The Potency of Toxic Cyanobacteria *Planktothrix agardhii* isolated from A Retention Pond in Palembang to *Cyprinus carpio* L: A Preliminary Study

The Potency of Toxic Cyanobacteria *Planktothrix agardhii* isolated from A Retention Pond in Palembang to *Cyprinus carpio* L: A Preliminary Study

R. Wulandari[1] M. Amalia[1,] , R. Aryawati[2], SA. Hamim[3] and M. Verawaty[1*]

[1]Biology Department, Faculty of Math and Natural Science, Sriwijaya University, South Sumatera, Indonesia
[2] Marine Department, Faculty of Math and Natural Science, Sriwijaya University, South Sumatera, Indonesia
[3]Faculty of Engineering, University of Indo Global Mandiri, South Sumatera, Indonesia
[*]*Corresponding author: marieskaverawaty@yahoo.com*

Abstract. Potential toxic filamentous cyanobacterium *Planktothrix agardhii* was detected to be dominated in one of the retention ponds located in Palembang city. It showed persistent domination in the location during observations. This species is known to produce a toxin that potentially causes environmental health problems. Hepatotoxin, microcystin (the cyanotoxin most commonly detected in the freshwater environment) are known types of toxin that is produced by this species. Its potential produced a toxin that is in concern. A *preliminary study* was conducted to study its potential toxic effect to fish (*Cyprinus carpio L.*); in this study, four different concentrations of *Planktothrix agardhii* biomass were used to study its effects to *Cyprinus carpio L.* gills. The results showed the higher the biomass concentrations (5,4086 x 10^7 cells/ml), the shorter the length of time for the fishes to be dead (± 2-2 hours 15 minutes). The fishes also showed swimming behavior changes such as swimming direction and excessive mucus excretion. Histopathological changes of the fishes' gills that exposed to *Planktothrix agardhii*., compared to the un-treated fishes (control) were observed such as edema, lamela gill hyperplasia, and necrosis.
Keywords: Cyanobacter: toxin; *Planktothrix agardhii*; *Cyprinus carpio L*; histopathology

Introduction

Cyanobacteria are one of freshwater phytoplankton that naturally parts of its community, however in a certain condition they can associate to eutrophication and water quality decline. Eutrophication in ponds frequently also associated with cyanobacterial blooms that can relate to cyanotoxins released into the water when the decomposition of algal cells occurred [1]. Some of the cyanobacteria released known toxins such as *Microcystis aeruginosa*, produce hepatotoxins such as microcystin-LR (MC-LR) [2]. Several reported cases of deadly cyanotoxins had been published, such as high mortality in channel catfish, *Ictalurus punctatus* (Rafinesque), and production ponds in South Carolina, USA because of a toxic bloom of *Anabaena flos-aquae* was reported by English, Schwedler, and Dyck [3]. Many of the global cases have also been summarized in a study done by Blaha et al., [4] such as the case in Ohio River in 1931, Palm Island, Australia in 1979, Finland (2002-2003), Rio de Janeiro (2001), etc. It also has been suggested by Chorus, [5] and Blahova *et al.*, [6,7] that 25 to 75 % of cyanobacterial blooms are toxic. Importantly, for our concern that the cyanotoxins potentially cause

illness and mortality at relevant concentrations that become current human and animal health hazards [8].

In a study conducted by Walsby et al. [9], the filamentous cyanobacteria *Planktothrix* was originally classified into the genus *Oscillatoria*, due to it solitary trichomes grow that they live without sheaths, , and akinetes. However, because the *Planktothrix spp* showed different ultra-structure, life strategy and phenotypic appearance, the genus was later separated from the *Oscillatoria* genus [10, 11, 12]. According to the study done by Fastner et al. [13], it is one of the most important microcystin producing genera found in freshwater habitats in the Northern hemisphere. The Planktothrix group is one of the groups producing microcystin. This group consists of nine species, one of which is *Planktothrix agardhii*. The nine species suggested by Komarek [14] are *Planktothrix agardii, P. iwanoffiana, P. lacustris, P. miyadii, P. mougeotii, P. penornata, P. planctonica, P. pseudoargadii* and *P. rubescens*. In relation to that, among the nine species, *P. agardhii* is the most abundant species in shallow and eutrophic lakes [15]. Moreover, Blaha et al., [4] summarized and commpiled his study from Codd et al., [8] and Codd et al., [16] about the principal groups of cyanobacterial toxins, their acute toxicities, structures, and known producers. One of them is *Planktothrix* group, they suggested this group potentially produced microcystins with LD50 - acute toxicity of (25 to ~ 1000), the toxin activities include hepatotoxic, protein phosphatase inhibition, membrane integrity, and conductance disruption, and can act as tumor promoters.

