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Monitoring of algal species growth in drinking water reservoirs using onsite fluorescent probe: impact of suspended solids composition on cell counting

Shyh-Fang Kang[a], Fahrudin Sidik[b,c], Angga Aji[b,c] and Jr-Lin Lin[c,*]

[a] Department of Water Resources and Environmental Engineering, Tamkang University, New Taipei City, Taiwan, R.O.C.
[b] Department of Civil Engineering, Chung Yuan Christian University, Chung-Li, Taiwan, R.O.C.
[c] Department of Environmental Engineering, Chung Yuan Christian University, Chung-Li, Taiwan, R.O.C.
*Corresponding author. E-mail: jrlin@cycu.edu.tw

J-LL, 0000-0003-0915-0833

ABSTRACT

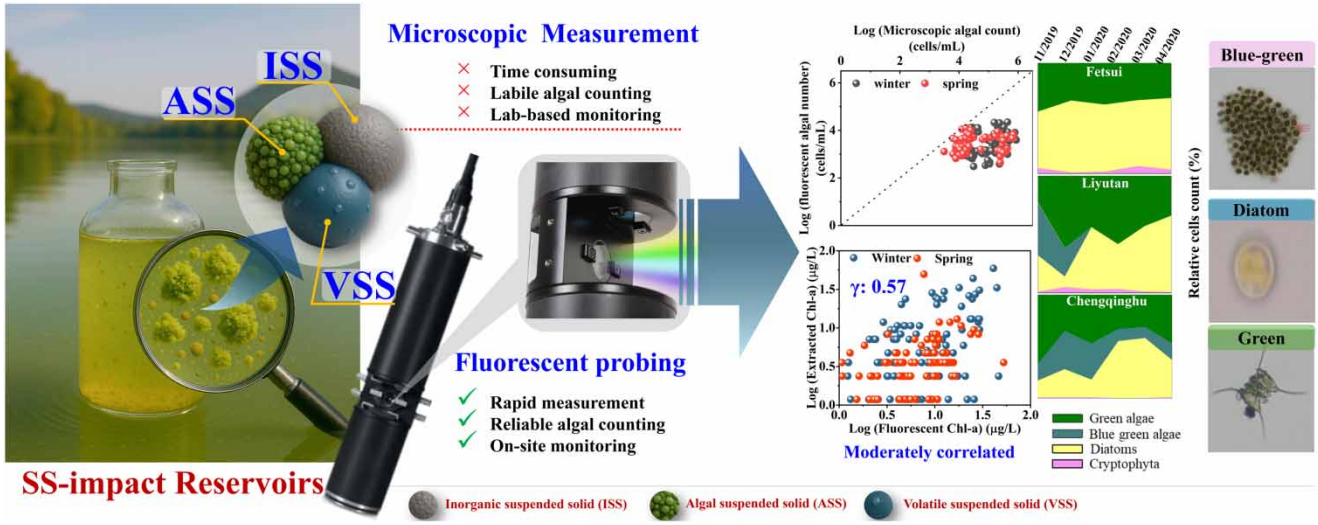
Evaluation of algal growth potential (AGP) is critical to prevent reservoir eutrophication. However, accurately and promptly monitoring algal species and counts via the microscopic method is challenging due to interference from suspended solids (SS). The study aimed to determine the composition and the impact of SS in six major drinking water reservoirs in Taiwan over a 6-month period (November to April). A fluorescent probe was used to quantify algal cells and to determine species diversity. Pearson's correlation was employed to assess the relationship among various indicators, including chlorophyll-a, total phosphate (TP), and SS. The results indicated that TP significantly enhances AGP, showing a moderate correlation ($\gamma = 0.54$) with algal suspended solid (ASS). The ASS can serve as an alternative and more robust indicator for assessing AGP in reservoirs. However, algal cell counting may be hindered by inorganic particles, resulting in lower accuracy compared to microscopic enumeration. Consequently, the 'relative algae count' emerges as a practical indicator to reach cost-effective species-specific monitoring for reservoir management. Furthermore, the fluorescence probe proved effective in distinguishing algal species fractions (e.g., diatoms, green algae, cyanobacteria, and cryptophyta), even under the impact of SS. It was concluded that fluorescent probing is a rapid-response method for AGP monitoring across diversified drinking reservoirs.

Key words: algal cell counting, chlorophyll-a, drinking water, reservoir

HIGHLIGHTS

- Algal suspended solids moderately correlate with total phosphate in eutrophicated reservoirs.
- Fluorescent probing shows moderate correlation ($\gamma = 0.57$) with extracted chlorophyll-a.
- 'Relative algae count' by fluorescent probing can be an indicator to evaluate dominant algal species across eutrophicated reservoirs.

GRAPHICAL ABSTRACT



INTRODUCTION

Water quality management has become a critical issue for drinking water reservoirs worldwide due to the lack of clean water resource under the impacts of climate changes. One of the major concerns about drinking water safety is the proliferation of harmful algal blooms, which generate toxins and unpleasant odors. Harmful algal blooms are primarily triggered by excessive nutrient accumulation in reservoirs from anthropogenic pollution (Wåhlström *et al.* 2020). Hence, accurate evaluation of algal growth potential (AGP) is essential to prevent eutrophication and to ensure safe and sustainable management of drinking water reservoirs. So far, monitoring algal species and quantifying their counts onsite remains a significant challenge due to unexpected interference under the impact of inorganic suspended solids (ISS) where it obscures optical measurements and distorts cell count. Traditional assessment tools, such as the Carlson trophic state index (CTSI), are commonly used to classify the trophic state and the AGP. However, their applicability is quite limited in reservoirs where optical or nutrient indicators are strongly influenced by nonalgal factors, such as suspended sediments, detritus, or colored dissolved organic matter (CDOM), rather than algal biomass, which is often the case in systems affected by seasonal turbidity or high inflow events (Lin *et al.* 2022). Likewise, the status index approach based on the empirical relationship among biological (chlorophyll-a (chl-a)), physical (Sechi Disk), and chemical (total phosphorus) parameters has been developed for shallow freshwater reservoirs, yet these methods heavily rely on laboratory analysis and are time consuming (Markad *et al.* 2019). However, water quality and ecological condition of reservoirs are highly sensitive to environmental evolution, especially in biodiverse and turbid waters affected by suspended sediments or increased algal concentrations. Therefore, an effective tool for onsite algal species counting is indispensable for managing water quality and controlling algal blooms in drinking water reservoirs.

