Distribution of saxitoxin producing algae in Jakarta Bay and the implication to saxitoxin concentration in green mussel

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Abstract. Harmful algae blooms (HABs) have been associated with an annual incidence in Jakarta Bay. The present study aimed to investigate the concentration of dinoflagellates producing saxitoxins (STXs) in Cilincing and Kalibaru regions and the STXs concentration in mussel species from this coastal water. A sampling of phytoplankton, green mussel (Perna viridis), and environmental parameters were measured during the transit from wet to dry season. The water nutrients of the Cilincing region, mainly nitrite and ammonia in some green mussel aquaculture, have exceeded the recommended concentration to support the eutrophication/algae bloom. The N/P ratio at the study area was between 10 and 22. Plankton identification found STXs producing dinoflagellates, i.e., Alexandrium sp. and Gymnodinium sp. Other species of dinoflagellates and diatom showed predominantly to trigger algae Bloom, such as Skeletonema sp., Chaetoceros sp., Prorocentrum sp., Gonyaulax sp., Protoperidinium sp., and Nitzchia sp. Further analysis of saxitoxin in mussel samples from Cilincing and Kalibar showed STX concentrations of 10.15 µg/100 g and 21.24 µg/100 g, respectively, which is fairly below the official maximum limit (80 µg/100 g) as set by FAO/WHO or national standard.

Keywords: algae bloom; dinoflagellate; green mussel; Jakarta Bay; saxitoxin

1. Introduction

Anthropogenic activities along Jakarta Metropolitan Area (JMA) have been associated with water quality degradation in Jakarta Bay. Several studies showed significant pollution load to Jakarta Bay, such as heavy metals, organic pollutants, plastic debris, and organic nutrients [1, 2, 3, 4]. With the increasing population in the coastal and urban cities of JMA, more land-based pollutants are potentially transported to the coastal regions and adversely affect the Jakarta Bay ecosystem, including water quality. Planktonic organisms are among the most sensitive compartments to water quality disturbance. As a result, Harmful algae blooms (HABs) have been linked with an annual HABs outbreak with adverse effects to the coastal environment in some coastal areas, including in Jakarta Bay [5, 6, 7]. Water quality degradation is the most common impact of HABs incidence due to eutrophication. This phenomenon could promote mass mortality of fish species which potentially affect the local fisherman, tourism activity and other economic loss [8, 9].

Food safety is an important issue associated with HABs outbreaks. This is due to some species of HABs are known to produce natural toxins that are accumulated in seafood species through bioaccumulation and biomagnification pathway. One of the severe toxins is saxitoxin, a group of toxins produced by certain species of dinoflagellates, such as Alexandrium spp., Gymnodinium catenatum, and Pyrodinium bahamense var. compressum [10]. Accordingly, saxitoxin intoxication or known as paralytic shellfish poisoning (PSP), may undergo various symptoms from non-lethal to death occurrence within 24 hours. A number of studies have investigated the occurrence of HABs in Jakarta Bay, as well as identified the water nutrient parameter and link to the related HABs potentially phytoplankton species [11, 12, 13]. However, no available studies assess the correlation between HABs producing plankton,
specifically saxitoxin producing plankton/algae, and the saxitoxin concentration in seafood species. The present study aimed to investigate the concentration of dinoflagellates producing saxitoxins (STXs) in connection with environmental parameter in Jakarta Bay and the STXs concentration in green mussel species which is cultured by local farmers, especially in Cilincing and Kalibaru regions.

2. Material and methods
Phytoplankton, water, and green mussel samples were provided from 2 locations of green mussel aquaculture facilities in Jakarta Bay, i.e. Kalibaru (K1: -6.081888ᵒ, 106.92608ᵒ and K2: -6.073728ᵒ, 106.916392ᵒ) and Cilincing (C1: -6.065294ᵒ, 106.967804ᵒ and C2: -6.061371ᵒ, 106.966293ᵒ) as presented in Figure 1. The sampling campaign was conducted during transition season I (rainy to dry season) in April. Phytoplankton samples were horizontally collected with a Ø30cm plankton net of 50 µm mesh size [12]. For composition and abundance analysis, plankton samples were conserved with 4% formaldehyde prior to microscopic identification following [14]. The concentration of phytoplankton was calculated following the Sedgwick Rafter method [15].

