MYANMAR

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I. Introduction

Biotoxin Monitoring Survey and procedure has been carried out in Myanmar since 2009 by Department of Fisheries (DoF) through SEAFDEC, MFRD under the Japanese Trust Fund II (JTF II) Program. Myanmar participated in the 1st project (2009 – 2012) as well as the 2nd project under the Japanese Trust Fund VI (JTF VI) Program (2013 – 2017).

Most of the shellfish species are located and distributed along the coastal regions of Myanmar. Although most people in Myanmar do not prefer to eat shellfish, many visitors from overseas eat them at the coastal beaches. There is also a small amount of shellfish being exported, but there has been no outbreak of poisoning concerning Biotoxins in Myanmar.

In the 2nd Biotoxin monitoring project (JTF VI), Myanmar divided the monitoring period into three quarters and used Green Mussel and Oyster to test for Amnesic Shellfish Poisoning (ASP) Domoic Acid.

Monitoring period:

- 2015 June to August (1st Quarter)
- 2015 October to December (2nd Quarter)
- 2016 April to June (3rd Quarter)
- Domoic Acid for Amnesic Shellfish Poisoning(ASP) in Green Mussel and Oyster are monitored.

Monitoring Area - Kyune Su Township, Tanintharyi Region (Southern parts of Myanmar)

Sample Collection - Wild catch of Green Mussel and Oyster from the market area of Kyune Su Township were collected to test ASP.

II. Objectives and Goals

Our Objectives and Goals are as follows;

- To analyse the ASP (Domoic acid) levels in shellfish especially Green Mussel and Oyster
- To practice and train skillful laboratory staffs for the analysis of Biotoxin in bivalves molluscs
- To protect and raise awareness of consumer's health in the event of any Biotoxins outbreak.
- To ensure harmonization and compliance in accordance to the standard of Biotoxins in ASEAN countries

III. Survey Methodologies

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

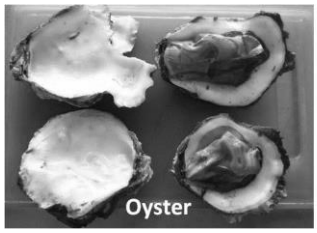
Sampling Method – Sample collection, collect the sample directly at sampling site and place into insulated box, control the temperature about 0˚C to +4˚C (chilled) and send to Analytical Laboratory Unit (Yangon Region) by air.

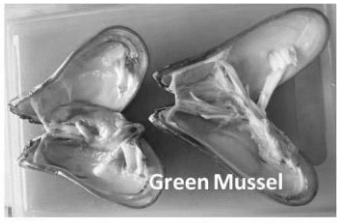
Sampling Site – Kyune Su Township, Tanintharyi Region (Southern Parts of Myanmar) Coastal Regions Area (wild caught).

Target Species – Common Name (Scientific Name)

Green Mussel (Perna viridis)

Oyster (Crassostrea beicheri)





Number of samples and size – 10 samples per testing and marketable size.

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

Sample Preparation Method:

According to the testing procedure of Bioo Scientific Chemical Test Kit, U.S.A for sample preparation of Mussels:

1. Remove the mussel tissue samples from shells, wash with deionized water.
2. Drain the excess liquid and homogenize the sample to a soupy texture.
3. Weigh out 0.5 g of homogenized tissue, add 2 mL of 50% methanol (in water) and vortex for 5 minutes.
4. Centrifuge tube for 10 minutes at 4,000 rpm.
5. Transfer 0.5 mL of supernatant to a new tube, heat the sample 75°C for 5 minutes.
6. Centrifuge 10 minutes at 4000 rpm.
7. Transfer 50 µL of the clear supernatant to a new tube, add 950 µL of 1X Sample Extraction Buffer/Methanol (90/10, V/V), mix well. The sample is ready for the assay.

Analytical Method Used:

Bio Scientific Test Kit by ELISA Method

ELISA Testing Protocol

1. Add 50 µL each Domoic Acid Standard into different wells.
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of Domoic Acid-HRP Conjugate to each well.
4. Add 50 µL of Anti-Domoic Acid Antibody to each well **IMMEDIATELY FOLLOWING MIXING IN THE WELL BY PIPPETING UP AND DOWN ONCE**. After add the Antibody to all wells, mix wells by gently rocking the plate manually for 1 minute.
5. Incubate the plate for 30 minutes at room temperature (20 - 25°C / 68 - 77°F) in the dark.
6. Wash the plate 3 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels.
7. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while

incubating. Incubate for 15 minutes at room temperature (20 - 25°C / 68 - 77°F) in the dark.

