# Rapid counting method for 2-MIB-producing cyanobacteria (*Pseudanabaena* sp.) using fluorescence detection of phycocyanin pigments in algal cells

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## Abstract

The sudden occurrence of odor in drinking water is one of the significant challenges for drinking water utilities. The concentrations of 2-methylisoborneol (2-MIB)-producing cyanobacteria, *Pseudanabaena* sp., can be a surrogate indicator for alarming the potential of odor occurrence. This study aimed to develop a rapid and automatic method to accurately measure *Pseudanabaena* sp. concentrations using fluorescence detection of auto-fluorescent chemical in cyanobacteria, phycocyanin. This study first identified that increases in 2-MIB concentrations in a lake almost simultaneously occurred according to the rise in the counts of *Pseudanabaena* sp., which was only <0.5% of the entire algae population. The developed method using a phycocyanin filter and threshold settings in fluorescence microscopy (algal cell width and length, fluorescence intensity, and shape) confirmed that almost exclusively cyanobacteria appeared in fluorescent photos, unlike another commonly-used method using a chlorophyll filter that captured all algae. As a result, a high correlation ($R^2$=*0.998*) was observed between manually-counted and automatically-counted concentrations of *Pseudanabaena* sp. The detection limit of this method was determined at 11 units/mL. Further, the new method's accuracy using a phycocyanin filter was 88%, while the previously developed method using a chlorophyll filter was approximately 62%. These results suggest that the developed method with a phycocyanin filter can provide a more reliable warning of potential 2-MIB occurrence in drinking water sources.



**Keywords:** algae counting; 2-MIB; cyanobacteria; phycocyanin; drinking water; *Pseudanabaena* sp.

# Introduction

The occurrence of odor in drinking water is a growing concern worldwide. The main odorous compounds from algae in drinking water sources are geosmin and 2-methylisoborneol (2- MIB). These compounds are secondary metabolites mainly originating from cyanobacteria, and the human detection limit of those two compounds is low (<10 ng/L) [1-3]. Musty odor and earthy off-flavors caused by geosmin or 2-MIB in drinking water are significant causes of consumer complaints[4, 5]. Various cyanobacteria such as Aphanizomenon, Pseudanabaena, Planktothrix can produce 2-MIB and cause odor and taste problems [6-8]. Among the numerous 2-MIB-producing cyanobacteria, *Pseudanabaena* sp. has been notably identified as a significant odor-causing alga in rivers and lakes globally [9] and particularly in Asia [10-12]. In 2019, *Pseudanabaena* sp. was detected in Lake Y. (Japan) and identified as responsible for the 2-MIB odor events [13]. The production of 2-MIB by *Pseudanabaena* sp. can vary from 14 to 147 fg/cell depending on various factors (e.g. strain, light, and growth medium) [14, 15]. Although the pre-treatment process using powdered activated carbon (PAC) can remove 2-MIB concentrations [16, 17], the start of PAC doses can be frequently delayed due to the difficulty of determining odor concentrations on-site at drinking water treatment plants, resulting in periods of non-compliance with the 2-MIB regulations (e.g., 10 ng/L in Japan).

The concentrations of odorous compounds are typically determined using gas chromatography-mass spectrometry (GC/MS). Because GC/MS is costly and requires a high-skilled technician, 2-MIB analysis is only regularly performed (e.g., once a week), even during the odor-occurring period. Any fast detection of odor occurrence can minimize the level and frequency of non-compliance. Recently developed techniques such as artificial olfactory or gustatory systems are gaining interest for the analysis of 2-MIB: a bioelectronic nose can detect 2-MIB in water without any pretreatment [18], and a potentiometric E-tongue system can analyze

2-MIB concentrations in drinkable water [19]. However, the capacity of these methods for detecting 2-MIB concentrations below 10 ng/L has yet to be established. Quantitative real-time polymerase chain reaction (q-PCR) is also a promising tool for 2-MIB monitoring. The presence and quantity of genes necessary to produce 2-MIB can be analyzed in the water using specific primers [15]. However, when 2-MIB concentration is below 5 ng/L, the genes coding for 2-MIB synthetase cannot be detected. As a result, the rapid analysis of 2-MIB concentrations in surface water (<10 ng/L) remains a challenge.