Considering all of the information has been previously described, this study aims to give an information about the preliminary potential toxic effects of *Planktothrix agardhii.,* isolated from a retention pond in the city of Palembang to Cyprinus carpio L., especially its swimming behaviors and histopathological changes after the fishes were exposed to several levels of biomass *Planktothrix agardhii.,* concentrations. Information from this preliminary study can be used for a further study related to this cyanobacteria.

2. Methods

2.1 Algae Planktothrix agardhii characterization, identification, and biomass preparation for toxicity assay

Water samples from a retention pond in Palembang city was collected for algae identification. The collected samples were stored with an addition of 4% formalin solution before being observed at Laboratory for classification and identification according to Mizuno [17] and web page landcareresearch.co.nz. [18] Fresh biomass of the *Planktothrix agardhii* from retention pond was collected by centrifugation at a speed of 4000 rpm in 2 minutes. The wet biomass was counted to measure the algal cells per ml (cell/ml) according to Sedgewick Rafter manual instructions.

2.2 Preliminary assay of Planktothrix agardhii biomass effect to Cyprinus carpio L

Before being exposed to *Planktothrix agardhii.*, healthy fishes with sizes between 10-12 cm of length were subject to several days of adaptation; only healthy fishes were used for treatments assays. The healthy fish can be seen by its active movement, eating activity, bright color, and have normal external organs (fins, scales, and operculum). Experiments for the Preliminary assay to study the effect of *Planktothrix agardhii* biomass to *Cyprinus carpio* L consisted of six treatments namely K0: control; K1: control negative was used non-toxic algae (*Scenedesmus sp*.) with a concentration of 1,8 x 10^5 cells/ml; K2: *Planktothrix agardhii.* with concentration of 3,6 x 10^5 cells / ml; K3: *Planktothrix agardhii.*, with concentration of 1,4 x 10^7 cells / ml; K4 : *Planktothrix agardhii* with concentration of 2,7 x 10^7 cells / ml; K5: *Planktothrix agardhii* with the concentration of 5,4 x 10^7respectively. All the treatments were repeated for three times. The fishes have then exposed to the six treatments assays; the fishes swimming behaviors were observed by video recorder and visualization (time zero was the time when the fished dead were recorded). The dead fishes were then subjected to the histopathological analyses.

2.3 Histology Preparations

Organ especially gills of the dead fishes were subjected to these following steps for histopathology analyses: *Fixation*: the dead fishes' gills were then fixed in formalin solution (10% v/v) for 24 hours. *Cutting*: The gills that have been soaked into 10% buffer formalin subjected to the cutting process (2 cm in size) and then they were placed into 2x2x0.5 cm cassettes and then closed for further processing. *Dehydration*: the cassettes contained the organs were then loaded into a basket and then soaked into 70% alcohol solution (2 times, 30 minutes each). Then the immersion continued with 80% alcohol (30 minutes), 96% alcohol (30 minutes, 2 times), 100% alcohol (30 minutes) and finally into 100% alcohol for an hour. *Clearing*: tissue in the cassette was soaked into xylol 1 and xylol 2 solutions (30 minutes). *Infiltration*: the tissue was then immersed in paraffin 1 and paraffin 2 (2 hours each). *Embedding*: the tissue from cassette was added into a mold and arranged in transverse position. Then, it was filled with paraffin liquid until its full covering the cassette and placed in a cooling plate at 35°C. In this process is used liquid paraffin with a temperature of 65°C. *Slicing*: the tissue's block was sliced by using microtome (Shandon Finesse 325). *Coloring*: Cover glass that has been dried by hot plate was washed with xylol 1, xylol 2, and xylol 3 for 5 minutes each, and then left it dried. The cover glass was soaked into alcohol 96% (1), alcohol 96% (2), and alcohol 96% (3) for 3 minutes each. Then, it was cleaned with running water for ±2 minutes. Then, the cover glass was soaked into Mayer's hematoxylin for 1-7 minutes and then washed with running water 2 minutes, let it dried. After that 80% alcohol was immersed in 3 dips, followed by 2 minutes of eosin dye. Then it was rinsed with ±5 dip water. Next, the cover glass was soaked in alcohol 96% (1), alcohol 96% (2), and alcohol 96% (3). After that, it was placed into a solution of xylol 1 and xylol 2, each for 2 minutes and then let it dried. *Mounting*: the preparation was then dripped with Canada balsam and covered with cover glass and can be visualized with a microscope.

3. Results

3.1 Cyanobacteria Planktothrix agardhii cultivation and biomass collection

The filamentous cyanobacterium *Planktothrix agardhii* image used in this experiment is presented in Figure 1. The biomass being used was directly collected from the retention pond. During this study, we observed that *Planktothrix agardhii* was dominated by the retention pond ecosystem for several sampling periods. The concentration at the sampling location showed a slight fluctuation during the rainy and dry season but mostly stable during the year. The concentration of *Planktothrix agardhii.* Observed in the retention pond during the study was around 2,9 $x10^5$ cells/ml. Before being used for the preliminary assay to study its potential toxic effect to *Cyprinus carpio* L., the *Planktothrix agardhii*., biomass was prepared as it has been described in method section. Four different biomass concentrations were tested to study its effects on fishes swimming behavior, the results are presented in section 3.2. The histopathological effects of the biomass to fishes' gills are also presented in section 3.3.