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\text{Plankton concentration (cell mL)} = \frac{\text{Cell number (cell)}}{\text{Counted volume (mL)}} \times \frac{\text{Sample volume (mL)}}{\text{Filtered volume (mL)}}
\]  

(1)

In order to evaluate the water nutrient profile, 1 L of water was collected with a Nansen bottle at 50-100 cm depth. Water samples were kept at 4°C in a cool box prior to analysis of nitrate, nitrite, ammonia, and phosphate using HACH colourimeter DR-890. Other in situ parameters were measured directly from the boat, i.e. temperature, pH, salinity, and DO, with a portable refractometer (Atago), a pH-meter (Hanna HI 98107), and a DO-meter (HACH HQ950D). At least 1 kg of green mussels was collected from 3 aquaculture sites in Kalibaru and Cilincing. The mussels were culture at 5-9 m depth for 3-6 months. Mussels samples were conserved at chilling temperature for further analysis at the laboratory in Jakarta. Saxitoxin analysis was performed using an ELISA kit [16]. Saxitoxin analysis was confirmed with HPLC (Waters), following a method of [17]. For saxitoxin analysis, 0.5 g of composite mussel tissue from 3 aquaculture sites were separated from the shell and rinsed with pre-filtered water. In a falcon tube of 15mL, the tissue was vortexed with 1mL of 50% methanol for 3 minutes, followed by centrifugation at 4000 rpm for 10 minutes. 0.1 mL supernatant was transferred to a new falcon tube 15
mL and added with 1.9 mL 1X sample extraction buffer. 50µL of the mix was used for the saxitoxin ELISA analysis by following the manufacturer’s protocol.

3. Results and discussion

3.1. Water nutrient

Analysis of water nutrients at the study areas showed that nitrate, nitrite, and phosphate are found at various concentrations. However, ammonia was only identified at the Cilincing station at a relatively high concentration in Table 1. In contrast, the PO\textsubscript{4} concentration in Kalibaru was higher than that in Cilincing. This could indicate that there is different characteristic of nutrient load between Kalibaru and Cilincing waters. In comparison to the water quality index, the physical parameters in the study area were relatively normal for (salinity, temperature, pH, and DO). Likewise, nutrient concentration (NO\textsubscript{2}, NO\textsubscript{3}, NH\textsubscript{3}, and PO\textsubscript{4}) was also below the maximum recommended level, except NO\textsubscript{2} and NH\textsubscript{3} in the Cilicing region, which was over the maximum recommended level [18, 19].

Nitrate and phosphate are the main nutrients to support water productivity. Based on the concentration of nitrate (4-6 ppm) and phosphate (0.2-0.5 ppm) indicated the study area is productive water, i.e. PO\textsubscript{4} >0.2 ppm and NO\textsubscript{3}>5 ppm [20]. The different profiles on nutrient concentration might be associated with the different nutrient loads from the terrestrial rivers to the region of Cilincing and Kalibaru. Cilincing region is known to receive terrestrial loads from 2 big rivers, i.e. Banjir Kanal Timur/Eastern Flood Canal (BKT) and CBL River. On the contrary, the Kalibaru region receives terrestrial load through 2 relatively small rivers, namely Cakung and Sunter Rivers. The significant load of these rivers has been reported by [21].

In general, the N/P ratio in the study area was between 10 and 22, with a higher ratio in Cilincing than that in Kalibaru of 21.43±0.84 and 11.00±1.51, respectively. The difference in N/P ratio might consequently affect the concentration and composition and concentration of microalgae in the study area. Dinoflagellates prefer in the water with an NP ratio >16. A relatively lower N/P ratio was reported from Cilincing water, at a range of 6-12.5, with Chaetoceros as the dominant plankton [22, 23]. However, the growth of microalgae could also be influenced by other environmental conditions that support plankton productivity, such as water depth, turbidity, salinity, DO, and pH. In the present study, water turbidity might contribute significantly to the microalgae photosynthesis in Kalibaru as the water clarity was 1.5-1.8 m compared to that in Cilincing (0.75-1.0 m).

3.2. Profile of microalgae

The abundance of microalgae (phytoplankton) was focused on the major group reported earlier in the study area, i.e. diatom (Bacillariophyceae) and dinoflagellate (Dynophyceae). Diatom is a group of

