

Water Environmental Assessments for Bactericidal Effect of Copper Ions and Reduction Effect of Nutrients Inflow Loadings on Controlling Algal Blooms in a Hypertrophic Reservoir

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**Water Environmental Assessments for Bactericidal Effect of
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ABSTRACT

Many agricultural reservoirs have recently received excessive inflow loadings of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorous (DIP) resulting from fertilization of farmlands and have experienced serious eutrophic phenomena, such as the abnormal proliferation of phytoplankton. In particular, mat-like algae blooms flocculated on water surfaces have caused serious environmental problems, such as damaging landscapes, obstructing agricultural water use, generating fetid water, and damaging aquatic ecosystems, owing to a dysoxic or anoxic state derived from low transparency. Therefore, effective techniques for suppressing phytoplankton overgrowth are required for the environmental remediation and conservation of eutrophic water areas. This study presents significant insights regarding water environmental assessment for preserving agricultural reservoirs by focusing on two research approaches: a method for inhibiting algal overgrowth using the bactericidal effects of copper ions (Cu^{2+}) and the influence of nutrient inflow loading on the increase in phytoplankton chlorophyll-a (Chl-a).

In the first approach, water quality experiments were conducted using water tanks to examine the effectiveness of Cu^{2+} in controlling algal overgrowth under hypertrophic high levels of DIN and DIP while exploring the inhibitory effect on the increase in Chl-a level, the reduction effect of high phytoplankton concentrations, and the elimination effect of mat-like algal blooms. In the experimental condition characterized by low initial Chl-a level and high nutrient concentrations, Cu^{2+} concentrations over 200–400 $\mu\text{g/L}$ effectively suppressed the proliferation of cyanobacteria, which was more susceptible to its bactericidal action. However, high DIN and DIP concentrations promoted the growth of Chlorophyceae, which was insusceptible to the bactericidal action of Cu^{2+} . To adequately control algae under high nutrient concentrations, Cu^{2+} concentrations exceeding 600 $\mu\text{g/L}$ were necessary as the suitable concentration. In addition, for elevated initial concentrations of nutrients and Chl-a with a high composition ratio of cyanobacteria, it was possible to inhibit phytoplankton proliferation over a long period of approximately four weeks by adjusting the Cu^{2+} concentration to a high level of approximately 1000 $\mu\text{g/L}$. Although Chl-a increased owing to the overgrowth of Chlorophyceae four weeks or later after Cu^{2+} addition, a high-level supplementation of Cu^{2+} reduced nitrogen and phosphorous return rates from organic matter to available DIN and DIP for algal photosynthesis. Furthermore, cyanobacteria, which form

mat-like algal blooms on the water surface, was effectively eliminated by the bactericidal effects of Cu^{2+} in environments with extremely high concentrations of DIN and DIP. However, Chlorophyceae dominated the cyanobacteria because of interspecific competition owing to its non-susceptibility to bactericidal action, resulting in a notable increase in Chl-a levels under high concentrations of DIN and DIP, supplied by the extinction of the excessive cyanobacterial population. The first research approach provided significant outcomes by quantitatively evaluating the risks and advantages of applying the bactericidal effects of Cu^{2+} . Therefore, for the aquatic environmental remediation of hypertrophic water resources in agricultural areas, a hybrid countermeasure of reducing nutrient concentrations together with the bactericidal effect of Cu^{2+} is required to control algal blooms.

In the second approach, water environmental analyses in a hypertrophic nitrogen-limited reservoir were conducted based on field observations and numerical simulations to evaluate the influence of nutrient inflow loadings on the abnormal proliferation of phytoplankton. First, the periodic water quality survey focused on seasonal changes in the Chl-a level, the abundance ratio of the dominant algal class, and concentrations of DIN and DIP in the reservoir where long-term cyanobacterial blooms occurred at the water surface during autumn. The field observation results indicated that cyanobacteria particularly had an exceptionally sensitive response to the temporary supply of nitrate-nitrogen ($\text{NO}_3\text{-N}$) via the inflow loadings derived from heavy rainfall events while maintaining high DIP concentrations exceeding 0.1 mg/L, resulting in a significant increase in Chl-a level beyond 1000 $\mu\text{g/L}$. Therefore, the control of $\text{NO}_3\text{-N}$ elevation during the summer and autumn periods in nitrogen-limited eutrophic water bodies could link to one of the crucial countermeasures for inhibiting cyanobacterial blooms. Next, in the numerical simulation, the water quality prediction model based on the one-box-type ecosystem model was applied to scenario analyses to assess the extent to which reductions in inflow loadings of DIN and DIP influenced the inhibition of phytoplankton overgrowth. When simulating seasonal changes in Chl-a based on the ecosystem model, it is necessary to uniquely determine the parameters related to photosynthesis for seasonal changes, such that the dominant species would alter significantly at algal class-differentiated levels corresponding to water temperature and solar radiation. In this study, several temperature zones were set, and the optimal values of the model parameters for phytoplankton growth were determined for each zone. In particular, the difficulty of model application to the hypertrophic reservoir was solved by deciding the

dependencies on the water temperature, underwater light intensity of photosynthesis, and maximum algal growth rate, characterized by a dominant species corresponding to the water temperature zone. The validity of the proposed ecosystem model was confirmed because it could reproduce seasonal changes in Chl-a according to the abrupt appearance or disappearance of algal blooms in response to meteorological conditions for the observed results over two years. The scenario analyses provided the numerical target of reduction rates in the inflow loadings of DIN and DIP from the current situation for lowering Chl-a in the hypertrophic level (Ave: over 25 µg/L, Max: over 75 µg/L) to eutrophic level (Ave: 8–25 µg/L, Max: 25–75 µg/L) or mesotrophic level (Ave: 2.5–8 µg/L, Max: 8–25 µg/L). The outcome of the second approach demonstrates that the reduction of nitrogen in hypertrophic nitrogen-limited reservoirs has a substantially more pronounced effect on curbing phytoplankton growth than reducing phosphorus levels.

This research provides important knowledge on water environmental engineering for quantitatively assessing the bactericidal effect of Cu^{2+} on inhibiting mat-like algal blooms and the reduction effect of nutrient inflow loadings on preventing an increase in Chl-a levels to hypertrophic status in nitrogen-limited reservoirs. These results can be used to develop a technique for suppressing phytoplankton overgrowth and to integrate existing methods into a hybrid approach for more effective algal control in eutrophic agricultural reservoirs from excessive inflow of phosphorous and nitrogen. Therefore, they will contribute to the environmental remediation and conservation of regional water resources, ensuring sustainable agricultural development in rural areas.

Chapter 1 Introduction

In Japan, securing irrigation water for agriculture in low-lying farmlands is necessary because rivers exhibit limited storage capacities owing to their steep gradients, short flow paths, and small storage capacities. Therefore, closed water bodies such as lakes and reservoirs are important water resources for agricultural production activities (Thach et al., 2018). Many agricultural reservoirs have recently received excessive inflow loadings of nitrogen and phosphorous resulting from fertilization in farmlands and have experienced serious eutrophic phenomenon, such as cyanobacterial blooms (Paerl et al., 2018; Wurtsbaugh et al., 2019). Especially, cyanobacteria form harmful algal blooms which concentrate and float on the water surface like green color scum (Aparicio Medrano et al. 2016, Ostrovsky et al. 2020). Algal blooms can reduce underwater light intensity owing to solar light shielding at the water surface, inhibiting oxygen production through phytoplankton photosynthesis (Jahan et al., 2010; Chu et al., 2007). The overgrowth of phytoplankton leads to increased oxygen consumption via the respiration and aerobic decomposition of naturally dead algae (Huisman et al., 2018; Wurtsbaugh et al., 2019). Therefore, eutrophication in reservoirs causes a dysoxic or anoxic state because oxygen consumption exceeds oxygen production in the water bodies. In addition, prolonged anaerobic conditions can harm aquatic ecosystems, promote pathogenic bacteria, accelerate eutrophication owing to the elution of nutrients from the bottom, and generate toxic hydrosulfide and sludge accumulation resulting from metallic sulfide and undecomposed organic materials. Additionally, cyanobacteria-produced toxins severely damage aquatic ecosystems, and countermeasures against eutrophic phenomena, such as cyanobacteria bloom, are urgently needed for sustainable agricultural development in rural areas (Chia et al., 2018). Therefore, effective techniques for suppressing phytoplankton overgrowth are required for environmental remediation and conservation of eutrophic water areas.

The primary objective of countermeasures for the preservation of closed water bodies is to remove specific factors that cause water environmental issues. Eutrophication driven by increased nitrogen and phosphorous levels in water bodies from organic and inorganic inflows necessitates measures to reduce dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) through the implementation of water treatment, agricultural drainage, and sewage treatment facilities (Kadokami et al., 2021). Methods such as screening water surfaces with light-shielding floats (Gulden et al., 2019) and introducing ultrasound

(Xu et al., 2023) have been proposed to control algal blooms. While the float screening method has demonstrated advantages in aquatic environmental conservation and restoration in closed water bodies, challenges such as high initial costs and limited effectiveness against cyanobacterial blooms have been identified. Despite efficient algal bloom control, such as the removal of *Microcystis aeruginosa*, ultrasound increases water toxicity and greenhouse gas emissions (Xu et al., 2023). Therefore, developing a technique and integrating existing methods into a hybrid approach for more effective algal control in eutrophic water bodies is required, ensuring the sustainability and comprehensive improvement of the aquatic environment.

Chemical methods utilizing algaecides such as aluminum sulfate, copper sulfate, copper chelates, and potassium permanganate have been extensively employed to manage cyanobacteria in water reservoirs intended for human consumption (Xu et al., 2019). Among the various chemical algaecides, copper sulfate is most frequently utilized because of its cost-effectiveness, efficiency, relatively low risk to human health and the environment, and simplicity of application (García-Villada et al., 2004; Zhou et al., 2013). In freshwater environments such as lakes and reservoirs, Cu^{2+} naturally exist and serve as an essential trace element for aquatic organisms (Lu et al., 2015). Heavy metal ions, including Cu^{2+} , in waterbodies polluted with organic materials readily form chelate complexes with suspended organic matter, causing the ions to disappear from the water (Negishi and Matsugana, 1983; Grassi et al., 2000; Rader et al., 2019). The formation of an organic copper chelate complex masks the bactericidal properties of Cu^{2+} , which hinders algal growth. Previous studies have suggested that the apparent loss of Cu^{2+} owing to organic pollution significantly contributes to phytoplankton proliferation (Nanjo et al., 2000; Rader et al., 2019). Consequently, the masking effect of the bactericidal action, attributed to the coordination compounds of Cu^{2+} , contributes to the abnormal increase in phytoplankton in eutrophic reservoirs. Hullebusch et al. (2002) administered copper sulfate at a concentration of 63 $\mu\text{g/L}$ as Cu^{2+} from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to prevent the proliferation of cyanobacteria in a eutrophic lake in France. They showed that this treatment effectively controlled the growth of cyanobacteria throughout summer, leading to the disappearance of *Microcystis spp.* and enabling swimming activity in the lake during the tourist season. However, *Microcystis spp.* reappeared two months after treatment. Fawaz et al. (2018) conducted a study on a eutrophic irrigation canal that had algal proliferation during summer and found that applying copper chelates was necessary for adequate inhibition of overgrowth of high algal concentrations in the target

irrigation canal during periods of intensive algal proliferation. Although the bactericidal effect of Cu^{2+} has the potential to control phytoplankton overgrowth, studies in the field of water environment preservation and improvement are notably limited, and many uncertainties remain regarding the capacity to suppress cyanobacterial blooms by introducing Cu^{2+} solutions. Previous studies have primarily focused on using Cu to reduce high algal concentrations without considering its effects on inhibiting phytoplankton proliferation at low levels. Furthermore, although numerous studies have been conducted on eutrophic lakes, research on the inhibition of algal overgrowth in hypertrophic water bodies with long-term cyanobacterial blooms is lacking.

According to a trophic status classification based on the OECD standard (Organization for Economic Cooperation and Development 1982), many closed freshwater bodies can be classified eutrophic or even hypertrophic (Bhagowati et al. 2019). Lakes and reservoirs tend to receive over-nutrient overloads from DIN rather than DIP (Guildford & Hecky, 2000; Havens & Walker, 2002). So, limnology studies concerning water environmental conservation have primarily focused on eutrophic and hypertrophic water bodies in a phosphorus-limited state (Correll 1999). However, some lakes and reservoirs in rural areas tend to be in nitrogen-limited status because of an increase in inflow loading of DIP derived from excessive fertilizer application of phosphate on farmlands, resulting in the occurrences of prolonged cyanobacteria bloom (Downing & Mc Cauley 1992, Kotani 1992, Sharpley et al. 2006). In addition, due to climatic change, the inflow loadings of nutrients derived from agricultural activities would frequently increase in response to localized heavy rainfall, resulting in over-nutrient water areas (Hassanzadeh et al. 2019). Consequently, many lakes and reservoirs shift towards a hypertrophic state, representing the highest trophic level. In this state, the water bodies exhibit dense concentrations of algae and macrophytes, rendering them unsuitable for agricultural use and recreational activities.

Therefore, this study focused on urgent countermeasures to hypertrophication that are required to address inhibiting methods for algal bloom. The study presents significant insights regarding water environmental assessment for preserving agricultural reservoirs by focusing on two research approaches: a development of method for inhibiting algal overgrowth using the bactericidal effects of Cu^{2+} , and a quantitative estimation of the influence of nutrient inflow loading on the increase in phytoplankton chlorophyll-a (Chl-a). To achieve the research objectives, the selected water body was a hypertrophic reservoir with a long appearance of algal blooms on the water surface during autumn. The targeted

closed water body was a water resource for upland irrigation and was strongly affected by agricultural drainage from the farmland on the upstream. Especially, supply of nutrients via the inflow loadings derived from heavy rainfall events would increase Chl-a, and the targeted reservoir could be characterized by extremely high concentration of phosphorous and mat-like cyanobacteria blooms. Throughout this entire study, DIP is equivalent to $\text{PO}_4\text{-P}$. The detailed of study area would be explained in Chapter 2.

In the first approach, laboratory-scale water quality experiments were conducted to acquire fundamental knowledge regarding the feasibility and effectiveness of inhibiting cyanobacterial blooms through Cu^{2+} supplementation in organically polluted water bodies. The outcome of this laboratory experiment was described in Chapter 3. The specific objective of this chapter was to apply this technique as a countermeasure against the long-term appearance of cyanobacterial blooms in hypertrophic reservoirs resulting from the excessive inflow of phosphorous and nitrogen. To achieve the research aim, quantitatively assessing the efficacy of Cu^{2+} as a specific anti-eutrophication measure for restoring aquatic environments in hypertrophic water bodies is necessary. The experiments were carried out by monitoring water quality parameters such as Chl-a, DIN, DIP, and Cu^{2+} while cultivating phytoplankton in water tanks with a volume of 40 L under nutrient-rich conditions using artificial light irradiation. The experiments were divided into three series characterized by the initial concentrations of Chl-a, DIN, DIP, and Cu^{2+} according to the experimental purposes. The first objective was set as Series 1 to investigate the influence of Cu^{2+} concentration on the inhibition of phytoplankton growth from the initial low level under high concentrations of DIN and DIP. Second, a water quality experiment was conducted in Series 2 to estimate the reduction effect of phytoplankton concentration from the initial high Chl-a level with a high cyanobacteria composition ratio. Finally, the objective of Series 3 was to examine how much algal blooms flocculated on a water surface with a high Chl-a concentration, which would interfere with the bactericidal effects of Cu^{2+} under elevated levels of DIN and DIP.

In the second approach, water environmental analyses in a hypertrophic nitrogen-limited reservoir were conducted based on field observations and numerical simulations to evaluate the influence of nutrient inflow loadings on the abnormal proliferation of phytoplankton. First, the field observations were conducted to understand the actual status of water environment in the hypertrophic agricultural reservoir from the viewpoint of the seasonal change characteristics of Chl-a concentration according to meteorological factors.

The aquatic environment was estimated by not only the general water quality parameters related with eutrophication, including Chl-a, DIN, and DIP, but also the species composition of phytoplankton at algal class level. To grasp the reality in hypertrophic reservoir, this study focused on the influence of increase in the phytoplankton amount on other water quality dynamics from viewpoint of organic polluted phenomena represented as an anoxic water generation at the bottom. Following this, the influences of nutrient inflow loading on the water environment were numerically estimated using the one-box type ecosystem model, recognized as one of the most typical and fundamental water quality prediction models. The outcome of this research approach was described in Chapter 4. In this study, when simulating seasonal changes in Chl-a based on the ecosystem model, it is necessary to uniquely determine the parameters related to photosynthesis for seasonal changes, such that the dominant species would alter significantly at algal class-differentiated levels corresponding to water temperature and solar radiation. In this study, several temperature zones were set, and the optimal values of the model parameters for phytoplankton growth were determined for each zone. In particular, the difficulty of model application to the hypertrophic reservoir was solved by deciding the dependencies on the water temperature, underwater light intensity of photosynthesis, and maximum algal growth rate, characterized by a dominant species corresponding to the water temperature zone. This proposed ecosystem model was applied to scenario analyses to assess how much the reduction of inflow, and initial loadings of DIN and DIP influence the inhibition of phytoplankton overgrowth. The outcomes from these scenario analyses would play a vital role in establishing a specific numerical target for the aquatic environment restoration of hypertrophic reservoirs.

Finally, general conclusions and recommendations were pointed out from the viewpoints of water environmental engineering for quantitatively assessing the bactericidal effect of Cu^{2+} on inhibiting mat-like algal blooms. In addition, the reduction effect of nutrient inflow loadings on preventing an increase in Chl-a levels to hypertrophic status in nitrogen-limited reservoirs in Chapter 5. The outcomes in this study would be used to develop a technique for suppressing phytoplankton overgrowth to contribute to the environmental remediation and conservation of regional water resources, ensuring sustainable agricultural development in rural areas.