Monitoring the concentrations of 2-MIB-producing algae is an alternative method for predicting odor events. Odor-producing cyanobacteria are commonly measured by microscopic enumeration in bright-field observation. However, it is time-consuming and requires trained personnel. Although digital flow cytometry can be applied to enhance the speed without trained personnel [20], the channel of the flow cytometer can be readily clogged. Moreover, it has been mainly used to monitor large marine algae; and despite recent progress, data on freshwater cyanobacteria are limited [21, 22, 23]. Although a recent study [24] aimed to sort river water cyanobacteria into major genera, including *Pseudanabaena*, the classification was only semi-automated due to the requirement of manually checking the photos to identify wrongly counted or uncounted particles. As a result, automated counting of only odor-causing algae in surface waters by digital microscopy has yet to be successful.

A recent study by the authors [13] developed an automated counting technique for 2-MIB-producing *Pseudanabaena* sp. Firstly, algae photos are captured, and each alga in the pictures is analyzed based on auto-fluorescence emitted from chlorophyll. After separating cyanobacteria from other types of algae based on their high fluorescence intensity, the unique dimensions (length and width) of *Pseudanabaena* sp. are utilized to enumerate their concentrations. However, long and narrow algae (such as Ankistrodesmus and Nitzschia), often

present in high concentrations in lake waters, cause an overestimation of *Pseudanabaena* sp. concentrations [13]. The method's accuracy can be improved by eliminating the impact of algae other than cyanobacteria. This study suggests an alternative approach that utilizes phycocyanin, a pigment specific to cyanobacteria, instead of chlorophyll a and b. Phycocyanin is commonly used to detect cyanobacteria [25]. The phycocyanin concentration in *Pseudanabaena* sp. cells varies depending on photoperiod, light intensity, and growth states [14, 26, 27].

This study aims to develop a high-precision and rapid analytical protocol for automatically measuring *Pseudanabaena* sp. concentrations using phycocyanin in algal cells. The accuracy of the developed method was evaluated using the manually-counted algal concentrations in the bright field and the auto-counted algal concentrations using fluorescence emitted by (a) phycocyanin or (b) chlorophyll a and b. The developed method can enhance the reliability of automatically counting *Pseudanabaena* sp. to predict 2-MIB occurrence in drinking water sources.

# Materials and Methods

## *Water samples*

The occurrence of 2-MIB indicated by an increase in *Pseudanabaena* sp. concentrations was evaluated in a lake in Nagasaki Prefecture, Japan. The lake (denoted as Lake Y) is used as a drinking water source. Water samples were collected weekly at a lake water intake, from which the lake water was transported to a drinking water treatment plant. The samples were collected from mid-March to July 2022. It should be noted that the weekly monitoring of 2-MIB and *Pseudanabaena* sp. concentrations continued until the end of September 2022. However, *Pseudanabaena* sp. was absent in the samples from July, and there was no increase in 2-MIB

concentration. It was consistent with the trend of odor occurrence in Lake Y; 2-MIB peaks are detected in spring from March to June over the last four years. The water quality is available in **Table S1**. In addition, river water (denoted as River U) in Nagasaki Prefecture, Japan, was also used for verification. The collected water samples were stored at 4 °C and analyzed within four days after collection.

## *Odor quantification*

The samples collected from Lake Y were analyzed to detect geosmin and 2-MIB [13]. In brief, a purge and trap concentrator (PT7000, GL Science, Tokyo, Japan) was used to extract the two compounds in each sample. Then, concentrations of geosmin and 2-MIB were determined using a gas chromatograph–mass spectrometer (GCMS-QP-2010Plus, Shimazu, Kyoto, Japan).

## *Microscope and sample preparation*

Lake Y water samples were analyzed weekly to determine algae concentrations. Observations were conducted using an all-in-one Fluorescence Microscope (BZ-X800, Keyence Co.; Osaka, Japan) with objective lenses (Nikon, Japan). A sample volume of 300 µL of water was inserted into a plankton counting polycarbonate slide (MPC-200, Matsunami Glass Industry; Osaka, Japan). The microscope slide has a specimen capacity of 0.1 mL and a chamber depth of 1 mm. A resting time of 10 min is sufficient to allow the algae to settle at the bottom of the slide.

Firstly, the bright-field observation was conducted to identify the accurate concentration of each alga. The natural counting unit (single cell, filament, or colony) determined the number of algae in 0.1 mL. *Pseudanabaena* sp. and *Pseudanabaena limnetica* were separately counted in the bright-field observation. Secondly, fluorescence observations were performed using two filters targeting auto-fluorescence emitted by chlorophyll and phycocyanin. The filter targeting chlorophyll a and b (Chroma Technology Japan; Yokohama, Japan) has excitation and emission wavelengths of 440 ± 20 nm and ≥590 nm, respectively. In addition, another filter with excitation and emission wavelengths of 592 ± 11 nm and 630 ± 15 nm, respectively (Chroma Technology, Yokohama, Japan) was used to identify phycocyanin fluorescence in algal cells **(Fig. S1)** [28].

