Presence of Organochlorines in the Waters of Singapore

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Little Green Dot Student Research Grant
PROJECT REPORT

submitted to

Nature Society (Singapore)

Junior College Category 2011
3237 words

Abstract

The aim of this project was to investigate the presence and percentage of organochlorines in Singapore's waters through the collection of the cockle, *Fimbria fimbriata*, from two sites. The sites were specifically chosen to represent a polluted site and an unpolluted site to ensure that further comparisons can be made. The sites chosen were Sembawang and Pulau Hantu specifically. Collection was carried out at low tides and the samples were treated to filtration, blending and lipids were extracted to determine the amount of organochlorines found to be stored in the lipids of the cockles. The final analysis was carried out using gas chromatography mass spectrometry analysis at National University of Singapore (NUS) Chemistry Laboratories. The results showed that 1-chloroheptacosane may be present in cockles collected from the samples taken at Sembawang Park and 2-Bromododecane (an organobromine) may be present in the samples taken from Pulau Hantu. However in both cases, the percentages were statistically insignificant (91% chance for the presence of 1-chloroheptacosane and 87% for the presence of 2-Bromododecane). This may be due to experimental limitations or that Singapore waters might indeed be low in organochlorine distribution. Methods to keep the concentration low are discussed as well in the discussion.

(200 words)

INTRODUCTION

Much effort has been placed into cleaning Singapore's shores. However, it seems that the cleaning efforts were not very effective. In the past, the beaches were conducive for the survival of various marine life such as dolphins and dugongs (Chua, 2011). Being at the top of the local food chain, the presence of marine mammals is a reliable indicator of the quality of the water. Despite much effort being placed into cleaning our shores, many are still puzzled by their absence. This means the cause of this phenomenon goes beyond the naked eye.

With many different pollutants in the sea, this project focused on a type of pollutant known as organochlorines. Organochlorines are a form of organic compound that contains at least one chlorine atom. Organochlorines commonly originate from pulp and paper factories that use chlorine-based bleaches such as chlorine dioxide (Johnston, *et al.*, 1996).

These compounds cannot be digested by animals and causes bioaccumulation. Bioaccumulation is a process by which certain toxic substances (in this case organochlorines) accumulate in living organisms, posing a threat to health, life, and to the environment (BusinessDictionary.com, 2012). When such bioaccumulation occurs, it poses a greater threat to the apex predator of the local marine food chain; human beings.

The apex predators in many food chains are human beings. When humans consume these marine animals, they will increasingly store organochlorines in them. This creates a negative impact on their health. Organochlorines may adversely affect semen quality and cause testicular cancer in males, induce menstrual cycle abnormalities and spontaneous abortions in females, and cause prolonged pregnancies, reduced birth weight, skewed sex ratio, and altered age of sexual development (Toft *et al.*, 2004).

In addition, this bioaccumulation of organochlorines might cause stress to marine animals that once roam free in the waters of Singapore. Organochlorine pesticides (dieldrin, DDT and its compounds, aldrin) have been identified as endocrine disrupting substances (Lyons, 1999). The presence of high concentrations of organochlorine pesticides (and polychlorinated biphenyl, PCBs) or their residues in marine mammals have been suggested as the cause of pathological changes and reproductive failures in marine organisms in other parts of the world such as in Baltic seals (Helle *et al.*, 1976) sea lions, seals and beluga whales (Addison, 1989). It also leads to immunity suppression (and hence the possibility of being more susceptible to disease) in harbour porpoises (Kuiken *et al.*, 1994) and seals (Reijnders, 1986; Swart *et al.*, 1994). Other examples include changes in the development stability of the Baltic grey seal (Zakharov and Yablokov, 1990); and premature pupping in California sea lions (Delong *et al.*, 1973).

300 different types of organochlorines have been identified including dioxins, furans and other highly toxic, persistent substances (Greenpeace, 1992). Tonnes of these by-products are released into local waterways and the air (Joe, 2000). Another source of organochlorine pollution is pesticides, which are extremely persistent in the environment and organisms (Ongley, 1996). Despite its use being banned by the Singapore government since the 1980s, there is a possibility that the organochlorines may originate from our neighbouring countries where chlorine-based pesticides are still widely used as farming is the basic source of income for these countries (Tan *et al.*, 2007). This is the main reason why this research will be investigating on the concentration of organochlorines in our waters.