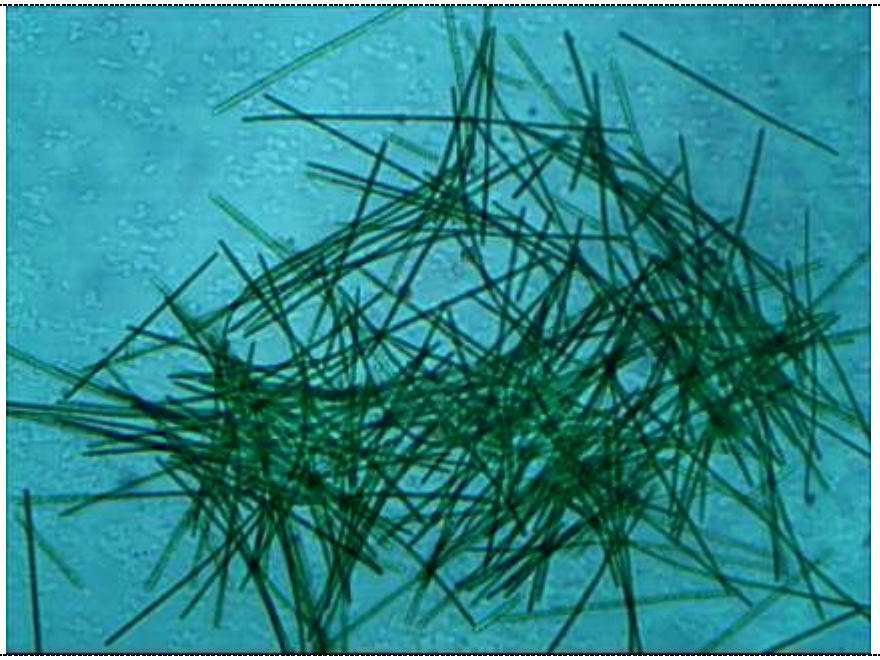

Figure 1. Images of Planktothrix agardhii isolated from a retention pond in Palembang city used in this study (magnification 100 x).

3.2 Length of time after cyanobacterium Planktothrix agardhii exposure to the fish dead

Four different concentrations of *Planktothrix agardhii* biomass used in this study were labeled as K2, K3, K4, and K5; and then they were compared to K0 (control), and K1 (as a negative control), each of them was repeated for three times. Observations were conducted that were started at time zero (when the fishes were started to be exposed to the experimental treatments) until the time when the fishes were noted to be dead. Observations of swimming behavior changes and length of time of the fishes to be dead were video recorded; the result is presented in Table 1. The result showed that there were no fishes were dead in both K0 and K1 treatments. A significant time length of the fishes dead were recorded after they were exposed to the biomass at four different concentrations; the trends showed that the higher the *Planktothrix agardhii.,* biomass concentration, the shorter the lethal time of the fishes, it was observed for the concentrations more than 10^7 cells/ml the fishes were dead below 7 hours (an acute effect). A further effect studied by its histopathological changes that are presented in section 3.3 below.

In relation to that, types of swimming behavior were also recorded in this study; compared to both the negative control (K1) and blank control (K0), fishes that were exposed to the *Planktothrix agardhii.,* biomass showed abnormal swimming behavior; the higher the cells concentrations the more obvious the swimming behavior changes were observed. The fishes showed normal swimming behaviors both in K0 and K1 such as they swam actively in the middle and from top to bottom and reversed, there were no dead fishes in these treatments. The abnormal swimming behavior of fishes was observed after they were exposed to *Planktothrix agardhii.,* such as in K3 mostly the fishes swam in the surface after one hour of exposure. More dramatic abnormalities were observed in K5; the fishes swam upside down and in tilted positions before they finally died after around 2 hours. The fishes in both K3 and K4 treatments showed swimming behavior changes such as they swam at the surface, showed hyperactive moves and started to secrete mucus.

Table 1. Length of time of fishes dead after exposure to *Planktothrix agardhii*.

