A study of microplastic contamination in water and white shrimp (*Litopenaeus vannamei*) in shrimp ponds in Songkhla Province, Thailand.

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Abstract

Microplastics contaminated in the water are harmful to aquatic lives. Shrimp farming uses contaminated water from the sea. Plastics are also used in the shrimp ponds, so it is very important to conduct research on microplastics in shrimp ponds. The objective of this research was to study water quality, the number, type, shape, and color of microplastics contaminated in water and shrimp in shrimp ponds in Songkhla Province, Thailand. Water samples were collected from 3 sources in shrimp farms which are water preparation ponds, shrimp ponds. and water treatment ponds. And we also collected shrimp samples from shrimp ponds. The results showed that the water treatment ponds had the highest number of microplastics (28±1.41 pcs/1000 ml), followed by the water preparation ponds (26±2.82 pcs/1000 ml) and the shrimp ponds (13±3.53 pcs. /1000 ml) respectively. The average amount of microplastics found in shrimp was 435 pieces per kilogram. Most of the microplastics were found in water and shrimp are 300µm-1mm in size, fragment shape, and white color. The most common type of microplastic is polystyrene, followed by polypropylene and polyethylene, respectively. We can conclude that in shrimp farming there are microplastics contamination in both water and shrimp. The microplastics found may come from seawater used for shrimp farming or may be from materials used in shrimp ponds. This study will help farmers to plan for microplastic-free shrimp farming.

Keywords: *Shrimp Pond, Microplastic, Seawater, polystyrene*

Introduction

White shrimp are of great economic importance in Thailand, since 2003, Thailand is the 2nd in Asia for exporting over 170,000 tons of white shrimp per year (Office of agricultural economics, 2021). Cultivation can be done in water with a salinity level of 5 - 35 parts per thousand (PPT), but the salinity level that grows well is 10 - 22 PPT, which can only be cultivated in coastal areas or in deep inland areas that are county Areas with low salinity (Inland area) because seawater is needed for raising. To increase the average survival rate to about 30-65%, but at present, microplastics are found in seawater in large numbers. and shrimp farming which uses sea water as a main ingredient in raising including being in community areas makes it more likely to find microplastics. Microplastics can drastically harm animals, humans, and ecosystems. These plastic wastes are not biodegradable in a short period of time. While new waste is constantly being added to the oceans causing more accumulation over time, these plastic waste breaks down into smaller sizes, ranging from 5 millimeters down to the micrometer scale. Also known as microplastics. These microplastics are able to absorb organic and metal contaminants. These wastes are therefore a major factor in bringing toxins into the ecosystem and causing the accumulation of toxins within the organisms' bodies and increasing the level of concentration more and more according to the hierarchy of eating in the food chain. The research organizers found out the problem and therefore created a project to study microplastic contamination in shrimp farming facility in Ranode District, Songkhla Province, microplastics from water and shrimp from shrimp ponds will be studied. This is because Ranode District, Songkhla Province is the area where shrimp farm is commonly established. Furthermore, Songkhla Province is also a local distribution of shrimp. and exported to foreign countries and in this area is also a community where most people live. That's why the organizers are interested in studying the area of the water source. This is an area with higher levels of microplastic contamination than other areas in Songkhla Province.

Research questions

1. What is physical characteristic of microplastic found in water and white shrimp in shrimp pond, Songkhla Province, Thailand?
2. How many microplastic found in water and white shrimp in shrimp pond, Songkhla Province, Thailand?
3. What is water quality in shrimp ponds?

The Objectives

1. To investigate the number of microplastic presence in water and white shrimp (*L. vannamei*) in shrimp ponds in Songkhla Province, Thailand.
2. To investigate the microplastic numbers, size, shape and color in water and white shrimp in shrimp ponds in Songkhla Province, Thailand
3. To test water quality in shrimp pond.

Methodology

1. Study sites

Study contamination of microplastic in water and shrimp in shrimp pond at Songkhla Province, Thailand, take samples from 3 types of ponds, preparation pond, shrimp pond and treatment pond.

2. Sample collection

2.1 Collection of sea water sample

Collect water sample by using a manta net and a flow meter at the surface near the pond edge parallelly, then keep the sample 1000 ml in the beaker and repeat 3 times, for each pond.

2.2 Collection of white shrimps

30 white shrimps (1 Kilogram) from each pond were collected, measured weight and size.

3. Sample preparation

3.1 Water sample preparation

(1) The water sample was poured on a 1 mm sieve (filter paper) and thus the microplastics more than 1mm were attached on the sieve. Afterwards the sieve was dried at 60 °C for 24 hours to observe the microplastics clearly.