The establishment of an algae growth monitoring technique would be beneficial to manage algal growth in reservoirs to reach a low trophic state. To estimate the composition and quantity of different groups of algae, there is a need to develop some techniques and algorithms. Multiple methodologies have been successfully adapted to monitor algae growth for managing water quality, such as nephelometry, gravimetry, spectrophotometry, and fluorescence (Qin *et al.* 2015). Fluorescence detection offers several advantages over traditional algal counting techniques in algal counting, including rapid, noninvasive, and cost-effective measurement of algal biomass based on the unique excitation and emission wavelengths of algal pigments (Leloup *et al.* 2013; Pivokonsky *et al.* 2014). In some cases, fluorescence has been used to estimate chl-a concentration in Bi Lake in Taiwan, as a quick indicator of eutrophic water (Chen & Chen 2021). Recently, multisensor fluorometers have been successfully tested for *in situ* monitoring of complex algal communities, offering advanced detection across multiple phytoplankton groups (Kring *et al.* 2014; Khan *et al.* 2019).

Despite the promise of fluorescence-based algal quantification techniques for algal monitoring, fluorescence-based accuracy can be significantly affected by suspended solids (SS) in natural waters. These particulates interfere with fluorescence signals by scattering and adsorbing light, leading to signal distortion, misclassification, and biases in the calculation of

algal cells. A previous study has reported that correction protocols for SS interference are species dependent, with varying degrees of accuracy depending on cell diameter and pigment properties (Choo *et al.* 2019). To enhance the precision of algal counting, Zuse *et al.* (2022) implemented the laboratory culture-based approach to reduce the biases generated from the varied size and species, which established reliable conversions from raw fluorescence signals to biomass or biovolume. However, most of the existing studies only emphasized SS effects on the monitoring of single algal species in laboratory trials. Further investigation is still required to determine the composition of SS in drinking water reservoirs. In particular, it is important to understand how SS affects fluorescence-based algal cell monitoring across various algae species under different seasonal conditions in SS-impacted reservoirs.

The aim of this study is to determine the composition of SS and investigate its impacts on algal cell counting and growth monitoring. The important indicators, including nutrients (i.e., nitrogen/phosphorus), SS, and chlorphyll-a, were analyzed to evaluate the water quality of six drinking water reservoirs (i.e., Shihmen, Feitsui, Liyutan, Mingde, Renyitan, and Chengqing Lake) in Taiwan. A fluorescent probe was further used to count the algal species and cells in these reservoirs during six months of algal growth monitoring from November to April. All important water quality indicators were collected and analyzed statistically to further understand their relationship using Pearson's correlation. The implications of the fluorescent probe were finally evaluated for onsite algal growth monitoring under SS impact.

MATERIALS AND METHODS

Characterization of reservoirs

Taiwan has faced yearly issues with the quality of water sources even though its annual rainfall can be achieved up to 2,350 mm in recent years, which is above the average world water rainfall. To store available resources of water extensively, 28 reservoirs have been operated with a total capacity of nearly 1 billion m^3. These reservoirs supplied approximately 3,017 million cubic meters per day (CMD) to northern Taiwan, 2,669 million CMD to central Taiwan, 2,943 million CMD to southern Taiwan, and 397,000 CMD to eastern Taiwan. In addition, erosion and siltation in reservoirs and accumulation of nutrients (total nitrogen (TN) and phosphate) in reservoirs from agricultural, industrial, and residential areas facilitate algae growth potential (AGP) and algae proliferation (Li *et al.* 2018). Notably, nonpoint pollution sources, such as agricultural activities and landslides, contribute nutrients and SS to reservoirs, raising eutrophication risks. To identify eutrophication potential, all reservoirs in Taiwan have been assessed by using the CTSI index, which is divided into oligotrophic ($CTSI < 40$), mesotrophic ($40 \leq CTSI \leq 50$), and eutrophic ($CTSI > 50$).

In this study, six major drinking water reservoirs in Taiwan, categorized into three classes, namely, oligotrophic (i.e., Feitsui reservoir), mesotrophic (Liyutan, Shihmen, and Renyitan reservoirs), and eutrophic (Chengqinghu and Mingde reservoirs) (Lin *et al.* 2022), were selected to be monitored in terms of water quality on a monthly basis for half a year. All of the reservoirs were selected as they are major drinking water reservoirs in Taiwan from North to South. The detailed water capacity and supply water loading rate, and functionality are illustrated in Supplementary material, Figure S1.

Water quality of reservoirs

Water quality variations in each reservoir were monitored in water quality variations during a half-year investigation by surveying several physicochemical parameters, namely, concentration of nutrients (TN and total phosphate (TP)), pH, turbidity, conductivity, dissolved oxygen, chl-a status, transparency (SD), total organic carbon (TOC), SS composition, and algal cell count. Samples were taken once per month for six months sequentially (November 2019–April 2020). To ensure consistency and representativeness, triplicate sampling procedures were conducted at an approximate depth of 0.5 m below the surface within the epilimnion for three designated zones across reservoirs, namely, the inlet, center, and outlet points. Thereafter, all samples were immediately analyzed within 24 h by following the Taiwan Environmental Protection Agency (EPA) standard methods. All data for water quality parameters (i.e., pH, turbidity, conductivity, dissolved oxygen, chl-a, transparency, TOC, TN, and TP) of six reservoirs have been investigated and organized in Table S1.