## *Auto-counting method development and validation*

Counting algal concentrations using the photos captured under fluorescence observations were performed similarly to a previous study [13]. Firstly, 12 pictures of the sample were captured using the phycocyanin filter. Each image has a total area of 9.85 $mm^2$, equal to a sample volume of 118 μL. Then using the chlorophyll filter, the same area was divided into 49 sequential photos captured in auto-focus and auto-moving stage modes. Secondly, using hybrid cell count software (BZ-H3C, Keyence Co.; Osaka, Japan), the characteristics of each alga were analyzed, and they were sorted based on their dimensions (e.g., width and length), fluorescence intensity, and shape (circularity). The counting software utilizes the minimum bounding box method to determine length and width. In addition, the software calculated circularity as 4π multiplied by object area divided by object perimeter. Finally, the software extracted the target algal cells with pre-set object properties, and they were recognized as the targeted 2-MIB-producing cyanobacteria. Analysis of each sample was performed in duplicate. The image capture and

149 software analysis were completed within 5 min (excluding the resting time) using phycocyanin
150 fluorescence and within 1 h for chlorophyll fluorescence. The auto-counting method was
151 evaluated by correlating manually and automatically counted algal concentrations in Lake Y
152 and River U samples.

153

154

# 155 Results and Discussion

## 156 *Variation of 2-MIB concentrations*

157 Weekly analysis of algal concentrations in Lake Y found that increases in
158 *Pseudanabaena* sp. and 2-MIB concentrations occurred concurrently from mid-March **(Fig.**
159 **1A)**. On March 25, *Pseudanabaena* sp. concentrations were identified at 370 units/mL, and 2-
160 MIB concentrations were also high at 7.4 ng/L. At the maximum concentration of 370 units/mL,
161 *Pseudanabaena* sp. accounted for only 0.49% of the entire algal community **(Fig. S2)**. No other
162 2-MIB-producing cyanobacteria were identified. This is consistent with a previous study [13]
163 where an odor event was attributed to the minor population of 2-MIB-producing
164 *Pseudanabaena* sp. Due to the increased 2-MIB concentrations identified on March 25, the
165 water treatment plant dosed copper sulfate, an algicide, into the lake on March 29, and the
166 concentration of *Pseudanabaena* sp. rapidly decreased to 9 units/mL after ten days (April 8)
167 **(Fig. 1A)**. However, on April 1 (three days after copper sulfate treatment), 2-MIB
168 concentration reached as high as 13.4 ng/L **(Fig. 1A)**. Because copper (Cu) can cause damages
169 on algal cells, toxin or odor compounds can be released from the algal cells [29-31]. Therefore, it
170 can be assumed that the intracellular bound 2-MIB was released in the water after damaging

*Pseudanabaena* sp. cells [32, 33]. Following the reduction of *Pseudanabaena* sp. concentrations, the 2-MIB concentration also decreased and stabilized at approximately 3 ng/L **(Fig. 1A)**.

