

INDONESIA

Ms. Tri Handayani

Deputy Director

Surveillance and Products Certification

Fish Quarantine and Inspection Agency

Ministry of Marine Affairs and Fisheries

I. Introduction

In Indonesia, since 1997-2016, there were several reported food poisoning cases due to the consumption of contaminated shellfish. Between June – September 2010, more than 30 people were hospitalized after consuming clams and fish from Teluk Lasongko and Bau-Bau in Southeast Sulawesi. They showed similar symptoms, i.e. vomiting, diarrhea, tongue and lips paralysis. The presence of *Peridinium sp.* was confirmed from the fish caught in those areas, there was a lack of clinical data from patients. On 16 July 2010, another case of food poisoning in Lampung due to consumption of contaminated shellfish and fish' resulted in nausea and vomiting. Phytoplankton *Pirodinium bahamense* was identified as the species causing the shellfish poisoning.

Monitoring of shellfish and its environment (water quality) aims to protect consumers from chemical and microbiological hazards that may arise from consuming shellfish products. Sufficient data on the safety status of shellfish also aims to increase the utilization of its resources for domestic and export markets.

The government of Indonesia has established several regulations, namely, The Government Regulation No. 57/2015 and Decree No. 52A/KEPMEN-KP/2013 which lay down the requirements for Quality and Safety Assurance of Fishery Product. These regulations could help to maintain and protect the culturing area of shellfish from the domestic and industrial sewage which may contaminate shellfish.

In Indonesia, the biotoxin monitoring activity which is also known as shellfish sanitation program has been conducted since 1997 as part of general monitoring program designed to identify and evaluate biological toxins as well as chemical and microbiological contamination of shellfish and the water quality.

National Regulatory Limits for Analytes tested:

No	Parameter	Regulatory Limit	Frequency
1.	ASP (<i>Domoic Acid</i>)	2 mg/100 g	Once every 2 months, at sampling point
2.	AZP (<i>Azaspiracid</i>)	1,6 µg/100 g	
3.	NSP (<i>Brevetoxin</i>)	0,8 mg/100 g	

Indonesia has a monitoring programme for ASP, AZP and NSP under the Japanese Trust Fund VI (JTF VI) in Tanjung Balai Asahan (North Sumatra) and Teluk Lampung (Lampung). The project is located in Tanjung Balai Asahan because this is largest production area in Indonesia while according to the previous monitoring result, there has been PSP occurrence around Lampung area.

II. Objectives and Goals

The objectives of shellfish sanitation program are to ensure the quality and safety of shellfish harvested in the production area, as well as to provide data and information required for setting policies or regulations in terms of implementation of quality assurance and safety of fishery products.

III. Survey Methodologies

a. Sampling Method, Sampling Site, Target Species (include scientific name), Number of Samples and Sampling Size

Sampling for shellfish was conducted on sampling site of shellfish monitoring area of ± 7500 ha in Tanjung Balai Asahan where the coordinates of the sampling sites are chosen on largest production area:

- 1 = 3°01'59.6"N 99°51'48.9"E
- 2 = 3°02'45.4"N 99°52'23.1"E
- 3 = 3°03'14.2"N 99°52'59.2"E
- 4 = 3°03'08.8"N 99°53'35.5"E
- 5 = 3°02'48.2"N 99°53'52.1"E
- 6 = 3°02'11.8"N 99°54'15.2"E

In Teluk Lampung, sampling for shellfish was conducted on sampling site of shellfish monitoring area where the coordinates of the sampling sites are chosen according to the previous monitoring result i.e. positive result for PSP:

- 1 = -5.4793737, 105.2537337
- 2 = -5.4805782, 105.2608285
- 3 = -5.4665515, 105.2608285
- 4 = -5.4651093, 105.2615561

The targeted species for JTF VI project is Baby Clam (*Meritrix meritrix*) in Tanjung Balai Asahan and Green Mussel (*Perna viridis*) in Teluk Lampung. The samples were tested for the following toxins ASP, AZP, and NSP.

b. Method of Analysis (*e.g. sample preparation method, analytical method used, quality control measures*)

The shellfish sampling for Baby Clam (*Meritrix meritrix*) in Tanjung Balai Asahan was conducted through the following steps:

1. Sampling was carried out with scallop shells dredge gear (scallop rakes) which consists of two types namely : garuk or tojok¹. In this monitoring, garuk is most often used.
2. After arriving at the sampling area, garuk was lowered to scrape into the sand layer. The shaft was held firmly and the boat was turned until the garuk contained the shellfish.
3. Garuk was then removed from the water and the shellfish were washed to clean off sand and any other foreign material by shaking the gear several times on the surface of sea water.
4. Shellfish were then placed on the deck of the boat waiting to be sorted. The sorted shellfish were washed again with clean sea water and packed into a sack. Samples were collected at per sampling point and selected with individual weight of ± 50 g/

¹ Traditional sampling equipment

shellfish (minimum weight accepted by company/exporter to get \pm 1kg shellfish that yield > 100 g of meat.

5. Samples were prepared according to AOAC (2000). Shells were cleaned and opened. The inner part was also cleaned to remove any foreign material. Meat was handled carefully to prevent damage or truncation. Meat was then drained and frozen at -20° C until analysis.

The shellfish sampling for Green Mussel (*Perna viridis*) in Teluk Lampung was conducted through the following steps:

1. Sampling was carried out from the ropes in floating net on area of marine culture.
2. Shellfish were then placed on the deck of the boat waiting to be sorted. The sorted shellfish were washed again with clean sea water and packed into a sack. Samples were collected at per sampling point and selected with individual weight of \pm 50 g/ shellfish (minimum weight accepted by company/exporter to get \pm 1kg shellfish that yield > 100 g of meat.
3. Samples were prepared according to AOAC (2000). Shells were cleaned and opened. The inner part was also cleaned to remove any foreign material. Meat was handled carefully to prevent damage or truncation. Meat was then drained and frozen at -20° C until analysis.

Meat of shellfish for Azapiracids and Domoic Acid analysis were extracted as follows:

1. 9 ml of MeOH/distilled water was added to 1 g of shellfish meat (9:1 v/v)
2. Extract was homogenized for 3 minutes.
3. Homogenized extract was then centrifuged at 3000 rpm for 5 minutes.
4. The aliquot of supernatant was passed through a 0.5 μ m filter.
5. 5 μ L aliquot of the filtrate was injected into the LC-MSMS.

Meat of shellfish for Brevetoxins analysis were extracted as follows:

1. Meat were removed from their shells, washed with deionized water, thoroughly dried and homogenized (Waring blender, Polytron or equivalent).

2. 1.0 gm of homogenized mussels was then placed in a 40 mL glass vial.
3. 9.0 mL of a methanol/deionized water solution was added (9:1 v/v).
4. Vial was capped and hand shaken vigorously for 2 minutes.
5. Mixture was then centrifuged for 10 minutes at 3000 rpm. Supernatant was collected.
6. 20 uL of collected extract was diluted to 1.0 mL with Sample Diluent (equals a 1:50 dilution).
7. Diluted extracts were analyzed as samples.
8. ELISA method was used for analysis (the result were obtained in units of ng/g and converted to $\mu\text{g}/100$ g of meat).

c. Limit of Detection and Limit of Quantification

No	Parameter	Analytical Method	LOD	LOQ	Recovery
1	ASP (<i>Domoic Acid</i>)	LC-MSMS	0,1 $\mu\text{g/g}$	0,2 $\mu\text{g/g}$	94,0%
2	AZP (<i>Azaspiracid</i>)	LC-MSMS	0,01 $\mu\text{g/g}$	0,02 $\mu\text{g/g}$	91,9 %
3	NSP (<i>Brevetoxin</i>)	ELISA	22,5 ng/g	45 ng/g	90,0%

d. National Regulatory Limits

No	Parameter	Regulatory Limits
1	ASP (<i>Domoic Acid</i>)	2 mg/100g of meat
2	AZP (<i>Azaspiracid</i>)	1.6 $\mu\text{g}/100$ g of meat

3	NSP (<i>Brevetoxin</i>)	0.8 mg/100 g of meat
---	---------------------------	----------------------

IV. Results and Discussions

a. Participation in Inter-Laboratory Proficiency Testing and Results (*if any*)

Did not participate in inter-laboratory proficiency testing.