Chapter 2 Study Area

The targeted closed water body is the No. 7 regulating reservoir (**Figure 2.1**) constructed in 2017 in the Ito Campus of Kyushu University, Japan. This reservoir, where the bottom is flat without a slope and water depths are almost uniform, has a maximum water depth of approximately 4.5 m, a water surface area of ca. 2 700 m², and a capacity of ca. 12 150 m³ under a water-filling state, and it is used as a water resource for upland irrigation. Because the benefited farmland is located on the upstream side, a storage pump is installed at the bottom bed in the reservoir to supply the irrigation water. Additionally, the targeted reservoir has two spillways and one inflow entrance for extrinsic water. In the former, the reservoir's water flows out by overflowing broad-crested weirs with a width of 0.45 m, as shown in **Figure 2.1C**. The latter means that agricultural drainage from the farmland on the upstream side flows into this reservoir through a box culvert used as a closed conduit drain channel. The targeted reservoir, subject to a robust influx of nutrients, particularly due to the extensive fertilization of the farmland upstream, receives a substantial nutrient load through the box culvert during rainfall.

Kobayashi et al. (2019) reported the water environmental characteristic of the targeted reservoir via the weekly water quality observations from May to September in 2018 as follows. The No. 7 regulating reservoir was characterized by high concentrations of Chl-a (Ave.: 38.2 µg/L and Max.: 189.3 µg/L), total nitrogen (TN) (Ave. 1.23 mg/L), and total phosphorous (TP) (Ave. 0.17 mg/L). Also, the mean transparency was 1.1 m with the lowest value being 0.3 m. Therefore, the observations of TP, Chl-a, and transparency indicated that the targeted reservoir was in a hypertrophic state. This is the highest state in the trophic status classification based on the OECD standard and can be detailed further later on. The seasonal variations in Chl-a were categorized as a period of low concentration from May to July, growth from August to early October, and decline beginning from mid-October, with cyanobacteria being dominant during each period. Chl-a increased linearly from August to early October with an increasing rate of 0.7 µg/(L d). The increase of Chl a was attributed to the presence of optimal environmental conditions for the dominant cyanobacteria, such as high-water temperatures and high levels of solar radiation (Robarts & Zohary 1987). In addition, the fact that the N/P ratio (N: nitrogen, and P: phosphorus) remained in the range of 10 to 18 starting from August indicated that nitrogen limitation was relaxed compared to the period of low Chl a.

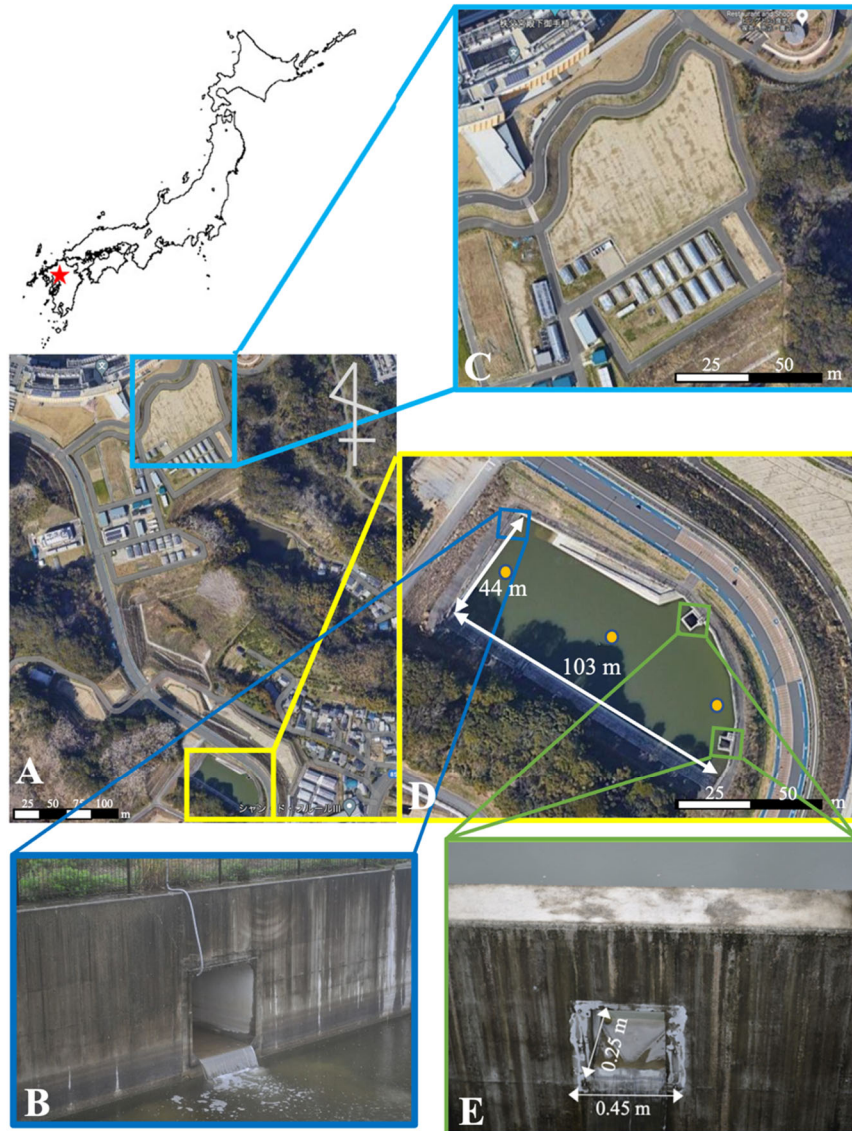


Figure 2.1 The location of the No. 7 regulating reservoir at Ito campus, Kyushu University, Japan (**A**: on campus agricultural field, **B**: a box-culvert, **C**: upstream farmland, **D**: target reservoir, **E**: two spillways)

The effect of the nitrogen supply to the waterbody through the box culvert during rainfall in early July on the increase in Chl-a concentration manifested since early-August. The changes in DIN over time indicated the selective uptake of $\text{NO}_3\text{-N}$ over $\text{NH}_4\text{-N}$ as a nitrogen source for photosynthesis. In particular, as cyanobacteria can prefer $\text{NO}_3\text{-N}$ for photosynthesis, relaxation of the nitrogen limitation due to $\text{NO}_3\text{-N}$ was considered as one of the causes for the increase in Chl-a levels. During the same period, cyanobacterial Chl-a exceeded $100\text{ }\mu\text{g/L}$ was followed by extensive algal blooms for approximately 1 month as shown in **Figure 2.2**. As a result of the increase in phytoplankton, DO became supersaturated in the surface layer of the water with concentration exceeding 200 %. In addition, transparency was extremely low at 0.3 m due to the overgrowth of phytoplankton in the surface layer, indicating that the waterbody had experienced insufficient underwater light conditions during summer. Furthermore, despite a shallow water depth of approximately 2 m from August to October, the area near the bottom was strongly reductive with ORP (oxidation-reduction potential) $< -150\text{ mV}$ due to anoxia. Such strong reductive state might lead to generation of hydrogen sulfide as well as elution of $\text{PO}_4\text{-P}$ and $\text{NH}_4\text{-N}$ from bottom sediment.

Since the reservoir's initial impoundment in 2017, the high Chl-a concentration, indicative of algal bloom, has been identified by observing the color at the water surface. Additionally, numerous phytoplankton in this reservoir adversely impacted water utilization for upland irrigation, as documented in the following manner. The reservoir's water, including high Chl-a concentration, is pumped up and temporarily stored in an underground embedded farm pond to supply the irrigation water forward on the upstream side. Many organisms attributed from algal death in a light-shielding farm pond caused extreme oxygen consumption, resulting in the generation of hydrogen sulfide under anaerobic conditions with



Figure 2.2 Cyanobacteria blooms observed in 2018
(left: mid-September, and right: early-October)

a strong reductive state. The high sulfide concentration (over 150 $\mu\text{g/L}$) has been detected with high frequency in the benefited farmland. In the targeted reservoir, the organic pollution confirmed grey sludge deposition over a wide area at the bottom. Irrigation water with high sulfide concentrations can harm plants by interfering with nutrient uptake, causing damage to the root system, and reacting with metal ions to form toxic metal sulfides (Lamers et al. 2013; Zhang et al. 2017). Therefore, it is urgent to suppress the proliferation of phytoplankton in agricultural reservoirs from the viewpoint of harmful influence on irrigation water use.

Chapter 3 Experimental Study on the Control of Algal Overgrowth in a Hypertrophic State Using Bactericidal Effect of Copper Ions

3.1 Introduction

One of the aquatic environmental issues in closed freshwater bodies, such as lakes and reservoirs, is the abnormal proliferation of phytoplankton associated with eutrophication. In recent years, eutrophication has worsened, leading to the deterioration of the aquatic environment under the hypertrophic state, causing the long-term appearance of cyanobacteria blooms throughout the water body. Upon the consideration of environmental remediation and conservation in the eutrophic water area, effective technology for water quality improvement to suppress the overgrowth of phytoplankton is urgently needed. The loss of Cu^{2+} due to the formation of complexes with suspended organic materials may be an important causal factor of phytoplankton proliferation in organically polluted water bodies. (Yunkun et al., 2024) Generally, Cu^{2+} exists in water bodies under natural environmental conditions and is an essential trace element for numerous aquatic organisms. Meanwhile, Cu^{2+} possesses bactericidal properties and inhibits the growth of microorganisms, including phytoplankton. Therefore, the masking effect of the bactericidal action of Cu^{2+} due to coordination compounds leads to algal overgrowth in eutrophic water bodies.

This study focused on algal control by employing the bactericidal effects of Cu^{2+} . In this context, “algal control” encompasses inhibiting the increase in phytoplankton from low levels, reducing high algae concentrations, and eliminating mat-like cyanobacteria blooms. The specific objective of this study was to apply this technique as a countermeasure against the long-term appearance of cyanobacterial blooms in hypertrophic reservoirs resulting from the excessive inflow of phosphorous and nitrogen. To achieve the research aims, quantitatively assessing the efficacy of Cu^{2+} as a specific anti-eutrophication measure for restoring aquatic environments in hypertrophic water bodies is necessary. Laboratory-scale water quality experiments were conducted to acquire fundamental knowledge regarding the feasibility and effectiveness of inhibiting cyanobacterial blooms through Cu^{2+} supplementation in organically polluted water bodies.

3.2 Materials and methods

3.2.1 Outline of water quality experiments

Water quality experiments were conducted using water samples from the surface area of the No. 7 regulating reservoir, which is in a hypertrophic state. After adjusting the concentrations of Cu^{2+} , Chl-a, phosphate-phosphorus ($\text{PO}_4\text{-P}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), and ammonium-nitrogen ($\text{NH}_4\text{-N}$) in the water tank according to the experimental conditions, experiments were performed by monitoring the changes in water quality parameters related to eutrophication over time. The target reservoir in 2020 was characterized by high concentrations of Chl-a (Ave. 108.3 $\mu\text{g/L}$), TN (Ave. 3.12 mg/L), and TP (Ave. 0.57 mg/L), and a cyanobacterial bloom appeared over a wide range on the water surface, with a high Chl-a concentration of over 1000 $\mu\text{g/L}$ from September to November (**Figure 3.1**). Therefore, it was possible to easily prepare environmental water in which the Chl-a concentration was significantly high owing to the abnormal growth of cyanobacteria. In this study, experimental conditions were determined based on water quality data observed from spring to autumn (April to November) in 2020 to determine the feasibility and effectiveness of controlling phytoplankton overgrowth using the bactericidal effects of Cu^{2+} in a hypertrophic water body.

The laboratory experiments at the water tank scale largely consisted of three series of experiments. Depending on the research objective of each series, water samples will be collected at different times of the year for each series. The water samples were collected in different seasons: Series 1 in spring, Series 2 in summer, and Series 3 in autumn. Temporal changes in water quality were monitored using light irradiation for phytoplankton photosynthesis as the following preparation. First, the sampled environmental water was divided into 2–6 rectangular water tanks ($0.6\text{ m} \times 0.3\text{ m} \times 0.36\text{ m}$, $L \times W \times D$) with a volume



Figure 3.1 Cyanobacteria blooms in the targeted agricultural reservoir

of 40 L. Next, the concentrations of Cu^{2+} , DIP ($= \text{PO}_4\text{-P}$), and DIN ($= \text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) were respectively adjusted using a copper sulfate aqueous solution (CuSO_4 solution), sodium dihydrogen phosphate aqueous solution, and a mixed solution of ammonium chloride and sodium nitrate in which the ratio of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ was approximately 1.0. The sampled water in the rectangular water tanks was kept in a thermostatic temperature-controlled room at a temperature of 25 °C to prevent the water temperature from becoming a limiting factor of the photosynthesis for Chlorophyceae and cyanobacteria, which were dominant algal classes in the target reservoir. In addition, each water tank was covered with four fluorescent lamps to irradiate light immediately above the water's surface (**Figure 3.2**). Fluorescent lamps (FL20SBR-A, NEC) were used as artificial light sources for growing ornamental aquatic plants. The underwater light intensity in each tank was set to the light quantum of approximately $100 \mu\text{mol}/(\text{m}^2 \text{ s})$ using four fluorescent lamps and water quality experiments with a 24-hours cycle of 12 hours on/12 hours off for approximately eight weeks. According to LED irradiation experiments (Honglikith et al., 2022), photosynthesis in phytoplankton, mainly composed of Chlorophyceae, is not strongly inhibited by a light intensity of approximately $100 \mu\text{mol}/(\text{m}^2 \text{ s})$; however, its light quantum is not always the most suitable lighting condition for cyanobacterial photosynthesis. Despite this limitation, $100 \mu\text{mol}/(\text{m}^2 \text{ s})$ is the highest light intensity could be set. Therefore, four fluorescent lamps were used to replicate the optimal environmental conditions for algae growth, similar to their natural habitat.

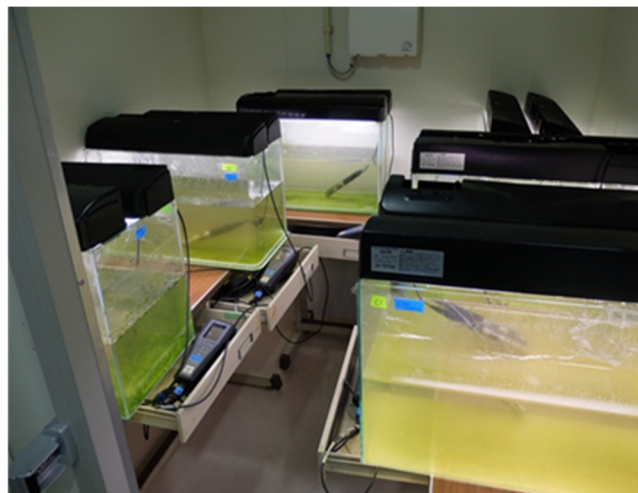


Figure 3.2 Water quality experiments in water tank

3.2.2 Water quality monitoring

Water quality parameters related to organic pollution and eutrophication were measured periodically at two- or three-day intervals for approximately eight weeks after

setting the initial experimental conditions. After sufficiently stirring experimental water in the water tanks and ensuring a uniform phytoplankton concentration, samples were collected for measurements of water quality parameters: Chl-a, class-differentiated *in vivo* Chl-a, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, TN, TP, Cu^{2+} , dissolved organic carbon (DOC), and total organic carbon (TOC). This study used TN, TP, DOC, and TP as reference data.

The analytical methods for the major water quality parameters were as follows. Chl-a was analyzed using a fluorometer (Aquaflour, TURNER DESIGNS) via solvent extraction of chlorophyll with N,N-dimethylformamide. In addition, *in vivo* Chl-a concentrations in class-differentiated algae were measured using a multi-wavelength excitation fluorometer (FluoroProbe, bb-Moldaenke). In this analysis, the class composition of phytoplankton was roughly classified into four algal classes: Chlorophyceae, cyanobacteria, diatom/dinoflagellates, and cryptophytes based on the fluorescence excitation spectra obtained by exposing chlorophyll to the light-emitting diodes of six different wavelengths (Beutler et al., 2002). Because the excitation spectra of diatoms and dinoflagellates are similar and cannot be distinguished, total Chl-a was the sum of their concentrations. $\text{NH}_4\text{-N}$ was measured using a colorimetric titration-type ammoniacal nitrogen meter (Quick Ammonia AT-2000, Central Kagaku Corporation), and ion chromatography (Dionex Integrion HPIC, Thermo Fisher Scientific K.K.) was used to measure $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$. In addition, Cu^{2+} was measured via porphyrin detection using an absorption spectrophotometer (DR5000, HACH).

In addition to the scheduled measurements, continuous dissolved oxygen (DO) measurements were performed at 20-min intervals using a fluorescent DO meter (ProODO, YSI) in each tank. As shown in the measured results in the figures below, a change in DO with time showed a fluctuation of a 24-hours cycle according to illumination with 12 hours on/12 hours off. That is, DO increased due to the photosynthesis of phytoplankton during the lighting period and decreased due to plankton respiration and the decomposition of organic matter during the non-lighting period. When the Chl-a concentration was as high as the eutrophic status, the amplitude of the DO fluctuation was an extremely large value, and it was regarded as an oxygen production rate (a photosynthetic rate). Therefore, the continuous measurement of DO aims to examine the controlling effect of phytoplankton growth by additional Cu^{2+} through the amplitude of daily fluctuations.

3.2.3 Experimental conditions

This study focused only on the feasibility of controlling the overgrowth of phytoplankton by the bactericidal action of Cu^{2+} as a basic approach and did not consider the influence of irrigation water and aquatic life on plant cultivation in water bodies. Therefore, the Cu^{2+} concentrations were set over a wide range, and the upper limit was determined based on the following considerations: In the Water Pollution Control Law of Japan, copper and its compounds are not designated as hazardous materials in general effluent and environmental quality standards concerning the protection of human health. In addition, the copper level is legally controlled to be less than 3 000 $\mu\text{g/L}$ as a common count in a general effluent standard and less than 1 000 $\mu\text{g/L}$ as the tap water quality standard. Considering the above-described legal restrictions concerning Cu, the upper limit of the concentration range was not over 1 000 $\mu\text{g/L}$, and its lower limit was 50–100 $\mu\text{g/L}$.