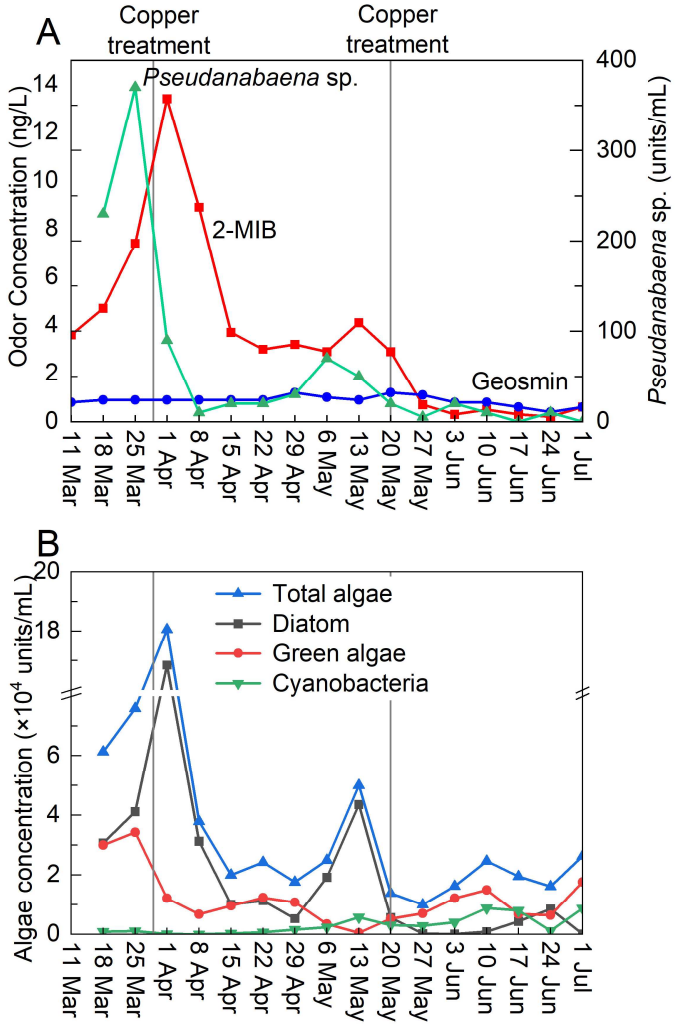


**Fig. 1** Concentration of (A) 2-MIB, geosmin, and *Pseudanabaena* sp., and (B) all algae in Lake Y from March 11 to July 1, 2022. Algal concentrations were quantified via bright-field observations. Vertical grey lines represent copper sulfate treatment.

From April 15, increases in *Pseudanabaena* sp. and 2-MIB concentrations occurred concurrently **(Fig. 1A)**. On May 13, *Pseudanabaena* sp. concentrations were identified at 50 units/mL, and 2-MIB concentrations reached 4.1 ng/L. As the water temperature rose in Lake Y from 16.0 to 19.3 °C from May 13 to May 20 **(Table S1)**, another copper sulfate treatment to the lake was conducted on May 20. On May 27, one week after treatment, concentrations of *Pseudanabaena* sp. decreased to 8 units/mL. and 2-MIB to 0.7 ng/L. Cyanobacteria concentrations decreased slightly from 3,000 to 2,800 units/mL **(Table S2)**. However, the diminution of *Pseudanabaena* sp. and cyanobacteria concentrations had occurred one week before the copper sulfate treatment; thus, the decrease cannot be solely attributed to the algicidal treatment. From June, heavy rainfalls occurred **(Fig. S3)**, and the high precipitations likely flushed the lake water containing 2-MIB and *Pseudanabaena* sp. After that, 2-MIB concentration was relatively stable below 1.0 ng/L, and *Pseudanabaena* sp. concentrations remained below 23 units/mL **(Fig. 1A)**. The copper sulfate treatment can mitigate the proliferation of cyanobacteria, including 2-MIB-producing *Pseudanabaena* sp., avoiding a further increase in their population.

## *The potential of Pseudanabaena sp. to predict the 2-MIB occurrence*

This study identified that 2-MIB and *Pseudanabaena* sp. concentrations were somewhat correlated with an $R^2$ of 0.673 ($r$ = 0.820, $p$ = .00377) **(Fig. 2)**. In contrast, no correlation was found for the other odor-producing algae in Lake Y, such as *Pseudanabaena limnetica* and *Staurastum* sp. with $R^2$ of 0.042 and 0.015, respectively **(Fig. S4)**. Furthermore, no other 2-MIB-producing cyanobacteria, such as Aphanizomenon or Planktothrix, were identified during the study. This indicates that *Pseudanabaena* sp. is likely responsible for the odor event in Lake Y. Although the regulatory limit for 2-MIB in drinking water in Japan is

set at 10 ng/L, most water treatment plants maintain 2-MIB concentrations below 3 ng/L as a conservative measure to avoid customer complaints. In fact, during the odor event, a drinking water treatment plant managing Lake Y was mainly operated using the other drinking water sources not impacted by the odor event. Therefore, increasing *Pseudanabaena* sp. concentrations in lake water could indicate a 2-MIB concentrations increase and potential odor event occurrence.

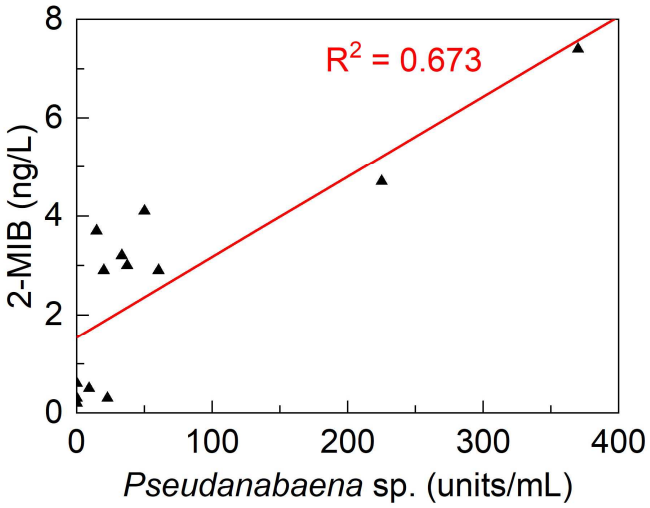


**Fig. 2** Correlation between concentrations of 2-MIB and *Pseudanabaena* sp. in Lake Y from March 18 to July 1, 2022. The samples collected after the copper sulfate treatments (April 1 and 8 and May 27) were not included because the copper sulfate treatment impacted the algal concentrations.