Analysis of SS composition

The presence of particulate SS and elevated turbidity can significantly hinder the accurate assessment of the AGP in drinking water reservoirs. The occurrence of heavy precipitation or typhoons would elevate the levels of SS and turbidity in various reservoirs, which contribute to the eutrophication level (Marsha *et al.* 2020). Our previous study has also suggested that SS and turbidity are major influencing factors for the evaluation of AGP (Lin *et al.* 2022). At such a condition, the total

suspended solid (TSS) in reservoir consists of ISS, volatile suspended solid (VSS), and algal suspended solid (ASS). Each fraction of TSS was measured based on the gravimetry method (EPA 1999), as shown in Figure 1. To measure the ASS content, the gravimetry method was modified by dividing it into two steps, namely, without digesting algal content (Equation (1)) and by digesting algal content (Equation (2)):

Step 1:

$$\mathrm{TSS}_{\text{without digested algal content}}\ (\mathrm{W1}) = \mathrm{ISS(W2)} + \mathrm{VSS} + \mathrm{ASS} \tag{1}$$

Step 2:

$$\mathrm{TSS}_{\text{digested algal content}}\ (\mathrm{W3}) = \mathrm{ISS(W2)} + \mathrm{VSS} \tag{2}$$

In the first step, water samples were filtered through glass fiber filters (Whatman 934-AH, Germany). The filtered volume was adjusted according to the expected SS content, based on visual observations: 1,000 mL for low SS content and 200 mL for high SS content with the total SS weighing at least 1 mg. Residue-containing filter was then dried in the oven at 105 °C for 120 min and weighed to obtain ISS, VSS, and ASS content (W1). In Step 2, a similar amount of water sample was prepared, and the sample was heated at 60 °C for 30 min to dissolve the algal content. Thereafter, the sample was immediately filtered through a glass fiber filter and dried in the oven at 105 °C for 120 min. The sample was taken out of the oven and weighed to obtain ISS and VSS (W3). The sample was transferred to the furnace for further heating at 550 °C for 60 min to obtain the constant weight of ISS (W2). After that, each fraction of SS can be calculated by Equations (3) and (4):

$$\mathrm{VSS\ (W4)} = \mathrm{W3} - \mathrm{W2} \tag{3}$$

$$\mathrm{ASS\ (W5)} = \mathrm{W3} - \mathrm{W1} \tag{4}$$

To reduce the potential biases, triplicate analyses were implemented for each test. Before each test, filter blanks were corrected for background mass and potential residue. After that, each weighing procedure was conducted with an error margin of ± 0.02 mg for each sample at triplicate measurements. Under such a condition, the relative percent difference of triplicate samples did not exceed an absolute value of 5% for an acceptable method accuracy, as regulated by the American Public Health Association (APHA) standard method (APHA 2017).

Algal cell counting

Fluorescent probing

Fluorescence-based probing is an easy-to-use and real-time measurement tool for algal cell counting. This approach could differentiate a spectral group of phytoplankton based on their relative internal fluorescence (Garrido *et al.* 2019). In this

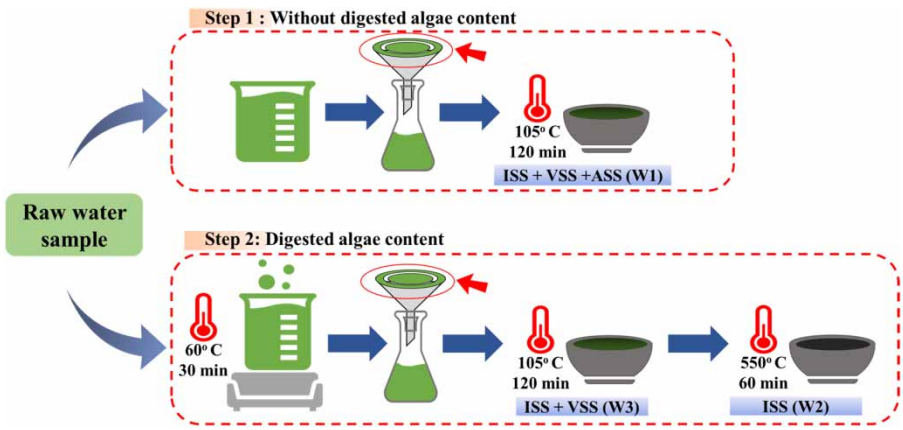


Figure 1 | The diagram of classified total suspended solids from reservoirs.

study, algal cell counting was carried out by a FluoroProbe (bbe^{++} moldaenke, Germany), as shown in Figure S2. This device was equipped with five LEDs (340, 525, 570, 590, and 610 nm) to evaluate a different class of algae based on their chl-a distribution (e.g., brown algae, green algae, blue-green algae, and red algae) within 35 fingerprint references of algal spectra. In addition, a ultraviolet-light wavelength at 370 nm was used to measure chromophoric dissolved organic matter. All the raw data were evaluated using bbe^{++} software. Despite its rapid *in situ* measurement capability, the sensitivity of fluorescent probing is constrained in low-biomass environments due to insufficient levels of CDOM and algal biomass, particularly at concentrations below the detection limit of 0.01 μg chl-a/L or outside the measurable range of 0–500 μg chl-a/L. The fluorescent probing used the built-in fluorescence 'fingerprints' of various standard algal species along with the corresponding calibration data. However, this fluorescent approach may introduce species-specific biases and result in inaccurate classifications when unregistered algae are present. To avoid this failure in algal counting, the new calibration curve can be employed additionally to load the database of specific algae species.

Microscopy observation

The algae cell count was carried out by using a light microscope (Olympus CX-31, Tokyo, Japan) equipped with a digital camera (Olympus EP50, Tokyo, Japan). A sample of 1 μL was dropped onto a Sedgewick-rafter glass plate and observed at various magnifications (10, 40, and 100×) with Lugol's solution to enhance the resolution of the cell tissue. The microscope was connected to EPview software to further the process of captured images.