One way to identify the presence of organochlorines in Singapore's waters is to process filter feeders such as shellfish. Filter feeders are organisms that consume large amounts of phytoplankton, which contributes to the maintenance of the water quality (Bryant). They filter large amounts of water and thus take in a huge amount of phytoplankton (Wijgerde, 2008). Through this process, toxic substances may accumulate in them (Allie, 2011). In addition, organochlorines are known to be stored in the lipids of cockles (Scobie *et al.*, 1998). As such, filter feeders are the best organisms to work with in the context of this project.

The species of bivalves chosen for this investigation was *Fimbria fimbriata*, a type of cockle that can be locally found on Singapore beaches (both on Singapore's mainland and offshore islands). Therefore, it was easier to obtain as compared to other types of cockles.

METHODS

Site Selection

Two sites were chosen to represent the polluted and relatively unpolluted regions of Singapore. The sites were Sembawang Park and Pulau Hantu respectively.

Sembawang Park is considered to be relatively polluted as many ships pass along the area, due to the presence of Sembawang Shipyard (refer to Fig. 1). On the other hand, Pulau Hantu is relatively cleaner.

Thus a comparison of the levels of organochlorines in *F. fimbriata* obtained from these two areas would show a good distinction between the presence and levels of organochlorines in polluted and unpolluted waters of Singapore.

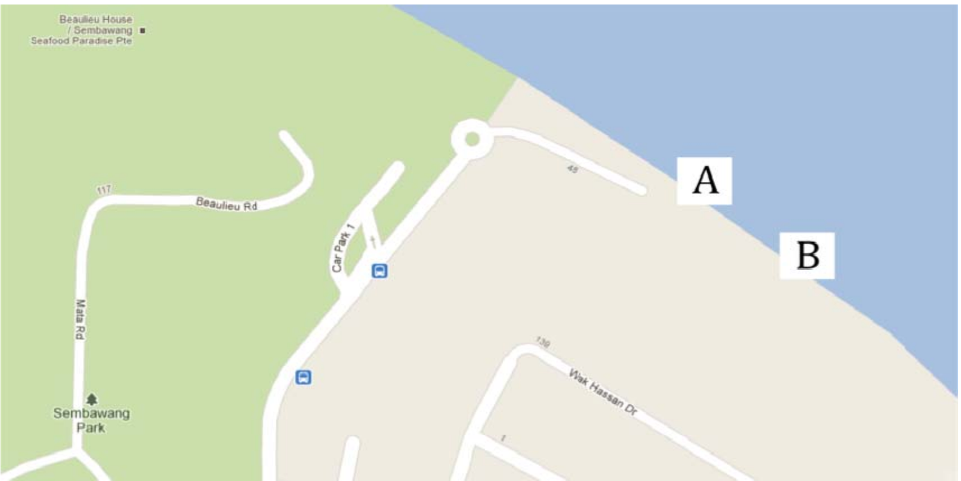


Fig. 1: A map of Sembawang Park and its surroundings (Google Maps, 2012) [Edited]

Fig. 2: Side view of Spots A and B at Sembawang Park (Virtual Tourist, 2012) [Edited]

Fig. 3: Sembawang Park at low tide

Fig. 4: This water catchment at Sembawang Park is suitable for the growth of shellfish such as crabs and clams