8. After incubation, add 100 µL of Stop Buffer to stop the enzyme reaction.
9. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength.

Quality Control Measures:

1. Every batch used standard series for calibration curve
2. Duplicate analysis for each sample (with separate extraction)
3. Zero (0) standard used for OD (standard blank)
4. Sensitivity (LOD = Detection Limit) – ng/g (or) ppb
    - Mussels – 30 (according to Bio Scientific Chemical Test Kit)
5. The European Commission Directive 2002/226/EC implemented a domoic acid (ASP), the Maximum Permitted Level (MPL) of 20µg/g (ppm) in shellfish intended for human consumption.

c. Limit of Detection and Limit of Quantification

1st quarter		LOD	LOQ
ASP	(G.M)	1.34015 µg/100g	4.46716 µg/100 g
	(O)	4.59141 µg/100g	15.30468 µg/100g

2nd quarter			
ASP	(G.M)	0.09487 µg/100g	0.31623 µg/100g
	(O)	0.50596 µg/100g	1.68655 µg/100g

3rd quarter			
ASP	(G.M)	1.16790 µg/100g	3.89301 µg/100g
	(O)	3.63112 µg/100g	12.10372 µg/100g

d. National Regulatory Limits

There are currently no national regulatory limits for ASP in Myanmar. Department of Fisheries (DoF) is mainly adopting and complying the limits based on ASEAN and EU Standards, following the international conformity for food safety of imported and exported fishery products. The maximum tolerance levels established by the EU criteria is as follows;
ASP is 20 µg/g (ppm) = 2000 µg/100g

IV. Results and Discussions

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

Did not participate in inter – laboratory proficiency test.

b. Survey Results and Discussion

Table for Survey Results

Sampling Location	Month & Year of Sampling (MM/YYYY)	Analyte Tested	No. of Samples Analysed	Min Concentration (ug/100g of meat)	Max Concentration (ug/100g of meat)	Average Concentration (ug/100g of meat)
Kyune Su Township, Tanintharyi Region, Southern Parts of Myanmar, Coastal Region Area (wild)	June – August, 2015	ASP	GM 10	Not Detected	1.4	0.18
			O 10	Not Detected	4.6	0.67
	October– December, 2015	ASP	GM 10	Not Detected	0.1	0.01
			O 10	Not Detected	0.4	0.08
	April- June, 2016	ASP	GM 10	Not Detected	0.9	0.24
			O 10	Not Detected	2.7	0.75

Discussion Results

The average concentration of Domoic acid (ASP) for all test results including 1st, 2nd and 3rd quarters of Biotoxins survey, are less than the maximum concentration stipulated by EU. In addition, all the test results can be considered as Not Detected (ND) because each average concentration of Domoic acid (ASP) is less than LOD. The findings of this survey suggested

that ASP toxins were not detected for the bivalve mollusc samples in Kyune Su Township, Tanintharyi Region of Myanmar. In the last survey project (JTF II, 2009-2012) at Kawthaung Township, Tanintharyi Region, ASP toxins were shown to be higher than current results (JTF VI, 2013-2017) at Kyune Su Township, Tanintharyi Region. However, all results are Not Detected (ND) since they are under the EU limit. According to the survey results, the coastal area is considered non-hazardous and safe for human consumption.

c. Corrective Actions

Since ASP toxins were not detected in all green mussel and oyster samples tested for, no corrective actions were required.

Problems and Challenges Encountered

At present, we could only use ELISA method of biotoxins analysis for these survey as LC-MSMS method is more expensive than ELISA method.

Project funding is very small and insufficient to cover the whole monitoring survey, therefore Myanmar decided to test only ASP (Domoic acid) but not AZA and BTX.

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

We would like to suggest to continue this project with more Biotoxins Training. Through the training offered in this project, it has equipped the laboratory staff with testing experiences and helped developed capability for the laboratory. We suggest the training to be provided on an annual basis for biotoxin analysis and principle and procedure of biotoxin monitoring system.

We have participated in two Biotoxins monitoring surveys, (2009- 2013) and (2013 – 2017). We would like to continue the research as there are still other species of shellfish/ bivalves not covered. We hope to conduct Biotoxins testing for these seafood and that MFRD conduct more Proficiency Tests (PT). We need to conduct research study in other coastal regions such as Rakhine, where most tourists concentrate. This is to ensure that all shellfish/ bivalves are safe for human consumption. HABs trainings conducted were also very useful to identify types of algae and red tide and others. We would like to do research with other ASEAN countries.