Statistical analysis

Pearson's correlation was conducted to identify the important factors of algae growth potential. This approach was performed by logarithmically transforming several parameters such as TP, Chl-a, SS, and algal count. The Pearson correlation coefficient is represented in Equation (5):

$$\gamma = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\left[\sum_{i=1}^{n}(x_i - \bar{x})^2\right]\left[\sum_{i=1}^{n}(y_i - \bar{y})^2\right]} \tag{5}$$

Pearson's correlation coefficient is symbolized as 'γ', and it is commonly used when the variables are normally distributed. The aforementioned coefficient could be interpreted as $1 \leq \gamma \leq +1$, where there is no linear relation between x and y axis, while γ is about zero. Otherwise, y is close to -1 or $+1$ means that there is a strong correlation or strong positive correlation between variables, respectively. Lin *et al.* (2022) reported that Pearson's correlation is divided into three categories, namely, $\gamma > 0.7$ (high correlation), $0.4 < \gamma < 0.7$ (medium correlation), and $\gamma < 0.4$ (low correlation). In addition, principal component analysis (PCA) was used to determine the predominant load factors from selected parameters such as depth, Chl-a, DO saturation, TN, TP, TSS, cell count, and temperature.

RESULTS AND DISCUSSION

Effect of nutrients on algae growth potential

The previous study has revealed that nitrogen and phosphate in the reservoir act as a source of nutrient in accelerating AGP (Marsha *et al.* 2020). The ideal algal growth requires at least three important nutrients of carbon (C), nitrogen (N), and phosphorus (P), with a ratio of C:N:P around 106:16:1. Among them, nitrogen and phosphorus are commonly determined as limiting factors in algal growth. However, there are still a lot of discrepancies on whether phosphorus or nitrogen is the dominant factor affecting algal growth. Figure 2 presents TN/TP ratios for six reservoirs. A ratio of TN/TP exceeding 16 indicated phosphorus limitation, indicating that algal growth was primarily constrained by TP availability (Li *et al.* 2022). In this study, most drinking reservoirs, such as Feitsui, Shihmen, and Chengqinghu, exhibited a TN/TP ratio higher than 16, while other reservoirs (i.e., Liyutan, Mingde, and Renyitan) showed a TN/TP ratio less than 16. According to Lin *et al.* (2022), the Feitsui reservoir demonstrated a consistently low AGP, remaining at oligotrophic levels based on long-term monitoring via CTSI investigation. In contrast, Mingde, Liyutan, and Renyitan reservoirs have been classified as either eutrophic or mesotrophic over the past decade. This indicated that TP significantly affect algal growth regardless of the trophic state. In other words, TP acted as a limiting factor in the AGP for most major drinking reservoirs in this study.

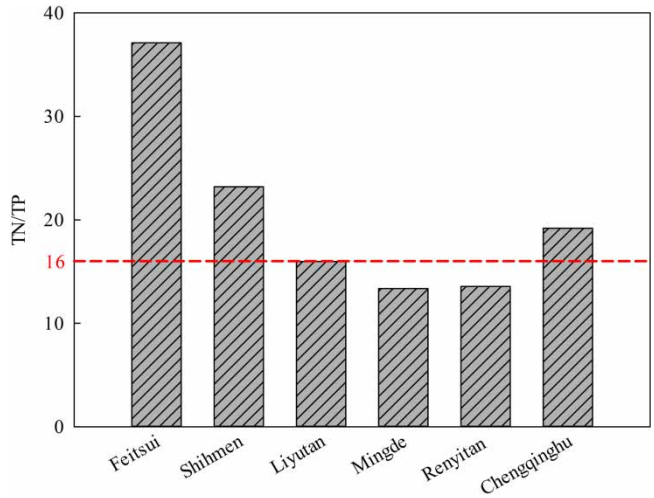


Figure 2 | TN/TP nutrient ratio for six reservoirs in Taiwan (dash red line is the ideal N:P ratio).

The concentration of Chl-a could be associated with the level of algal biomass density in reservoirs. The Chl-a/TP, representing an indicator of AGP attributed to TP for six reservoirs, was calculated to further understand the TP effect on AGP. Figure 3(a) shows that the trendline of TP concentration was highly correlated with Chl-a concentration for each reservoir. As TP concentration increased, it corresponded to an increase in Chl-a concentration. The lowest TP and Chl-a concentrations were found for the Feitsui reservoir, while the highest levels of TP and Chl-a occurred for the Chengqinghu reservoir. It indicated that the presence of higher TP leads to algal biomass proliferation because TP could enhance the concentration of the chlorophyll pigment which can be found in algae cells (Chen & Chen 2021). In addition, the fluctuation of Chl-a and TP varied at different seasons for each reservoir. As shown in Figure 3(b), Liyutan reservoir showed the highest mean value of Chl-a/TP compared to other reservoirs. This phenomenon could occur occasionally due to anthropogenic activity surrounding this reservoir. Two major factors, including household wastewater and tourist wastewater, significantly contributed to large variations in the Chl-a/TP ratio for Liyutan reservoir (Chen *et al.* 2019). In summary, the presence of TP as a limiting factor could cause a high AGP for drinking water reservoirs in Taiwan.

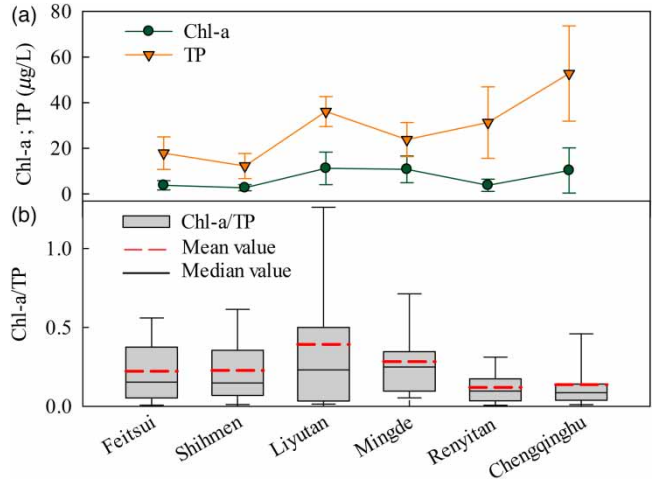


Figure 3 | Variations of Chl-a and total phosphorus at six reservoirs in Taiwan (a) and chlorophyll-a/TP ratio (b) as the important factor of algae biomass.