b. Survey Results and Discussion

Table for Survey Results

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
May 2015	TL1	28	Not Detected	Not Detected
	TL2	29	Not Detected	Not Detected
	TL3	27	Not Detected	Not Detected
	TL4	35	Not Detected	Not Detected
	TL5	16	Not Detected	Not Detected
	TL6	27	Not Detected	Not Detected
	TL7	21	Not Detected	Not Detected
	TL8	26	Not Detected	Not Detected
	TL9	29	Not Detected	Not Detected
	TJB1	30	Not Detected	Not Detected
	TJB2	11	Not Detected	Not Detected
	TJB3	10	Not Detected	Not Detected
	TJB4	10	Not Detected	Not Detected
	TJB5	18	Not Detected	Not Detected
	TJB6	19	Not Detected	Not Detected
	TJB7	18	Not Detected	Not Detected
	TJB8	26	Not Detected	Not Detected
TJB9	20	Not Detected	Not Detected	

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
July 2015	TL10	13	Not Detected	Not Detected
	TL11	12	Not Detected	Not Detected
	TL12	12	Not Detected	Not Detected
	TL13	13	Not Detected	Not Detected
	TL14	11	Not Detected	Not Detected
	TL15	9	Not Detected	Not Detected
	TL16	13	Not Detected	Not Detected
	TL17	16	Not Detected	Not Detected
	TL18	17	Not Detected	Not Detected
	TJB10	Not Detected	Not Detected	Not Detected
	TJB11	Not Detected	Not Detected	Not Detected
	TJB12	141	Not Detected	Not Detected
	TJB13	37	Not Detected	Not Detected
	TJB14	130	Not Detected	Not Detected
	TJB15	74	Not Detected	Not Detected
	TJB16	86	Not Detected	Not Detected
	TJB17	104	Not Detected	Not Detected
	TJB18	23	Not Detected	Not Detected

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
October 2015	TL19	34	Not Detected	Not Detected
	TL20	37	Not Detected	Not Detected
	TL21	33	Not Detected	Not Detected
	TL22	48	Not Detected	Not Detected
	TL23	27	Not Detected	Not Detected
	TL24	19	Not Detected	Not Detected
	TL25	34	Not Detected	Not Detected
	TL26	15	Not Detected	Not Detected
	TL27	19	Not Detected	Not Detected
	TJB19	Not Detected	Not Detected	Not Detected
	TJB20	Not Detected	Not Detected	Not Detected
	TJB21	Not Detected	Not Detected	Not Detected
	TJB22	Not Detected	Not Detected	Not Detected
	TJB23	Not Detected	Not Detected	Not Detected
	TJB24	Not Detected	Not Detected	Not Detected
	TJB25	Not Detected	Not Detected	Not Detected
	TJB26	Not Detected	Not Detected	Not Detected
	TJB27	Not Detected	Not Detected	Not Detected

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
December 2015	TL28	12	Not Detected	Not Detected
	TL29	11	Not Detected	Not Detected
	TL30	12	Not Detected	Not Detected
	TL31	9	Not Detected	Not Detected
	TL32	10	Not Detected	Not Detected
	TL33	13	Not Detected	Not Detected
	TL34	12	Not Detected	Not Detected
	TL35	12	Not Detected	Not Detected
	TL36	11	Not Detected	Not Detected
	TJB28	11	Not Detected	Not Detected
	TJB29	12	Not Detected	Not Detected
	TJB30	12	Not Detected	Not Detected
	TJB31	20	Not Detected	Not Detected
	TJB32	18	Not Detected	Not Detected
	TJB33	17	Not Detected	Not Detected
	TJB34	26	Not Detected	Not Detected
TJB35	18	Not Detected	Not Detected	
TJB36	20	Not Detected	Not Detected	

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
February 2016	TL37	24	Not Detected	Not Detected
	TL38	26	Not Detected	Not Detected
	TL39	18	Not Detected	Not Detected
	TL40	29	Not Detected	Not Detected
	TL41	31	Not Detected	Not Detected
	TL42	31	Not Detected	Not Detected
	TL43	18	Not Detected	Not Detected
	TL44	31	Not Detected	Not Detected
	TL45	27	Not Detected	Not Detected
	TJB37	22	Not Detected	Not Detected
	TJB38	15	Not Detected	Not Detected
	TJB39	13	Not Detected	Not Detected
	TJB40	23	Not Detected	Not Detected
	TJB41	28	Not Detected	Not Detected
	TJB42	10	Not Detected	Not Detected
	TJB43	26	Not Detected	Not Detected
	TJB44	38	Not Detected	Not Detected
TJB45	24	Not Detected	Not Detected	