Water quality experiments with a 24-hours irradiation cycle were conducted for three series with different initial conditions for three parameters: Chl-a, nutrients ($\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$), and Cu^{2+} . When preparing the environmental water in the tanks, the initial concentrations of Chl-a and nutrients were determined as specialized trophic conditions according to the experimental objective in each series, and the initial Cu^{2+} was adjusted in multiple conditions in each series from the perspective of the abovementioned lower and upper limit concentrations. Each series had 4–6 cases, including a control condition, and these cases were characterized by the initial Cu^{2+} concentration. The initial concentration of nutrients in each series was set to a relatively high level because it was assumed that the water quality environment in the tank to be the actual targeted reservoir, where DIN and DIP concentrations could increase due to excessive inflow loading of an inorganic material for fertilizers. The initial conditions concerning water quality are summarized in **Table 3.1**. The experimental objective for each series was as follows:

Series 1 was set to examine how much Cu^{2+} would influence the inhibitory effect on the overgrowth of phytoplankton resulting from the high DIN and DIP concentrations and was divided into five cases where the initial Cu^{2+} concentrations varied in the range of zero (control) to approximately 700 $\mu\text{g/L}$ (upper limit). The initial DIP concentration was adjusted to a notably high level (approximately 0.1 mg/L according to the water environmental characteristics in the targeted reservoir, and the initial DIN concentration

Table 3.1 Initial conditions of water quality experiments

Experiments		Cu ²⁺ (µg/L)	NH ₄ -N (mg/L)	NO ₃ -N (mg/L)	DIN (mg/L)	PO ₄ -P (mg/L)	Chl-a (µg/L)	B-ratio (%)
Series 1	control	0.0	0.75	0.50	1.25	0.10	2.1	20.2
	1	75.5	0.70	0.48	1.18	0.10	1.6	53.9
	2	245.0	0.70	0.48	1.18	0.10	1.1	30.7
	3	375.0	0.70	0.48	1.18	0.07	1.1	31.6
	4	677.5	0.70	0.48	1.18	0.06	1.6	29.4
Series 2	control	0.0	0.10	1.44	1.54	0.02	156.2	85.0
	1	131.5	0.10	1.40	1.50	0.02	176.6	88.5
	2	344.0	0.11	1.40	1.51	0.02	159.4	85.0
	3	932.5	0.09	1.41	1.50	0.02	155.8	88.1
	1 (control)	0.0	0.51	2.01	2.52	0.16	69.7	71.1
Series 3	1 st ; 1	230.0 2 nd ; 305.0	0.50	1.95	2.45	0.16	60.2	70.3
	2 (control)	0.0	0.49	1.94	2.43	0.16	214.7	81.4
	1 st ; 2	229.0 2 nd ; 430.0	0.49	1.94	2.43	0.15	185.9	81.5

B-ratio: abundance ratio of cyanobacteria

was determined such that the N/P ratio (=TN/TP) was approximately 12.0 to make the trophic condition of neither the nitrogen-limitation nor phosphorous-limitation for algal growth. In addition, the initial Chl-a concentration was set to a relatively low level of less than 2 µg/L by sampling the environmental water before the cyanobacteria increased and formed a bloom. Therefore, the experimental aim of Series 1 was to examine the amount of Cu²⁺ that could effectively inhibit cyanobacterial blooms derived from sufficient nutrient concentrations. Unlike Series 2 and 3, the water quality experiments in Series 1 were conducted under initial conditions such that the Cu²⁺ concentration would hardly decrease owing to the adsorption of metal ions to suspended particulate organic matter, including phytoplankton, and the Cu²⁺ concentration calculated from the amount of added CuSO₄ solution would be substantially coincident with the actual concentration.

Next, water quality experiments labeled Series 2 and 3 were conducted to examine the reduction effect of the high concentration of phytoplankton, in which cyanobacteria existed in the initial sampled water as the dominant species with a high composition ratio. The initial condition for Series 2 was a high Chl-a concentration of approximately 160 µg/L, which was divided into four cases with different Cu²⁺ concentrations. Because the

initial Chl-a level in Series 2 was much higher (over 100 g/L) than that in Series 1, the maximum initial Cu^{2+} concentration in this series was set to approximately 1000 $\mu\text{g/L}$. In Series 2, the initial condition of the water did not have algal blooms, unlike in Series 3. When adjusting the Cu^{2+} concentration in the water tank to a targeted level in Series 2, the actual added amount of CuSO_4 solution was required to exceed the calculated addition amount because some of the added Cu^{2+} disappeared owing to the formation of an organic copper chelate complex with suspended organic matter, unlike Series 1. Series 3 was an extended experiment of Series 2 to examine eliminating the effects of cyanobacteria blooms under high levels of DIN and DIP, as well as the effect of decreasing the high phytoplankton concentration to a low level. To acquire a basic understanding concerning the control of cyanobacteria, this study aimed to examine how much of the mat-like algal bloom that flocculated on a water surface with a high Chl-a concentration interfered with the bactericidal effects of the provided Cu^{2+} . To quantitatively examine the influence of Chl-a concentration on the eliminating effects of phytoplankton over a long period of eight weeks, Series 3 was conducted under two different conditions of the initial Chl-a being set to an intermediate level of approximately 60 $\mu\text{g/L}$ (Series 3-1), and a high level of approximately 200 $\mu\text{g/L}$ (Series 3-2). The experimental water in Series 3-1 was prepared by diluting the environmental water in Series 3-2, which was obtained by directly sampling the cyanobacterial bloom generated on a large scale in autumn. Because the initial phytoplankton in Series 3 accumulated only on the water surface as a mat-like bloom, the Chl-a concentration in the entire water tank in Series 3 was slightly higher than that in Series 2. Similarly, as in Series 1, the DIN and DIP concentrations in Series 2 and 3 were adjusted such that the N/P ratio was approximately 10–12.

As described in the next section, Series 2 results showed that the elimination of a large amount of phytoplankton required a high Cu^{2+} concentration. Therefore, the experimental conditions regarding the Cu^{2+} concentration in Series 3 were divided into the control (zero) and a high concentration with an upper limit of approximately 1 000 $\mu\text{g/L}$. Series 3 focused on the timing of the addition of Cu^{2+} to the water tank as a specialized experimental condition. Unlike the other series, the concentration of Cu^{2+} in Series 3 was adjusted in two steps: at the beginning of the water quality experiment and after four weeks. Although the CuSO_4 solution was added to the environmental water in the tank such that the calculated Cu^{2+} concentration was approximately 500 $\mu\text{g/L}$ at each

step, the actual concentration was approximately 200–400 $\mu\text{g/L}$ as shown in **Table 3.1**. The low concentration of Cu^{2+} measured in the water tank compared to the calculated concentration resulted in the formation of an organic copper chelate complex with a scum-like algal bloom. As in Series 2, some of the Cu^{2+} seemed to disappear because of chelate complexes with suspended organic matter. However, an excessive addition amount of the CuSO_4 solution was required because a large number of suspended organic matter resulting from cyanobacterial blooms would significantly decrease the concentration of Cu^{2+} , and it was practically impossible to adjust the actual Cu^{2+} concentration to approximately 500 $\mu\text{g/L}$, unlike in Series 2.

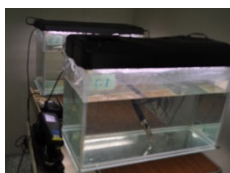
3.3 Results and discussions

The inhibitory effects of Cu^{2+} on phytoplankton growth were examined by considering the initial conditions of Chl-a, Cu ions, and nutrient concentrations based on the measured results, as shown in **Figures 3.3–3.12**, where t denotes the time elapsed from the start of the water quality experiments (after adjusting the Cu^{2+} concentration in the water tanks). The photographs shown in **Figure 3.3** are vertically arranged in four rows from top to bottom to roughly comprehend the time course of phytoplankton appearance in Series 1 to 3. In addition, photographs in each row show, from the left side to the right side, the initial, intermediate, and final statuses in the control condition and two statuses at the end of the first and last halves in the case with the highest concentration of initial Cu^{2+} .

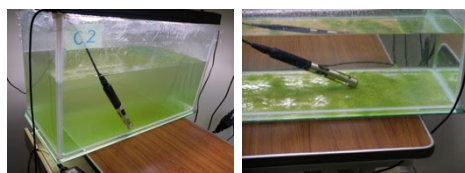
3.3.1 Inhibitory effects on algal overgrowth under notably high nutrient concentrations

Figure 3.4 shows the changes in DO with time obtained by continuous monitoring. Algal growth rates by photosynthesis were quantitatively estimated by focusing on the amplitude of daily fluctuations in DO. **Figure 3.5** shows the periodic monitoring results of the Chl-a concentration and class-differentiated composition ratios in phytoplankton. **Figure 3.6** shows the temporal changes in nutrient ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$) and Cu^{2+} concentrations obtained by periodic monitoring.

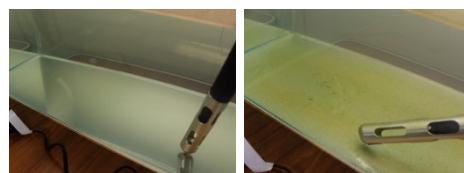
The effects of Cu^{2+} on restraining the overgrowth of phytoplankton at the eutrophic level with high nutrient concentrations (DIN: 1.2 mg/L, DIP: 0.09 mg/L) are discussed by comparing five cases in Series 1, including the control condition. In Series 1, Chl-a at $t = 0$ d was set to a very low concentration (approximately 2.0 $\mu\text{g/L}$), as shown in the initial status in **Figure 3.5**.



Initial status ($t = 0$ d)



Control (left: $t = 31$ d, right: $t = 59$ d)

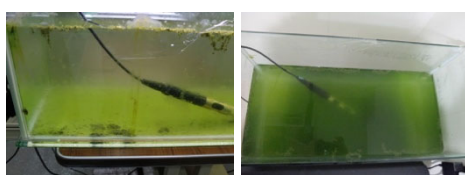


Series 1-4 (left: $t = 31$ d, right: $t = 59$ d)

(1) Control in Series 1 and Series 1-4



Initial status ($t = 0$ d)



Control (left: $t = 32$ d, right: $t = 60$ d)



Series 2-3 (left: $t = 32$ d, right: $t = 60$ d)

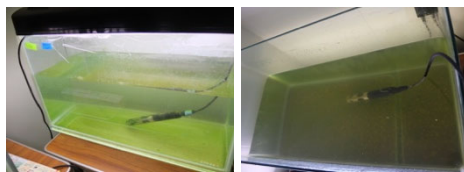
(2) Control in Series 2 and Series 2-3



Initial status ($t = 0$ d)

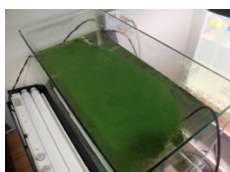


Control (left: $t = 28$ d, right: $t = 56$ d)



Series 3-1 (left: $t = 28$ d, right: $t = 56$ d)

(3) Series 3-1 and its control



Initial status ($t = 0$ d)



Control (left: $t = 28$ d, right: $t = 56$ d)



Series 3-2 (left: $t = 28$ d, right: $t = 56$ d)

(4) Series 3-2 and its control

Figure 3.3 Appearance of phytoplankton in the initial, intermediate, and final statuses in Series 1, 2 and 3

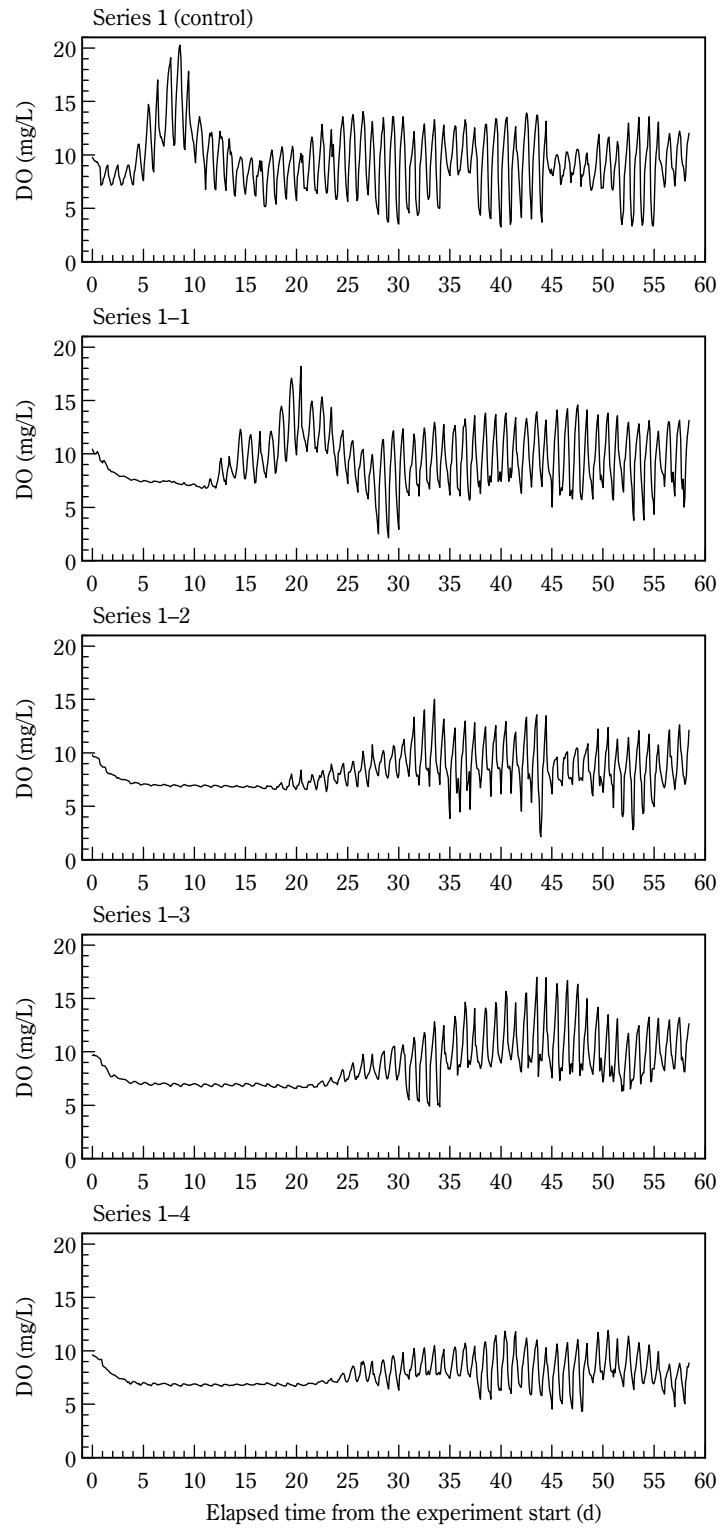


Figure 3.4 Continuous measurements of DO in Series 1

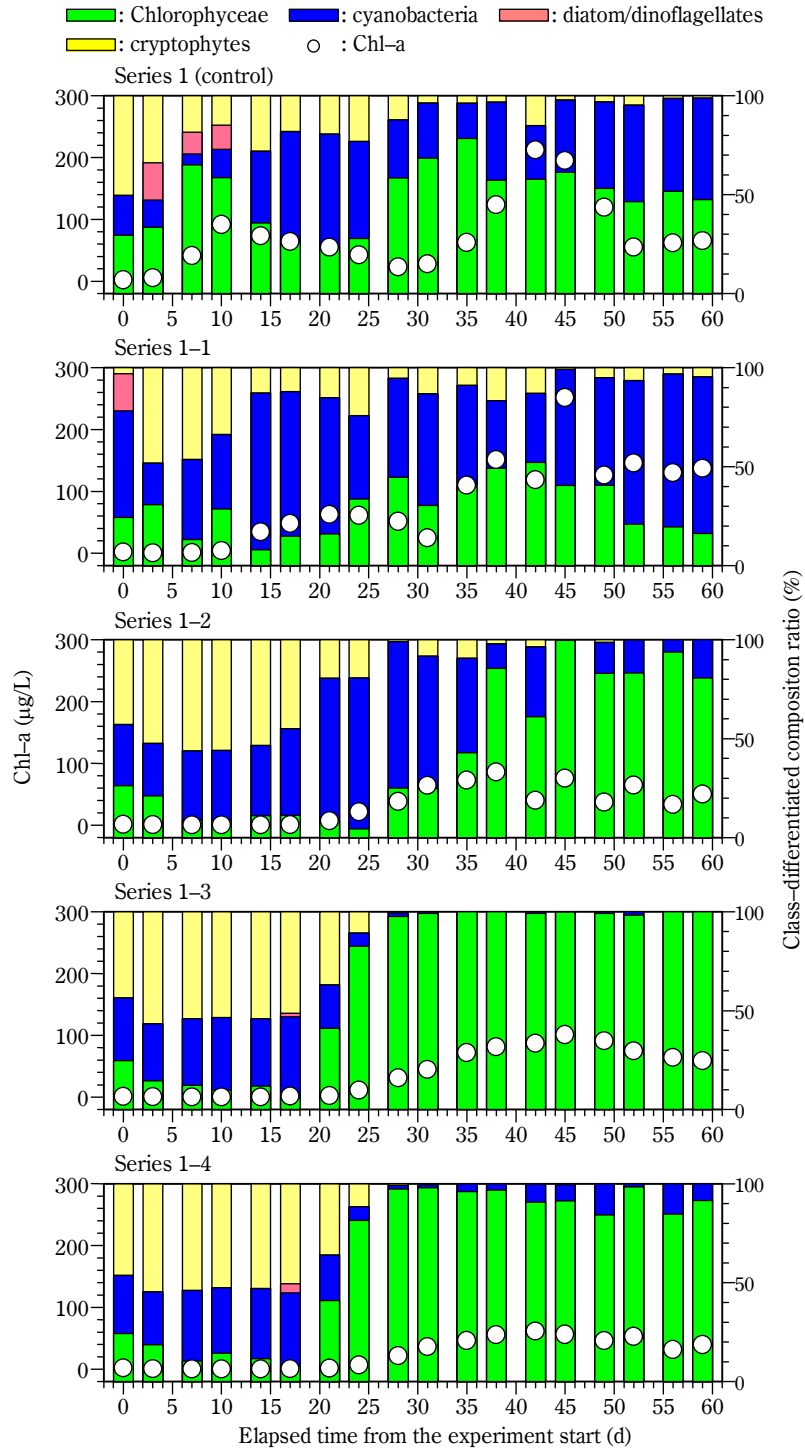


Figure 3.5 Periodical measurements of Chl-a concentration and class-differentiated composition ratio in Series 1

