

# VIET NAM

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## I. Introduction

Bivalve molluscs are important aquatic products in Viet Nam. There are 4 species (baby clam, *Metrix lyrata*; Undulated surf clam/ yellow clam, *Paphia undulata*; Antique ark, *Anadara antiquata*; and scallop, *Chlamys nobilis*) that are mainly harvested for domestic consumption and export. Among these species, undulated surf clam, *Paphia undulata*, has been monitored since 2000 for biotoxin contents in clam meat. The samples were collected from wild undulated surf clam in southern province of Kien Giang according to sampling scheme of the Sanitation Monitoring Program for Bivalve Molluscs Production areas in Viet Nam. Ba Lua harvesting area has been observed to have higher probability of ASP and marine lipophilic toxins detected in bivalve molluscs.

## II. Objectives and Goals

The objective of this project is to monitor marine biotoxins including ASP, AZA in bivalve molluscs in harvesting areas in 8 provinces/cities in Viet Nam.

## III. Survey Methodologies

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

#### 1. Setup of sampling plan

- Define sampling date upon tidal zone
- For production areas on tidal flat, sample shall be collected on day of largest tidal range (highest and lowest tide on a day)
- For offshore production areas, sampling date may be any weekday.
- Samples were not collected during the period from June to November due to closed season for resource protection purpose.

- Define sample collecting time
  - For inshore production area:
    - Water sample collecting time: at highest tide of a day ( $\pm 1$  hour);
    - Bivalve mollusc sample collecting time: at lowest tide of a day.
  - For offshore production area: sample (water and bivalve molluscs) collecting time: at any time.
- Identify bivalve mollusc species and size of sampling:
 

Bivalve mollusc samples shall be at commercial size, as follows:

<b>TT</b>	<b>Species</b>	<b>Minimum size (mm –in length)</b>
1.	Baby clam ( <i>Meretrix lyrata</i> )	30
2.	Blood clam ( <i>Tegillarca granosa / Arca granosa</i> )	30
3.	Antique ark ( <i>Anadara antiquata</i> )	55
4.	Scallop ( <i>Chlamys nobilis</i> )	60

- Sampling points
  - Sampling point for biotoxin: at representative sampling point (defined on map and at field).
- Sample volume and quantity:
  - Bivalve mollusc volume need to meet testing requirements but must be sufficient for extracting at least 50g of flesh and include at least 10 LBM pieces.
  - Bivalve mollusc sampling for analysis of biotoxins: 01 sample per representative sampling point.
- 2. Bivalve mollusc sample collecting:
  - For inshore production area: sample shall be collected by manual methods (by hand or wooden rake).
  - For offshore production area: sample shall be collected by hand (diving to pick), trawl or other appropriate means.
  - Undulated surf clam samples were collected for testing ASP and marine lipophilic toxins including AZA and transferred to the National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD) Branch 6 in Can Tho city for analysis.

- Sample collection was carried out in compliance with the Guideline for implementation of the Sanitation Monitoring Program for Bivalve Molluscs Production areas adopted by NAFIQAD as above mentioned procedures. Total number of collected samples is 71 (2016: 24 samples, 2017: 22 samples, 2018: 25 samples).
- Sample preparation was carried out by the method developed and validated by NAFIQAD.

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

- ASP was analysed by the HPLC method (European Union Reference Laboratory for Marine Biotoxins, “EU-harmonised Standard Operating Procedure for determination of Domoic acid in shellfish and finfish by RP-HPLC using UV detection”, version 1, June 2008).
- Lipophilic toxins were analysed by the LCMS/MS method. (European Union Reference Laboratory for Marine Biotoxins, “EU-harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS”, version 5, January 2015).
- PSP was analysed by Mousebioassay method (AOAC 959.08).

**c. Limit of Detection and Limit of Quantification**

- Limit of detection for ASP by HPLC is 0.2 mg/kg. Limit of quantification is 0.6 mg/kg.
- Limit of detection for lipophilic toxins by LCMS/MS.

<b>Biotoxin</b>	<b>LOD (µg/kg)</b>
45 OH Yessotoxin (45 OH YTX)	10 µg/kg
45OH homo Yessotoxin (45 OH homoYTX)	10 µg/kg
Azaspiracid 1 (AZA1)	1 µg/kg
Azaspiracid 2 (AZA2)	1 µg/kg
Azaspiracid 3 (AZA3)	1 µg/kg
Dinophysistoxin 1 (DTX1)	20 µg/kg

Dinophysistoxin 2 (DTX2)	20 µg/kg
Homo Yessotoxin (homoYTX)	10 µg/kg
Okadaic acid (OA)	20 µg/kg
Pectenotoxin 1 (PTX1)	5 µg/kg
Pectenotoxin 2 (PTX2)	5 µg/kg
Total OA and ester/DTXs	20 µg/kg
Yessotoxin (YTX)	10 µg/kg

- Limit of detection for PSP by LCMS/MS is 400µg/kg.