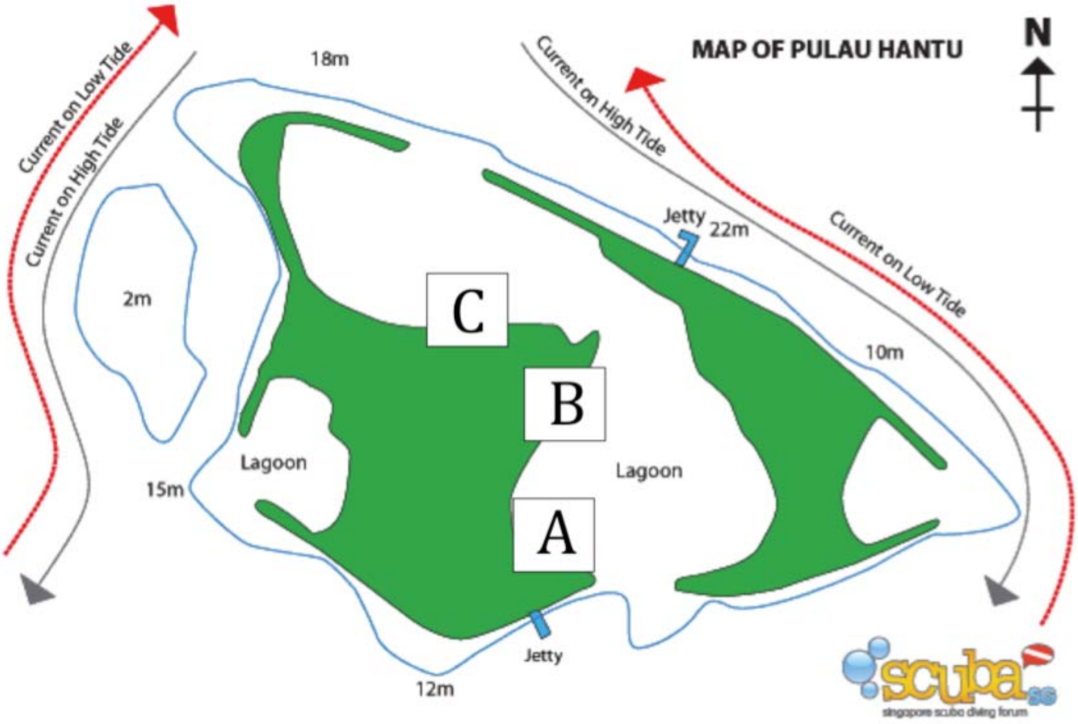


Fig. 5: A map of Pulau Hantu which is surrounded by other offshore islands and in the Northeast of Pulau Hantu is Bukom Kechil. Bukom Kechil is home to the SHELL oil refinery (Din, 2012)

Fig. 6: The SHELL oil refinery can be seen from the background of this image of Pulau Hantu Besar (Ng, 2011)

Method of obtaining organochlorine from the samples

Organochlorines are highly fat soluble (Bräuner *et al.*, 2011) and as a result build up in the fatty tissues and stay in the body for a long time because they are only slowly metabolised and excreted (Ellis & Buckland). Therefore to obtain the organochlorines, the lipids of the shellfish will need to be extracted. In order to extract the lipids from the cockles, the Soxhlet method was used with hexane as a solvent.

Field work - Cockle collection:

Materials used:

1) Mini-Shovels
2) Gardening spoon
3) Sieve
4) Storage containers

Fig. 7: Picture demonstrating the method of collecting *F. fimbriata* at Pulau Hantu

1) Visit the site at low tide.
2) Select one spot and label the spot 'A'.
3) Dig into the sand with the shovel as the cockles dig themselves into the sand to shield away from the sun.
4) Once a cockle is found, place it in a storage container containing seawater in order to keep it alive.
5) Continue collecting the cockles from spot 'A' until 30 cockles are collected.
6) We collected from two spots from Sembawang Park and from three spots from Pulau Hantu Besar. The spots we chose to collect the cockles from were randomly selected.
7) Label the new spot 'B'.
8) Repeat steps 2 to 6 until the aim of collecting from three different spots is met.

Filtration method:

Materials used:

1) Test tube rack
2) Boiling tubes
3) 6 filter funnels
4) Filter paper
5) Blender
6) 250ml beaker

Procedure:

1) Tare a dry, empty 250ml beaker using an electronic weighing balance.
2) Remove 25.000g of the insides of the cockles and place it in the beaker.
3) Place the contents of the beaker into a blender and blend it till liquefied.
4) Line 6 filter funnels each with a damp filter paper and place the neck of the filter funnels into boiling tubes.
5) Pour equal volumes of the liquefied cockles into each filter funnel.
6) Let the liquefied cockles drain overnight.
7) Repeat steps 1 to 6 for the remaining cockles from the four other spots.