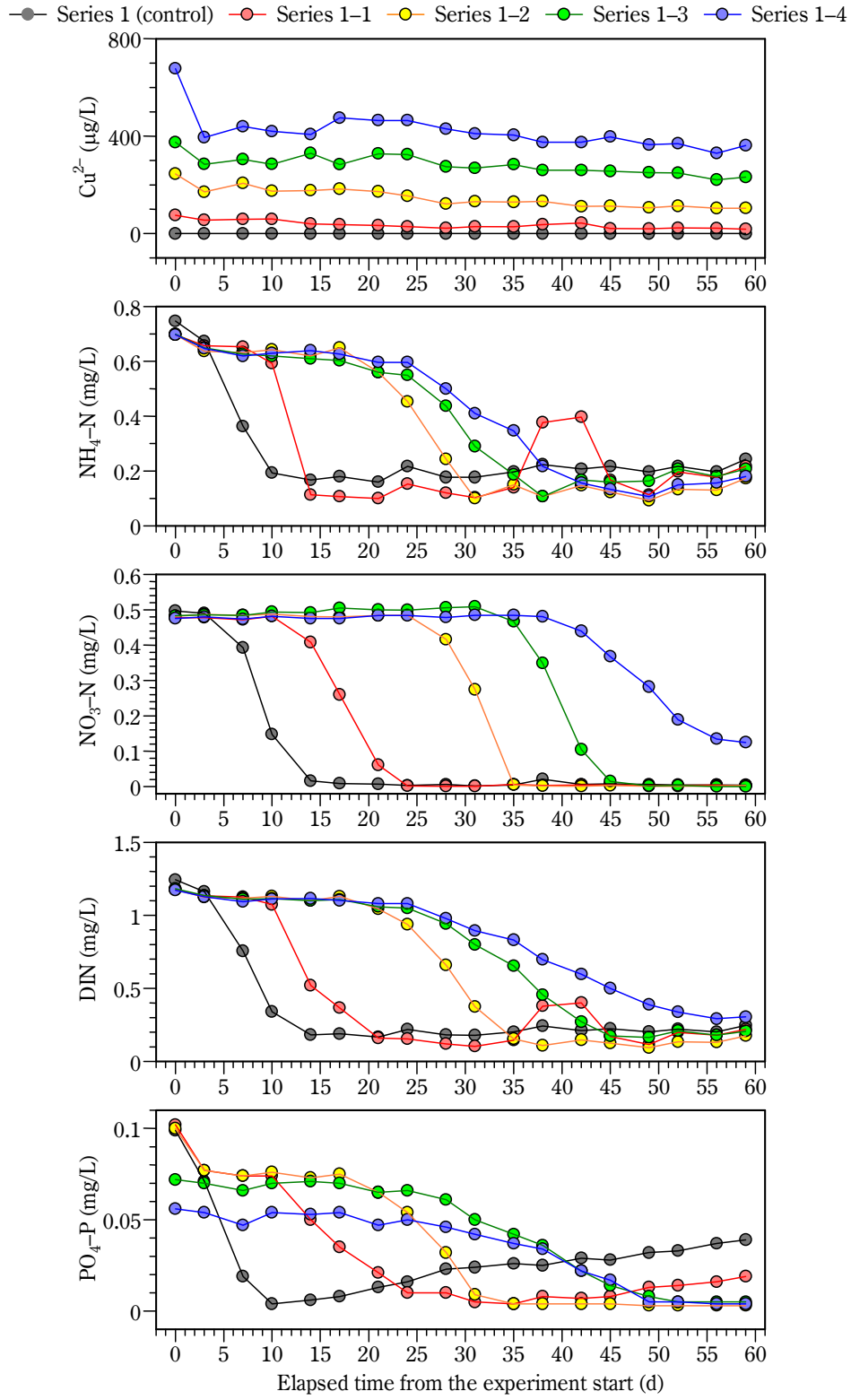


Figure 3.6 Periodical measurements of nutrients and Cu^{2+} in Series 1

According to the results of the control condition, Chl-a began to increase almost immediately after the experiment started, together with a decrease in nutrient concentrations, and showed a two-step increasing process, such that Chl-a increased to approximately 90 $\mu\text{g/L}$ at $t = 10$ d and rapidly proliferated to a hypertrophic level of over 200 $\mu\text{g/L}$ at $t = 42$ d via a decreasing period of approximately three weeks. Evidently, from the photograph of the intermediate status of the control in **Figure 3.3(1)**, the water color in the tank changed from transparent to green at $t = 31$ d owing to algal growth. Eventually, the Chl-a settled to a concentration of approximately 60 $\mu\text{g/L}$, which was much higher than the initial concentration. In addition, many small green grains were observed at the bottom of the water tank, indicating the final status of the control at $t = 59$ d in **Figure 3.3(1)**. In addition, DIN and DIP decreased to a lower level than the initial concentration during the first two weeks, and $\text{NO}_3\text{-N}$ decreased to almost zero. In addition, the average amplitude of DO fluctuations was as high as 6.6 mg/L. The above results indicate that the initial high level of nutrients highly promoted the growth rate (photosynthesis rate) and was linked to an increase in phytoplankton amount to the eutrophic level, in which approximately 30–50 % cyanobacteria of the total phytoplankton appeared continuously, and Chlorophyceae occupied at a rate of 30–75 % as a dominant species. The gradual increase and decrease in Chl-a appeared to be derived from the unbalanced relationship between the demand and supply of nutrients for photosynthesis. A certain amount of time may be required to provide DIN and DIP through the mineralization of organic compounds by biodegradation. Therefore, a rapid increase in phytoplankton causes a lack of available nutrients due to the uptake of large amounts of DIN and DIP by algal photosynthesis, resulting in a decrease in Chl-a.

In Series 1-1, where the Cu^{2+} concentration was the lowest, Chl-a was maintained at the initial low concentration without increasing the amplitude of DO fluctuation or decreasing the nutrient concentration from $t = 0$ d to 12 d. Phytoplankton growth was inhibited by adding a Cu^{2+} solution during the first two weeks, unlike in the control. Although the temporal length inhibiting the phytoplankton growth in Series 1-1 was longer than that in the control, the Chl-a increased gradually via the change in two stages like the control since $t = 15$ d. Chl-a reached a level of approximately 150 $\mu\text{g/L}$ at a high growth rate at $t = 37$ d (the average amplitude of DO fluctuation: 5.4 mg/L) together with a decrease in nutrients, and cyanobacteria multiplied as a dominant species (composition rate: approximately 80 %). The concentration of Cu^{2+} slightly and gradually decreased from the

initial value and was maintained at approximately 50 µg/L throughout the experimental period. However, from the results of Chl-a and DO fluctuations, it could be considered that the bactericidal effect with Cu²⁺ of 50 µg/L was not sufficient in this hypertrophic state and had a short-term effect on algal overgrowth inhibition. Suppressing the proliferation of cyanobacteria over the long term is impossible.

Meanwhile, in Series 1-2 to Series 1-4, the Chl-a concentration maintained a low initial value without increasing for approximately three weeks from the initial Cu²⁺ concentration, and the bactericidal effect completely inhibited phytoplankton overgrowth. Although Cu²⁺ decreased from its initial concentration immediately after the start of the water quality experiments (in particular, there was a notable decline in Series 1-4), the Cu²⁺ concentration was approximately constant or slightly decreased after $t = 3$ d. Evidently, from the photograph of the intermediate statuses of Series 1-4 in **Figure 3.3** (1), the watercolor in the tank remained transparent without a change to green. However, the Chl-a concentration evidently and gradually increased for approximately two weeks in the last four weeks despite the inhibition of cyanobacteria, and the high level of phytoplankton resulted from the proliferation of Chlorophyceae. During the same period, the Cu²⁺ concentrations remained almost unchanged at high concentrations of 150 µg/L, 300 µg/L and 400 µg/L in Series 1-2, 1-3, and 1-4, respectively, from $t = 25$ d until the end of the experimental period. This phenomenon showed that the bactericidal effect of Cu²⁺ weakened over a later period of time. Specifically, the influence of the Cu²⁺ level on the inhibition of phytoplankton growth was summarized from the perspective of the water quality dynamics of Chl-a, DO, and nutrients as follows: Cyanobacteria were suppressed by 15 % or less. The overgrowth of Chlorophyceae resulted in an increase in Chl-a up to a hypertrophic level of 80–100 µg/L at $t = 38$ d in Series 1-2 and at $t = 45$ in Series 1-3, with an average amplitude of DO fluctuation of approximately 4.0 mg/L. The peak concentration of Chl-a in Series 1-4 was limited to approximately 60 µg/L and Chl-a eventually remained almost flat at 30–40 µg/L. At the end of the experiment in Series 1-4 ($t = 59$ d), the small green grains that precipitated at the bottom of the water were lighter than those in Series 1, including the control, as shown by the final status at $t = 59$ d in **Figure 3.3**(1). In addition, the DO in this series fluctuated with a relatively small amplitude within 3.5 mg/L. In Series 1-2 and 1-3, NH₄-N was lowered to a low level of less than 0.2 mg/L, and both NO₃-N and PO₄-P decreased to almost zero with increasing Chl-a. Unlike in the other cases, the nutrient concentrations in Series 1-4 were

maintained at a low level without being completely taken up by phytoplankton by photosynthesis. In particular, the timing of the decrease in $\text{NO}_3\text{-N}$ was delayed as the Cu^{2+} level increased, and the start of lowering in Series 1-4 required the longest time in this series. Because the difference in decreasing timings in nutrients among the experimental cases corresponded to one of increasing timing in Chl-a, the persistence of the inhibitory effect on phytoplankton growth increased as the concentration of Cu^{2+} increased.

The inhibitory effects by bactericidal action of Cu^{2+} on phytoplankton overgrowth when the initial Chl-a concentration was very low are summarized as follows: Under extremely high nutrient concentration conditions at the hypertrophic level, adjusting the Cu^{2+} concentration to 200–400 $\mu\text{g/L}$ was required to suppress the proliferation of cyanobacteria. However, high concentrations of DIN and DIP promoted the growth rate of Chlorophyceae. Although Cu^{2+} prevented the phytoplankton from increasing over a short period of four weeks, under high Cu^{2+} addition, Chlorophyceae still grew greatly. Therefore, Chlorophyceae was insusceptible to the bactericidal action of Cu^{2+} . While Chlorophyceae are essential for healthy aquatic systems due to their role in photosynthesis, excessive growth can lead to eutrophication, water quality degradation, and ecosystem imbalance. Under high nutrient concentrations, adjusting the Cu^{2+} concentration to over 600 $\mu\text{g/L}$ required algal proliferation, such as cyanobacteria and Chlorophyceae. Therefore, measures to reduce the excessive inflow of nutrients are necessary for long-term algae control.

3.3.2 Reduction effects of high cyanobacteria concentration

Figure 3.7 shows the changes in DO with time obtained by continuous monitoring. Algal growth rates by photosynthesis were quantitatively estimated by focusing on the amplitude of daily fluctuations in DO. Here, the missing DO data was due to a dead battery from $t = 38$ d to $t = 46$ d in Series 2-1, as shown in **Figure 3.7**. **Figure 3.8** shows the periodic monitoring results of the Chl-a concentration and class-differentiated composition ratios in phytoplankton. **Figure 3.9** shows the temporal changes in nutrient ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$) and Cu^{2+} concentrations obtained by periodic monitoring.

The reduction effects of a large amount of phytoplankton by adding Cu^{2+} are discussed by comparing four cases in Series 2. Water in the initial state was green, as shown in **Figure 3.3(2)**, because of the high Chl-a level of approximately 160 $\mu\text{g/L}$, and the composition rate of cyanobacteria was also very high, at approximately 85 %. In this series, maximum concentration of Cu^{2+} was set to the high level of approximately 1000 $\mu\text{g/L}$,

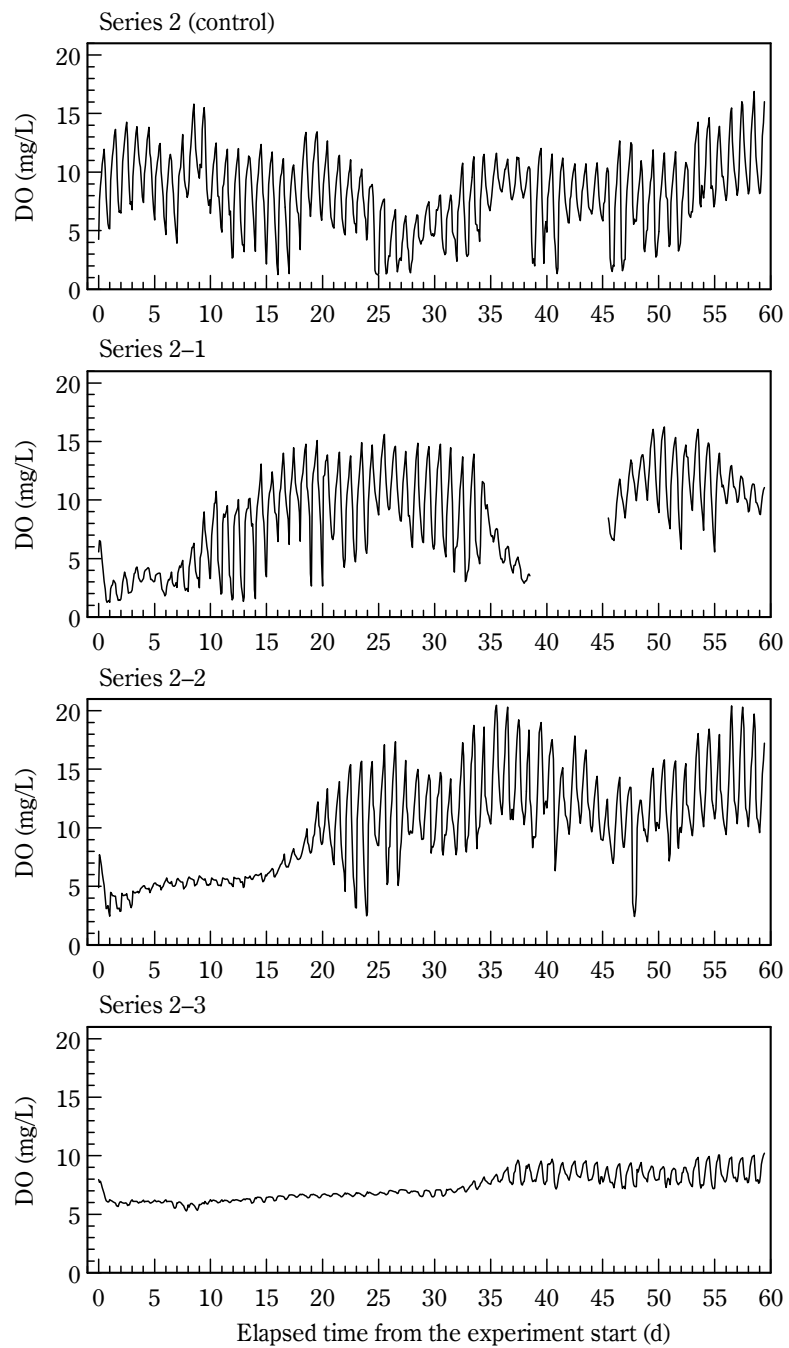


Figure 3.7 Continuous measurements of DO in Series 2

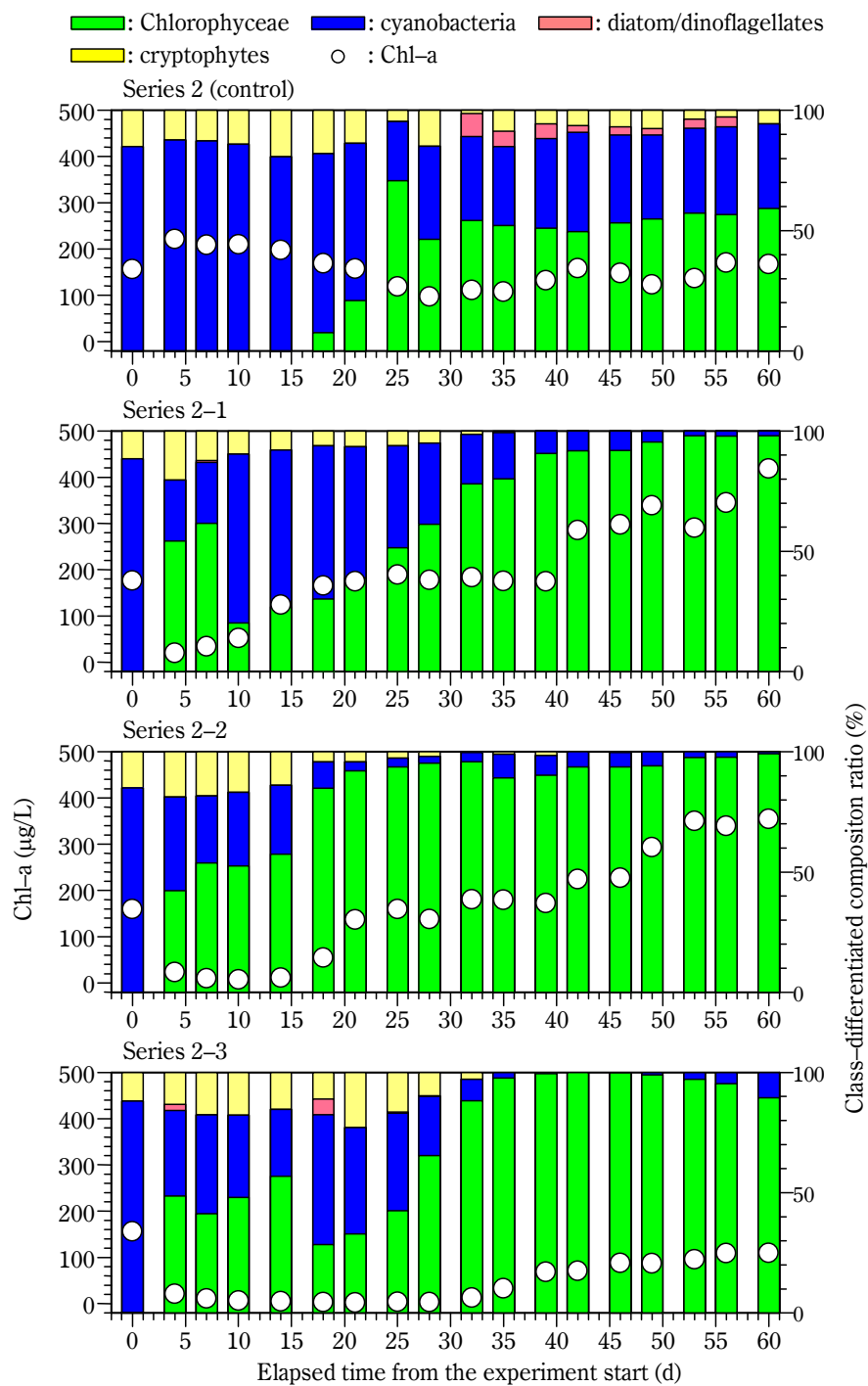


Figure 3.8 Periodical measurements of Chl-a concentration and class-differentiated composition ratio in Series 2

