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Concentration of PSP (*Paralytic Shellfish Poisoning*) Toxin On Shellfish From Inner Ambon Bay and Kao Bay North Halmahera

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Abstract The Inner Ambon Bay and Kao Bay have potential on fisheries resources which one of them is molluscs. Molluscs especially for class bivalve have economical values and are consumed by coastal community. The research had been done to analyze saxitoxin (STX) concentration on bivalves from Kao Bay and Inner Ambon Bay. The Saxitoxin Elisa Test Kit Protocol was used to determine saxitoxin concentration. The measurement showed that the highest concentration of saxitoxin (392.42 µg STXeq/100g shellfish meat) was *Gafrarium tumidum* from Ambon Bay, whereas concentration of saxitoxin (321.83 µg STXeq/100g shellfish meat) was *Macra mera* from Kao Bay

1. Introduction

The effects of *Pyrodinium* caused health problems to the coastal peoples in Inner Ambon bay and Kao bay, Indonesia. The worst was in 1994 has led to people mortality [12]. *Pyrodinium* natural habitat is close to mangrove ecosystem, where they receive almost continuously nutrients, therefore the achieve bloom concentrations. Waters of Inner Ambon bay and Kao bay are dominated by mangrove ecosystem and are influenced by fresh water. Those mangrove ecosystems fulfill an important role in marine ecosystem and fisheries, such as shrimp, baitfish (*Stolephorus* spp) and mollusk that provide a potential source to coastal peoples. In some part of Kao bay, experienced as red tide phenomena in 1930, caused by the blooming of toxic phytoplankton [11].

The bloom of toxic algal has threatened the human health through marine food chain. From those 5000 species of marine algal, about 300 species often has an extraordinary abundance and 40% of them reproduce toxin that can be accumulated to mollusks and fish and is harmful to human who consumes the organisms [3].

Commonly, toxin from algal can be divided into five groups based on the symptom, e.g., Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Ciguatera Shellfish Poisoning (CSP) and Neurotoxic Shellfish Poisoning (NSP) [2]. Paralytic Shellfish Poisoning (PSP) is famous for saxitoxin (STX) that produced by toxic algal, e.g., *Alexandrium tamarense*, *Pyrodinium bahamense* var *compressed*, *Gymnodinium catenatum* and other dinoflagellates [1].



The main goal of this study is to determine saxitoxin concentrations from several species of mollusks, which is consumed by people and to analysis the toxic Dinoflagellates from Inner Ambon bay and Kao bay.

2. Material and Method

The study was conducted in June 2016, in the inner Ambon bay, Province of Maluku and Kao bay of Halmahera, North Maluku. Geographically, sampling sites in inner Ambon bay is at 06,66° 39'29" – 03,63° 30'30"S and 128°19'4,03" – 128°24'33"E, while in Kao bay is 1°8'1.41"LU -1° 8'49.77" N and 127°49'32.88" - 127°54'14.29" E 'Figure 1'.

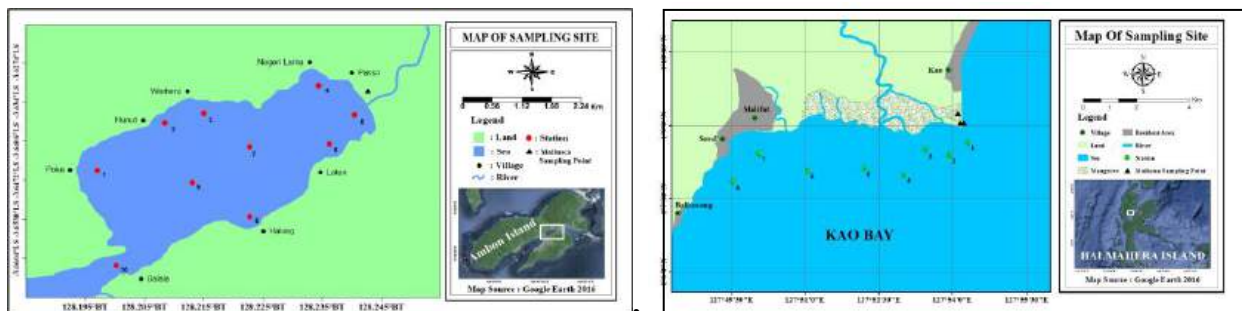


Figure 1. Map of sampling sites in inner Ambon bay, Region of Ambon and Kao bay, North Maluku.