Correlation between ASS and phosphorous nutrient

In this study, the fraction of ISS, VSS, and ASS in water samples from six drinking water reservoirs was classified and measured, as shown in Figure 4, during a half-year investigation to further understand the composition of SS. It was revealed that Feitsui, Shihmen, Liyutan, and Mingde reservoirs contained a low proportion of SS and exhibited narrow SS variations (small standard deviation). However, Renyitan and Chengqinghu reservoirs showed enormous SS variations (large standard deviation). Based on our investigations, SS levels of reservoirs were primarily governed by topography and hydrology, particularly during typhoons and heavy rainfall, across various types of streams connected to the reservoirs. On-stream reservoirs (i.e., Feitsui, Shihmen, and Mingde), which lie directly on natural river channels, receive immediate SS input from runoff in mountainous terrain and frequent landslides. In contrast, off-stream reservoirs (i.e., Renyitan and Chengqinghu) depend on managed, pumped inflows, allowing operators to divert or halt intake during high-turbidity events. It is anticipated that off-stream reservoirs could exhibit lower and more consistent SS variations in stormy circumstances. This is why the high concentration of SS dominantly disturbs the algal counting of reservoirs by fluorescent probing in Taiwan. VSS contributed the lowest fraction for each reservoir regardless of ASS and ISS variations. At such a condition, the ASS fraction was much higher for Renyitan and Chengqinghu reservoirs compared to other reservoirs, which differed from the Chl-a variations in each reservoir, as shown in Figure 3(a). This indicated that the algal growth and trophic status could significantly vary with reservoirs based on the ASS fraction instead of the Chl-a concentration. In this study, ASS offered a cost-effective and rapid method to estimate algal biomass through gravimetric analysis without requiring pigment extraction or fluorescence calibration. By using this method, ASS included all algal-derived solids containing pigmented and nonpigmented biomass. Unlike ASS, Chl-a merely reflected pigment-based algal biomass. Thus, Chl-a as an indicator for algal counting is only valid for green pigment-rich algal species. In addition, estimating algae growth based solely on Chl-a concentrations could introduce bias, particularly in high SS-impacted reservoirs, as it only reflects pigment-based biomass and may overlook nonpigmented algae. Based on a quantitative assessment of ASS as a biomass indicator against Chl-a, a moderate-to-high level of error, with a root-mean-square error (RMSE) of 0.365 and relative percent error (RPE) of 20% was revealed. Likewise, the assessment of correlation between ASS and microscopic cell count showed a limited agreement, with RMSE and RPE values of approximately 0.566 and 30%, respectively. These elevated error metrics likely resulted from the interference of high SS levels in both Chl-a detection and microscopic enumeration. Under such a condition, ASS analysis remained unaffected by light scattering or signal distortion. Therefore, ASS is a robust and practical alternative indicator to assess total algal biomass during algal growth for drinking water reservoirs in this study.

The presence of high phosphate could also contribute to ASS accumulation. The highest TP contributed to the largest fraction of ASS, as shown in Figures 3(a) and 4. TSS concentration in each reservoir depends greatly on the surrounding anthropogenic and natural activity. Taiwan topography was primarily classified as fragile slates, and also the state of soil

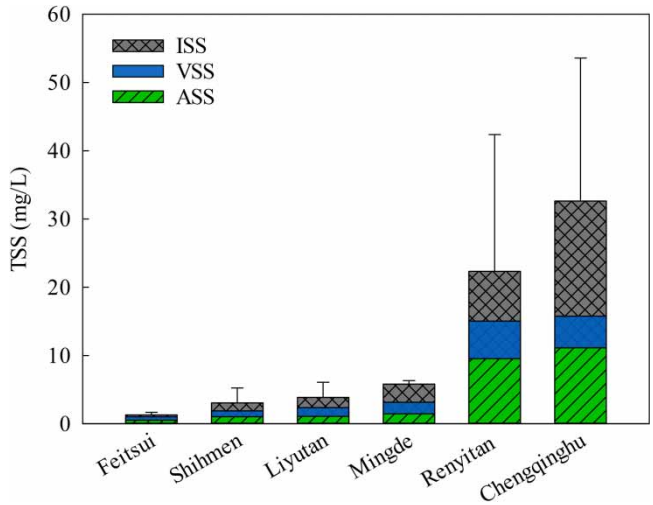


Figure 4 | TSS distribution for six major drinking water reservoirs in Taiwan.

with poor conservation allows gravel and sand upstream to be easily transported down to reservoirs by heavy precipitation, which then contributes to the majority of ISS. Moreover, the variations of endemic algal species had different growth rates, which eventually contributed to ASS in reservoirs (Hao *et al.* 2021). Algal growth rate has been reported to increase rapidly, while highly concentrated phosphorus is present in reservoirs in Taiwan (Lin *et al.* 2022). Owing to favorable environmental conditions and the presence of numerous biosynthetic pathways (including aromatics, alcohols, terpenoids, and others), organic substances in reservoirs are able to transfer and contribute to VSS. Furthermore, the water quality of reservoirs is easily affected by climate conditions, seasonal rainfalls, typhoons, and downpours. Under such a condition, stand-alone CTSI might fail to assess trophic status or AGP due to a significant increase in turbidity or ISS, resulting from the bias of SD reading. Proliferation of algae species in reservoirs also directly affects the classification of trophic status with CTSI because of the varied Chl-a concentration, specifically in high-level phosphorous. It implied that ASS could serve as an alternative indicator to evaluate AGP regardless of SS or TP variations for each reservoir.

