

## Hong Kong Student Science Project Competition 2023

Template of Extended Abstract (Investigation)

(Word Limit: 1,600 words, Pages: 3 pages only)

Team Number: SBBC215

Project Title: **Impact of microplastics on aquatic organisms**

Project Type: Investigation

*To our best knowledge, there are/are no \* similar works in the field; (if there are, ) related research links are as below:*

**Research about the toxic effect of microplastics can be found, but not mainly on contaminated microplastics. Besides, our report is the first to use atyid shrimp to test for toxic effect of contaminated microplastics.**

### I. Background

- Provide background information of project and/or state the problem to tackle
- Provide highlights of the **literature review** with the support of pertinent and reliable references
- Provide an overview of work and mention the **research gap that the project is trying to fill**

Microplastics refer to the plastic fragments of any type of plastic less than 5 mm in length. Microplastics impose risks on human health and ecosystems, because they are not readily biodegradable. Because of its micro size, it may enter into the environment, food chain and then human body. It could take physical and biological effects on human body. Moreover, Microplastics absorb a lot of environmental pollutants due to their high surface area to volume ratio. Organic pollutants and heavy metals may accumulated along food chain and affect human health and ecological balance. The food safety risk is rinsing when people eat contaminated food.

### II. Objectives

- State the **aim(s)** of project

We aim to research the biological effect of microplastics on the brine shrimp ,including the different concentrations of the microplastics with OXY and BPA , the different sizes of microplastics, and the birth rate of brine shrimp.

### III. Hypothesis

- Propose an explanation for a phenomenon and stating how the **hypothesis** can be tested by experiments
- Brine shrimp is a more sensitive and better biomarker, than Atyid shrimp . Also the death rate of the experiment that tests the toxicity of Oxybenzone and Bisphenol A with PP Microplastics is increasing with the higher concentration. The activity of the brine shrimp is less active with the smaller size of the man made PP Microplastics

### IV. Methodology

- List out the materials used
- Describe the **experimental protocol** including the set-up of **control experiment** (if any), **repeated experiment** (if any), and its scientific theory
- Indicate with the support of reasons, the **analysis** used in the investigation

For testing the toxicity of microplastics with OXY and Babine shrimp nauplii, polluted, unpolluted 13 μm of PP Microplastics beads solutions, and BPA, OXY solutions (10%, 1%, 0.1% 0.01%) were used and added to the petri dish containing brine shrimps.

The death rate of the samples was then counted, so as to calculate the death rate from 0 hour to 24 hours. We will also be testing brine shrimp samples with different sizes and types of microplastic particles.

For understanding the effects of microplastics on the birth rate of brine shrimp, 1 g 13 μm PP MPs 1% in a 50 ml sample: 47.5 ml DI water and 500 μl 1% Triclosan, Bisphenol A, Oxybenzone, Octinoxate, Copper. The polluted microplastic samples were added to 0.02 g Brine Shrimp cysts samples.

Experimental sets 13  $\mu\text{m}$  PP MPs 1%, and control samples were added to Brine Shrimp cysts and waited for two days to allow it to hatch, the results were then compared.

The effect of polluted and unpolluted microplastic is further investigated. We investigated whether the size of the microplastic bread would affect the death rate of brine shrimps. Adult brine shrimp samples were selected as indicator species for the above experiment.

Adult brine shrimps were purchased in local markets, and 30 adult brine shrimps were added to a 0.1% of 30 ml PP MPs in size of 6.5  $\mu\text{m}$ , 13  $\mu\text{m}$  and 150  $\mu\text{m}$ . The samples were settled and Counted for its death rate at 48 h.

## V. Results

- Present the **data** with figures, tables or photos
- **Data analysis** (if any, with emphasis on data reliability and the reproducibility based on statistics)
- Interpret the results and its implication
- Discuss **limitation** and compare with existing related works (if any)
- Discuss the importance or impact of the research and how it is applicable to real problems

A higher concentration of pollutants resulted in a higher death rate than samples containing lower concentrations of pollutants. PP Microplastics themselves alone cause acute death in brine shrimp samples after around two hours of exposure to PP MPs. PP

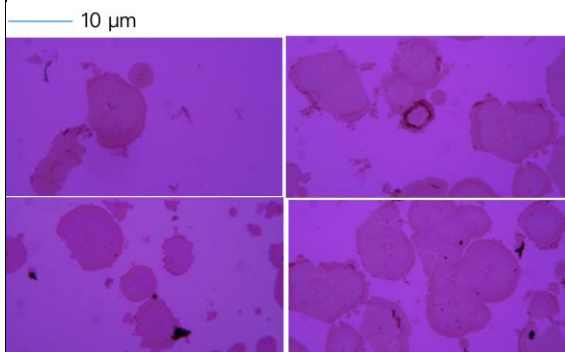
All samples have a death rate greater than that of control. Samples with a higher concentration of pollutants have a higher death rate than that of samples containing a lower concentration of pollutants. However, at low concentrations, the differences between the death rate of samples treated with polluted and polluted microplastics were less significant.