(2) The same water sample was filtered again through a 300-micrometer filter paper to observe the microplastics less than 1 mm.

(3) The microplastic samples with filter were taken into beakers and were digested by a modified method of Mathalon and Hill (2014) which includes ferrous sulfide ($FeSO_4$) and hydrogen peroxide solution (H_2O_2). After the reaction, they were kept at room temperature for 5 minutes.

(4) Then we put a magnetic stirrer into the solution beakers. The beaker was placed on hotplate magnetic stirrer. Afterwards we added 20 ml of H_2O_2. This time temperature was not more than 75 °C as this solution can boil violently if heated >75 °C. We heated the solution to 75 °C on the hotplate.

(5) Then we added NaCl (6g per 20 mL of sample) and heated the mixture to 75 °C until the salt was dissolved.

(6) Afterwards we poured the solution into a container and kept it for 24 hours.

(7) After 24 hours, we filtered the solution through a 300-micrometer filter paper again and dried at 60 °C for 24 hours to observe the microplastics.

(8) Afterwards microplastics were observed under microscope. Examinations of size, shape and color of the microplastics were done.

3.2 Shrimp sample preparation

(1) Measure samples weight.

(2) Cut sample into small pieces.

(3) The shrimps were cut into small pieces and were taken into the beakers.

(4) Then we poured 180 ml of hydrogen peroxide (30% volume) and added 20 ml of potassium hydroxide (9: 1) into each beaker and waited until the sample was digested.

(5) Afterwards we put a magnetic stirrer into each beaker and stirred until the meat pieces were completely digested. During stirring, the beaker was covered with foil paper.

(6) When the digestion was completed, we added NaCl (6g per 20 ml of the solution) and stirred the solution until dissolved. Then we poured the solution into a container and kept for 24 hours.

(7) After 24 hours, the sample was filtered through a filter paper (20 microns diameter) and dried at 60 °C for 24 hours.

(8) Afterwards microplastics were observed under microscope. Examinations of size, shape, and color of the microplastics were done.

4. Calculation of water volume and microplastic numbers

4.1 Observe samples under stereo microscopic, then analyze volume size shape and color.
4.2 Microplastic numbers were calculated as:

$$\text{The sample per 1000 ml water} = \frac{1000 \times \text{number of microplastics}}{\text{water volume (ml)}}$$

5. Water quality test

We test water quality from 3 ponds, preparation pond, shrimp pond and treatment pond, by using GLOBE Hydrology protocol. Water indexes are pH, temperature, salinity, and dissolve oxygen. After that submit data to GLOBE date entry.

5. Statistical analysis

Two-way ANOVA tests were used to determine the effects of (1) study sites and microplastic sizes, (2) study sites and microplastic shapes, and (3) study sites and microplastic colors. Numbers of microplastics color in water and white shrimp in shrimp ponds, water quality in 3 ponds were tested using one-way ANOVA. Data were analyzed using SPSS and all data were reported as mean ± standard error (SE). All tests were considered statistically significant at P<0.05.

Results

1. Microplastic contamination in shrimp pond experiment result

1.1. Numbers of different-sized microplastics in three types of ponds.

In this study, we divided the microplastics into three different sizes (<300μm, 300μm - 1 mm, >1mm), and the numbers of microplastics from different sizes were different among three types of pounds ($F_{2,18}$ = 13.48, P<0.001) (Table 1). The microplastics more than 1mm in size were less in numbers. In the case of ponds, microplastic numbers of any size were higher in treatment ponds and shrimp ponds than in preparation ponds ($F_{2,18}$ = 9.17, P<0.005) (table 1).

Table 1: The number of microplastics of different sizes in three type of ponds.

Sites	Microplastic numbers / 1000ml water		
	<300μm	300μm-1mm	>1mm
treatment ponds	5±0.707	23±0.707	0
shrimp ponds	7±2.121	19±0.707	0
preparation ponds	5±2.121	8±1.41	0

1.2. Numbers of different-shaped microplastics in three types of ponds.

In the case of shapes of microplastics, we found two different shapes (fragment, fibers) (Figure 1A-B).

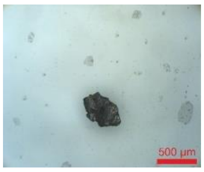
(A)  (B) 

Figure1. two different-shaped microplastics (fragment (A), fibers (B)) were observed in this study.

Among the shapes, the numbers of fragments was higher than fibers numbers ($F_{2,72}$ = 100.13, P<0.001) (Table2). Among the sites, the numbers of fragments was higher than fibers numbers in treatment ponds and shrimp ponds than in preparation ponds ($F_{2,18}$ = 9.17, P<0.005) (Table 2)

Table 2: the numbers of different-shaped microplastics water ponds.