As mentioned earlier, TP was an important nutrient that significantly contributed to the AGP for most drinking water reservoirs in Taiwan. In general, it contains orthophosphate, polyphosphate, and organophosphate in these water reservoirs. A previous study has reported that orthophosphate significantly affects the AGP because orthophosphate is preferable for the algal growth (Shi *et al.* 2011). Thus, a further investigation of the correlation between ASS and orthophosphate by Pearson regression was logarithmically plotted, as shown in Figure 5. Pearson's correlation analysis showed that ASS and orthophosphate were moderately correlated ($\gamma = 0.54$), indicating that the presence of orthophosphate in reservoirs would certainly improve the algal growth and correspond to algae biomass accumulation. It also demonstrated that high orthophosphate concentration in reservoirs aligned with the eutrophication status of reservoirs. For instance, Mingde and Chengqinghu reservoirs were classified as eutrophic status, and yet Feitsui reservoirs with low orthophosphate were classified as oligotrophic status (as shown in Figure S3). Our previous study has indicated that phosphorus concentration is highly correlated with Chl-a, which directly represents algal biomass and eutrophication status (e.g., Mingde and Chengqinghu are eutrophic reservoirs, and Feitsui is the oligotrophic reservoir) (Lin *et al.* 2022). ASS offered a cost-effective and rapid method to estimate algal biomass through the gravimetric analysis without requiring pigment extraction or fluorescence calibration. ASS included all algal-derived solids containing pigmented and nonpigmented biomass. Unlike ASS, Chl-a merely reflected pigment-based algal biomass. Thus, Chl-a as an indicator for algal counting is only valid for green pigment-rich algal species. In addition, using Chl-a would generate biases in the evaluation of algal growth, particularly in SS-impacted reservoirs. Instead, ASS is much more suitable for determining the total algal biomass to clearly understand the growth of various algae in drinking water reservoirs. Therefore, the variations in SS composition for drinking water reservoirs, especially for the ASS fraction, could significantly influence the trophic status determination. It is inevitable to rely on accurate cell counting for further monitoring of algal growth under varied SS impact.

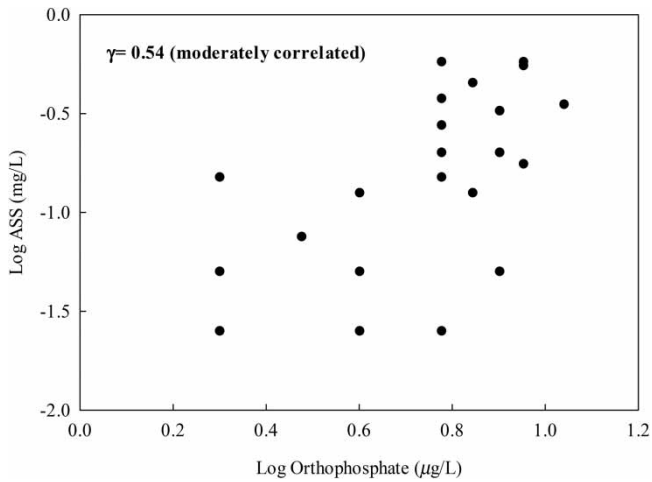


Figure 5 | Correlation between ASS and orthophosphate (total data number: 23).

Algal cell counting and Chl-a measurement by fluorescent probing

Algal cell enumeration and Chl-a measurement are essential components of reservoir monitoring to assess the eutrophication status. Previous studies have employed various methods for algal cell counting, including manual counting, fluorescence-based techniques, staining methods, and flow cytometry (Lee *et al.* 2015; Camacho-Fernández *et al.* 2018; Cheung *et al.* 2020). However, these methods are not applicable for *in situ* and portable measurements. Fluorescent-based measurements offers a relatively rapid *in situ* method for algal cell counting, which enables early warning for algal proliferation in reservoirs. Thus, most studies have focused on investigating the accuracy of fluorescent cell counting by quantifying its correlation with manual counting (Camacho-Fernández *et al.* 2018; Lundgreen *et al.* 2019). Figure 6(a) shows a correlation between microscopic enumeration and fluorescent measurements for algal cell counting on collected samples from six drinking water reservoirs. Microscopic enumeration consistently obtained higher algal densities compared with fluorescent measurement. This phenomenon was particularly pronounced during the winter season, where microscopic counts ranged from 10^4 to 10^6 cells/mL, while fluorescent measurement showed cell counts between 10^3 and 10^4 cells/mL (Figure S4a and b), indicating significant biases between microscopic enumeration and fluorescent measurements. This divergence suggested that fluorescent counting has inherent limitations in cell counting, potentially due to algal species overlapping with each other (Simis *et al.* 2012). Another contributing factor was the attenuation of fluorescence intensity caused by SS. As illustrated in Figure 4, ISS constituted the dominant fraction in collected samples from most of the drinking water reservoirs in this study. In Taiwan, the complex topography and variability in rainfall patterns often lead to a sudden increase in SS concentration where the turbidity could exceed 10,000 NTU at monsoon or typhoon events. Under these extreme conditions, optical

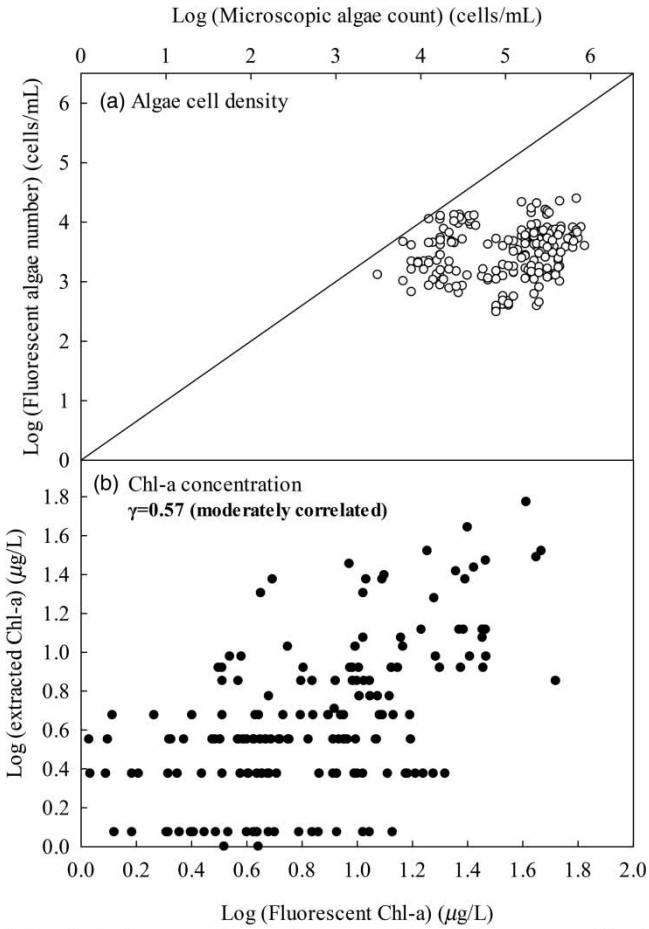


Figure 6 | Correlation between microscopic enumeration and fluorescent probing in terms of (a) algal counting and correlation between fluorescent probing and chemical extracting in terms of (b) Chl-a concentration with p-value <0.0001 and confidence level of 95%.

algal counting methods, such as fluorescence-based techniques and flow cytometry, are especially vulnerable to interference. High SS concentrations scatter and absorb light, leading to inaccurate fluorescence signals and distorted cell counts. As a result, the significant bias in algal counting occurs during monsoon/typhoon events, causing inaccuracies in algal monitoring.