| No. | Station | Coordinate | Depth (m) | Sal (ppt) | Temp (°C) | pH | DO (ppm) | NO\textsubscript{2} (ppm) | NO\textsubscript{3} (ppm) | NH\textsubscript{3} (ppm) | PO\textsubscript{4} (ppm) | N/P |
|-----|---------|------------|----------|-----------|----------|----|----------|----------------|----------------|----------------|----------------|-----|
| 1   | C1      | -6.065294\textdegree 106.967804\textdegree | 5.5      | 30        | 31.0     | 7.0| 7.3      | 0.08           | 4.70           | 0.16           | 0.24           | 20.58 |
| 2   | C2      | -6.061371\textdegree 106.966293\textdegree | 6.0      | 31        | 32.0     | 8.0| 8.0      | 0.11           | 4.57           | 0.22           | 0.22           | 22.27 |
| 3   | K1      | -6.081888\textdegree 106.92608\textdegree | 8.0      | 29        | 31.0     | 8.0| 8.0      | 0.01           | 6.07           | <0.01          | 0.58           | 10.48 |
| 4   | K2      | -6.073728\textdegree 106.916392\textdegree | 9.0      | 30        | 32.0     | 8.5| 8.6      | 0.02           | 4.30           | <0.01          | 0.32           | 13.50 |
|PP RI No.82/2001 Kepmen LH No.51/2004| -27-33 15-32 | 6.0-9.0 | 3-8 <0.06 | <20 | <0.10 | <1.00 | - |
plankton that present dominantly in both freshwater and marine water. Additionally, dinoflagellate is among the dominant plankton to promote HABs. Diatoms and dinoflagellates have been reported to generate HABs in Jakarta Bay. Specifically, certain species of dinoflagellate have been subjected to produce natural toxins, including saxitoxins. Based on the plankton identification of the most dominant species, it can be seen that diatoms are more abundant (>99%) than dinoflagellates, either in Cilincing or in Kalibaru in Table 2. Additionally, species richness in Kalibaru is more superior as presented by 9 species of diatom and 7 species of dinoflagellate, in comparison to 6 and 5 species respectively in Cilincing. Furthermore, diatom species were more concentrated in Cilincing than that in Kalibaru, which is in contrast to dinoflagellate concentration. The most dominant species of diatom were *Skeletonema* sp., *Chaetoceros* sp., *Nitzschia* sp., and *Thalassionema* sp., while the dinoflagellate was dominated by *Ceratium* sp., *Protoperidinium* sp., and *Alexandrium* sp.

In general, the concentration and composition of diatoms and dinoflagellates in the present study showed that microalgae related to HAB species are more dominant than that of saxitoxin-producing algae. *Skeletonema* sp. was identified as the most dominant diatom in Cilincing and Kalibaru, which contributes to 68 and 82% of the total phytoplankton, respectively. In contrast with dinoflagellate species in Cilincing, *Gymnodinium* sp. and *Pyrophacus* sp. are identified at Kalibaru station. *Alexandrium* sp. and *Gymnodinium* sp. are known as dinoflagellates associated with saxitoxins. However, the presence of both species was only around 10% of the total dinoflagellates, which was dominated by 80% of *Ceratium* sp. and *Protoperidinium* sp. The diatom concentration in the present study can be categorized as blooming, as the concentration was more than 5000 cell/L [24]. A similar result was reported from earlier studies in Jakarta Bay. In a study between Muara Karang and Ancol region, Nasution et al. (2021)

| No. | Phytoplankton        | Cilincing Cell/m³ | Cilincing % | Kalibaru Cell/m³ | Kalibaru % |
|-----|----------------------|-------------------|------------|-----------------|------------|
| 1   | *Bacteriostrum* sp.  | -                 | -          | 84,745          | 0.05       |
| 2   | *Coscinodiscus* sp.  | 33,898            | 0.01       | 50,847          | 0.03       |
| 3   | *Chaetoceros* sp.    | 3,593,220         | 15.05      | 20,559,322      | 13.03      |
| 4   | *Lauderia* sp.       | -                 | -          | 101,694         | 0.06       |
| 5   | *Nitzschia* sp.      | 26,915,254        | 11.71      | 4,203,389       | 2.66       |
| 6   | *Skeletonema* sp.    | 157,779,661       | 68.65      | 129,474,576     | 82.09      |
| 7   | *Thalassiothrix* sp. | -                 | -          | 50,847          | 0.03       |
| 8   | *Thalassionema* sp.  | 10,305,084        | 4.48       | 1,728,813       | 1.10       |
| Total Diatom |                     | 229,627,118       | 99.91      | 156,254,237     | 99.07      |
| 9   | *Alexandrium* sp.    | 16,949            | 0.01       | 169,491         | 0.11       |
| 10  | *Ceratium* sp.       | 67,796            | 0.03       | 762,711         | 0.48       |
| 11  | *Gymnodinium* sp.    | -                 | -          | 16,949          | 0.01       |
| 12  | *Pyrophacus* sp.     | -                 | -          | 33,898          | 0.02       |
| 13  | *Prorocentrum* sp.   | 33,898            | 0.01       | 16,949          | 0.01       |
| 14  | *Protoperidinium* sp.| 84,745            | 0.04       | 474,576         | 0.30       |
| Total Dinoflagellate |                | 203,389           | 0.09       | 1,474,576       | 0.93       |
| Diversity Index |                   | 0.94              |            | 0.64            |            |
| Index Of Evenness |                  | 0.43              |            | 0.24            |            |
| Species Richness |                  | 0.42              |            | 0.69            |            |
| N/P Ratio |                  | 21.43±0.84        |            | 11.99±1.51      |            |
reported that dinoflagellate abundance was \textit{Noctiluca} > \textit{Ceratium} > \textit{Gonyaulax} > \textit{Gymnodinium} > \textit{Dinophysis}, with \textit{Noctiluca} concentration, was 85,279,547 cells/m$^3$. Additionally, \textit{Gymnodinium} also presented only at some stations. Likewise, \textit{Dinophysis}, which also presented minor. Another study at Lampung Bay reported 13 harmful dinoflagellates with \textit{Ceratium} sp. was the predominant genus with a concentration of 1800 cells/L\footnote{25}.