#### d. National Regulatory Limits

Sampling frequency, testing criteria and maximum permitted levels for biotoxins thereof in the Sanitation Monitoring Program are showed in the following table:

No.	Criteria	Sampling frequency	Maximum Permitted Limits (MPLs)	Reference analysis method
1	Diarrhea-causing toxins (Lipophilic toxins)	Twice a month <sup>(1)</sup> Once a week <sup>(2)</sup>	Negative, or - Total Okadaic acid + Dinophysis toxins + Pecteno toxins: 160 µg/kg - Yessotoxins: 3,75 mg/kg - Azaspiracids: 160 µg/kg	LC-MS/MS
2	Muscle-paralyzing toxins (Paralytic Shellfish Poison - PSP)	Twice a month <sup>(1)</sup> Once a week <sup>(2)</sup>	Negative, or 800 µg/kg	Mousebioassay or LC-MS-MS
3	Dementia-causing toxins (Amnesic Shellfish Poisoning - ASP)	Twice a month <sup>(1)</sup> Once a week <sup>(2)</sup>	20 mg domoic acid/kg	HPLC

(1): Production areas affected by tides (with tidal flat)

(2): Production areas not affected by tides (without tidal flat)

## 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 (samples detected with marine biotoxin only)*

Sampling Location	Month & Year of Sampling (MM/YYYY)	Analyte Tested	No. of Samples Detected	Average Concentration (ug/100g of meat)
Kien Giang	Jan 2016	PTX2	01	13.05 µg/kg
Ben Tre	Mar 2016	ASP	01	970 µg/kg (9.7mg/kg)
Kien Giang	May 2016	PTX2	01	149.63 µg/kg

#### *Discussion Results*

Of the 71 samples collected for analysis of ASP and marine lipophilic toxins (including AZA), only one (01) sample (ID: S17) was detected to contain ASP (970 µg /100g of meat) in 2016. However, the detected value was under the regulatory limit of 20 mg/kg. Among all samples (298 in 2016, 289 in 2017, 295 in 2018) collected in the other bivalve molluscs harvesting areas, there is no sample detected with ASP even though there were several times toxin-producing planktons, *Pseudonitzschia spp.* and *Dinophysis caudata*, was detected to exceed warning limits of 100,000 cell/l and 500cell/l respectively, in water samples in 2017 and 2018.

It was also noticed that AZA was not detected in undulated surf clam sample collected in Ba Lua harvesting area of Kien Giang province even though 2 samples were detected to contain pectenotoxins (PTX2) at lower levels (13.05µg/kg in January 2016 and 149.63 µg/kg in May 2016) than regulatory limit (160 µg/kg).

### c. Corrective Actions

When the concentration of marine biotoxins exceed the maximum regulatory limits, NAFIQAD, as an inspection body, shall:

- Deliver warning to concerned production area, in which requires the following handling measures:

- Bivalve molluscs not meeting biotoxin criteria are not allowed to harvest and placed on the market.
- Local Competent Authorities in charge of fishery quality assurance (monitoring body): conduct intensive sampling for toxin-producing plankton and biotoxins with the sampling frequency of every 2-3 days at sampling points of bivalve molluscs detected with unsatisfactory biotoxin level.
- Handling/processing establishments: consignments processed from bivalve molluscs harvested before sampling occasion are placed on the market or exported only when the biotoxin testing results are satisfactory.
  - Update status of concerned production area on the its website.
  - Bivalve molluscs in alert production areas may be harvested (accompanied with Certificate of Origin) for futher process if analysis results of toxin-producing plankton and biotoxins are satisfactory in 02 consecutive supplementary sampling occasions.
  - Update harvesting regime and post-harvesting handling on its website.

## **V. Problems and Challenges Encountered**

The monitoring program of marine biotoxins in bivalve molluscs and harmful algae are comprehensive and consecutive, and require resources to implement.

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

Capacity building is necessary for people, particularly fishermen, to provide understandings of HABs and measures taken for the occurrence of HABs.

Advanced methods and equipment are recommended for quantitative analysis of harmful algae.

Member States should formulate a monitoring program for marine biotoxin in bivalve molluscs in order to ensure food safety of bivalve mollusc products for domestic consumption and export.