Fluorescent measurement of the Chl-a content is principally based on the pigments from algae cells, even in the presence of high-level SS. The correlation between Chl-a concentrations measured via fluorescence and those obtained through chemical extraction was moderate ($\gamma = 0.57$), as shown in Figure 6(b). This level of correlation was strong enough to state that the Chl-a fluorescence-based approach is reliable for Chl-a detection in algae cells (Schober *et al.* 2018). On the other hand, seasonal variations could influence the fluorescence-based approach to Chl-a measurements. The correlation between fluorescent Chl-a and extracted Chl-a was moderate in winter ($\gamma = 0.56$) and weak in spring ($\gamma = 0.36$), as illustrated in Figure S4c and d. Meanwhile, temperature was found to be insensitive to the variation of both algal cell count and Chl-a measurement, as evidenced by PCA inference in Figure S5. In summary, the fluorescent probe could be used easily and powerfully for routine onsite algal monitoring (e.g., daily, weekly) due to its rapid ability to infer cell counts for groups of algal species though its measurement level was not close to microscopic counting. In contrast, microscopy observation was best suited to periodic algae identification and counting (e.g., monthly).

Chl-a measurement and algal species counting by onsite fluorescent probing

Numerous techniques have been developed to identify and count cells of algae species in waters, including a standard microscope, fluorescent-based method, Chl-a-based approach, and turbidity-based counter. Algae counting requires consideration of several essential issues, including counting time, the influence of a sample after counting, application, and affordability. It has reported that the conventional microscope method, with or without a hemocytometer, provides a high accuracy yet it is time consuming and causes subjectivity issues when counting algae species (Park *et al.* 2018). Flow cytometry, optical density, and fluorescent methods offer several advantages, such as rapid measurement and various data variations, but they require high costs for equipment procurement and maintenance (Liu *et al.* 2022). Several straining-based techniques rely on incredibly expensive dyes. In addition, limited equipment for algae counting can be used for the onsite measurement because those techniques are primarily intended for the laboratory work. Previous studies have revealed that the development of fluorescent devices incorporated various light-emitting diodes to magnify the measurement of algal species that are present differently in a reservoir (Garrido *et al.* 2019). In this study, the fluorescent instrument equipped with six LEDs was utilized to instantly measure diatom, green algae, and cyanobacteria onsite (Rosero-López *et al.* 2021). These algal species emitted various fluorescent spectral groups and were further built into fluorescent probes as references (Garrido *et al.* 2019).

To further verify the performance of fluorescent probing on algal counting, three representative reservoirs, including Feitsui, Liyutan and Chengqinghu, at various trophic statuses (i.e., oligotrophic, mesotrophic, eutrophic) were selected, respectively, to evaluate their algal species growth. Figure S6 shows a variation of algal species in three representative reservoirs that fluctuated during the field investigation from November to April. Algal cell density was lower during December to February, especially for Feitsui and Liyutan reservoirs. The algal density steeply increased from March to April afterward. This reduction in the algal cell density could happen due to the changes in temperature of reservoir water (i.e., thermal stratification) at different depths in the water column, as shown in Figure S7. This phenomenon was consistent with a previous study where Chl-a concentration increased at the epilimnion–hypolimnion region (Xu *et al.* 2019). It also contributed to a Chl-a concentration decline beneath the reservoir (Reiss *et al.* 2007; Li *et al.* 2012), as illustrated in Figure 7(a) and 7(b). However, the quantification of Chl-a concentration at Chengqinghu reservoir could be limited by the relative depth due to high TSS occurrence resulting from shadow water (below 3 m), as illustrated in Figure 7(c). Chengqinghu reservoir contains higher levels of turbidity compared to other reservoirs (Table S1). It is suggested that high turbidity could reduce Chl-a content in a specific depth due to lower light incidence (Nunes *et al.* 2022).

In Taiwan, a large amount of ISS is occasionally retained in specific drinking reservoirs, which strongly affected algal cell counting, which results in variations in algal abundance across different seasons. Therefore, a relative algal cell counting approach was proposed and implemented by fluorescent probe measurement, as shown in Figure 7(d)–7(f). It was observed that a relative algal cell count on selected species varied with reservoirs. Feitsui reservoir was categorized as oligotrophic status, and it contained two dominant algal species fractions (green algae and diatom) and a small amount of cryptophytes. In this situation, the relative algal cell count in each fraction was relatively constant. Furthermore, blue-green algae was not observed because of the limited TP nutrient in Feitsui reservoir, as proven in Table S2 and Figure S6. A previous study has