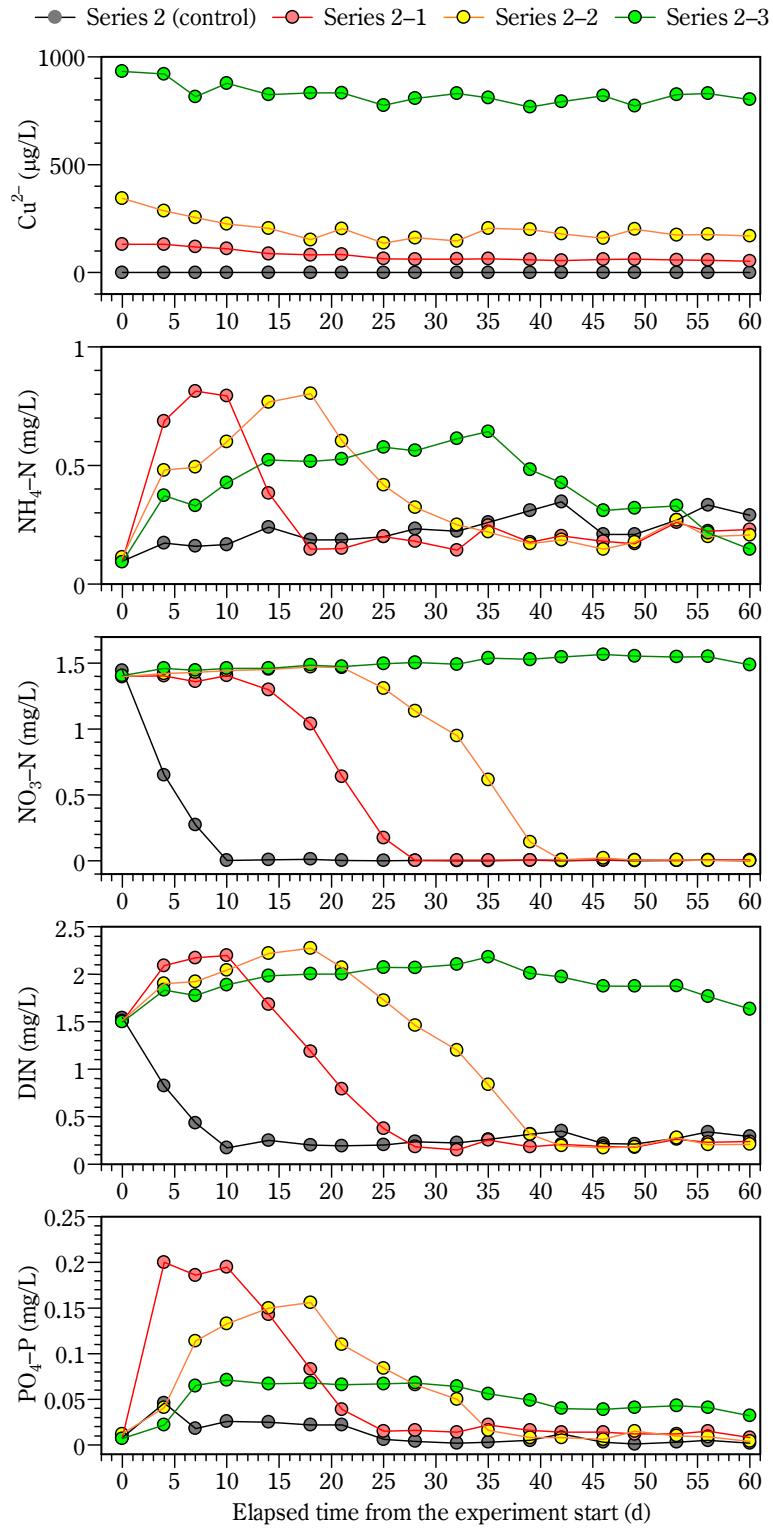


Figure 3.9 Periodical measurements of nutrients and Cu²⁺ in Series 2

and water quality monitoring was conducted under DIN (approximately 1.5 mg/L) and DIP (approximately 0.02 mg/L) eutrophic state as the initial conditions. The results of the control conditions are summarized as follows: Chl-a maintained a high concentration of over 200 $\mu\text{g/L}$ (except for the temporal decrease to approximately 125 $\mu\text{g/L}$) with a high growth rate, represented as the average amplitude of approximately 10 mg/L with DO fluctuation until the end of the experiment. The water color in the control condition of Series 2 became darker green compared with the initial status over time, as shown in **Figure 3.3(2)**, owing to the increase in Chl-a. Because of the initially high level of Chl-a, the concentrations of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ decreased to almost zero via phytoplankton uptake immediately after starting the water quality experiments. In addition, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ remained at low levels during high algal growth rates until the end of the monitoring period, although $\text{NH}_4\text{-N}$ was maintained at its initial concentration. The difference in the degree of lowering between $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ could result from cyanobacteria preferentially utilizing $\text{NO}_3\text{-N}$ in DIN as a nitrogen source necessary for photosynthesis (Issa et al., 2020). In addition, the high concentration of cyanobacteria was maintained as a dominant species with a composition ratio of over 70 % for approximately three weeks, and Chlorophyceae appeared at a high abundance ratio of approximately 50 % from $t = 25$ d and coexisted with cyanobacteria until the end of the experiment. The reason why the concentrations of cyanobacteria decreased, and the composition ratio of Chlorophyceae increased can be explained as follows: The optimal light intensity and temperature of photosynthesis for cyanobacteria are higher than those for Chlorophyceae, and cyanobacterial blooms tend to occur during the heating season with high solar radiation, such as summer and early autumn (Li et al., 2023). The light quantum of approximately 100 $\mu\text{mol}/(\text{m}^2 \text{ s})$ in this study was not necessarily a sufficient light intensity for cyanobacteria. Therefore, cyanobacteria appear to receive little light inhibition, resulting in a natural shift from the main algal class to Chlorophyceae.

In Series 2-1 and 2-2, Chl-a decreased to approximately 20 $\mu\text{g/L}$ immediately after starting the water quality experiments, and the proportion of cyanobacteria notably decreased from 85 % to less than 10 %. In particular, Series 2-1 demonstrated a removal effect of the cyanobacterial bloom, in that the low level of Cu^{2+} (60 g/L) immediately reduced the Chl-a concentration from 160 $\mu\text{g/L}$ to 20 $\mu\text{g/L}$. During the decreasing period of Chl-a (Series 2-1: $t < 7$ d and Series 2-2: $t < 15$ d), the DO concentration temporally changed with a very small amplitude of 1.0 mg/L compared to the control, and the temporal change patterns of nutrients

in both cases differed from those in the control. Specifically, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ increased from their initial concentrations, and $\text{NO}_3\text{-N}$ remained constant. In particular, the increase in nutrient concentrations and the decrease in Chl-a revealed that the supply of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ to water via the aerobic degradation of organic substances, including dead algae, exceeded their consumption by photosynthesis. Therefore, the addition of Cu^{2+} instantaneously inhibited the growth of cyanobacteria from $t < 7$ d in Series 2-1 and $t < 15$ d in Series 2-2, resulting in a decrease in phytoplankton abundance. However, the time required to inhibit algal overgrowth in Series 2-1 and 2-2 was extremely short at 7 and 15 days, respectively. The Cu^{2+} in Series 2-1 maintained an approximately constant value without a decrease in the initial concentration. However, Cu^{2+} in Series 2-2 decreased gently from approximately 350 $\mu\text{g/L}$ to approximately 200 $\mu\text{g/L}$ during the two weeks following the start of the water quality experiments. Chl-a eventually increased to high levels (420 $\mu\text{g/L}$ in Series 2-1 and 350 $\mu\text{g/L}$ in Series 2-2) beyond the peak concentration in the control. The temporal changes in DO with large fluctuating strokes of 10–15 mg/L and the lowering of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ to almost zero indicated that the notable increase in Chl-a was caused by phytoplankton's uptake of a large amount of nutrients. This proved that the inhibitory effect on algal growth disappeared after $t = 7$ d in Series 2-1 and after $t = 15$ d in Series 2-2.

In Series 2-3, where the initial Cu^{2+} concentration was set to a very high level of approximately 1000 $\mu\text{g/L}$, Chl-a rapidly decreased immediately after starting the water quality experiment, and it remained at almost zero without fluctuations in DO in the first half of the four weeks, as in the case of Series 2. The Cu^{2+} concentration in Series 2-3 declined from approximately 950 $\mu\text{g/L}$ to approximately 850 $\mu\text{g/L}$ in the short time from $t = 3$ d to 6 d. In both Series 2-2 and 2-3, a significant decline in Cu^{2+} was not observed throughout the water quality experiment for eight weeks, and the influence of the Cu^{2+} decrease on the experimental results appeared to be small. In addition, the increasing tendencies of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ appeared to result from the supply of particulates and dissolved organic matter to the water during the decreasing period of Chl-a. Specifically, the reduction effect of high cyanobacteria concentrations could be proven from the perspective of the biochemical dynamics of nutrients over a prolonged period of four weeks compared to other cases. Moreover, this effect was confirmed by the fact that the green color in the initial status disappeared immediately after starting the water quality experiment, and the transparent shade with a slightly pale blue color, resulting from high Cu^{2+} concentration,

was maintained during eight weeks as shown by the intermediate and final statuses in **Figure 3.3(2)**. However, Chl-a in Series 2-3 increased from zero to a high concentration of approximately 100 µg/L during the last two weeks. Although the timing of Chl-a from the almost zero level to the increasing process forward, the hypertrophic level, was similar in Series 2, the timing of the increasing process and rate (growth rate of phytoplankton) in Series 2-3 were later and lower, respectively than those in Series 2. In addition, DO fluctuated during the increasing period of Chl-a with a relatively smaller amplitude within 2.5 mg/L compared to others. The initial concentration of NO₃-N with a high level of 1.4 mg/L was maintained constant without lowering to zero throughout the water quality monitoring of eight weeks, and the PO₄-P was maintained at a higher concentration of 0.04 to 0.06 mg/L compared to other cases. Specifically, the promotion of phytoplankton growth rate under high nutrient concentrations was controlled to a certain extent. Therefore, the advantages of the high Cu²⁺ concentration (approximately 1000 µg/L) were to maintain persistence over a long-term period of approximately four weeks to inhibit phytoplankton proliferation and to keep the maximum concentration below 100 µg/L after the suppression effect for the increases in Chl-a disappeared.

In Series 2, Chl-a increased with an increase in the abundance of Chlorophyceae. However, the timing of the increase differed from that of the initial concentration of Cu²⁺; $t = 7$ d in Series 2-1, $t = 18$ d in Series 2-2, and $t = 35$ d in Series 2-3. In addition, the Chl-a concentration of Chlorophyceae apparently increased to a higher level (Series 2-1: 420 µg/L, Series 2-2: 350 µg/L, and Series 2-3: 100 µg/L) as the adjusted concentration of Cu²⁺ decreased. However, the abundance ratio of Chlorophyceae reached a high value of over 90 % in the last two weeks, regardless of the Cu²⁺ concentration. The shift in the dominant algal class from cyanobacteria to Chlorophyceae resulted from differences in susceptibility to the bactericidal action of Cu²⁺. Chlorophyceae, which were insusceptible to the bactericidal action of Cu²⁺, dominated instead of cyanobacteria due to interspecific competition. In addition, the excessive increase in Chlorophyceae Chl-a to a hypertrophic level of over 100 µg/L was believed to have been caused by the increase in DIN and DIP. A large amount of nitrogen and phosphorus compounds from cyanobacteria that died because of the bactericidal effect of Cu²⁺ was reduced to DIN and DIP via the aerobic decomposition of organic matter under sufficient DO conditions. Until the Chl-a began to increase, NH₄-N and PO₄-P reached the maximum levels of beyond 0.7 mg/L and 0.07 mg/L respectively. The

maximum $\text{NO}_3\text{-N}$ corresponded to an initial concentration of approximately 1.4 mg/L in all three cases. Therefore, extremely high levels of nutrients, in which $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were particularly higher than the initial concentrations, promoted the exclusive proliferation of Chlorophyceae. Notably, the maximum concentrations of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ increased with decreasing Cu^{2+} concentrations (**Figure 3.9**). For example, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ in Series 2-1 had higher maximum values of 0.8 mg/L and 0.2 mg/L, respectively, compared to Series 2-3 ($\text{NH}_4\text{-N}$: 0.65 mg/L and $\text{PO}_4\text{-P}$: 0.07 mg/L). This result indicates that the return rates of nitrogen and phosphorous from organic matter to available $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ for algal growth were reduced proportionally to the Cu^{2+} concentration. Therefore, the addition of Cu^{2+} to water provides an advantage in that the chemical death of phytoplankton caused by high Cu^{2+} concentrations can reduce the supply of available nutrients for algal photosynthesis.

3.3.3 Elimination effects of cyanobacteria bloom by two-step addition of Cu^{2+}

Figure 3.10 shows the changes in DO over time, as obtained through continuous monitoring, and these changes were used to quantitatively estimate algal growth rates by photosynthesis, focusing on the amplitude of daily fluctuations in DO. **Figure 3.11** shows the periodic monitoring results of the Chl-a concentration and class-differentiated composition ratios in phytoplankton. **Figure 3.12** shows the temporal changes in nutrient ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$) and Cu^{2+} concentrations obtained by periodic monitoring.

The aim of Series 3 was to examine the bactericidal effects of Cu^{2+} for eliminating cyanobacterial blooms in two different cases. The mat-like algae floating on the water surface in Series 3-2 spread more extensively and thickly than those in Series 3-1, as shown in **Figure 3.3(3)** and **Figure 3.3(4)**, under the condition that the rich nutrients promoted algal overgrowth (DIN : 2.43 mg/L and DIP : 0.16 mg/L). Series 3 also differed from the other series in that the Cu^{2+} concentration was adjusted in two steps: at the beginning of the water quality experiment and after four weeks from the start. Therefore, this series included the influence of the step-by-step addition of Cu^{2+} on inhibiting the increase in phytoplankton.

In the control condition of Series 3-1, the initial concentration of Chl-a increased to a maximum level of approximately 140 $\mu\text{g/L}$ until $t = 7$ d, with a daily average amplitude of 2–5 mg/L in DO. Even though Chl-a decreased from its peak concentration at $t = 25$ d, it was maintained at a high concentration of approximately 100 $\mu\text{g/L}$ with a high growth rate, represented as a DO amplitude of 5–10 mg/L, throughout the water quality experiment.