Extraction method: Soxhlet Method

Materials used:

- Soxhlet apparatus:

1) Condenser
2) Cellulose extraction thimble (1 per experiment)
3) Glass wool
4) 500ml flat-bottomed boiling flask
5) Heating mantle
6) Soxhlet extractor
7) Dried blended/grinded cockles
8) Hexane

Procedure:

1) Remove 5.000g of the dried and filtered liquefied cockles from the filter paper and place it into a cellulose extraction thimble.
2) Cover the top of the thimble with glass wool to prevent the samples from floating.
3) Weigh the flat-bottomed boiling flask.
4) Pour 136.0ml of hexane into the flat-bottomed boiling flask.
5) Place the thimble in the extraction chamber of the Soxhlet extractor.
6) Grease the bottom of the condenser and the bottom of the Soxhlet extractor.
7) Attach the flat-bottomed boiling flask to the bottom of the Soxhlet extractor and the top of the Soxhlet extractor arm to the bottom of the condenser. Place the flat-bottomed boiling flask in the heating mantle. The setup should look like this:

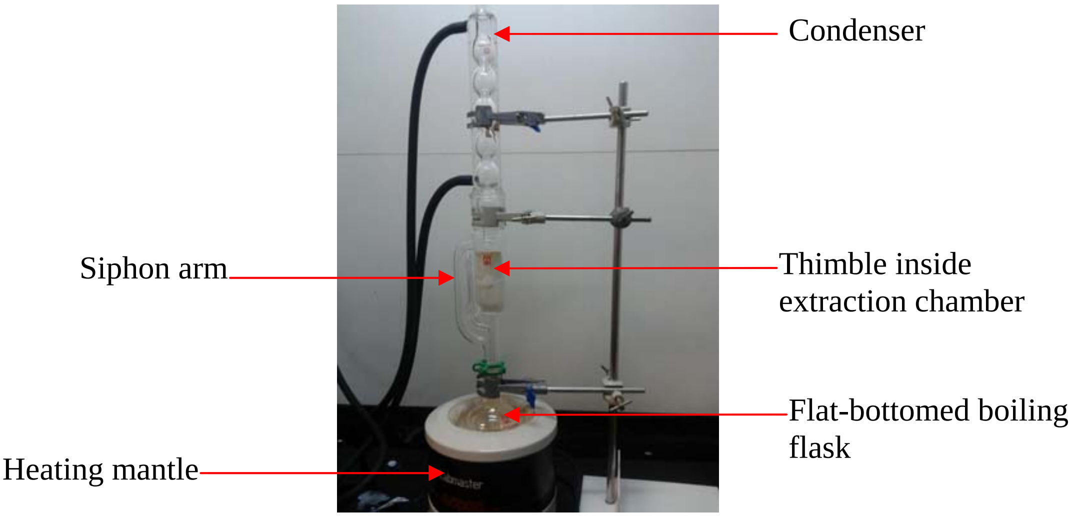


Fig. 8: A set-up of a Soxhlet, an equipment used for the lipid extraction

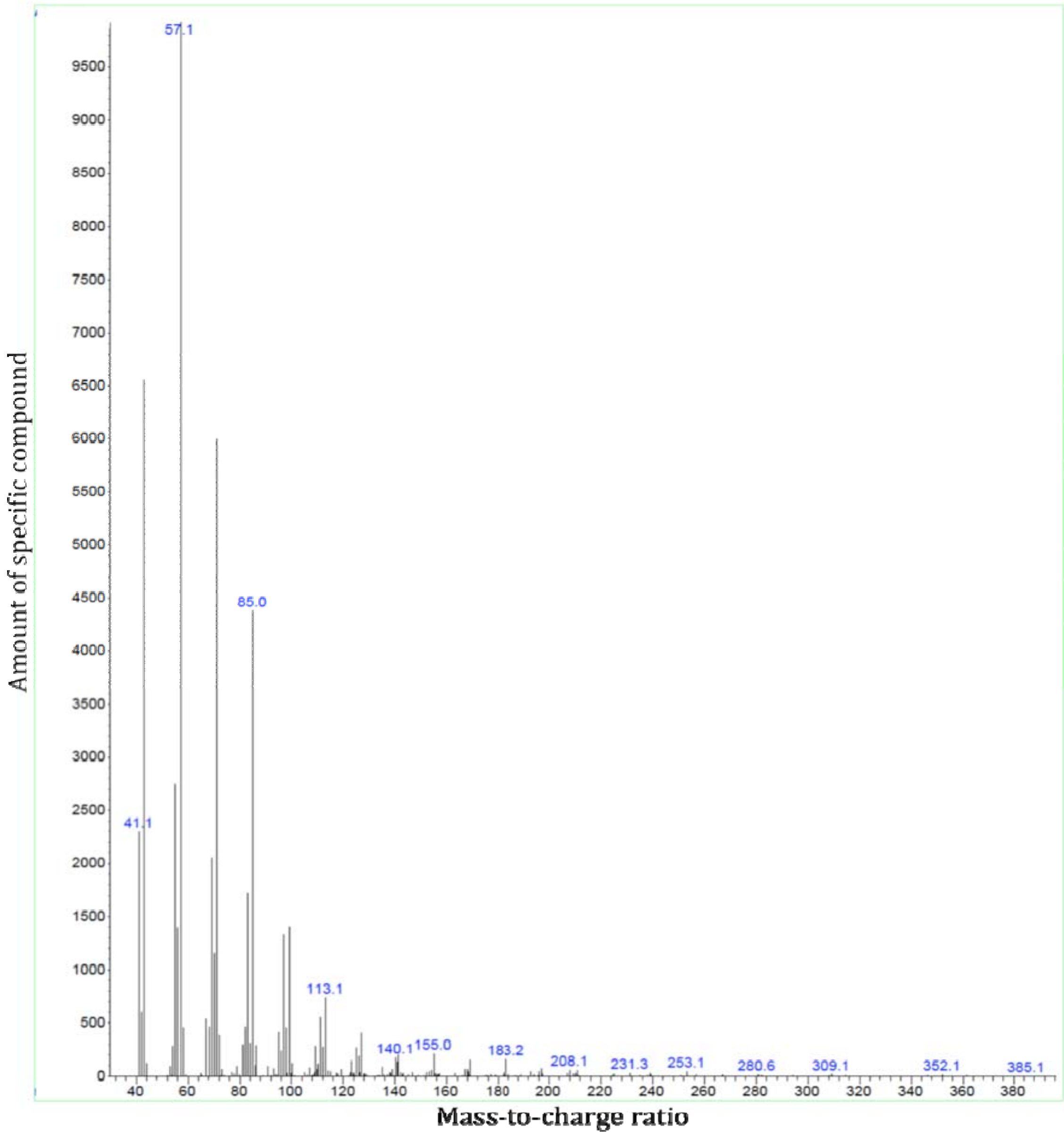
8) Allow the Soxhlet apparatus to extract lipids from the dried cockles at the boiling point of hexane for 7 hours.
9) Allow the contents of the flat-bottomed boiling flask (extract) collected at the end of 7 hours to cool.
10) Remove the solvent from the extract and place it in a boiling tube.
11) Use a cork stopper to prevent any spillage and evaporation.
12) Repeat steps 1 to 11 for the remaining samples.
13) Send the extracts for gas chromatography mass spectrometry analysis at the National University of Singapore (NUS) Chemistry Laboratories.

RESULTS and CONCLUSIONS

After analysing the results from the Gas Chromatography (GC) graphs, the conclusions made is that the samples collected are considered to be free from organochlorine pollution.

The data obtained from the GC analysis represents the identity of chemical compounds and the probability of their presence in the samples. A presence of probability 95% and above confirms the presence of that particular chemical compound in the sample.

Below is the graph (refer to Graph 1) and an accompanying table (refer to Table 1), which shows the GC analysis, showing 10 possible chemical compounds present in cockle samples from Sembawang Park. Out of these 10 possible compounds, there is only one chlorine compound identified with a 91% presence possibility in the samples. This chlorine compound is called 1-chloroheptacosane.