No	Treatment		Swimming Behaviours	Lethal time
1	K0U1 K0U2 K0U3	Control	Fish swim actively in the middle and from top to bottom and reversed.	Alive until end of the experiment (20 days)
2	KIU1 KIU2 KIU3	Negative Control (Non-toxic algae)	Fish swim actively in the middle and from top to bottom and reversed.	Alive until end of the experiment (20 days)
3	K2U1 K2U2 K2U3	PA (3,56 $x10^5$ cells / ml)	Fish swim from the bottom to the middle and mostly in the surface after 1 hour	Dead at day 8, 10 and 12
4	K3U1 K3U2 K3U3	PA (1.35 $x10^7$ cells / ml)	Fish swim on the surface of the water	Dead after 5 hours 29 minutes, 6 hours 39 minutes and 6 hours 41 minutes
5	K4U1 K4U2 K4U3	PA (2.70 $x10^7$ cells / ml)	Fish act hyperactively Fish swim to the surface Fish secretes mucus	Dead after 4 hours 27 minutes, 4 hours 30 minutes, 4 hours 52 minutes
6	K5U1 K5U2 K5U3	PA (5.41 $x10^7$ cells / ml)	Fish act hyperactively Fish swim to the surface Fish swim upside down and swim in a tilted position	Dead after 1 hour 50 minutes, 2 hours 2 minutes, and 2 hours 15 minutes

3.3 Histopathological changes of fishes' gills after the exposure of Planktothrix agardhii at different concentrations

The potential toxic effect of the *Planktothrix agardhii*., biomass was further studied; this study compared the histopathological changes of the fishes' gills amongst six treatments as suggested in Table 1In the experiments there were various algal biomass concentrations that caused the deathly effect to the fishes were observed. The dead fishes organ were then fixed and subjected to the histopathological analysis. The dead fishes organ were then fixed and subjected to the histopathological analysis. The results showed in these following images. The gill structure of K1 (control) shown normal structure; it is shown in figure 2. This is indicated by each of its filament in the gill structure has several parts that are called lamellae, primary lamellae, and secondary lamellae. The primary lamellae consist mainly of epithelial tissue, cartilage, and vascular system. And then secondary lamellae lined neatly, it protrudes along the entire length of primary lamellae; about the study done by Fujaya [19], lamellae composed of thin epithelium that located in the outside layer, and membrane base and pole cells as a buffer that located in the inside. In the normal gills, its lamellar fringe that does not stick to the gill's arch is very thin, it is covered by epithelium and contains capillary vascular tissues.

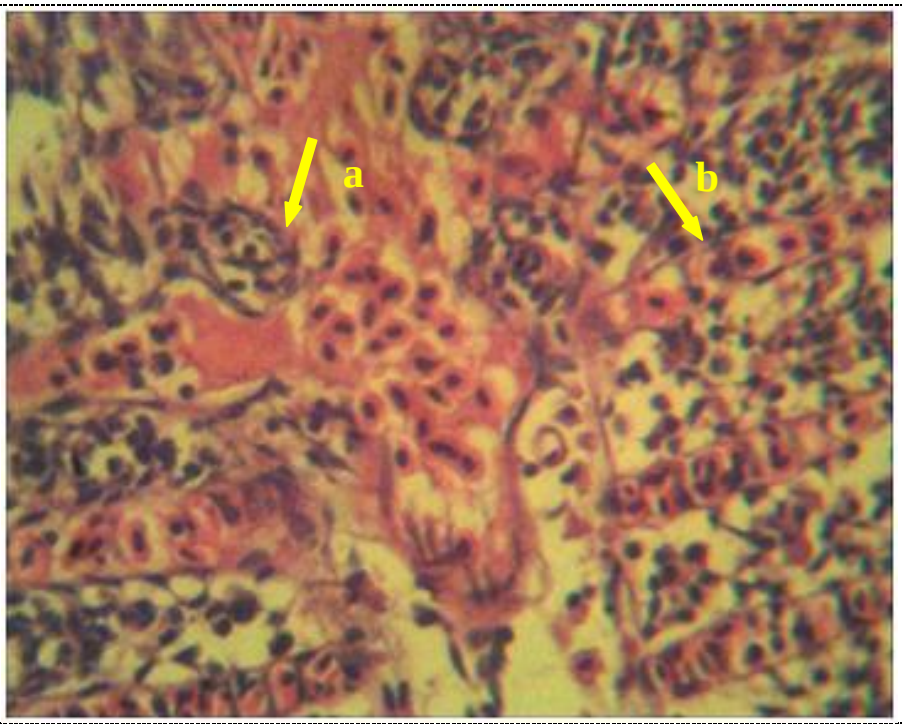

Figure 2. Cross section of gill's histology of *C. carpio* L. (control) (40x, HE). (a) Primary lamellae; (b) secondary lamellae