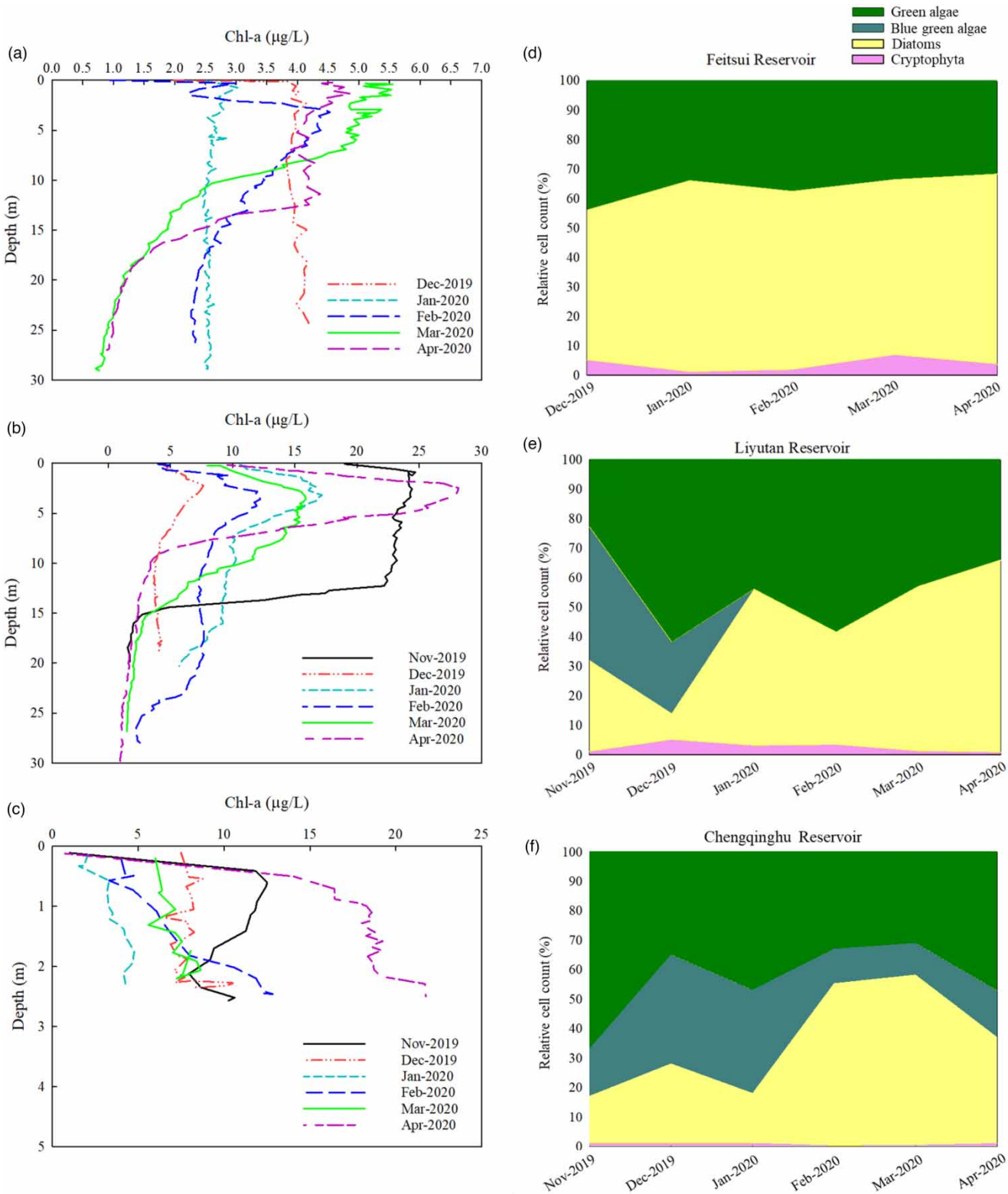


Figure 7 | Variations in Chl-a concentration and relative cell count of various algal species in Feitsui (a, d), Liyutan (b, e), and Chengqinghu (c, f) reservoirs (sampling from November 2019 to April 2020).

suggested that a reservoir with lower phosphate concentration is more favorable for green algae (*Chlorella sp.*) to grow up in reservoirs compared with blue-green algae (*Microcystis aeruginosa*) (Shi *et al.* 2011).

In contrast, the algal species observation on Liyutan reservoir showed that blue-green algae were present because the concentration of phosphate increased from November to January, which is similar to a previous study (Chen *et al.* 2019). Thereafter, the blue-green algae fraction decreased and was absent from January to April. On the other hand, the observation of the eutrophic Chengqinghu reservoir presented a relatively variable level of algae distribution fraction during an investigation. An obvious growth in blue-green algae fraction appeared during November to December, and then the growth varies from January to April, which could be attributed to concentrated TP formation in Chengqinghu reservoir, as shown in Table S1. Based on this 6-month field evaluation, the fluorescence probe was found to be a reliable and practical tool for onsite monitoring of algal species growth, even under conditions with variable ISS concentrations.

It is important to note that the reliability of fluorescence-based identification is influenced by the presence of unregistered or regionally unique algal taxa. As reported by Bertone *et al.* (2019), the response of sensor-based devices may lead to potential underestimation or overestimation of algal biomass. To minimize such biases, region-specific calibration datasets derived from lab-cultivated local algae should be developed and integrated into devices as new algal fingerprint databases. In addition, adaptive classification algorithms via those incorporating machine learning techniques can enhance probe accuracy by adjusting for pigment variability and spectral overlap during field measurements. These approaches are expected to allow fluorescence-based probes under multialgal groups and various SS conditions.

CONCLUSIONS

ASS obtained by the gravimetry method can be used as an alternative indicator to represent the total biomass of algal species in drinking water reservoirs with different trophic status (oligotrophic, mesotrophic, eutrophic) regardless of varied ISS. However, the ISS fraction had certain impacts on the accuracy of algal cell counting by Chl-a-based fluorescent probing compared with microscopic observation. In this regard, the data of cell counting by fluorescent probing were turned into 'relative algae count,' and it is powerful to compare the fractions among algal species (i.e., diatom, green algae, cyanobacteria, and cryptophyta) in three representative drinking water reservoirs based on the results of a half-year field investigation. In addition, TP was the major influencing factor for AGP in major drinking water reservoirs in this study, regardless of the eutrophication level, especially for blue-green algae growth. It was concluded that onsite fluorescent probing is a rapid and low-cost method with reasonable accuracy for algal species growth monitoring of drinking water reservoirs, even though the composition of SS varies significantly.

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AUTHORS' CONTRIBUTIONS

Jr-Lin Lin: conceptualization, resources, visualization, writing – original draft, writing – review and editing, validation, and supervision. Fahrudin Sidik: writing – original draft, writing and editing, methodology, and formal analysis. Angga Aji: writing – original draft, methodology, and formal analysis. Shyh-Fang Kang: conceptualization and supervision.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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