## *Advantages of using a phycocyanin filter in fluorescence observation*

During the assessment from March 18 to July 1, photos of algae containing autofluorescent pigments (e.g., chlorophyll and phycocyanin) were captured using filters

219 targeting chlorophyll or phycocyanin fluorescence. Phycocyanin is a pigment present only in
220 cyanobacteria, including *Pseudanabaena* sp., red algae (Rhodophytes), and Cryptophytes such
221 as Cryptomonas [34]. However, red algae are relatively rare in freshwater [35], and Cryptophytes
222 are non-filamentous [36]. Thus, the filamentous cyanobacteria (e.g., *Pseudanabaena* sp.) and
223 Cryptomonas were separated based on shape. Green algae and diatoms contain only
224 chlorophyll, whereas cyanobacteria contain chlorophyll and phycocyanin. As a result, green
225 algae (e.g., Scenedesmus) and diatoms (e.g., Nitzschia) were not visible in the photos taken
226 using the phycocyanin filter **(Fig. 3)**. In some cases, when the filament was trapped in
227 aggregates with other algae or particles, the obtained photos became blurry, leading to the
228 miscounting of *Pseudanabaena* sp. (i.e., underestimation). In addition, all algae appeared in
229 the pictures using the chlorophyll filter, whereas the phycocyanin filter specifically targeted
230 cyanobacteria.

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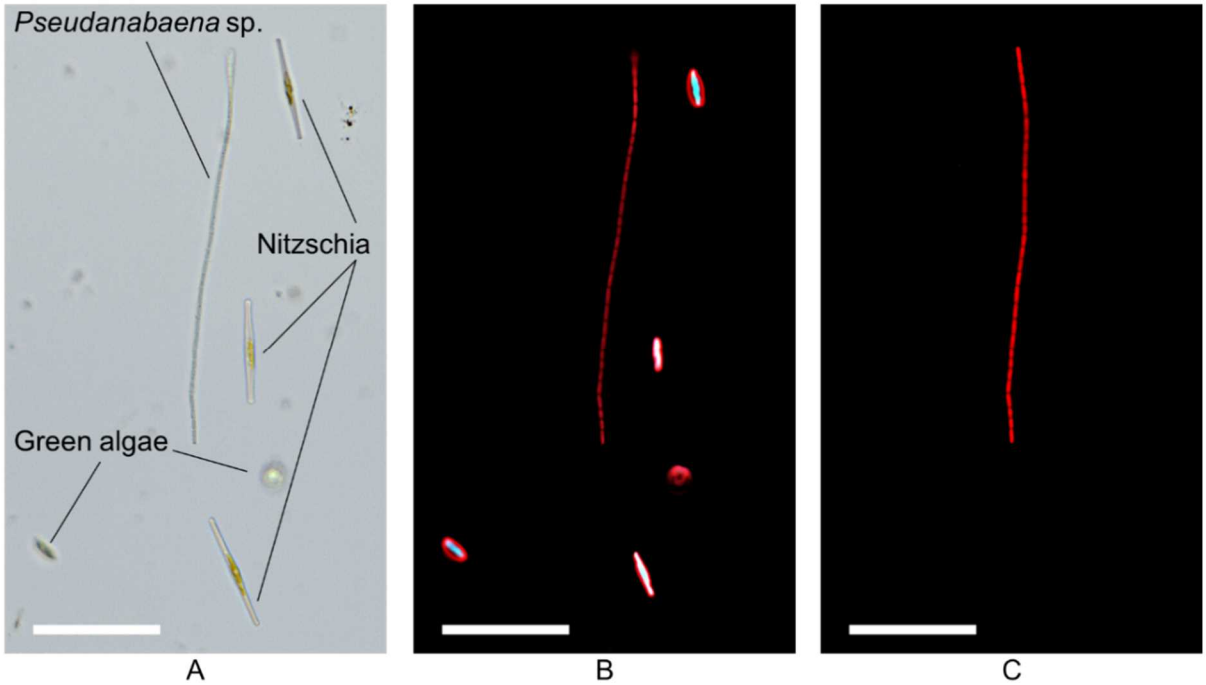


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**Fig. 3** Microscopic photos of *Pseudanabaena* sp. and other algae in Lake Y: (A) bright-field, (B) chlorophyll fluorescence, and (C) phycocyanin fluorescence. The white line is 50 μm wide.