Temperature, Salinity, and pH were measured. Data of saxitoxin concentration was detected by using Elisa test kit protocol. The phytoplankton was collected by hauling phytoplankton net of 48 μm mesh size from the depth of 5 meters to the water surface. Phytoplankton samples were preserved with formaldehyde of 4% immediately. Phytoplankton was identified according to [14], [8], and [10] to the lowest taxonomic as possible. The abundance of phytoplankton was calculated based on [9].

$$D = \frac{N_f \times V_p}{V} \quad (1)$$

Where: D = phytoplankton abundance (ind/ m^3)
 N_f = numbers of cell per 1 ml
 V_p = volume of diluted sample
 V = volume of filtered water (m^3)

Volume of filtered water was measured following [8].

$$V = \pi \cdot r^2 \cdot l \quad (2)$$

Where:

V = volume of filtered water (m^3)
 π = constant (3.14)
 r = half of the mouth diameter of the net
 l = the distance of net towing (m)

3. Results and Discussion

3.1. Paralytic Shellfish Poisoning (PSP) concentration

Four species of molluscs have been identified from inner Ambon bay, and Kao bay (Figure 2, Figure 3 and Table 1) and the high concentration of saxitoxin in inner Ambon bay was found from *Gafrarium tumidum* with the value of 392.42 $\mu\text{g STXeg}/100\text{ g}$ of mollusc meat, whilst the lowest concentration was from *Psammotea elongata*. From four species of mollusks collected from Kao bay, two of them have saxitoxin. The high concentration of saxitoxin was from *Mactra mera* (321.83 $\mu\text{g STXeg}/100\text{ g}$ of meat and followed by *Polymesoda erosa*, while *Atactodea striata* and *Telescopium telescopium* have not saxitoxin.