The gill's histopathology of *C. carpio L.* that was exposed to the *Planktothrix agardhii* with concentration 3,56 x 10^5 is shown in figures 3 and 4. There are same pathological changes were observed from the image such as congestion and secondary lamellae breakage when it is compared to the control (in figure 2). There also congestion (abundance of blood in blood vessels) is observed In figure 3, the arrow (a). Congestion can be triggered by the breakdown of cell structure pillars which increases blood flow in lamellae. Congestion that is obtained can be caused by the presence of substances that are suspected to be toxic to the respiratory system of *C. carpio L.* According to Alifia et al. [20], congestion can cause blood vessels swelled. Congestion can occur due to the evidence of an increasing amount of blood and vasodilatation of blood vessels caused by the inflammatory reaction after changes in the cell's biochemical structure by toxic substances. Congestion that observed is suspected because of toxic substances that interfered the circulatory system, so the cells experienced the lack of oxygen; this is also in accordance with the study by Muhartono et al. [21], they revealed that congestion might occur through two mechanism; firstly it happened when an increasing amount of blood flows to sinusoid or when a decreased amount of blood flows to sinusoid. If the flow of blood into the sinusoid area increases and causes congestion, it called active congestion. If congestion is caused by a disturbance in blood flow, it is called passive congestion.

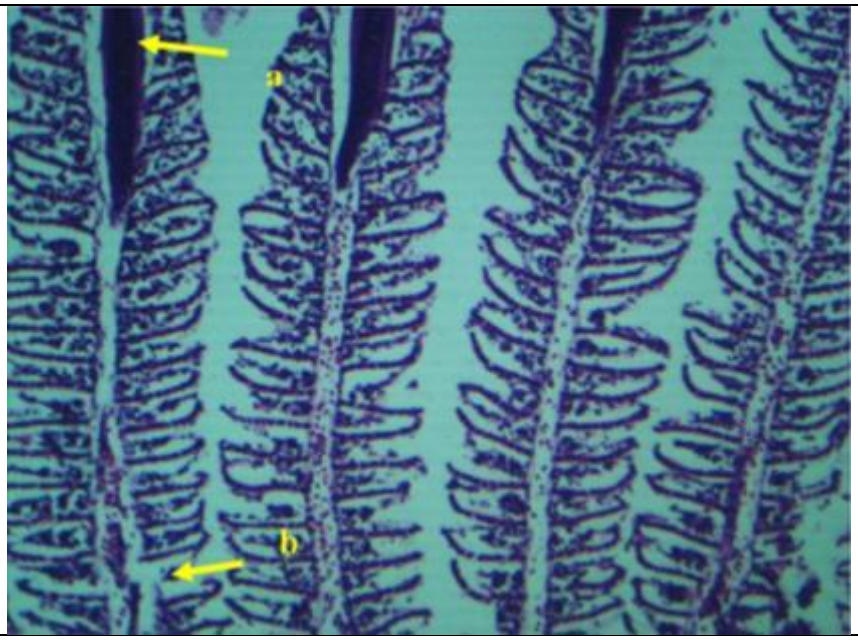

Figure 3. Cross section of gills' histology with *Plankthothrix agardhii* exposure concentration 3,56 x 10^5 cells/ml (a). congestion; (b). secondary lamellar break (10x, HE).

In figure 4, compared to the normal gill in figure 2, the arrow (a) shows pathological changes such as secondary lamellar hyperplasia. Usually, hyperplasia is initiated by some released of epithelial cells on the gills' lamellae. Hyperplasia is a state where is an increase in secondary lamellae due to excessive chloride cells. In this study, the cause of hyperplasia is suspected to be the presence of toxic substances. According to Robert [22] and Sudaryatma et al., [23] gill's secondary lamellar hyperplasia can be caused by the uncontrolled epithelial cell division. It makes secondary lamellae stick and make a group. According to Alifia et al. [20], these may act as the adaptation response; that may function as a defense mechanism, as a result of this, it can increase the distance between blood and external environment. Sudaryatma et al. [23] also suggested that the gill's secondary lamellar hyperplasia is one of the defense's mechanisms from toxic substances. In relation to that, according to Hayati et al. [24], hyperplasia is occurred due to several cells damaged; therefore some level of proliferation occurred in the cells to replace the damaged cells. In relation to this, Robert [25] suggested that the proliferation of chloride and epithelial cells was normally followed by both degenerative changed and inflammatory cells infiltration.