The main benefit of using the phycocyanin filter over the chlorophyll filter is the improved accuracy due to the capacity to capture cyanobacteria only. Green algae and diatoms do not appear on the captured photos with the phycocyanin filter, significantly reducing the number of algae to analyze, minimizing counting errors, and overestimating. Further, the accuracy was maintained even when algae with shapes similar to *Pseudanabaena* sp., such as Nitzschia, were in abundance., For example, samples contained high concentrations of Nitzschia (up to $16.8\times10^4$ units/mL) on April 1. With the chlorophyll filter, Nitzschia caused many errors and overestimation of auto-counted concentrations of *Pseudanabaena* sp. **(Fig. 5)**. In fact, the leading causes of miscounts in the previous study [13] included the miscounting of other green algae or diatoms with similar dimensions, moving plankton, double counting in segmented photos, and other cyanobacteria. In contrast, high concentrations of Nitzschia did not impact accuracy with the phycocyanin filter.

## *Auto-counting method development*

A method for determining *Pseudanabaena* sp. concentrations from the captured photos was established based on the capability of the cell counting software, as described in **Fig. 4**. After image acquisition, each fluorescent object in the captured image was set to be automatically sorted based on its properties (e.g., fluorescence intensity, dimensions, and shape) using the cell counting software. Firstly, all objects on the image with fluorescence intensity above 20 (a.u.) are selected. Then objects with dimensions (length and width) outside

the targeted range are removed from the selection. Last, the objects with a circular shape are removed from the selection, keeping the more elongated objects (filaments) in the selection. The final conditions to automatically count *Pseudanabaena* sp. include a length of ≥10 µm, a width of 1.5–12 µm, and circularity <0.9 (i.e., not circular objects) **(Table S4)**. Because more suitable images can be attained due to the use of the phycocyanin filter, the developed method in this study allows for the counting of shorter filaments (≥10 µm) in comparison with the previous technique (≥15 µm) [13]. During the study, only two *Pseudanabaena* sp. filaments shorter than 10 µm were observed, indicating the validity of the length setting. The other causes of failing to count *Pseudanabaena* sp. include weak fluorescence intensity or folded form (i.e., wider width). Throughout 16 sampling occasions in Lake Y in the study from March 18 to July 1,209 filaments of *Pseudanabaena* sp. were manually identified and counted in bright observation. In contrast, as high as 184 filaments (88% accuracy) were correctly and automatically calculated based on the fluorescent-based method established using the phycocyanin filter in this study. It is noted that the technique in this study analyzed a sample volume of 0.1 mL within 15 min, including the resting time, which is by far faster than a previous study [13] using the chlorophyll filter. Further, the improved technique did not use sequential photos for image capturing; thus, there was no error due to double counting.

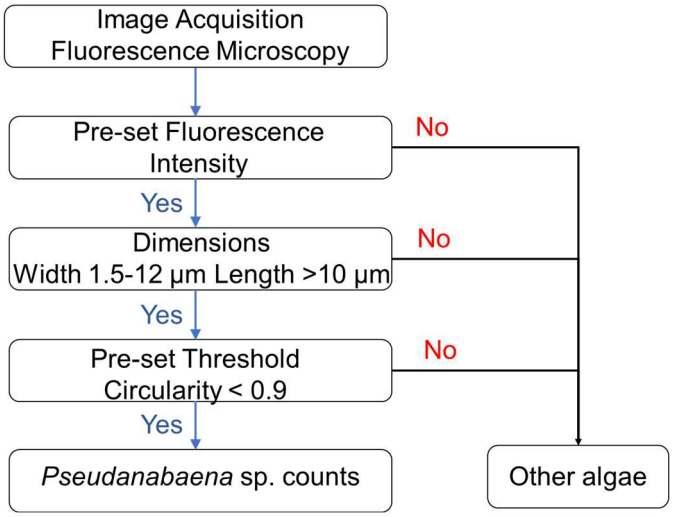



**Fig. 4** Flowchart describing the technique determining *Pseudanabaena* sp. counts using fluorescence microscopy, phycocyanin filter, and cell counting software.