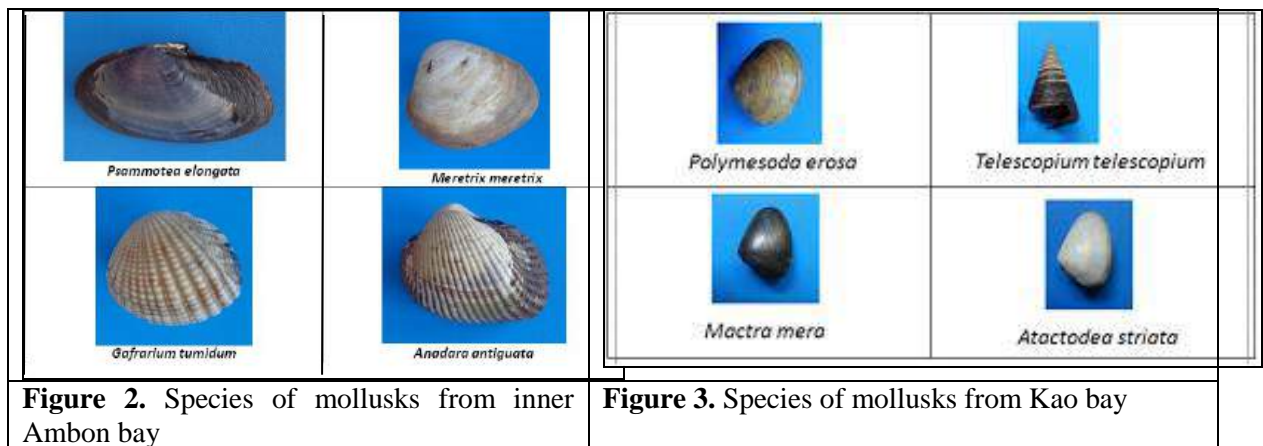


Table 1. Saxitoxin concentration of molluscs from inner Ambon bay and Kao bay

Locations	Species of Molluscs	Saxitoxin concentration ($\mu\text{g}\cdot 100\text{g}^{-1}$)
Kao bay	<i>Polymesoda erosa</i>	196.11
	<i>Atactodea striata</i>	0
	<i>Mactra mera</i>	321.83
	<i>Telescopium telescopium</i>	0
Inner Ambon bay	<i>Gafrarium tumidum</i>	392.42
	<i>Anadara antiquata</i>	136.4
	<i>Psammotea elongata</i>	131.97
	<i>Meretrix meretrix</i>	142.53
	Limit of saxitoxin concentration (FAO,2004)	80

According to SNI 3460.1 year of 2009, saxitoxin allowed in frozen meat of mollusks is 80 $\mu\text{g}/100\text{ gr}$. The result of this study showed that the concentration was above of the limit. The concentration of saxitoxin in the meat of mollusks correlates with the abundance of toxic Dinoflagellates [4].

3.2. Composition and Abundance of phytoplankton

At least 64 species of phytoplankton belonging to 4 classes, i.e., Bacillariophyceae (Diatom), Dinophyceae, Cyanophyceae dan Chrysophyceae were found from 10 stations in inner Ambon bay. Bacillariophyceae has 36 species, and Dinophyceae has 26 species, while Cyanophyceae and Chrysophyceae only have one species. There were 72 species of phytoplankton from Kao bay belonging to 4 classes as recorded in inner Ambon bay. Bacillariophyceae consisted of 46 species, while, Dinophyceae has 23 species, Cyanophyceae and Chrysophyceae consisted of 1 and two species respectively. The abundant of Dinoflagellates in inner Ambon bay reached 22.71% of total number phytoplankton, while in Kao bay was 58.14% (Table 2).

Table 2. The abundance of phytoplankton and Dinoflagellates (cell/m³) in inner Ambon bay and Kao bay

Inner Ambon Bay			Kao Bay		
Stations	Phytoplankton	Dinoflagellates	Stations	Phytoplankton	Dinoflagellates
1	141,847	44,459	1	53,651	33,810
2	150,000	62,389	2	30,698	14,698
3	130,987	37,898	3	39,476	8,238
4	147,994	27,898	4	31,587	22,857
5	494,331	116,369	5	81,810	43,206
6	346,051	62,532	6	32,190	15,746
7	215,924	25,318	7	61,143	46,794
8	771,847	132,293	8	49,175	35,429
9	61,576	38,169			
10	54,411	23,742			

Ten of 23 Dinoflagellates species found in Kao bay were present in all sampling sites, namely *Prorocentrum micans*, *Dinophysis caudata*, *Ceratium furca*, *Ceratium fucus*, *Ceratium lunula*, *Ceratium macroceros*, *Ceratium tripos*, *Ceratium trichoceros*, *Phyrophacus stenii* and *Protopteridinium depressum*. The most species which contributed to Dinoflagellates assemblage whereas *Dinophysis caudata* and *Ceratium trichoceros* (Table 3).

Table 3. Percentage of 5 dominant species of Dinoflagellates in Kao bay

Dinoflagellates	Stations							
	1	2	3	4	5	6	7	8
<i>Dinophysis caudata</i>	47.42	48.81	13.87	38.06	32.26	18.15	24.15	13.71
<i>Ceratium trichoceros</i>	33.52	30.67	39.88	26.94	24.76	44.05	46.27	40.50
<i>Ceratium tripos</i>	5.45	4.97		6.11		8.47	10.45	12.63
<i>Ceratium furca</i>	4.23		11.56	8.06	5.88	10.38	3.39	
<i>Ceratium fucus</i>	4.23		7.51	12.22	10.29	3.73	2.85	11.02
<i>Ceratium lunula</i>		6.05						
<i>Ceratium macroceros</i>		2.59	6.36					
<i>Protopteridinium depressum</i>					6.61			
<i>Phyrophacus stenii</i>								6.99

