceus*	Fallen *Hibiscus tiliaceus*
6	Fallen unknown non-mangrove plant	Fallen unknown non-mangrove plant

Result of the experiment

1 a	  Fresh *Aegiceras corniculatum* leaf was not eaten by Zophobas	*1 b*	  Fallen *Aegiceras corniculatum* leaf was not eaten by Zophobas
2 a	  Fresh *Excoecaria agallocha* leaf was not eaten by *Zophobas*	*2 b*	  A small part of fallen *Excoecaria agallocha* leaf (tip of the leave) was eaten by *Zophobas*

3 a	  Fresh *Bruguiera gymnorrhiza* leaf was not eaten by *Zophobas*	*3 b*	  Fallen *Bruguiera gymnorrhiza* leaf was not eaten by *Zophobas*
4 a	  Fresh *Kandelia obovata* leaf (the bites existed before the experiment) was not eaten by *Zophobas*	*4 b*	  Fallen *Kandelia obovata* leaf was not eaten by *Zophobas*

5 a	  Fresh *Hibiscus tiliaceus* leaf was not eaten by *Zophobas*	5 b	  A large part of fallen *Hibiscus tiliaceus* leaf was eaten by *Zophobas*
6 a	  Part of the fresh unknown non-mangrove leaf was eaten by *Zophobas*	*6 b*	  Part of the fallen unknown non-mangrove leaf was eaten by *Zophobas*

7. <u>Analysis</u>

Tannin test

In this test, mangrove leaf extractions showed positive results. There are significant colour changes when ferric chloride is added to the mangrove sample. This proves that mangrove leaves contain tannin. Also, it is observed that the colour intensity results of different mangrove samples are different. This shows that different species of mangrove plants store different amount of tannin. As it is a semi-qualitative test, it is difficult to determine how much they differ from each other. A quantitative experiment can be carried out for further investigation.

For non-mangrove plants, *Hibiscus tiliaceus* contains an extremely small amount of tannin while unknown non-mangrove leaves contain no tannin. Therefore, it is believed that one special characteristic of mangrove plants is the storage of tannin.
Besides, the experimental result of fallen leaf samples and that of fresh samples are very similar. No conclusion can be drawn in these two set of results.

Test for chloride ion

From the results table, it is observed that the volume of silver nitrate required for the titrations with mangrove leaves extractions is large. Mangrove leaf extractions require 4-8 times of the volume of silver nitrate used in non-mangrove leaf titration to reach the end point. This indicates that the concentration of chloride salt stored in mangrove leaves is 4-8 times higher than non-mangrove leaves. It is believed that one of the functions of mangrove leaves is to store large amounts of salt.

Concentration of salt stored in fresh leaves and fallen leaves in the same species shows significant difference. Except *Excoecaria agallocha* and unknown non-mangrove plant, fallen leaves contain more salt. For mangrove plants, a possible reason for this result is that the plants store excess salt in leaves so that they can be removed once the leaves fall. But for the non-mangrove plant *Hibiscus tiliaceus*, the concentration of salt in fallen leaves is abnormally high. It is believed that the salt comes from the salty environment such as soil and sea water as the samples are collected near the sea.

Effect of different kinds of leaves on *Zophobas*

At the beginning of the experiment, superworms were evenly distributed in the box, allowing them to climb on different leaves samples freely. As the experiment proceeded, it was observed that more and more superworms moved towards *Hibiscus tiliaceus* and stayed under the leaf eventually. A few worms stayed on unknown non-mangrove plant leaves. Also, it was observed that a large part of fallen *Hibiscus tiliaceus* leaf and part of the fresh and fallen unknown non-mangrove leaves had been eaten by the worms. However, no worm stayed on the mangrove leaves and the leaves are not eaten up except the fallen *Excoecaria agallocha* leaf.

Based on all the three experiments, the possible reason for which worms will not eat mangrove leaves is suggested as follows. As mangrove leaves usually contain a large amount of salt and tannin, these substances are not beneficial to worms and may affect the health of them. Instead, worms tend to eat leaves with a lower concentration of salt and tannin such as *Hibiscus tiliaceus* and other non-mangrove leaves.

8. Discussion

8. 1 Sources of error

- The leaves were not washed by distilled water in order not to wash away any salts that may have adhered on them. Therefore, some may have many impurities on the surfaces and affect the results.

Tannin Test

- Ferric chloride might react with other unknown substance inside the samples, not only with tannin acid.

Test for chloride ion

- The titration should be carried out under alkaline condition of pH 7 to 10. However, for the presence of tannins in mangrove leaf extracts, this may not have been achieved. The accuracy of end point is affected. To improve, we may add sodium hydrogen carbonate into the samples in order to keep an alkaline condition.

- The silver nitrate titration can be used for testing the presence of both chloride and bromide ions, which means the result is not only showing the concentration of SODIUM CHLORIDE but also other chloride or bromide-containing impurities.

- The colour change in titration is not sharp enough because the leaf extracts are coloured. Therefore, there may be error in determining the end point.

Animal Test

- The *Zophobas morio* may not represent any other animals' choice of food.

8.2 Precautions

- We should be careful when handling silver nitrate solution as it will stain clothes and skin.

- We should pay attention when handling iron(III) chloride as it is corrosive when dissolved in water.

- Make sure the *Zophobas morios* have enough water or they will die of dehydration.