Samples with 10%, 1%, 0.1% & 0.01 PP MPs show death rates of 43.636%, 34.711%, 32.71% & 33.708% respectively. By the use of AAT Bioquest Calculator, the LC 50 (24h) is determined. The LC 50 of PP is 39.8%.

However, the experiment results compared with brine shrimp nauplii samples treated with PP MPs only do not show significant increase in death rate. Therefore it is estimated that polluted microplastic with BPA does not increase the risk of acute death in brine shrimp nauplii samples significantly.

The effect of polluted and unpolluted microplastics on the birth ratio of Brine Shrimps were also investigated. Brine Shrimp cysts treated with polluted and unpolluted microplastics showed a birth rate of 116/0.01g +/- 5, 154.5/0.01g +/- 5 respectively, and control samples with 181.5/0.01g +/- 5 of birth ratio. It shows that the presence of microplastics in Brine Shrimp cyst samples lowered the birth or hatching ratio to 0.01g of cyst. Samples treated with polluted microplastics further decrease the hatching ratio of brine shrimps.

With PP MPs in size 6.5 $\mu\text{m}$ , 13 $\mu\text{m}$ , and 150 $\mu\text{m}$ : The death rate with particle size 13 $\mu\text{m}$  is significantly higher than the others and is also the highest. 13 $\mu\text{m}$  PP is similar to the size of food particles obtained by adult brine shrimps, the microplastic may be ingested and stuck in the digestive tract, causing acute death in the adult brine shrimps.



**Figure 1** 13  $\mu\text{m}$  PP MPs in Nile Red stain (in DMSO)

The microphotograph in **Figure 1** 13  $\mu\text{m}$  PP MPs in Nile Red stain in DMSO. Ethanol evaporates rapidly, and the solution is quickly reduced back to powder-form. Therefore, Ethanol is not an able solvent for Nile red stain. As captured in the figure above, DMSO is an able solvent for Nile red stain for MPs.

**VI. If your team will compete the Sustainable Development Award, please indicate the specific sustainable development goal the project is related to, and provide justification for competing for this award. (Word limit: 300 words)**

Microplastics are the plastic fragments less than 5 mm in length. They are the raw materials for the production of various plastic products, and can be produced by the fragmentation from large plastic wastes. As the result of our previous research shown, MPs are ubiquitous pollutants in two catchment systems in the Deep Bay oyster culture region. Microplastics impose risks on human health and ecosystems, because they are not readily biodegradable, and they absorb a lot of environmental pollutants due to their high surface area to volume ratio. The wider usage of microplastics by mankind, the more severe contamination of microplastics to the environment. Based on the previous result of the microplastics research in Deep Bay, the effects of microplastics on organisms should be understood as well. Therefore, we research it by the use of the biomarker, brine shrimp through testing its death rate and birth rate under the effects of microplastics. The difference of the size of microplastics is tested with the adult brine shrimp as one of the possible reasons of the higher toxicity of microplastics. We aim to make people pay more attentions on microplastics issue on the environment and human's health, and raise the public awareness on the environmentally friendly concept.

## **VII. Conclusion**

- Make a **data-driven** conclusion of the project and the way forward of the research
- Justify if the proposed project meets the objective(s)

MPs are ubiquitous pollutants in two catchment systems in the Deep Bay oyster culture region. Exposure to contaminated PP MPs led to a higher death rate of atyid shrimp and brine shrimp. Brine shrimp is a more sensitive, and better Bioindicator for Microplastic toxicity tests. The difference between the death rate of polluted and unpolluted microplastic samples were also more significant in high-concentration samples in brine shrimp nauplii. Oxybenzone caused a higher death rate in brine shrimp nauplii compared to Bisphenol A. It is evident that the presence of microplastics affect the birth and hatching of aquatic organisms, and that pollutants absorbed on the surface of microplastics further decrease the ratio of birth to brine shrimp cysts. Particle size of 13  $\mu\text{m}$  PP MPs causes the greatest negative impact to adult brine shrimps. And Nile red stain could be applied in identification of microplastics.

**□ Our project is developed based on previous project and the enhancement is below:**

We have tested the toxicity of MPs by the use of atyid shrimp. Unlike brine shrimp nauplii, which can be cultured in the school lab, *Caridina cantonensis* cannot be obtained in large numbers. Brine shrimp nauplii can be cultured easily, and each sample can contain a relatively high number of nauplii, which facilitates to reduce result deviation due to individual difference in samples. Brine shrimp nauplii is also a more well-established and acclaimed indicator species. Therefore, Brine shrimp nauplii in comparison is a better model organism and it is used in the following experiments.

In addition, we tested the toxicity using atyid shrimp with the mixture of different pollutants so we adjust to test it by brine shrimp with only OXY and BPA respectively for knowing the toxicity of the microplastics with OXY and BPA on the brine shrimp individually.