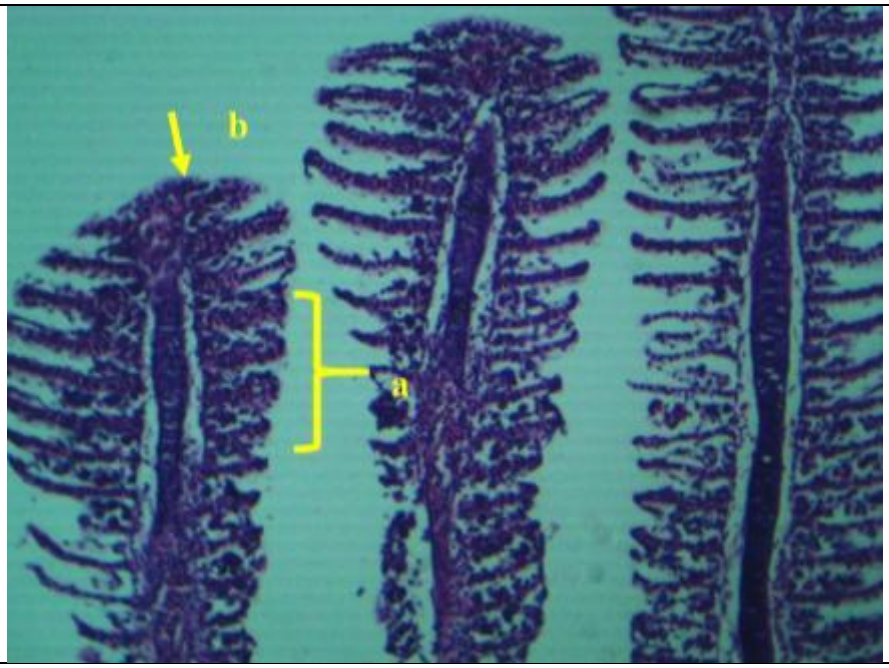

Figure 4. Cross section of gill histology with *Plankthothrix agardhii* exposure concentration 3,56 x 10^5 cells/ml. (a). secondary lamellar hyperplasia; (b). lamellar fusion (10x, HE)

Another pathological change that was observed is lamellar fusion. Both previously suggested pathological changes that occurred have linkages between them. Hyperplasia that occurred was normally accompanied by an increase of mucus cells production that located at the base of lamellae, this can cause the lamellar fusion. The lamellar fusion occurred due to the increase of hyperplasia which continuously occurred and caused the space of secondary lamellae is filled by new cells and stick together. Bhuvaneshwari et al. [26], reported the histological changes such lamellar fusion of secondary lamellae in gills may be an indication of either reaction to toxicant substance such a toxic algal entry through the gill's surface. According to Benli et al., [27], the lamellar fusion is the signal of a severe level of the damage; this is because the lamellar fusion is an advanced stage of lamellar hyperplasia damage. Lamellar fusion is massive hyperplasia. This condition makes lamellar capillaries within mass epithelium hyperplastic. In relation to this, Robert [25] suggested that lamellar fusion occurred during the less proliferation condition. The physiological effect of lamellar fusion is difficult to quantify, however, it can cause the loss of large areas of respiratory epithelium.

Pathological changes in figure 5 are congestion and atrophy. Pathological changes occur due to the intrusion of harmful substances into the fish' gill. Atrophy is the cells' shrinkage that normally caused by foreign substances; according to Saber [28], this also is due to the shrinkage of branchial blood vessels and cellular atrophy. In line to that, Alifia et al. [20], also suggested that atrophy can occur if the fish is exposed to toxic substances for a long time, thus the cells will experience shrinkage, if it is severe, then the cell will disintegrate and experience necrosis. Strzyzewska et al. [29], also reported that complete atrophy in the gill filaments that cause uncovering cartilaginous elements might cause necrosis.

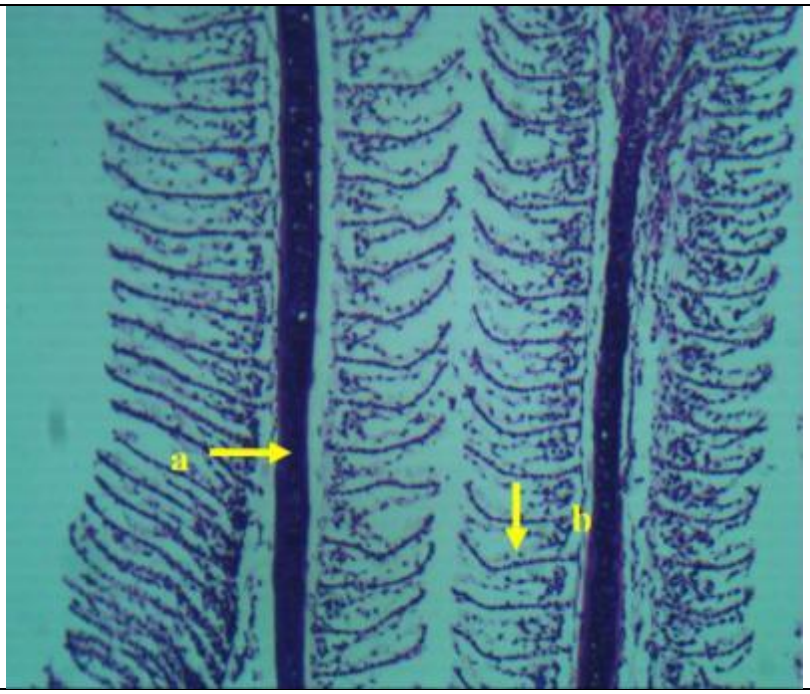

Figure 5. Cross section of fish' gill histology with *Plankthothrix agardhii* exposure concentration of 2,70 x10^7cells/ml. (a). congestion (b). atrophy (10x, HE)

Figure 6. Cross section gill histology with *Plankthothrix agardhii* exposure concentration 5,41 x 10^7 cells/ml. (a). lamellar fusion (*clubbing*); (b). congestion; (c). secondary lamellar breakage (4x, HE).