Graph 1: GC identification of compounds from samples obtained from Sembawang Park

No.	Name	Molecular Mass	Compound formula	Probability of presence of compound/ %
01	Tetratetracontance	619	$C_{44}H_{90}$	91
02	Octacosane	394	$C_{28}H_{58}$	91
03	Tritetracontane	605	$C_{43}H_{88}$	91
04	Heptacosane, 1-chloro-	414	$C_{27}H_{55}Cl$	91
05	Heptadecane, 9-octyl-	352	$C_{25}H_{52}$	90
06	Pentadecane, 8-heptyl-	310	C_{22}H46	87
07	Hexadecane	226	$C_{16}H_{34}$	87
08	Pentadecane	212	$C_{15}H_{32}$	87
09	Pentadecane	212	$C_{15}H_{32}$	87
10	Octadecane	254	$C_{18}H_{38}$	87

Table 1: Compounds identified from the samples from Sembawang Park

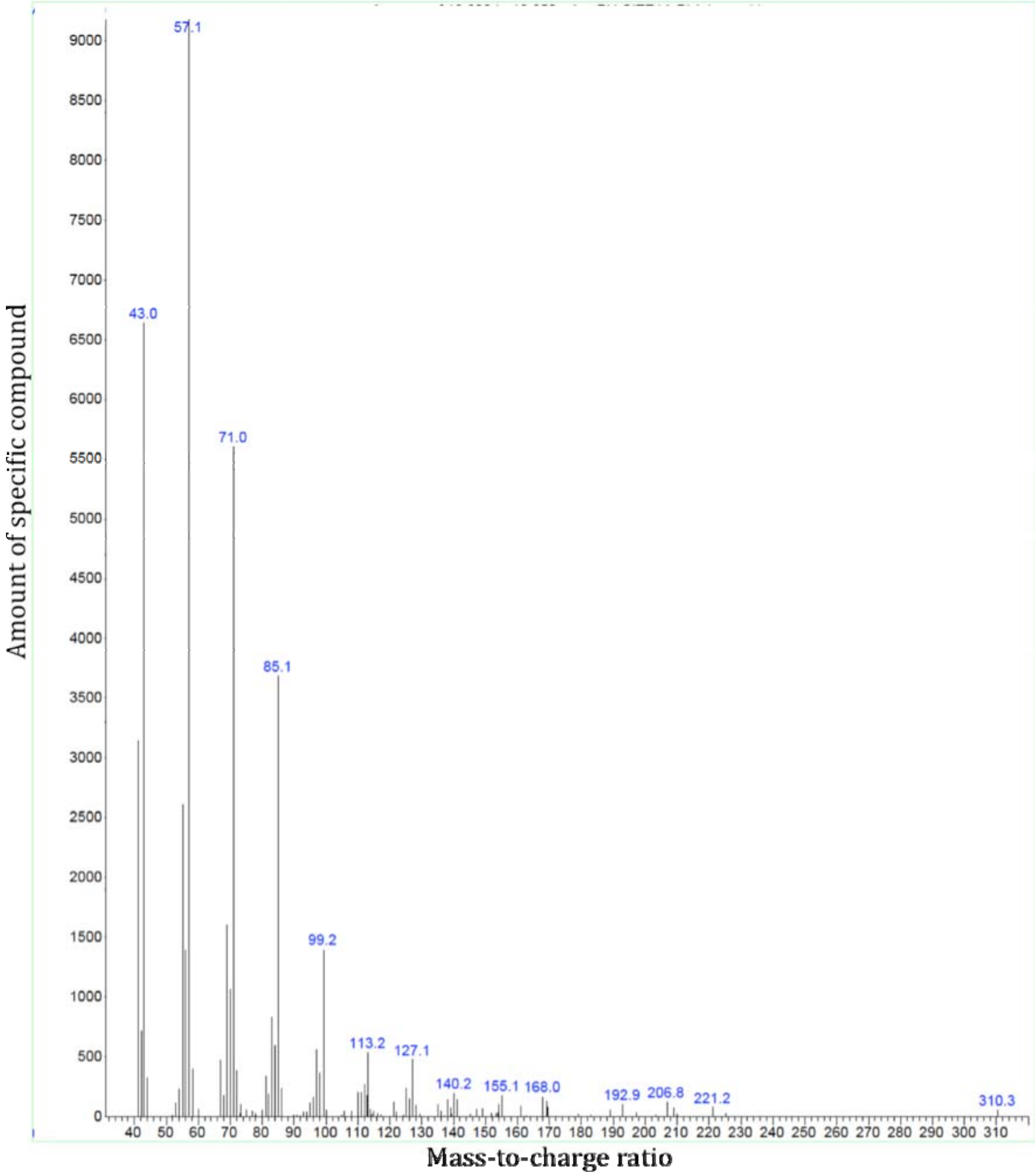
The main reason why the samples are deemed to be free of organochlorines is because the results do not show at least a 95% probability of any chlorine compound's existence. 95% is the confidence level we are using because when a substance is present with a probability of 95%, scientists can conclude its presence. Hence, the presence of chlorine cannot be confirmed as the chances of the sample containing chlorine such as, $C_{27}H_{55}Cl$ is lower than expected with only a 91% chance.