The result also showed that 13 species of 26 species of Dinoflagellates recorded in inner Ambon bay were found in all stations, i.e. *Dinophysis caudata*, *Dinophysis miles*, *Ceratium furca*, *Ceratium fucus*, *Ceratium lunula*, *Ceratium macroceros*, *Ceratium tripos*, *Ceratium trichoceros*, *Ceratium pulchellum*, *Gonyaulax spinifera*, *Pyrocystis noctiluca*, *Phyrophacus stenii* and *Protopteridinium depressum*. *Dinophysis miles* contributed the high percentage of the Dinoflagellates assemblage at all stations except for station ten which was dominated by *Dinophysis caudate* (Table 4).

Table 4. Percentage of 5 dominant species of Dinoflagellates in Inner Ambon Bay

Dinoflagellates	Stations									
	1	2	3	4	5	6	7	8	9	10
<i>Dinophysis miles</i>	61.89	49.69	38.15	53.88	70.99	57.45	37.23	70.68	38.30	17.30
<i>Ceratium macroceros</i>	14.61	10.34	8.99	14.84	9.20	8.25	11.07	10.64	13.14	
<i>Phyrophacus stenii</i>	7.02	8.04	11.76	10.27	10.62	12.38	19.12	5.10	12.01	7.85
<i>Gonyaulax spinifera</i>	3.94	9.42	11.76	4.45			4.53			4.63
<i>Ceratium fucus</i>	2.36					2.60	3.14			
<i>Dinophysis caudata</i>		5.28	8.40					2.79	4.63	43.86
<i>Ceratium trichoceros</i>				3.42	1.53	5.35		2.65	6.88	
<i>Ceratium tripos</i>					1.81					
<i>Ceratium furca</i>										8.45

Some species of Dinoflagellates, such as *Alexandrium*, *Gymnodinium* and *Pyrodinium* produce saxitoxin [6], and the concentration of the toxin correlate with the abundance of toxic dinoflagellates[4]. Based on this study, the abundance of *Alexandrium tamarense* and *Alexandrium affine* was only 3.64% of total dinoflagellates in inner Ambon bay, while the abundance of *Pyrodinium bahamense* and *Alexandrium affine* was about 0.37% of total the abundance of dinoflagellates in Kao bay. However, the concentration of saxitoxin in the meat of mollusks from inner Ambon bay and Kao bay were above the limit of concentration stated by [2]. It is argued that the high concentration of saxitoxin is caused by the ability of the mollusks to accumulate the toxin day by day. On the contrary, the study of [13] showed that concentration of saxitoxin was low even at the highest abundance of toxic dinoflagellate *Pyrodinium bahamense* in Lampung bay.

3.3. Hydrology parameters

The average of temperatures, pH and salinities in inner Ambon were nearly equal to those in Kao bay (Table 5). According to KEPMEN LH No.51/2004, the values of hydrology parameters in inner Ambon bay and Kao bay were in standard values.

Table 5. pH, Temperatures and salinities in inner Ambon bay and Kao bay

Stations	Inner Ambon bay			Kao bay		
	pH	Temp. (°C)	Salinities (‰)	pH	Temp. (°C)	Salinities (‰)
1	6	28	35	6	31	29
2	6	28	35	6	30	30
3	6	28	35	6	31	30
4	6	28	35	7	31	30
5	6	27	35	7	29	29
6	6	27	33	7	30	31
7	6	28	35	7	31	31
8	6	27	34	7	30	32
9	6	27	35			
10	6	27	34			
	Standart values			7-8,5	28-30	33-34

(*) Standard value according to KEPMEN LH No.51/2004

4. Conclusions

Saxitoxin concentration in the meat of mollusks from inner Ambon bay and Kao bay were above the limiting concentration according to SNI 3460.1. The abundance of *Alexandrium tamarense* and *Alexandrium affine* were only 3.64% of the total dinoflagellates in inner Ambon bay, while in Kao bay, the abundance of *Alexandrium affine* and *Pyrodinium bahamense* were only 0.37%. Moreover, the high concentration of saxitoxin in the meat of mollusks is accumulated for a long time.

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