This study showed that there was an increasing trend of the level of gill damage if the fish was exposed to a higher *Plankthothrix agardhii.,* concentration. This can be seen in the gill structure when the fishes were exposed to higher cells concentration, especially for the secondary lamellae, in the treatment with *Plankthothrix agardhii* concentration of 3,56 x 10^5, the lamellae still can be seen even though some conditions are broken. But at higher concentration (5,41 x 10^7 cells/ml), its structure is invisible; some studies suggested, this may be due to the increase of secondary lamellar hyperplasia which can be caused by space between the secondary lamellar cells filled with sticky new cells. The pathological changes of fish's gill that exposed to *Plankthothrix agardhii* with a concentration of (5,41 x 10^7 cells/ml) are shown in figure 7, the observed changes in the gill for this treatment such as lamellar fusion (clubbing), congestion, and gill's lamellar breakage. The lamellar fusion (clubbing) in figure 4 (a) signed by the secondary lamella stick together that make the primary and secondary lamellae become visible and differentiate. According to Priosoeryanto et al. [30], lamellar fusion started by hyperplasia at a lower level of irritation and usually accompanied by the increase of mucous cells in the lamellae' base. The lamellar fusion may also cause several epithelial thickening at the end of the filament to form baseball-bat (clubbing), or it can also cause epithelial thickening that locates near the lamellae' base; that is known as basal hyperplasia. In relation to this, according to Hadi and Alwan [31], epithelial thickening of gill's filament may lead to a lamellar fusion. The fusion occurred may be due to the effect of the toxin. This can change glycoprotein on mucus which functions as cells covering, these may cause the epithelium to become negatively charge and support adhesion to adjacent lamellae. The clubbing of gill filament may lead an overall reduction of gill filament efficiency in aid for oxygen diffusion in lamellae, this may resulting in the development of hypoxic condition to the fish. Based on Adeogun et al. [32] study, this can be due to the loss of epithelial cells in the gill, so the normal gas exchange in these cells will be compromised, and this may result in hypoxia. Some authors have shown that gill lesions didn't just indicate the possibility of respiratory interference function but also osmo-regulation disorders function too. The other observed pathological change in this treatment is congestion. The gill's congestion located in gill lamellar can be caused by toxic substances exposure, this can cause irritations and make higher vessels osmotic, as well as blood capillary fluid out. According to Triadayani et al. [33], the congestion may also be caused by circulation disorder that can lead to the lack of oxygen in the cells. In figure 7 (c), it can be seen the evidence of secondary lamellae breakage occurred in the gills. According to Alifia et al. [20], fractures of gill lamellar can be due to a reduction of elasticity of epithelium that supporting internal organelles after the lamella experienced lesions and atrophy.