Sites	**Microplastic numbers/ 1000m^3 water**	
	Fragment	**Fiber**
treatment ponds	19 ±0.707	9± 0.707
shrimp ponds	15 ± 4.94	11 ± 2.121
preparation ponds	12 ± 1.29	1 ± 0.707

3. Numbers of different-shaped microplastics in three type of ponds.

In this study, we observed five different color microplastics (black, white, yellow, dark-blue, and red) (Table 3). Among different color microplastics, white color microplastics were higher in numbers than yellow, black, dark-blue, and red color microplastics ($F_{5,144}$ = 135.21, P<0.001) (Table 3). Among the sites, the numbers of white color microplastic were higher in treatment ponds and shrimp ponds than in preparation ponds ($F_{2,144}$=15.62, P<0.001)(Table 3).

Table 3: The number of different color microplastics in three ponds

Sites	**Microplastic numbers/ 1000m^3 water**				
	White	**Yellow**	**Black**	**Blue**	**Red**
treatment ponds	17.00 ±2.121	3.00 ±2.121	1.00 ±2.121	4.00 ±2.828	3.00 ±2.121
shrimp ponds	10.50 ±0.707	0	0	3.00 ±2.121	2.00 ±1.414
preparation ponds	12.00 ±4.242	0	0	0	1.00 ±0.707

4. Numbers of plastic-type microplastics in three types of ponds.

In this study, we observed three different types of microplastics (polystyrene (PS), polypropylene (PP) and polyethylene (PE)) (Table 4). Among different types of microplastics, polystyrene (PS) microplastics were higher in numbers than polypropylene (PP) and polyethylene (PE) ($F_{6,144}$ = 135.21, P<0.001) (Table 4). Among the sites, the numbers of polystyrene (PS) were higher in treatment ponds and shrimp ponds than in preparation ponds ($F_{2,144}$=15.62, P<0.001) (Table 4).

Table 4: The number of different microplastics in three ponds.

Sites	**Microplastic numbers / 1000ml water**		
	Polystyrene (PS)	polypropylene (PP)	polyethylene (PE)
treatment ponds	17.00±2.121	3.00±2.121	8.00 ±0
shrimp ponds	21.00±0.707	0	5.00 ±3.535
preparation ponds	12.00±4.242	0	1.00 ±0.707

2. Microplastics in shrimp.

5. Numbers of different-sized microplastics in white shrimp.

In this study, we divided the microplastics into three different sizes (<300μm, 300μm - 1 mm, >1mm), and the numbers of microplastics from different sizes .

Table 5: The number of microplastics found in e shrimp.

Sites	**Microplastic numbers / 1kg shrimp**		
	<300μm	300μm -1mm	>1mm
White shrimps	189.00 ±37.476	502.00 ±19.798	179.00 ±45.961

6. Numbers of different-shaped microplastics in shrimp.

In the case of shapes of microplastics, we found two different shapes (fragment, fibers) (Figure 1A-B).

(A) (B)

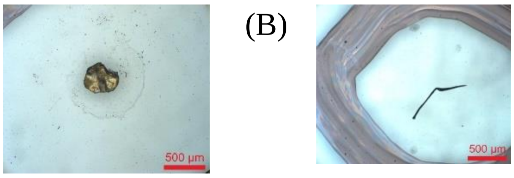


Figure 2. Two different-shaped microplastics (fragment (A), fibers (B)) were observed in this study.

Among the shapes, the numbers of fragments and fibers were higher than film numbers ($F_{2,72}$ = 100.13, P<0.001) (Table 6)

Table 6: the numbers of different-shaped microplastics found in shrimp.

Sites	**Microplastic numbers / 1kg shrimp**	
	Fragment	**Fiber**
White shrimps	760.00 ± 108.894	109.00 ±6.363

7. Numbers of different-shaped microplastics in shrimp.

In this study, we observed five different coloured microplastics (black, white, yellow, dark-blue, and red)(Table 7). Among different coloured microplastics, white coloured microplastics were higher in numbers than yellow , black , blue, and red coloured microplastics ($F_{5,144}$ = 135.21, P<0.001) (Table 7).

Table 7: The number of different-coloured microplastics in shrimp.

Sites	Microplastic numbers/ 1kg shrimp				
	White	**Yellow**	**Black**	**Blue**	**Red**
White shrimps	672.0±96.166	88.00±12.727	19.0±0.707	40.0±1.414	50.0±8.485

8. Numbers of different-type of microplastic in shrimp

In this study, we observed three different types of microplastics (polystyrene (PS), polypropylene (PP) and polyethylene (PE) (Table 8). Among different types of microplastics, polystyrene (PS) microplastics were higher in numbers than polypropylene (PP) and polyethylene (PE) ($F_{6,144}$ = 135.21, P<0.001) (Table 8).