## *Accuracy of the phycocyanin-based method*

Because of the improved accuracy, the concentrations of automatically-counted *Pseudanabaena* sp. were highly correlated with manually counted *Pseudanabaena* sp. **(Fig. 5)**. For Lake Y, the $R^2$ value of the phycocyanin-based method was 0.997 ($r = 0.998$, $p = 2.483 \times 10^{-19}$), while that of the chlorophyll-based method was 0.022 ($r = 0.148$, $p = 0.196$). For River U, the $R^2$ value of the phycocyanin-based method was 0.809 ($r = 0.899$, $p = 0.147$), while that of the chlorophyll-based method was 0.063 ($r = 0.263$, $p = 0.546$) **(Fig. 6)**. The abundance of Nitzschia was the leading cause of errors and overestimation for the chlorophyll method. The method detection limit (MDL) (11 units/mL) was calculated with 8 samples of 20 units/mL using the following formula: MDL = Value of the student's t distribution × standard deviation. Overall, the phycocyanin-based method developed in this study allows us to analyze a larger volume quickly and provides an accurate analysis of *Pseudanabaena* sp. concentrations.

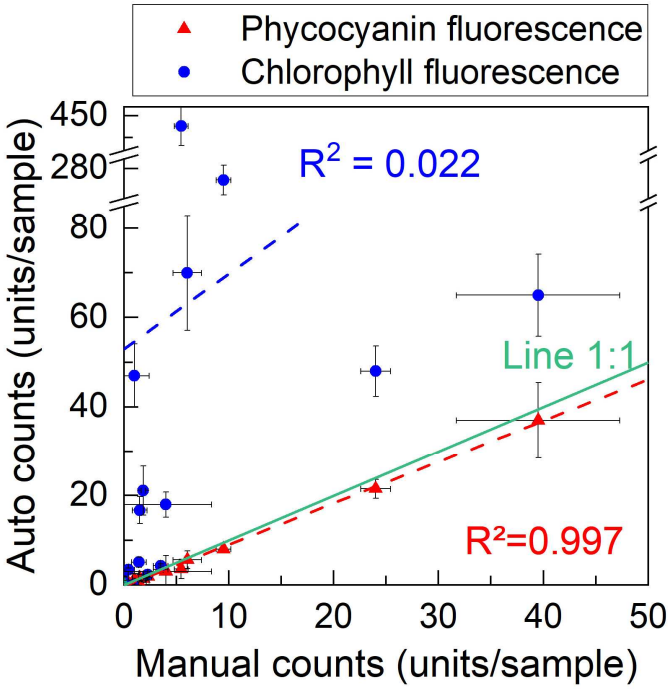
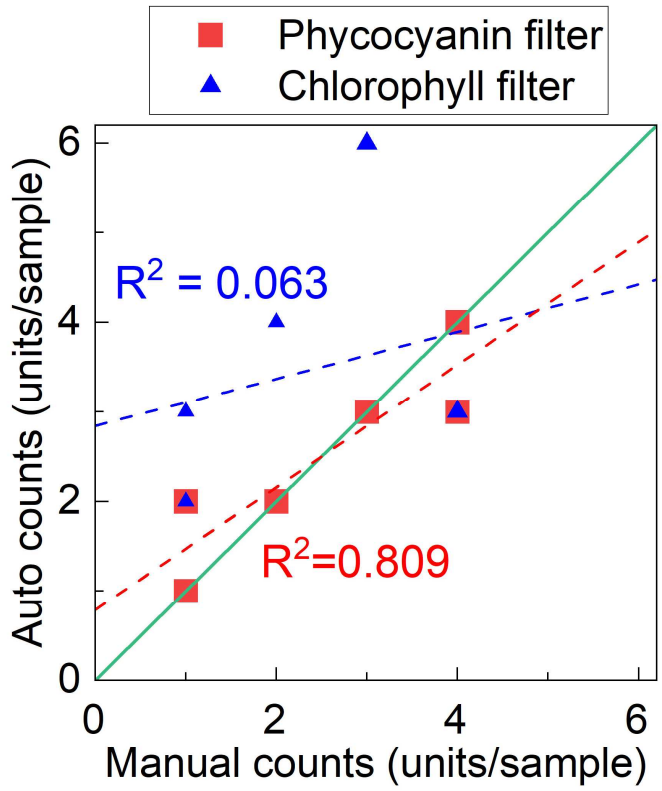



**Fig. 5** Correlation between manual and auto counts of *Pseudanabaena* sp. in Lake Y from March 18 to July 1, 2022, using the phycocyanin (red) or chlorophyll (blue) filter. A line of equality (in green) was added for comparison. The sample volume was 100 µL.

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297 **Fig. 6** Correlation between manual and auto counts of *Pseudanabaena* sp. in river U from
298 using the phycocyanin (red) or chlorophyll (blue) filter. A line of equality (in green) was added
299 for comparison. Sample volume of 100 μL.