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
April 2016	TL46	35	Not Detected	Not Detected
	TL47	31	Not Detected	Not Detected
	TL48	27	Not Detected	Not Detected
	TL49	36	Not Detected	Not Detected
	TL50	26	Not Detected	Not Detected
	TL51	17	Not Detected	Not Detected
	TL52	31	Not Detected	Not Detected
	TL53	14	Not Detected	Not Detected
	TL54	19	Not Detected	Not Detected
	TJB46	51	Not Detected	Not Detected
	TJB47	20	Not Detected	Not Detected
	TJB48	19	Not Detected	Not Detected
	TJB49	13	Not Detected	Not Detected
	TJB50	20	Not Detected	Not Detected
	TJB51	29	Not Detected	Not Detected
	TJB52	56	Not Detected	Not Detected
TJB53	30	Not Detected	Not Detected	
TJB54	55	Not Detected	Not Detected	

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
		ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
June 2016	TL55	35	Not Detected	Not Detected
	TL56	31	Not Detected	Not Detected
	TL57	27	Not Detected	Not Detected
	TL58	36	Not Detected	Not Detected
	TL59	26	Not Detected	Not Detected
	TL60	17	Not Detected	Not Detected
	TL61	31	Not Detected	Not Detected
	TL62	14	Not Detected	Not Detected
	TL63	19	Not Detected	Not Detected
	TJB55	51	Not Detected	Not Detected
	TJB56	20	Not Detected	Not Detected
	TJB57	19	Not Detected	Not Detected
	TJB58	13	Not Detected	Not Detected
	TJB59	20	Not Detected	Not Detected
	TJB60	29	Not Detected	Not Detected
	TJB61	56	Not Detected	Not Detected
TJB62	30	Not Detected	Not Detected	
TJB63	55	Not Detected	Not Detected	

Note : TL = Code of sample from Teluk Lampung
TJB = Code of sample from Tanjung Balai Asahan

Time of Sampling	Sampling Location	Code of Sample	Concentration ($\mu\text{g}/100\text{g}$ of meat)		
			ASP (Domoic Acid)	AZP (Azaspiracid)	NSP (Brevetoxin)
April 2017	Teluk Lampung	TL1	Not Detected	Not Detected	Not Detected
		TL2	Not Detected	Not Detected	Not Detected
		TL3	Not Detected	Not Detected	Not Detected
		TL4	0,017	Not Detected	Not Detected
		TL5	Not Detected	Not Detected	Not Detected
		TL6	0,011	Not Detected	Not Detected
May 2017	Tanjung Balai Asahan	TJ1	0,667	Not Detected	Not Detected
		TJ2	0,169	Not Detected	Not Detected
		TJ3	0,606	Not Detected	Not Detected
		TJ4	0,705	Not Detected	Not Detected

Note: TL = Code of sample from Teluk Lampung
TJB = Code of sampling from Tanjung Balai Asahan

Discussion Results

Samples of Green Mussel (*Perna viridis*) and Baby Clam (*Meritrix meritrix*) were analyzed for Domoic Acid, Azaspiracid, Paralytic and Brevetoxin from April to May 2017. The results showed that **Brevetoxins and Azaspiracid were not detected** in the shellfish samples from Tanjung Balai Asahan and Teluk Lampung which serve as the sampling site. Domoic Acid was detected with LCMSMS in six locations, but it is still below the regulatory limits in the 2 mg/100g of meat.

c. Corrective Actions

In the future, shellfish sanitation program need to be done more frequently.

V. Problems and Challenges Encountered

No significant problems were encountered except for the sampling process. The long distance between the sampling location and laboratory testing requires the samples to be frozen and sent by air to ensure that the testing is conducted within the schedule. The selection of species that serve as samples depended on its availability in nature at the time of sampling. Shellfish samples with individual whole weight of $\pm 50\text{g}$ were collected for the analysis, the smaller size shellfish were returned back into the water.

VI. Recommendations and Suggestions for Future Follow- Up Action(s)

This project should be continued periodically in the future to build a shared understanding of the occurrence of ASP, AZP and NSP in regional waters. It is also important to establish shellfish reference laboratory in ASEAN region in order to strengthen laboratories that have recently developed the shellfish monitoring programme.