Table 8: The number of different types microplastics in shrimp.

Sites	Microplastic numbers / 1kg shrimp		
	Polystyrene (PS)	**polypropylene (PP)**	**Polyethylene (PE)**
White shrimps	672.00 ±96.166	78.00 ±19.798	109.00 ±6.363

9.Water quality

In this study, we tested the water quality of the three ponds and found that the pH and salinity of the three ponds were not significantly different(p>0.05), but the dissolved oxygen in the treatment ponds was lower than the shrimp ponds and preparation ponds.

Table 9 : The quality of water in .

Sites	Water quality		
	pH	salinity (ppt)	Dissolved Oxygen (mL/L)
treatment ponds	7.63 ± 0.25	18.33 ± 0.58	4.97 ± 0.12
shrimp ponds	7.70 ± 0.10	19.67 ± 1.53	5.93 ± 0.15
preparation ponds	7.53 ± 0.31	18.67 ± 0.58	5.87 ± 0.06

Discussion

The results of microplastic contamination experiment in white shrimp and water showed that most of water contamination was found in the treatment ponds (28±1.41pcs/1000 ml), followed by shrimp ponds (26±2.82 pcs/1000 ml) and preparation ponds (13±3.53pcs. /1000 ml), respectively. Polystyrene (PS) plastic type were found the most, followed by polyethylene (PE) and polypropylene (PP) plastics type. Thus, it is assumed that microplastics found in water and white shrimp can be classified into two categories according to their source. Polyethylene (PE) plastic comes from tools that used in shrimp ponds such as nets, buoys, floaters, tarps, and other equipment. Polystyrene (PS) and polypropylene (PP) plastics, which are plastics found in everyday items such as foam food packaging and multi-purpose plastics is contaminated in the seawater even before the water preparation process.

The microplastic contamination in the water can affect several physiologies of white shrimp and all marine animals. Microplastics are plastic that small enough for marine life to ingest but gastric acid cannot digest them, so they're remained in their body, blocking the gastrointestinal tract leads to nutrient lacking, lower steroid hormone level, delays ovulation, unable to reproduction and eventually death.

The impact of such microplastic contamination will have a huge impact on the white shrimp farming business because of small shrimp, slow growth or death in large numbers will affect the efficiency and productivity of the white shrimp manufacturer. This leads to a serious loss of economic benefits to Thailand because white shrimp is one of Thailand's most important export products. In addition to directly affecting, it also affects indirectly. When marine ecosystems are destroyed by microplastic contamination. The charming scenery will be disappeared. Negatively affect the tourism business and other businesses involved, which also leads to serious negative effect on the country's economy. Therefore, microplastic contamination is a problem that should not be overlooked, and research studies should be expedited to find a solution as soon as possible.

Conclusion

The results showed that the water treatment ponds had the highest number of microplastics (28±1.41pcs/1000 ml), followed by the water preparation ponds (26±2.82pcs/1000 ml) and the shrimp ponds (13±3.53pcs. /1000 ml) respectively. The average amount of microplastics found in shrimp was 435 pieces per kilogram. Most of the microplastics were found in water and shrimp are 300µm-1mm in size, fragment shape, and white color. The most common type of microplastic is polystyrene, followed by polypropylene and polyethylene, respectively. The water quality of three types of ponds, pH, salinity and dissolved oxygen were within the shrimp water standards. We can conclude that in shrimp farming, there are microplastics contamination in both water and shrimp. The microplastics found may come from seawater used for shrimp farming or may be from materials used in shrimp ponds.

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Optional Badges

1. I make an Impact

We found problem when our family's shrimp pond business are getting less profit year by year because the shrimp we grown are more likely to sick, so we conduct experiment on shrimp and we found microplastic in one of sick shrimp's stomach, then we investigate further to find their source and try eliminate them. After research we found that we are not the only one who suffer from microplastic contamination but also the rest of the world so this research can help people who own a shrimp farming or other microplastic problem in general.

2. I am a Data Scientist

When we finished collecting all of the data we need, after that we need to find a way to make a good use of them. We observe them in number, size, color and what type of plastic they are using Fourier Transform Infrared (FT-IR), all to identify the source of microplastic and eliminate them.

3. I am a Collaborator

Of course, we are not able to do all of this on our own. After we collect all the sample, we contact MTEC, apart from NSTDA who specialize in conduct experiment on various material. They said they can verify the chemical structure of plastic sample we found by their custom made equipment for FT-IR, so we can know what plastic they are and find the source accurately.