### 3.3. Saxitoxin concentration

Concentrations of saxitoxin in green mussel species from Cilincing and Kalibaru were 10.15 µg/100 g and 21.24 µg/100 g, respectively. Figure 2. is the comparison result of STX analysis with HPLC. In general, the STX concentrations are relatively low in comparison to the national and global recommended limit of 80 µg/100 g\footnote{26, 27, 28}. However, the STX concentration has to be a concern as saxitoxin may promote a lethal effect at a concentration of 3 mg, or moderate disorder at a lower concentration of 0.5-$\sim$1 mg\footnote{16}. Tolerable intake of saxitoxin is 30 µg/60 kg body weight. Therefore, based on the saxitoxin concentration in Cilincing’s and Kalibaru’s mussels, the recommended mussel consumption is 290 g and 140 g tissue per serving, respectively\footnote{29}. The higher saxitoxin concentration in mussel from Kalibaru compared to that from Cilincing in the present study could be attributed to the relatively higher concentration of saxitoxin producing dinoflagellate in Kalibaru, particularly, \textit{Alexandrium} and \textit{Gymnodinium}. Notably, \textit{Gymnodinium} was not even detected in the Cilincing mussel site. The earlier study conducted by\footnote{30} reported relatively similar concentration of saxitoxin in green mussel from different traditional markets in Jakarta, i.e. Cilincing (6.92–17.34 µg/100 g), Dadap (7.75–11.76 µg/100 g), and Muara Karang (4.93 µg/ 100 g). This study also analysed

![Saxitoxin analysis with HPLC](image)
green mussels from Lampung Bay (7.29 µg/100 g) and Panimbang Bay (6.39 µg/100 g). A relatively lower concentration of STX in mussels was reported from Bagan siapiapi at a concentration of 5.59 µg/100 g [31].

4. Conclusion
Plankton identification showed that diatoms are more abundant (>99%) than dinoflagellates, either in Cilincing or in Kalibaru. Additionally, species richness in Kalibaru is more superior, as presented by 9 species of diatom and 7 species of dinoflagellate, in comparison to 6 and 5 species respectively in Cilincing. Furthermore, diatom species were more concentrated in Cilincing than that in Kalibaru, which is in contrast to dinoflagellate concentration. The most dominant species of diatom were Skeletonema, Chaetocero, Nitzschia, and Thalassionema, while the dinoflagellates were dominated by Ceratium, Protoperidinium, and Alexandrium. Analysis of water nutrients showed that ammonia was only identified at the Cilincing station, while PO₄ concentration in Kalibaru was higher than that in Cilincing. In comparison to the water quality index, the physical parameters in the study area were relatively normal for (salinity, temperature, pH, and DO). Likewise, nutrient concentration (NO₃, NO₂, NH₃, and PO₄) was also below the maximum recommended level, except NO₂ and NH₃ in the Cilincing region. Concentrations of saxitoxin in green mussel from Cilincing and Kalibaru were 10.15 µg/100 g and 21.24 µg/100 g, which are fairly low compared to the national and global recommended limit of 80 µg/100 g. However, the STX concentration has to be a concern since STX is bioaccumulative in seafood species and may pose various health effects from moderate symptoms to a lethal effect, depend on the concentration.

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