300

301 ***Practical implications***

302 The improved counting technique for *Pseudanabaena* sp. in lake waters is an easy and
303 fast way to monitor changes in *Pseudanabaena* sp. concentrations, especially when
304 *Pseudanabaena* sp. is a minority among algae (<1%). Rapid analysis using this technique can
305 allow drinking water treatment plants to take precautionary actions such as increased doses of
306 PAC for odor removal. In addition, fast monitoring of *Pseudanabaena* sp. could also help
307 manage the water source and plan preventive algicide treatment if the concentration of odor-
308 producing *Pseudanabaena* sp. rapidly increases within a week.

A limitation of the method developed in this study is the possibility of overestimation in case there is a high concentration of other filamentous cyanobacteria that have a similar size to *Pseudanabaena* sp. because the criteria used by the software are dimensions and fluorescence intensity only. As *Pseudanabaena* sp. is the dominating filamentous cyanobacteria in Lake Y, further studies would be necessary to adapt the technique to different water sources. Moreover, non-planktonic, benthic cyanobacteria are also known to be responsible for odor events [37]. However, as those cyanobacteria are periphytic (e.g., attached to submerged surfaces), they are not present in the sampled water. Algae with motility had little to no impact on the method as the exposure time before capture was short (0.33 sec/image); therefore, fixation pretreatment was not used in this study. Another limitation is that the developed method using only fluorescence microscopy photos cannot differentiate between odor-producing cyanobacteria and non-producers. The examples include *Pseudanabaena* sp. (2-MIB-producing cyanobacteria) and *Pseudanabaena limnetica* (some strains can produce 2-MIB). Despite the limitations, the technique could detect any increase in filamentous cyanobacteria concentrations, prompting further analysis of the source waters (e.g. odorous compounds or PCR analysis). It is noted that *Pseudanabaena limnetica* was not the major source of 2-MIB peaks in Lake Y during this assessment. In Lake Y, *Pseudanabaena limnetica* was detected at only <10 units/mL. More importantly, *Pseudanabaena limnetica* was not present in the sample during the odor event. Further studies will evaluate the possibility of overestimating *Pseudanabaena* sp. concentrations in water samples due to cyanobacteria with similar dimensions. The width of *Anabaena* sp. (6 to 14.6 µm [38]) is greater than that of *Pseudanabaena* sp. Therefore, overestimations are unlikely to occur. However, the filament shape of *Aphanizomenon* sp. and *Oscillatoria* sp. is similar to that of *Pseudanabaena* sp.; thus, the overestimations could happen because the software calculates the length and width of the bounding box method. Calculating the filament's average width could help separate

*Pseudanabaena* sp. from similar cyanobacteria such as *Aphanizomenon* sp. and *Oscillatoria* sp. Despite these limitations, the fast analysis developed here can trigger an alarm, allowing the operators of a drinking water treatment plant to act.

It should be noted that the same approaches can be adapted to monitor other algae other than *Pseudanabaena* sp. For example, some diatoms (e.g., *Synedra* sp.) are responsible for clogging sand filters in water treatment plants, resulting in more frequent backwashing and reducing drinking water production [39]. Concentrations of *Synedra* sp. could be monitored by using chlorophyll fluorescence and selecting suitable dimensions and fluorescence intensity. Another application could be monitoring of picophytoplankton concentrations. The presence of picophytoplankton in the water can increase the assimilable organic carbon concentration after disinfection, promoting microbial growth and degrading the water quality [40]. Picophytoplankton concentrations can be automatically determined by integrating their dimensions, chlorophyll, and/or phycocyanin fluorescence intensities into the criteria. Therefore, further research will focus on other problematic algae in drinking water applications.

# Conclusions

- The increases in 2-MIB concentrations in the studied lake concurrently occurred according to the rise in *Pseudanabaena* sp. concentrations, confirming that *Pseudanabaena* sp. can be a helpful indicator of odor occurrence.
- Automatically measured *Pseudanabaena* sp. concentrations using a phycocyanin filter were superior to those using a chlorophyll filter in speed and accuracy.
- A high correlation between manually and automatically counted *Pseudanabaena* sp. concentrations was observed in the lake water samples; among 209 filaments of

*Pseudanabaena* sp. observed during the study, the developed method achieved the identification of as many as 184 filaments with an accuracy of 88%.

- The phycocyanin filter-based technique developed in this study can predict the potential of 2-MIB occurrence in lakes by rapidly measuring the concentrations of 2-MIB-producing cyanobacteria, *Pseudanabaena* sp.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Acknowledgments

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