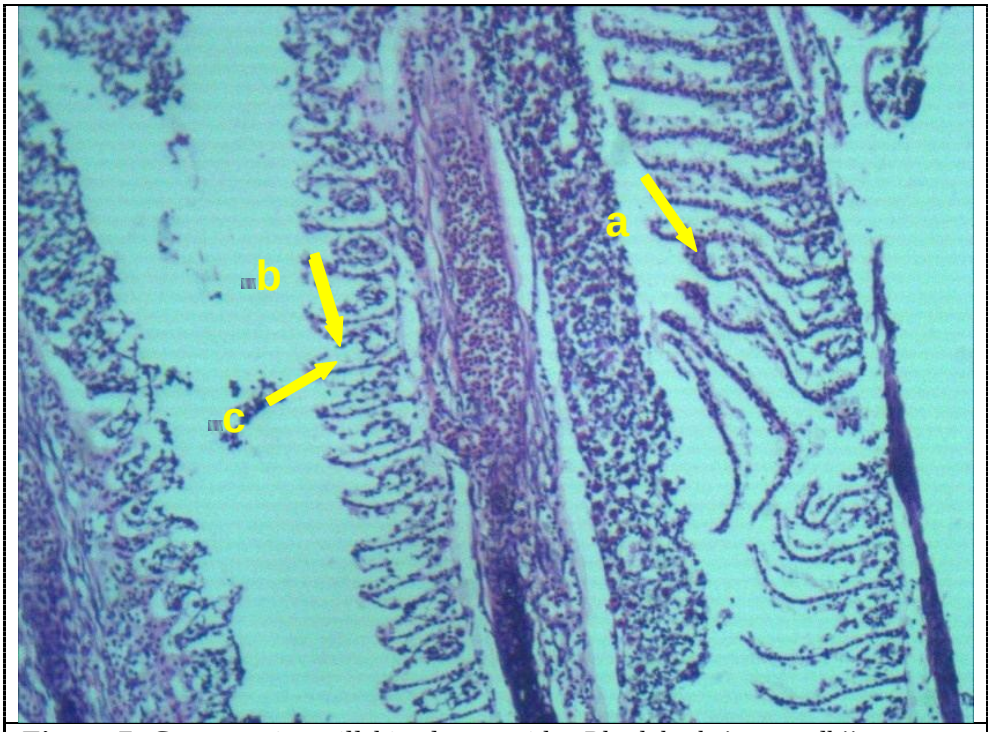

Figure 7. Cross section gill histology with *Plankthothrix agardhii* exposure concentration 5,4086 x 10^7 cells/ml (a). telangiectasis; (b). lifting respiratory epithelium; (c). edema (10x, HE)

Histopathological of gill that exposed to *Plankthothrix agardhii* with concentration 5,41 x 10^7 is shown in figure 7. The pathological changes observed such as telangiectasis (a) and edema (b). The changes occurred may be related to the fishes reaction to oxygen stresses. In relation to that, according to Roberts (2001), telangiectasis can be occurred by a normal process by which at low oxygen absorption, whereas normally the oxygen needs higher metabolism, these cause fish do homeostasis by accelerating the breakdown of pillar cells and expand their lacuna area at the end of secondary lamellae. In relation to that, according to Sudaryatma et al., [23] the closure lacuna by the epithelial cell of secondary lamellae can increase pressure in the lacuna and cause damage of pillar cells which its role is to serve in maintaining the stability of secondary lamellae. The damage of pillar cells can cause erythrocytes accumulation in blood vessels and also blood vessels dilatation, especially at the end of lamellae. A few telangiectases on secondary lamellae were observed. It showed by concentration 5,41 x 10^7, the higher concentration used, according to Hadi and Alwan [30] telangiectasis caused cellular degeneration which result is necrosis of gill epithelial tissues. In related to opinion Van Dyk et al. [34], telangiectasis or an aneurysm is dilated superficial blood vessel, inducing blood congestion. This phenomenon was identified in secondary lamellae of fish. An aneurysm as a common irritant that starts with lesions. After related to histologic study, 24% of studies related to acute, chronic lethal and chronic sublethal effects as well as physical irritation showed telangiectasis in the gill of exposed fish.

Another change can be observed in Figure 7 is lifting respiratory epithelium. It's earliest injuries found in fish. According to Santos et al. [35], Lifting respiratory epithelium is related to presence toxin. It characterized by displacement of the secondary lamellar layer, which space called edema to occur. This pathological change will endanger an oxygen exchange. Related to opinion Flores-Lopes and Thomaz [36], as consequences of epithelium lifting, there is an increase in distance between water and blood, so it'll be damaging absorption oxygen. However, in this condition fish increase their respiration rate by compensating for low oxygen entry.

The other pathological change is edema (b). Edema is one of pathological change to fishes' gill that is known to be caused by toxic substances exposures. Edema of secondary lamellae is characterized by detachment of epithelial cells of secondary gill lamellae due to the outflow of serous fluids into interstices of the gill tissue. According to Alifia (2013), edema will occur when there is an increasing amount of fluid occurred in the intracellular compartment, and also due to osmosis from increase natrium concentration in the cell. Related to opinion Bhagwant and Elahee [37], edema contributes to increase the diffusion distance from surrounding water to capillaries and increase the amount of tissue in secondary lamellae. (Susanah et al. 2013) [37], the presence of edema cause erythrocytes become fragile and deformed so that they will degenerate and makes fishes experience difficulty to control their respiratory (breath difficulty) and cause their body lack of oxygen, and this can cause the deadly effect.

4. Conclusion

This preliminary of study on the potential toxic effect of *Planktothrix agardhii.,* biomass effect to *Cyprinus carpio*. *L.,* concluded that there were symptoms of potential toxic effects caused by *Planktothrix agardhii.,* biomass containing potential cyanotoxin to *Cyprinus carpio. L.* The higher the biomass concentration, the shorter the time it needs to cause the dead effect to fishes. The biomass cells concentrations above 107 caused acute toxic effects symptoms that caused fishes dead below 6 hours, even the highest concentration in this study cause the fishes dead between 2 and 2 hours and 15 minutes. Compared to the negative control and the control that are alive until the end of experiment, a noticeable swimming behavior were recorded in the fishes treated with the high concentrations of the *Planktothrix agardhii.,* biomass such as swam in the water surface in the upside down and in tilted positions before they finally dead after around 2 hours. Further histopathological changes of the dead fishes compared to the un-treated fishes (control) were observed such as edema, lamela gill hyperplasia, and necrosis. The concentration of *Planktothrix agardhii.* Observed in the retention pond was around 2,9 $x10^5$ cells/ml during this study and the species was dominant, this suggested for a treatment priority to prevent its detrimental effects.

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