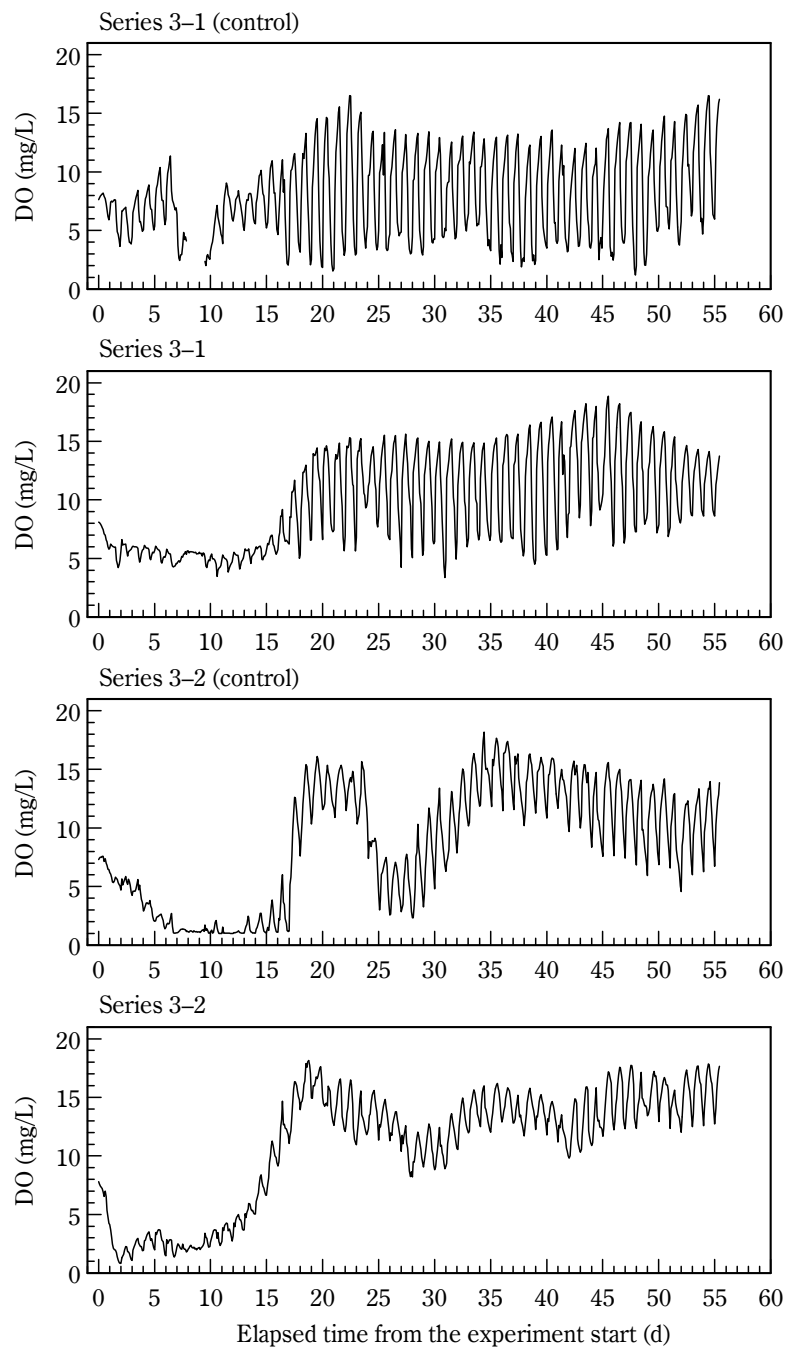


Figure 3.10 Continuous measurement of DO in Series 3

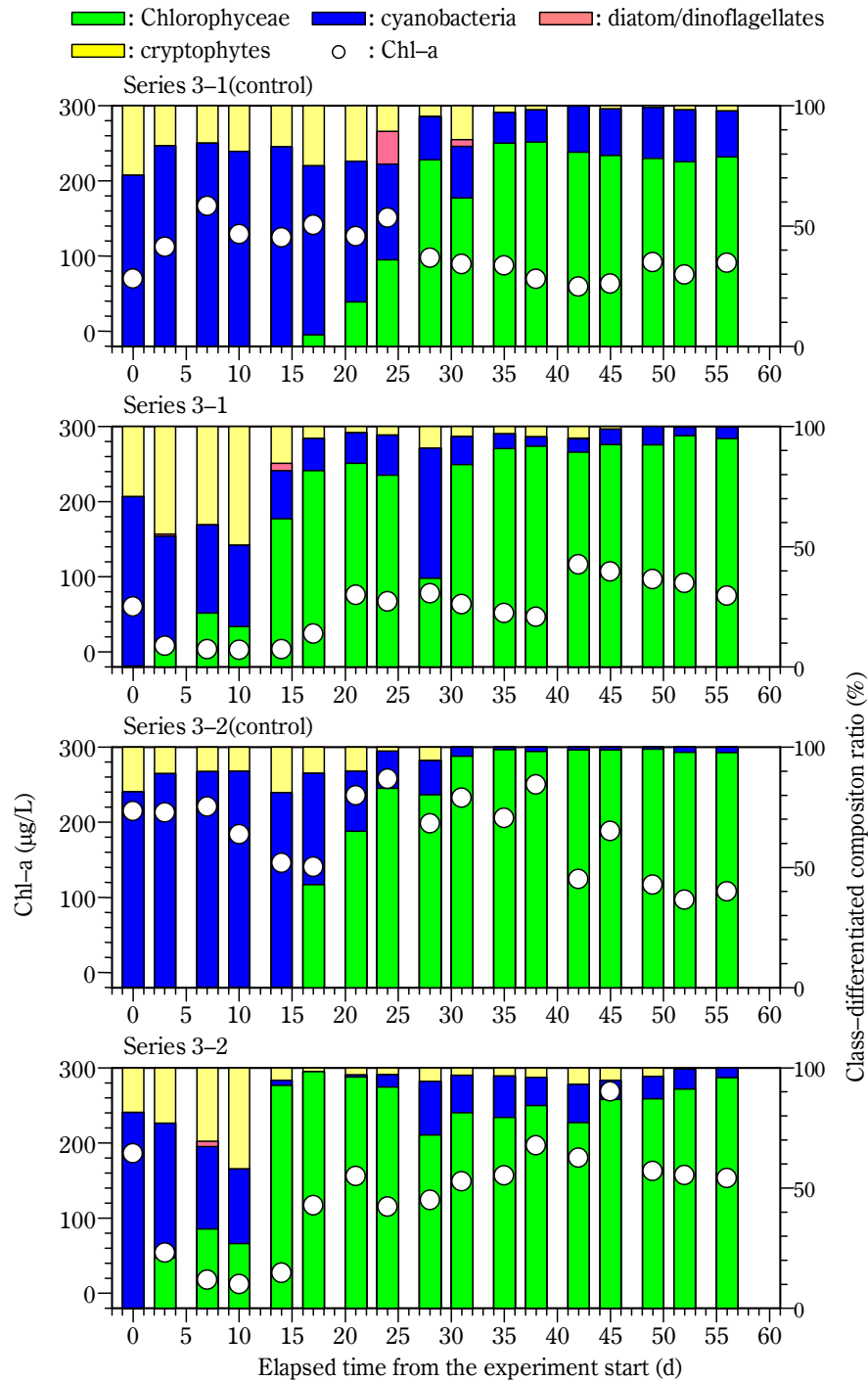


Figure 3.11 Periodical measurements of Chl-a concentration and class-differentiated composition ratio in Series 3

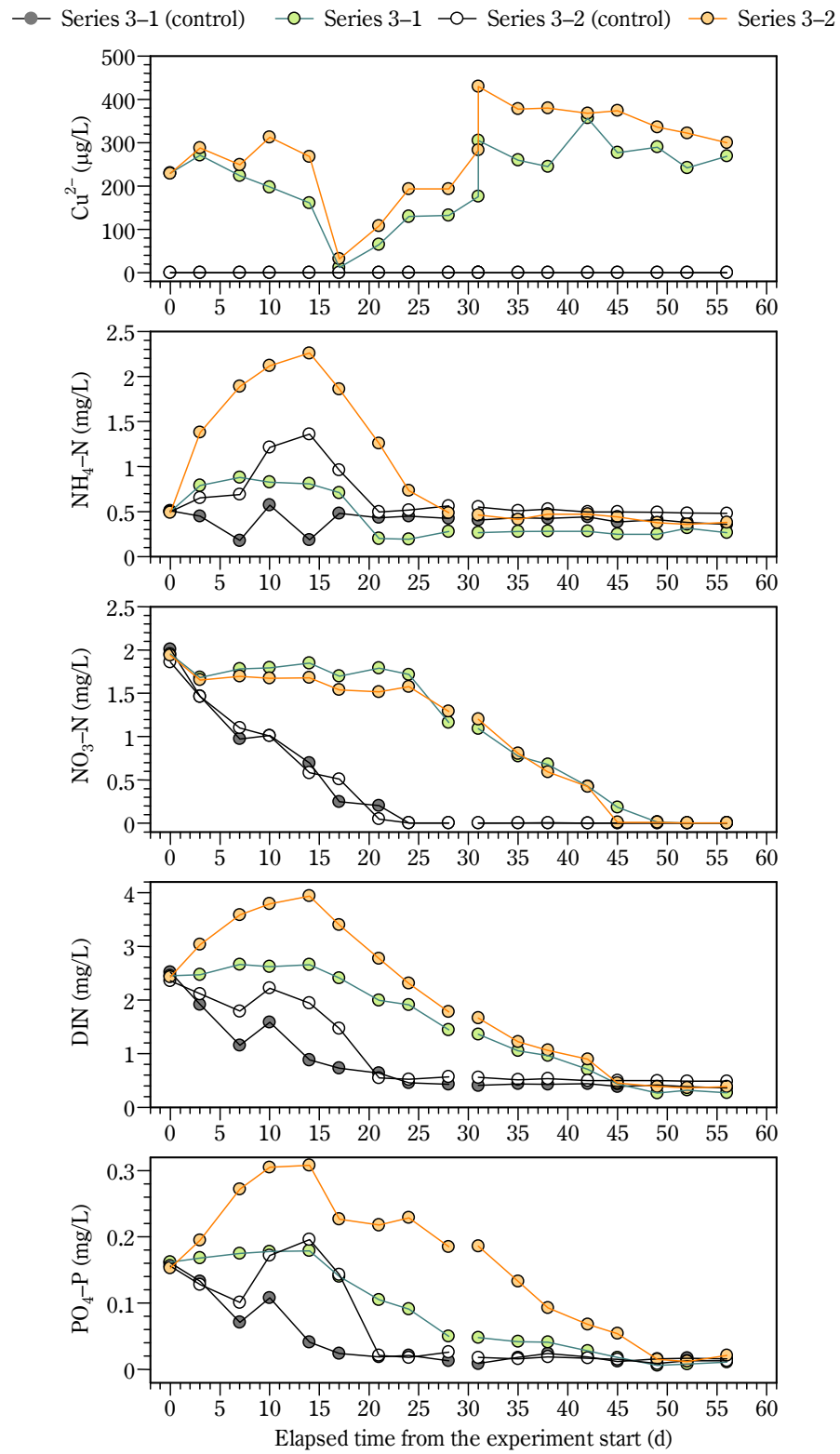


Figure 3.12 Periodical measurements of nutrients and Cu^{2+} in Series 3

As shown in **Figure 3.3(3)**, the entire water column had a uniform green color after four and eight weeks, although the algal bloom on the surface also disappeared. In the control of Series 3-2, the high Chl-a concentration was maintained the same as the initial condition of approximately 210 $\mu\text{g/L}$ until $t = 37$ d, even though it temporarily lowered to 140 $\mu\text{g/L}$ during $t = 7\text{--}17$ d. Although cyanobacteria blooms on the water surface were evidently observed by keeping up the initial status until $t = 15$ d, they disappeared as shown in **Figure 3.3(4)** together with the decrease in the abundance ratio of cyanobacteria from $t = 17$ d. Finally, Chl-a in the control of Series 3-2 reached a relatively high level of approximately 100 $\mu\text{g/L}$ through a gradual decrease during $t = 37\text{--}48$ d. Irrespective of the initial concentration of cyanobacteria bloom, the appearance of water in the control condition of Series 3 after eight weeks ($t = 56$ d) had a strong green color due to a high Chl-a concentration of approximately 100 $\mu\text{g/L}$ from Chlorophyceae. Here, DO fluctuated with a small amplitude of less than 1 mg/L until $t = 14$ d because the DO sensor was placed in the low Chl-a region under the water surface, on which mat-like algae floated. Meanwhile, the temporal change in DO from $t = 17$ d included a large amplitude of approximately 5 mg/L, corresponding to a high Chl-a concentration, because Chlorophyceae existed entirely and uniformly in the water tank instead of cyanobacteria.

Under both control conditions, cyanobacteria were the dominant species with a composition ratio of over 80 % until $t = 15$ d. During the algal bloom generation period in both control conditions, the concentrations of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ rapidly declined to near-zero levels due to uptake by algal photosynthesis immediately after starting the water quality experiments. Subsequently, the composition ratio of Chlorophyceae increased to become a dominant species instead of cyanobacteria and finally occupied the entire algal class at approximately 80–95 %. As mentioned above, the Chl-a concentration in the last half of the experiment was maintained at a high level of over 100 $\mu\text{g/L}$ with a high growth rate, even though it decreased from the initial concentration owing to the disappearance of the algal bloom. The significant increase from almost zero in Chlorophyceae appeared to be caused by the natural decay of cyanobacteria due to light inhibition, as mentioned in the results regarding the control in Series 2, due to interspecific competition at the algal class level.

Under Cu^{2+} addition conditions, the initially high concentration of cyanobacteria declined notably to a low level of less than 10 $\mu\text{g/L}$ in the first two weeks, unlike the control, and the disappearance of cyanobacteria was maintained over a long period of eight weeks,

as shown in **Figures 3.3(3)** and (4). In Series 3-1 and 3-2, Cu^{2+} in the first half ($t < 28$ d) decreased more prominently from the initial concentration than in the other series because of the formation of chelate complexes with a large amount of suspended organic matter from dead phytoplankton. In addition, the concentration of DIN and DIP in Series 3-1 was almost unchanged until $t = 15$ d, and these nutrients in Series 3-2 increased significantly immediately after Cu^{2+} addition and reached the peak (DIN: 4.0 mg/L, and DIP: 0.3 mg/L) at $t = 15$ d. Maintaining extremely high DIN and DIP concentrations without lowering them to zero showed that the bactericidal effect of Cu^{2+} effectively eliminated cyanobacteria and effectively inhibited phytoplankton growth. In particular, the increased DIN and DIP in Series 3-2 implied that the dead phytoplankton, due to the bactericidal effect of Cu^{2+} , supplied a large amount of available nutrients via the oxidative decomposition of organic substances because of the numerous cyanobacterial blooms compared to Series 3-1. Therefore, the Series 3 results demonstrated that the bactericidal effect of Cu^{2+} effectively eliminated algal blooms and suppressed the proliferation of cyanobacteria.

However, Chl-a increased significantly to a high level of over 100 $\mu\text{g/L}$ from a low level of less than 10 $\mu\text{g/L}$, with a gradual decrease in $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ from $t = 17$ d. Chlorophyceae became the dominant species at a high composition ratio of 90-95 %, unlike cyanobacteria in the first half (before the second addition of Cu^{2+}), which was similar to the control. Notably, the appearance of Chlorophyceae was confirmed approximately two weeks earlier compared to the control in Series 3-1 and 3-2. Although the algacide effect of Cu^{2+} was only effective on cyanobacteria, irrespective of the thickness of the algal bloom, its lack of effect on Chlorophyceae could be linked to the increase in Chl-a in the first half.

After the second addition of Cu^{2+} at $t = 31$ d, Chl-a was maintained at a high concentration of approximately 100 $\mu\text{g/L}$ in Series 3-1 and 150 $\mu\text{g/L}$ in Series 3-2. Maintaining a high Chl-a level entailed fluctuations in DO, with a daily average amplitude of approximately 15 mg/L in Series 3-1 and 4 mg/L in Series 3-2, similar to the control conditions. Unlike the first half in Series 3, Cu^{2+} in the last half was approximately constant at approximately 300 $\mu\text{g/L}$ without a significant decrease because suspended organic matter forming chelate complexes with Cu^{2+} significantly declined until the second addition of the CuSO_4 solution. As shown in **Figure 3.3(4)**, the water color in the final status (at $t = 56$ d) of Series 3-2 became darker green than in the intermediate status ($t = 28$ d), and it became worse compared to the final status in the control condition; the patch-like algae differed from

cyanobacterial blooms floating in the water. Specifically, the second treatment with the calculated Cu^{2+} concentration of 500 $\mu\text{g/L}$ was ineffective in inhibiting Chl-a growth from a low level as well as reducing the high initial concentration of Chl-a. Because of the difference in bactericidal effects between the two algal classes, the second addition of Cu^{2+} accelerated the timing of the change in the dominant species, and the growth period of Chlorophyceae was prolonged, resulting in a notable increase in Chl-a concentration. In addition, the initial high Chl-a concentration from an algal bloom results in a more notable proliferation of Chlorophyceae because of the high concentrations of DIN and DIP supplied by the extinction of excessive cyanobacterial populations.

3.4 Conclusions

Water quality experiments were conducted using water tanks to examine the effectiveness of Cu^{2+} in controlling algal overgrowth under hypertrophic conditions while exploring the inhibitory effect on the increase in Chl-a, the reduction effect of high cyanobacteria concentrations, and the elimination effect of mat-like algal blooms. The outcomes are summarized as follows:

In the experimental condition characterized by low initial concentrations of phytoplankton and high nutrient concentrations, Cu^{2+} concentrations over 200–400 $\mu\text{g/L}$ effectively suppressed the proliferation of cyanobacteria, which was more susceptible to the bactericidal action of Cu^{2+} . Although Cu^{2+} inhibited the overgrowth of cyanobacteria throughout the water quality experiment for eight weeks and prevented the Chl-a concentration from increasing in a short term of four weeks, the high DIN and DIP concentrations promoted the growth rate of Chlorophyceae, which was insusceptible to the bactericidal action of Cu^{2+} . To adequately control algae under high nutrient concentrations, Cu^{2+} concentrations exceeding 600 $\mu\text{g/L}$ were necessary as the suitable concentration. Therefore, long-term algal control under relatively low levels of Cu^{2+} requires countermeasures to reduce excessive nutrient input.

For elevated initial concentrations of nutrients and Chl-a with a high composition ratio of cyanobacteria, a high Cu^{2+} concentration of approximately 1000 $\mu\text{g/L}$ had the advantage of inhibiting phytoplankton proliferation over a long period of approximately four weeks. In addition, by adjusting the concentration of Cu^{2+} to such a high level, it was possible to suppress the maximum concentration to 100 $\mu\text{g/L}$ or less, although Chl-a increased owing

to the overgrowth of Chlorophyceae four weeks or later after Cu^{2+} addition. This effect can be attributed to a high Cu^{2+} concentration reducing nitrogen and phosphorous return rates from organic matter to available DIN and DIP for algal photosynthesis.

After adding Cu^{2+} , Cyanobacteria can be effectively eliminated under mat-like algal blooms and extremely high concentrations of DIN and DIP condition. Specifically, a Cu^{2+} concentration of approximately 200 $\mu\text{g/L}$ notably decreased the initial high concentration of Chl-a to 10 $\mu\text{g/L}$ or less in the first two weeks, even though adjusting the high level to 500 $\mu\text{g/L}$ was difficult because some of Cu^{2+} disappeared owing to chelate complexes with suspended organic matter. However, the following two factors significantly increased Chl-a from a low level of less than 10 $\mu\text{g/L}$ to a high level of over 100 $\mu\text{g/L}$. First, Chlorophyceae dominated the cyanobacteria because of interspecific competition due to its non-susceptibility to bactericidal action. Next, high concentrations of DIN and DIP, which were supplied by the extinction of the excessive cyanobacterial population, promoted a more notable proliferation of Chlorophyceae. Specifically, strategically providing Cu^{2+} as a countermeasure against cyanobacterial blooms in hypertrophic water bodies has a limited inhibitory effect on the increase in Chl-a in the long term due to the shift of algal dominance in nutrient-rich status.