Fig. 9: Chemical structure of 1-chloroheptacosane (Heptacosane, 1-chloro-, 2011)

In addition, if 1-chloroheptacosane were to really exist in Singapore's waters, it might be from the process of disinfecting drinking water, which poses risks to organisms that consume the water, instead of stemming from pollution via the release of such compounds intentionally into the seawaters of Singapore. Genotoxic compounds have been found in drinking water, most of which are chlorinated and/or brominated trihalomethans. A very potent chlorohydroxyfuranone, known as "mutagen X" has been found to be a potent carcinogen in rats.

A study of drinking water conducted in North-Eastern Romania revealed the presence of 1-chloroheptacosane in chlorinated drinking water. There were higher levels of "mutagen X" in chlorinated drinking water than in non-chlorinated drinking water. Under the Ames test, there was higher mutagenicity for chlorinated drinking water. Fish exposed to increasing concentrations of chemicals in both chlorinated drinking water and non-chlorinated drinking water displayed a higher lethality percentage in chlorinated drinking water (Mancas, *et al.*, 2002). Drinking water in Singapore is also disinfected with chlorine (Public Utilities Board, 2010). This may lead to the formation of the by-product 1-chloroheptacosane, which may enter the environment and water bodies in Singapore, thus posing mutagenic and toxic risks to marine life.

Below is the graph (refer to Graph 2) and an accompanying table (refer to Table 2), which shows the GC analysis, showing 10 possible chemical compounds present in cockle samples from Pulau Hantu. Out of these 10 possible compounds, there is only one bromine compound identified with a 87% presence possibility in the samples. Thus, its presence cannot be confirmed. This compound is called 2-bromododecane.



Graph 2: GC identification of compounds from samples obtained from Pulau Hantu

No.	Name	Molecular Mass	Compound formula	Probability of presence of compound/ %
01	Docosane	310	$C_{22}H_{46}$	91
02	Eicosane	282	$C_{20}H_{42}$	91
03	Heptacosane	380	$C_{27}H_{56}$	91
04	Tridecane, 1-iodo-	310	$C_{13}H_{27}I$	91
05	Heneicosane	296	$C_{21}H_{44}$	90
06	Eicosane	282	$C_{20}H_{42}$	87
07	Nonadecane	268	$C_{19}H_{40}$	87
08	Pentadecane	212	$C_{15}H_{32}$	87
09	2-bromododecane	248	$C_{12}H_{25}Br$	87
10	Heptadecane	240	$C_{17}H_{36}$	86

Table 2: Compounds identified from the samples from Pulau Hantu

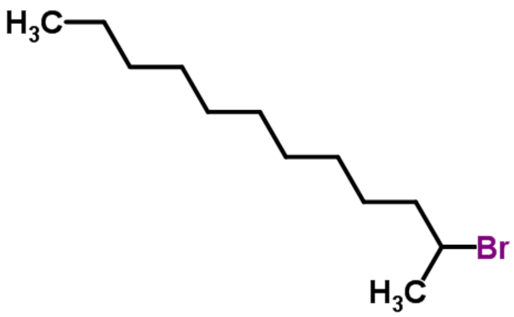


Fig. 10: Chemical structure of 2-Bromododecane (2-Bromododecane, 2011)