8.3 Suggestion for improvement

- We could wash the leaves' surfaces with distilled water if the leaf was covered with mud/any other apparent impurities, so as to lower the chances and the effect of possible impurities attached to the leaf surface. On top of that, we could have done the test with both cleaned and unclean leaves samples and compare their difference.

- We could also perform more titrations to get a more accurate result. Furthermore, we could have done a trial before each titration to estimate where the end point would be. Some control set-ups could be set too.

8.4 Difficulties

- The book that we read mentioned, 'leaves of mangrove contain tannin and some other chemicals which prevent insects from eating them.' At first, we merely decided to test for the presence of tannin. However, we thought the content of project might not be rich enough so we started to find out what kinds of chemical are present in the leaves. After conducting some researches, we learnt that mangrove leaves contain high concentrations of salt which can scare insects away. Yet, none of us knew how to test for tannin, and there was no equipment in our school that can be used for carrying out such tests. At first, the internet suggested a set of tannin test kit could be used, but it was too expensive. Fortunately, we discovered a simple method from a U.K research paper. It was quite useful and enabled us to determine the presence of tannin in leaves.

- We used to think that it is very easy to test the concentration of salt in leaves. Originally, we decided to use evaporation and weigh the mass of salt in different samples (As the higher the concentration of salt, the heavier the residue would be). However, we found it was impossible after testing the method. The crystals formed were not white but red, orange or brown in colour, i.e. they were not pure sodium chloride. After that, we thought of adding the sample to excess silver nitrate followed by filtration in order to obtain the precipitate and compare their amounts. But then we realized that the precipitate formed may also contain other chemicals like carbonate. On reflection and investigation, we chose the titration method (use of silver nitrate and potassium chromate) and it was successful.

- This was the first time we carried out the silver nitrate titration. Although we knew the procedures of the titration from the Internet, the lab manual only mentioned the procedures for testing the concentration of salt in sea water. We

did not know the exact concentration of salt in the leaf extract so it is difficult for us to decide the concentration of silver nitrate used for titration. Worse still, the samples were not colourless and some were very dark. Thus, it was difficult to carry out titration as the colour change was not sharp enough for us to determine the end point. Eventually, we were able to dilute the silver nitrate and leaf extracts suitable for titration by trial and error.

- It was hard to prepare the aqueous leaf extracts for experiments. We attempted to grind the leaves by mortar and pestle with the addition of a constant volume of distilled water. The residues were then filtrated by gravity filtration but failed as there were too much coarse residues. As a result, we tried to use vacuum filtration instead. However, the rate of filtration was slow and we found grinding by mortar and pestle was ineffective. In view of this, a blender was used to grind the leaves as an alternative. It was much more effective as it just took a few seconds for the leaves to be turned into fine pieces. Moreover, using muslin cloth can eliminate the problem of residue clogged up in filtration and the maximum amount of extracts can be squeezed out in this way.

8.5 Limitations

- Using a spotting tile could only provide a rough estimation of the difference in concentration of tannin.

- Only 4 kinds of mangrove leaves were used in this experiment, so the result was not fair to other mangrove species.

8.6 Further investigation

In the titration for concentration of salts, the mixtures turned into a very dark colour after they had reached the end point. We have asked our chemistry teacher for the reason behind and his answer was that there should be many other impurities in the leaves which caused the colour change. Therefore, we would like to try to find out what other chemicals are present in the leaves and which had caused the colour change.

We could also investigate on how the sea water's salinity in the area affects the salt concentration inside the leaves. In different seashore areas, the water's salinity might be different, and we may determine whether the salinity of the sea water would affect the types of mangroves present in those areas.

Furthermore, we can see if the other mangroves species also have a high salt concentration, not only the four kinds we have examined. For example, how the tannin concentrations vary in different kind of mangroves and the exact concentration of tannin inside those extracts could be found.

An experiment can also be conducted to see whether a higher concentration of salt would also prevent the leaves from being eaten by other herbivores.

Lastly, a quantitative experiment can be carried out to get a more accurate result and comparison between different mangrove and non-mangrove plants in tannin test.

9. Summary

After investigation, we found that mangrove leaves have a higher concentration of salt than non-mangrove leaves. Tannin is highly concentrated in mangrove leaves but absent or less in non-mangrove leaves. Moreover, worms prefer to eat non-mangrove leaves but not mangrove leaves.

10. Bibliography

- Forest in the water / Lee Shing-yip
- African Journal of Traditional, Complementary and Alternative Medicines—— Phytochemical Screening and Antiulcerogenic Effect of Moringa Oleifera Aqueous Leaf Extract http://www.bioline.org.br/request?tc06038
- Bark Tanning: Tannins and Tannin Sources http://www.braintan.com/barktan/2tannins.htm
- Trees On The Edge, Bill Amos http://caledonianrecord.com/main.asp?SectionID=5&SubSectionID=159&ArticleID=45910&TM=8454.068
- Zophobas morio, Wikipedia http://en.wikipedia.org/wiki/Zophobas_morio
- Phytexcell Green Tea, Crodarom http://www.croda.com/download.aspx?s=53&m=doc&id=179
- Determination of Chloride Ion Concentration by Titration (Mohr's Method) http://www.outreach.canterbury.ac.nz/chemistry/documents/chloride_mohr.pdf

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