This study provides extremely significant outcomes by quantitatively evaluating the risks and advantages of applying the bactericidal effect of Cu^{2+} . In particular, the suppression of the Chl-a increase in the long term is not expected under conditions of high nutrient concentrations, and a hypertrophic status results in the proliferation of Chlorophyceae, even though it is possible to inhibit the overgrowth of cyanobacteria and eliminate mat-like algal blooms. Therefore, a reduction in nutrient concentration is essential for solving eutrophic problems in closed water bodies. In conclusion, for aquatic environmental remediation of hypertrophic water resources in agricultural areas, a hybrid countermeasure with a reduction in nutrient concentration is required to control algal blooms based on the bactericidal effect of Cu^{2+} .

Chapter 4 Impacts of Inflow Loadings from Heavy Rainfall on Cyanobacteria Overgrowth in Nitrogen-Limited Reservoirs through Field Observation and Numerical Simulation

4.1 Introduction

A trophic status in a freshwater body can be classified by using 3 water quality parameters of Chl-a, TP, and transparency, based on the OECD standard (Organization for Economic Cooperation and Development, 1982). Recently, many agricultural reservoirs have been in the eutrophic status which the average and maximum concentrations of Chl-a are respectively 8–25 $\mu\text{g/L}$ and 25–75 $\mu\text{g/L}$, the averaged TP is 35–100 $\mu\text{g/L}$, and the mean and minimum values of transparency are respectively 3.0–1.5 m and 1.5–0.7 m. Also, the eutrophication in freshwater body generally would be not in nitrogen-limited state but rather phosphorous-limited state because the agricultural runoff contained a high concentration of DIN compared to DIP would strongly influence water bodies as the inflow loading (Havens and Walker 2002). So, limnological research concerning the water environmental conservation have mainly targeted the eutrophic water bodies with the phosphorous limited state. However, in rural area, an increase in inflow loading of DIP derived from excessive fertilizer application of phosphate beyond the adsorption ability of soil on farmland would result in the hypertrophication with nitrogen limited state in freshwater bodies (Sharpley et al. 2006, Downing and Mc Cauley, 1992). In recent years, prolonged cyanobacteria bloom could occur in the summer or autumn in many agricultural reservoirs, and the maximum concentrations of Chl-a and TP would be far beyond the criterion in the eutrophic status (Kotani, 1992). Also, as an influence of climatic change, the inflow loading of high nutrients concentrations derived from agricultural activities would frequently raise up by localized heavy rainfall, resulting in over-nutrient water areas. In addition, as one impact of global warming on aquatic ecosystem, the elevated water temperature would cause a large space scale and long-term algal bloom formed by cyanobacteria which prefers high water temperature. The Japan Meteorological Agency (2021) reports that annual precipitation, hourly rainfall intensity, and the number of days with heavy rainfall have increased in most parts of Japan from 1901 to 2021 due to climate change. Consequently, excessive nutrient inflow into lakes and agricultural reservoirs has worsened, leading to hypertrophication and

environmental degradation. Therefore, urgent countermeasures would be required to address preventing method for hypertrophication due to excessive inflow loading of nutrients and for generation of cyanobacteria bloom.

This chapter focuses on two distinct approaches to address inhibiting methods for algal bloom by reducing excessive DIN and DIP inflow loading. One is to understand the actual circumstances of the hypertrophic water environment to elucidate the generation mechanisms of algal bloom based on detailed field observations. The other one is to numerically approach the effects of environmental countermeasures on aquatic ecosystems using a water quality prediction model.

Over the years, numerous models have been developed and extensively applied to simulate eutrophication in freshwater bodies. In these models, the studies simulated phytoplankton growth using different state variables for each algae class. Aquatic Ecosystem Models from Zheng et al. 2024 research independently simulated and adjusted the biomass of blue-green algae, green algae, diatoms, and dinoflagellates (using ABM simulation) while tailoring the aquatic ecological cycling mechanism to replicate the eutrophication process in the Shanzai reservoir. In research of George and Michael 2005, the first trophic level of the model distinguished between three phytoplankton groups: diatoms, green algae and cyanobacteria. Similar discrimination of the phytoplankton assemblage was adopted in several studies such as Asaeda and Van Bon, 1997; Menshutkin et al., 1998; Savchuk, 2002. In these studies, the effectiveness of predicting structural changes in phytoplankton community composition under varying nutrient enrichment regimes has been well-demonstrated. However, an essential feature of eutrophication models is their ability to describe sudden shifts (increase/decrease) in phytoplankton populations due to changes in factors such as water temperature, light availability, and nutrients. In this context, the current methods of differentiating algae species within the models have not yet produced optimal results.

In our research, we uniquely determined photosynthesis-related parameters that change with the seasons. This ensures that the dominant species will significantly vary according to water temperature and solar radiation at different levels of algal differentiation. To replicate the abrupt changes in Chl-a, we established several temperature zones and determined the optimal model parameters for phytoplankton growth in each zone. To initially evaluate the effectiveness of applying different temperature zones in the numerical ecosystem model, we used a simple box model for this study.

This study conducted on the agricultural reservoir with the nitrogen-limited hypertrophic status, where a long-term cyanobacteria bloom would be formed on the extensive water surface in the autumn of September to November due to excess inflow loading of phosphorous derived from fertilizer application. First, the field observations were conducted to understand the actual circumstances of the hypertrophic water environment from the viewpoint of the seasonal change characteristics of Chl-a relating to inflow loadings of DIN and DIP stemming from rainfall events. To grasp the reality in hypertrophic reservoir, this study focused on the influence of increase in the phytoplankton amount on other water quality dynamics from viewpoint of organic polluted phenomena represented as an anoxic water generation at the bottom. Following this, the influences of nutrient inflow loading on the water environment were numerically estimated using the one-box type ecosystem model, recognized as one of the most typical and fundamental water quality prediction models. This study proposed the newly devised ecosystem model, which applies to the aquatic environmental analyses in the hypertrophic water area where Chl-a largely fluctuates depending on seasonal or daily changes in environmental factors. This proposed ecosystem model was applied to scenario analyses to assess how much the reduction of inflow and initial loadings of DIN and DIP influence the inhibition of phytoplankton overgrowth. The outcomes from these scenario analyses would play a vital role in establishing a specific numerical target for the aquatic environment restoration of hypertrophic reservoirs.

4.2 Methodology

4.2.1 Field observation

In this study, the periodic observations of water quality parameters related to the eutrophication index were conducted twice a week from April to November 2019 and 2021 to assess in detail the spatiotemporal changes in the aquatic environment. The on-site measurements and water sampling were carried out at the following four observation points: the water surfaces at the north side, south side, and center, and the bottom at the center of the reservoir (**Fig. 2.1D**). As on-site observations, the basic information was obtained using a multi-parameter water quality system (Model 6920, YSI Nanotech), and the transparency was measured using a Secchi disk at the center. The collected water samples were used for the laboratory analysis of two types of Chl-a, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, TN, TP, total organic carbon (TOC), and dissolved organic carbon (DOC). As an indicator representing the total

amount of phytoplankton without distinguishing algal classes, Chl-a concentration was measured by the solvent extraction method using N,N-dimethylformamide with a fluorophotometer (Aquaflour, Turner Designs). In addition, to roughly understand the phytoplankton composition, in-vivo Chl-a concentrations were analyzed at four class-differentiated algae levels, which consisted of Chlorophyceae, cyanobacteria, diatom/dinoflagellates, and cryptophytes, using a multi-wavelength excitation fluorometer (FluoroProbe, bb-Moldaenke). TOC and DOC were measured using a TOC analyzer (Sievers 900, GE Analytical Instruments) based on a wet ultraviolet oxidation reaction and selective membrane conductometric technology. The water quality parameters excluding Chl-a, TOC, and DOC were measured based on the testing methods for industrial wastewater (JIS K 0102) using an ion chromatograph (Dionex Integrion HPIC, Thermo Fisher Scientific K.K.) and an ultraviolet-visible light spectrophotometer (DR5000, HACH). The concentration of sulfide, which was determined as sulfide ions (S^{2-}) liberated from hydrogen sulfide, hydrogen sulfide ion, and soluble metallic sulfides after adding sulfuric acid, was measured using the ethylene blue method. Although the sulfide is not included in the general organic pollution and eutrophication index, this parameter was added as an organic polluted indicator focused on to evaluate how strong the reductive state on the bottom was due to generation of anoxic water.

In addition to periodic observations, continuous observations with 10-minute intervals were carried out to acquire data concerning water temperature, water depth, and inflow discharge to simulate the water environment using the ecosystem model. The water temperature approximately 1.5 m below the water surface was observed at the center as an overall average reservoir value. The water depth at the center was logged by a pressure sensor incorporating memory fixed on the bottom. The inflow discharge into the reservoir was continuously measured using a weir-type flowmeter installed inside the box culvert. Furthermore, the following irregular field surveys were conducted to examine the inflow loadings of DIN and DIP from the farmland on the upstream side. NO_3-N , NH_4-N , and PO_4-P in influent concentrations were measured seven times in 2019 and nine times in 2021 on heavy rainfall events. These results were provided for acquiring the LQ equations describing the relation between inflow loading ($L_x = C_x \cdot Q$) and inflow discharge of water (Q), where the subscript X indicates the water quality parameters of NO_3-N , NH_4-N , and PO_4-P , and C is a concentration of X parameter. Moreover, the basic data (air temperature, solar radiation,

and rainfall) was utilized at 10-minute intervals on a meteorological observation station near the reservoir's farmland.

4.2.2 Water quality prediction model based on a one-box type ecosystem model.

To quantitatively estimate phytoplankton's overgrowth related to DIN and DIP inflow from rainfall events, this study focused on the increase in Chl-a since summer or autumn as the targeted calculation period. When simulating seasonal changes in Chl-a using an ecosystem model, it is necessary to uniquely determine the photosynthesis-related parameters that vary with the seasons. This ensures that the dominant species will change significantly according to water temperature and solar radiation at different levels of algal differentiation. To reproduce the sudden change in Chl-a, several temperature zones were established and the optimal model parameters for phytoplankton growth were determined in each zone. Besides, the reservoir's shallow depth, approximately 4.5 meters, facilitated easy water mixing from bottom to top, increasing oxygen levels. To initially assess the effectiveness of applying different temperature zones in the shallow reservoir in the numerical ecosystem model, we chose a simple box model for this study.

The water quality prediction model in this study was a completely mixed-box model based on the ecosystem model (Bui & Dang, 2020) and included six state variables: phytoplankton (PP), zooplankton (ZP), particulate nonliving organic matter (POM), dissolved organic matter (DOM), DIP (= $\text{PO}_4\text{-P}$), and DIN (= $\text{NH}_4\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$). The basic idea of an ecosystem model is to define the biomass of all organisms in terms of the mass of carbon per unit volume and to formulate the matter cycle based on the biochemical interactions shown in **Figure 4.1** by using mass balance equations. When biochemical processes are represented by first-order kinetics, the resulting equations are given as a first-order ordinary equation for the concentration variation per unit time. In this target reservoir, the water depth changed over time due to factors such as inflows, outflows, and seasonal variations. When water depth varies, the volume of the reservoir also changes. If biochemical processes are described in terms of concentration (mass per unit volume), this variation in volume can complicate the calculations, as the concentration would need to be adjusted continuously based on the changing volume. Therefore, the mass balance equations are considered for the whole quantity in reservoir capacity. When the reservoir's volume V is represented by the product of representative water surface area (= 2 560 m²) and the water

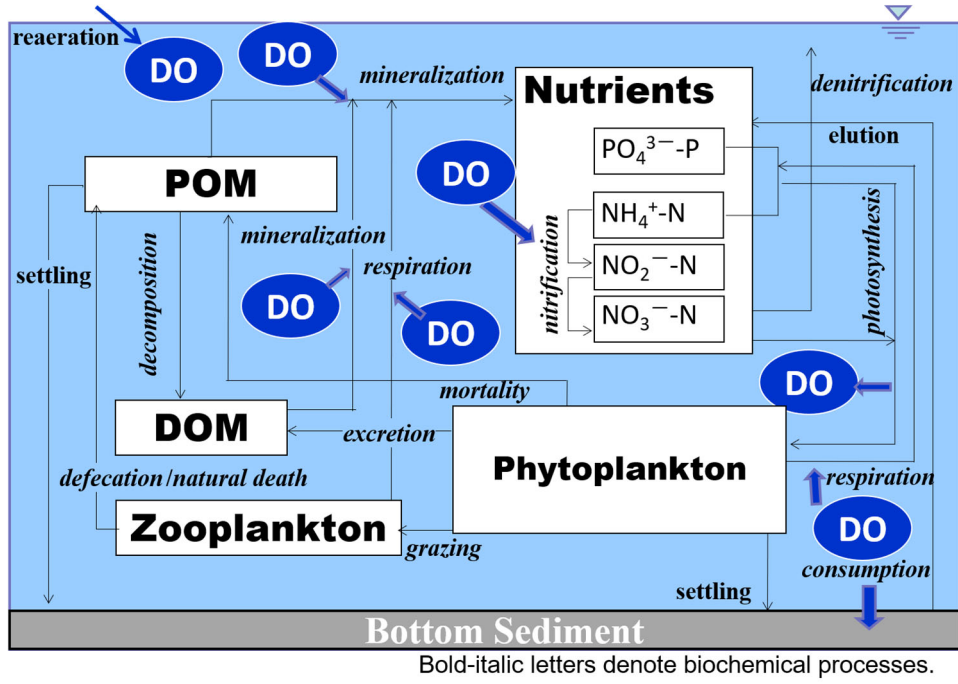


Figure 4.1 Schematic layout of the one-box ecosystem model

depth h continuously measured at the center, the mass balance equations of six state variables including PP, ZP, POM, DOM, DIN, and DIP were given as follows.

The carbon content of phytoplankton is gained by photosynthesis (growth) and lost by respiration, grazing by zooplankton, excretion, non-predatory mortality, and settling. As there is no inflow loading of algae from the upland farm, the mass balance for phytoplankton concentration C_{PP} is expressed as follows without considering the inflow loading of algae,

$$\frac{d(V C_{PP})}{dt} = v_g V C_{PP} - v_{rp} V C_{PP} - v_{gz} V C_{ZP} - v_e V C_{PP} - v_{npm} V C_{PP} - \frac{w_{PP}}{h} V C_{PP} - L_{out, PP} \quad (1)$$

where v_g is the photosynthesis rate, v_{rp} is the respiration rate, v_{gz} is the grazing rate for zooplankton, C_{ZP} is the concentration of zooplankton, v_e is the excretion rate, v_{npm} is the rate of non-predatory mortality, w_{PP} is the settling velocity, $L_{out, PP}$ is the outflow of phytoplankton.

As photosynthesis is mainly affected by water temperature, underwater light intensity, and nutrient concentration of DIN and DIP, these three environmental factors can be considered in the definition of growth rates. That is, v_g is represented in the consideration with limiting factors on photosynthesis as

$$v_g = a_g \cdot f_T \cdot f_I \cdot f_N \quad (2)$$

where a_g , the maximum growth rate, f_T , f_I , and f_N denote the limitation functions of water temperature, light, and nutrients, respectively. The limitation functions can be defined from

0 to 1, representing the positive influence against phytoplankton growth. For example, the smaller the value f_T , the greater the growth inhibition due to temperature. Generally, f_T is defined using an optimal temperature T_{opt} as follows (Do et al., 2010; Bui and Dang, 2020):

$$f_T = \left\{ \frac{T}{T_{\text{opt}}} \exp \left(1 - \frac{T}{T_{\text{opt}}} \right) \right\}^{\xi} \quad (3)$$

The relationship between the photosynthetic rate and light intensity can be represented with Steel's equation (Steel, 1965). When the vertical profile of underwater light intensity can be represented by the Lambert-Beer law, the light limitation function for the light intensity averaged for the whole depth can be expressed based on Steel's equation, as follows:

$$f_I = \frac{1}{\kappa h} \left\{ \exp \left(1 - \frac{I_0}{I_{\text{opt}}} \exp(-\kappa h) \right) - \exp \left(1 - \frac{I_0}{I_{\text{opt}}} \right) \right\} \quad (4)$$

where I_{opt} is the optimal light intensity, I_0 is the solar radiation at the water surface, and κ is the extinction coefficient of underwater light intensity. The above equation shows that the photosynthetic rate reaches a maximum at the optimal light intensity and that the phytoplankton growth has a strong light-inhibiting under the condition of $I > I_{\text{opt}}$. Furthermore, the growth limitation functions for nutrients of DIN and DIP can be described by the Michaelis–Menten equation and Liebig's law of the minimum, as follows:

$$f_N = \min \left(\frac{C_{\text{DIP}}}{K_{\text{DIP}} + C_{\text{DIP}}}, \frac{C_{\text{DIP}}}{K_{\text{DIN}} + C_{\text{DIN}}} \right) \quad (5)$$

where K_{DIP} and K_{DIN} are half-saturation constants. The above equation expresses that the least available nutrient determines growth. The first-order rate constants of other parameters, represented by the symbol of v , can be defined as functions of water temperature as $v = \alpha \cdot \exp(\beta T)$, where T is the water temperature, α is a rate constant at 0°C , and β is a temperature coefficient. The respiration rate v_{rp} can be define by:

$$v_{\text{rp}} = \alpha_{\text{rp}} \cdot \exp(\beta_{\text{rp}} T) \quad (6)$$

where α_{rp} is the respiratory rate, and β_{rp} is the temperature effect constant for respiration. The rate of non-predatory mortality v_{npm} can be define by:

$$v_{\text{npm}} = \alpha_{\text{npm}} \cdot \exp(\beta_{\text{npm}} T) \quad (7)$$

where α_{npm} is nonpredatory mortality rate, and β_{npm} is the temperature effect constant for mortality. The grazing rate for zooplankton v_{gz} can be define by:

$$v_{gz} = \alpha_{gz} \exp(\beta_{gz} T) \cdot (1.0 - \exp(\lambda \cdot (C_{th} - C_{pp}))) \quad (8)$$

where α_{gz} is the growth rate, β_{gz} is the temperature effect constant for growth, λ is the Ivlev constant and C_{th} is the phytoplankton threshold concentration for zooplankton predation.