Organobromine compounds are similar to organochlorine compounds in the sense that they are persistent environmental pollutants as they persist through humification of natural organic matter (Leri *et al.,* 2010). In addition, organobromine compounds do affect marine life adversely as well. A study done on Zebrafish embryos suggested that there is a possibility that brominated indoles may affect early life stages of marine fish species in the North Sea (Kammann *et al*,. 2006). This is the reason why there is a need to look out for organobromine compounds besides focusing on organochlorines as originally planned.

Thus the results obtained from both samples from Pulau Hantu and Sembawang indicated that there might be the presence of organochlorines and organobromine compounds in both sites but the percentages were not high enough to draw a firm conclusion. Further studies may need to be conducted to confirm the presence of these substances in the samples.

DISCUSSION

Initial hypothesis:
As stated in the introduction, the amount of pesticides present in the waterways in the region should have indicated a significant amount of organochlorines in Singapore's waters. As tides carry them in from the surrounding areas. Another source of organochlorine pollution is from the paper and pulp factories. Singapore use to be home to the largest paper and pulp factory in Southeast Asia in the 1900s. Even though this industry is depleting, Indonesia is now the ninth largest pulp and the 11^{th} largest paper factory in the world (Firework: Exhibition And Conferences, n.d.), According to research, organochlorines are non-biodegradable. And so, it was believed that significant traces of organochlorines can still be detected in the waters of Singapore.

However, this was not the case when the GC results were analysed.

Possibilities resulting in the results obtained

A possibility is that the level of organochlorine may be really low in the waters of Singapore.

This could be because of Singapore's agreement with the International Convention on the Control of Harmful Anti-Fouling Systems on Ships (AFS Convention) made on 31 December 2009. This agreement states that all Singapore bound ships are not allowed to use harmful organotin compounds, which are found in antifouling paints from 31 March 2010 (Cheon, 2010). Antifouling paints are used to coat the bottom of ships to prevent marine creatures such as molluscs from growing on the hulls. Unfortunately, the paint will leak into the waters as time passes. The dangers of organochlorine pollution in open waters have been recognised in 1989 by the International Maritime Organization (IMO) and since then encouraged countries to adopt the agreement in order to save the marine ecosystem before it is too late (Internaitonal Maritime Organization, n.d.).

Future improvements to keep waters low or free from organochlorines

In order to maintain the low concentration of organochlorines in Singapore's waters, these steps should be taken:

1. Create a suitable environment for the survival of filter feeders, especially molluscs such as clams and cockles. This is because filter feeders are known to trap any toxins in their body, which makes them important in maintaining the quality of our waters. With both the mangrove trees and filter feeders working together, Singapore's water can be cleaned at a faster rate.

2. Breed clams and cockles artificially and nurse them back to optimum health to increase their populations on mainland Singapore. This is due to the fact that despite the remaining conserved mangroves in Singapore, many local cockles and clams fail to thrive in our waters, as their loss of habitat was too great of a blow when industrialisation and developments were at their peak.

3. Reduce organochlorine pollution in the Southeast Asian region. Countries in the region, such as Thailand and Indonesia, still use cheap pesticides on their crops (Agnes and Prabhu, 1993). Farming has been their basic source of income and coming

from developing countries means income is little. With a small capita, many farmers have to resort to using DDT pesticides to prevent any damage on their crops. DDT pesticides are effective in killing unwanted bugs but compromise the wellness of the environment in order to maximize profits. The pesticides, insoluble in water, are transported to water bodies via agricultural runoff, bringing these organochlorines into the water.

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Acknowledgements

1. Nature Society (Singapore)
2. Mdm Tay Woon Ping
3. Mr Chua Chee Siang
4. Mr Lee Siu Kee
5. Mr Wong Yih Check
6. Yishun Junior College's Science Laboratory Technicians