The zooplankton was modeled as a single animal group whose dynamics is governed by the same general processes as seen for phytoplankton: algal predation, fecal excretion, respiration, and total mortality. The mass balance for zooplankton concentration C_{ZP} is expressed as follows:

$$\frac{d(V C_{ZP})}{dt} = v_{gz} V C_{ZP} - (1 - e) v_{gz} V C_{ZP} - (e - g) v_{gz} V C_{ZP} - v_{zm} V C_{ZP} \quad (9)$$

where e is the coefficients of assimilation efficiency; g is gross growth efficiency for zooplankton; v_{zm} is zooplankton total mortality. The rate of zooplankton total mortality v_{zm} can be define by:

$$v_{zm} = \alpha_{zm} \cdot \exp(\beta_{zm} T) \quad (10)$$

where α_{zm} is total mortality rate, β_{zm} is the temperature effect constant for mortality.

The POM concentration was primarily controlled by the following processes: nonpredatory mortality of phytoplankton, zooplankton fecal excretion, zooplankton mortality, mineralization to available nutrients, hydrolysis to DOM, and settling. The mass balance for POM is expressed as follows:

$$\frac{d(V C_{POM})}{dt} = v_{npm} V C_{PP} + (1 - e) v_{gz} V C_{ZP} - v_{zm} V C_{PP} - v_{mip} V C_{POM} - \xi v_{mip} V C_{POM} - \frac{w_{POM}}{h} V C_{POM} \quad (11)$$

where ξ is a constant generally determined in the range of 1 to 3, v_{mip} is the mineralization rate of POM, and w_{POM} is settling. The mineralization rate can be defined by the exponential curve in combination with an effect of the DO concentration. The mineralization rate of POM v_{mip} can be define by:

$$v_{mip} = \frac{\alpha_{mip} \cdot \exp(\beta_{mip} T) \cdot DO}{K_{DO1} + DO} \quad (12)$$

where α_{mip} is the rate constant of POM, β_{mip} is the effect of temperature on POM mineralization, DO is the concentration of dissolved oxygen, and K_{DO1} is the DO half-saturation constant for oxygen limitation of POM mineralization.

The processes for DOM include algal excretion, POM hydrolysis, and DOM mineralization. The kinetics equation for the DOM can be described as follows:

$$\frac{d(VC_{\text{DOM}})}{dt} = v_e VC_{\text{PP}} + \xi v_{\text{mip}} VC_{\text{POM}} - v_{\text{mid}} VC_{\text{DOM}} \quad (13)$$

where v_{mid} is the mineralization rate of DOM. The mineralization rate of DOM v_{mid} can be define by:

$$v_{\text{mid}} = \frac{\alpha_{\text{mid}} \cdot \exp(\beta_{\text{mid}} T) \cdot \text{DO}}{K_{\text{DO2}} + \text{DO}} \quad (14)$$

where α_{mid} is the mineralization rate, β_{mid} is the effect of temperature on DOM mineralization, and K_{DO2} is the DO half- saturation constant for oxygen limitation of DOM mineralization.

DIP and DIN are gained due to respiration from phytoplankton and zooplankton and mineralization of POM and DOM, they are lost by photosynthesis in phytoplankton. Furthermore, considering the elution of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ from the bottom sediment as well as inflow and outflow loadings, the mass balances for nutrient concentrations C_{DIPorDIN} can be written as follows:

$$\begin{aligned} \frac{d(VC_{\text{DIPorDIN}})}{dt} = & [\text{P or N:C}_{\text{PP}}] v_{\text{tp}} VC_{\text{PP}} - [\text{P or N:C}_{\text{PP}}] v_{\text{g}} VC_{\text{PP}} + [\text{P or N:C}_{\text{ZP}}] (e - g) v_{\text{gz}} VC_{\text{ZP}} \\ & + [\text{P or N:C}_{\text{POM}}] v_{\text{mip}} VC_{\text{POM}} + [\text{P or N:C}_{\text{DOM}}] v_{\text{mid}} VC_{\text{DOM}} + V \frac{F_{\text{DIPorDIN}}}{h} + L_{\text{in,DIPorDIN}} - L_{\text{out,DIPorDIN}} \end{aligned} \quad (15)$$

where $[\text{P or N:C}_{\text{PP}}]$, $[\text{P or N:C}_{\text{ZP}}]$, $[\text{P or N:C}_{\text{POM}}]$, and $[\text{P or N:C}_{\text{DOM}}]$ are the respective mass ratios of phosphorus-to-carbon or nitrogen-to-carbon in phytoplankton, zooplankton, POM, and DOM, F_{DIPorDIN} , the elution flux of DIP and DIN from the bottom sediments, $L_{\text{in,DIPorDIN}}$ and $L_{\text{out,DIPorDIN}}$ are the inflow and outflow loadings of DIP and DIN. In this research, the inflow load of nutrients due to deposition from the atmosphere was ignored because the site experiences minimal atmospheric nutrient deposition due to low industrial activity and geographical features. This decision was also made to isolate the impact of agricultural runoff without interference from atmospheric deposition, thus accurately assessing its contribution to nutrient loading. The elution flux of DIP and DIN from the bottom sediments occur when DO is lower than 4.0 mg/L. The elution flux of DIP occurs after denitrification ($\text{NO}_3\text{-N} < 0.1$). It can be defined by below equation:

$$F_{\text{DIP}} = \frac{\alpha_{\text{fp}} \cdot \exp(\beta_{\text{fp}} T)}{h} \quad (16)$$

$$F_{\text{DIN}} = \frac{\alpha_{\text{fn}} \cdot \exp(\beta_{\text{fn}} T)}{h} \quad (17)$$

where α_{fp} and α_{fn} are the elution flux of DIP and DIN at 0⁰ C, and β_{fp} and β_{fn} are effect of temperature on exchange flux.

The model parameters, for example, the biochemical reaction rate constant and the mass ratios such as [P: C_{PP}], were set in relation to previous studies (Do et al. 2010; Bui & Dang 2020). They were calibrated by trial-and-error adjustment to best match trends in the observed water quality data in 2019 and 2021. A dominant single species in the general ecosystem model would be considered as a typical phytoplankton through all seasons. It is difficult to consider algal species' diversity and dominant algae's seasonal change. However, it is hard to handle the typical phytoplankton in the long term over several months in the eutrophic water areas where actual dominant species drastically vary in multiple algal classes (Chlorophyceae, Cyanobacteria, diatoms, etc). Particularly, the difficulty of model application to the hypertrophic water area would be more conspicuous because algal blooms abruptly appear or disappear in response to meteorological conditions in the heating season. Therefore, it is required to uniquely determine the parameters related to the photosynthesis for the seasonal change such that the dominant species would alter significantly at algal class-differentiated levels corresponding to water temperature and solar radiation. In this study, several temperature zones were set, and the values of α_g , ξ , T_{opt} , K_{DIN} , K_{DIP} and I_{opt} were determined in each zone. By first setting the maximum growth rate α_g in the water temperature zones, it could be possible to suppose that typical phytoplankton is a dominant species determined to correspond to the water temperature. Also, it could be possible to reflect the typical phytoplankton to determine the model parameters, T_{opt} , K_{DIN} , K_{DIP} and I_{opt} characterize the limitation functions by temperature, light, and nutrients. By handling model parameters, it would be expected that the significant changes in phytoplankton concentration corresponding to meteorological environment factors could be reproduced.

The Runge-Kutta-Gill method was applied to the numerical solution of the ordinary differential equations at 10-minute intervals to reproduce the periodically

observed results of Chl-a. The initial condition of the model was set on July 2nd, 2019, and September 10th, 2021, and the calculation period was 151 days and 104 days in 2019 and 2021, respectively. In the targeted reservoir, macroalgae, such as filamentous algae, flourished from spring to summer. One-box ecosystem model inherently simplified the complexity of real ecosystems. It often focused on broad-scale dynamics and does not include detailed representations of specific organisms like macroalgae. By starting the model after the algae have disappeared, it avoids the need to represent the detailed growth, interactions, and seasonal variations of macroalgae, allowing for a more straightforward analysis of the remaining ecosystem components. Therefore, the initial condition was set after the disappearance of filamentous algae in both years. This simulation required observation data under the numerical conditions as follows. The time series data of water temperature, continuously measured at approximately 1.5 m below the water surface as an overall average value of the reservoir, was essential to consider the temperature dependency of biochemical rates. Also, the periodic observations of transparency D_s were applied to calculate an extinction coefficient of underwater light intensity by the relation of $\kappa = 1.7/D_s$ and was used to calculate the limitation functions expressed in Equation (4) together with the continuously observed results of solar radiation I_0 . Furthermore, the continuous measurements of water depth at the center were utilized not only for calculating the reservoir's volume V , but also for estimation of the outflow discharge through broad crested weirs by considering a difference of h from a weir height as an overflow depth to evaluate the outflow loadings of $L_{out,DIN}$, and $L_{out,DIP}$. In addition, the continuously measured inflow discharge via the box culvert was applied to the calculations of $L_{in,DIN}$ and $L_{in,DIP}$ using LQ equations derived from the observation data.

This proposed water quality prediction model was applied to scenario analyses to assess how much the reduction of DIN and DIP inflow loadings influence the inhibition of phytoplankton overgrowth. The scenario was set up for the same period as the calculation period in the model. The seriousness of algal bloom occurring in autumn might be affected by the timings when the nutrient concentration in the reservoir's water would increase due to inflow loadings during heavy rainfall. In 2019, heavy rains in summer caused a significant influx of nutrients, leading to high levels of both DIN and DIP by September. In 2021, despite low summer rainfall maintaining low nutrient inflow,

the concentration of both DIN and DIP increased from September due to substantial nutrient inflows from autumn rain. Based on the results of field observation in these two years, which will be discussed in more detail later, this study set up two cases for scenario analysis. The case in 2019 was determined by focusing on the excessive inflow-loadings due to local severe rain in late summer. This scenario analysis was applied to assess the influence of controlling the high DIN and DIP concentrations in late summer on the inhabitation of algal bloom in the case of little precipitations in autumn. The case in 2021 was set to evaluate how high concentrations of phytoplankton could be inhibited by reducing the excess inflow loadings of nutrients during the proliferation period of cyanobacteria in autumn. In each case, three scenarios were set up to quantitatively evaluate the responses of Chl-a concentration to changes in the inflow loading in 2019 and 2021 of 1) DIP only, 2) DIN (NH₄-N and NO₃-N) only or 3) DIP and DIN together. The increase in the inflow loading of DIP and DIN in scenarios was determined by multiplying the observed values with $(1 + \alpha/100)$, where α was the rate of change and was set from -100% to 50% at intervals of 10%.

4.3 Results and discussions

4.3.1 Seasonal characteristics of water quality parameters based on periodic field observations

Figure 4.2 shows periodically observed results of Chl-a concentrations and algal class-differentiated ratios of the average water surface points (north-side, center, and south-side) in 2019 and 2021. Additionally, the seasonal changes in the average of water quality observations (Chl-a, PO₄-P, NH₄-N, NO₃-N, and N/P ratio) at the water surface, the inflow discharge and inflow loadings of each type of nutrients, the temporal changes in daily rainfall measured on the farmland near the targeted reservoir were summarized in **Figure 4.3A** for 2019 and **Figure 4.3B** for 2021. In **Figure 4.3**, the N/P ratio means the ratio of TN and TP. **Figure 4.4** shows the water depth and transparency of the targeted reservoir in 2019 and 2021, and the LQ equations acquired based on the field observations are shown in **Figure 4.5**. The descriptive statistical characteristics of the above-observed results are summarized in **Table 4.1**. When comparing this table with the trophic status standard by OECD, the targeted reservoir was classified into the hypertrophic status (the average and maximum in Chl-a: more than 25 µg/L and more than 75 µg/L, the average in TP: more than 0.1 mg/L,

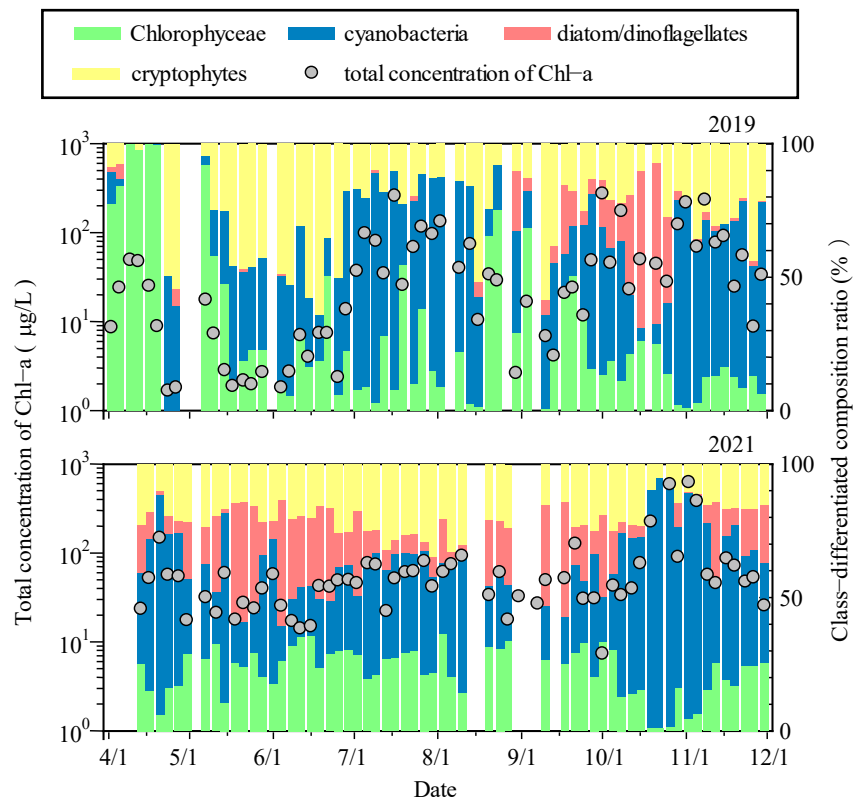


Figure 4.2 Periodically measured chl-a and algae class-differentiated ratio in 2019 and 2021 on the water surface

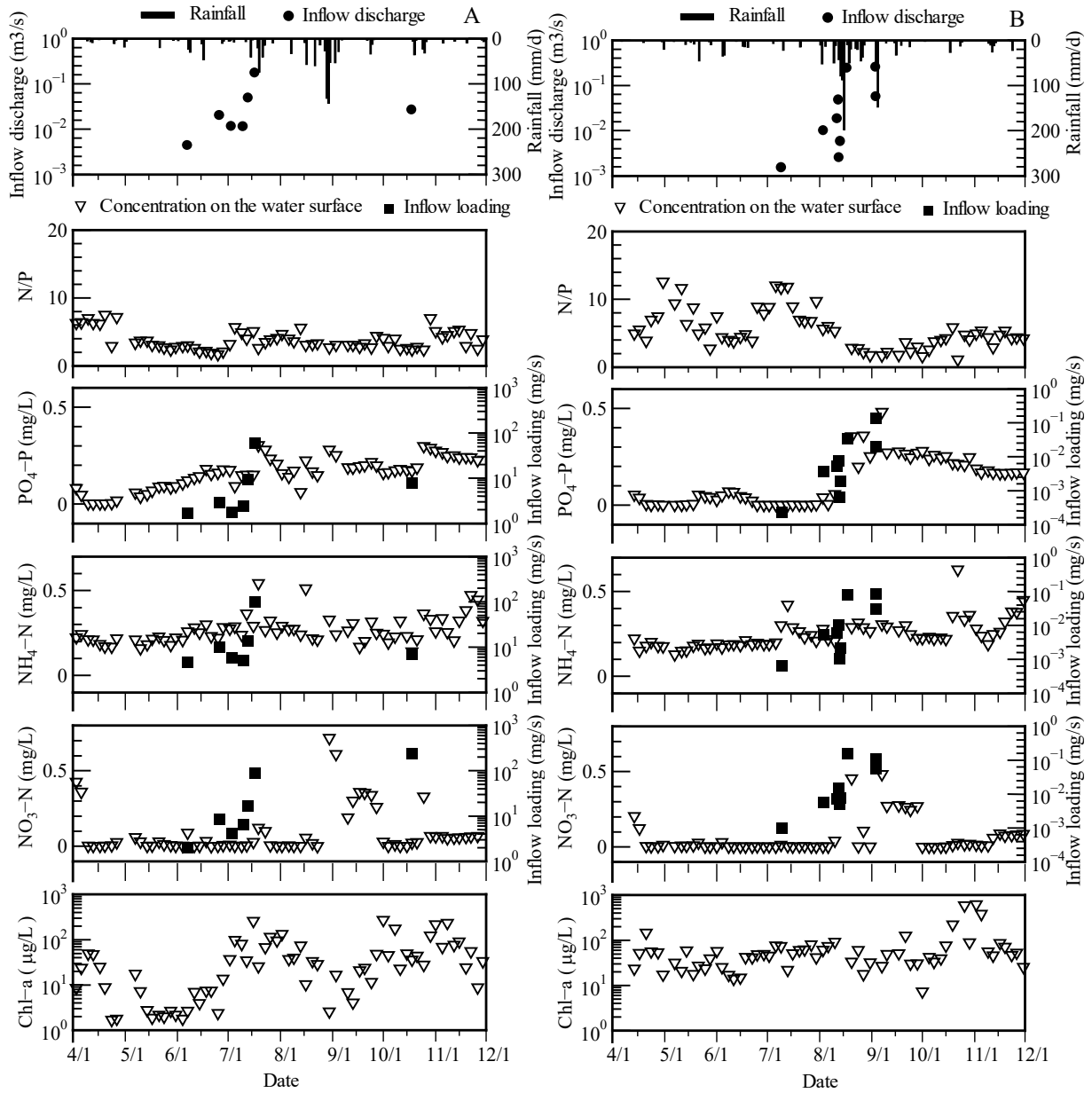


Figure 4.3 Rainfall, inflow discharge and inflow loading observed results and Ratio of N/P, PO₄-P, NH₄-N, and NO₃-N, and Chl-a concentration on the water surface in 2019 (A: left) and 2021 (B: right)

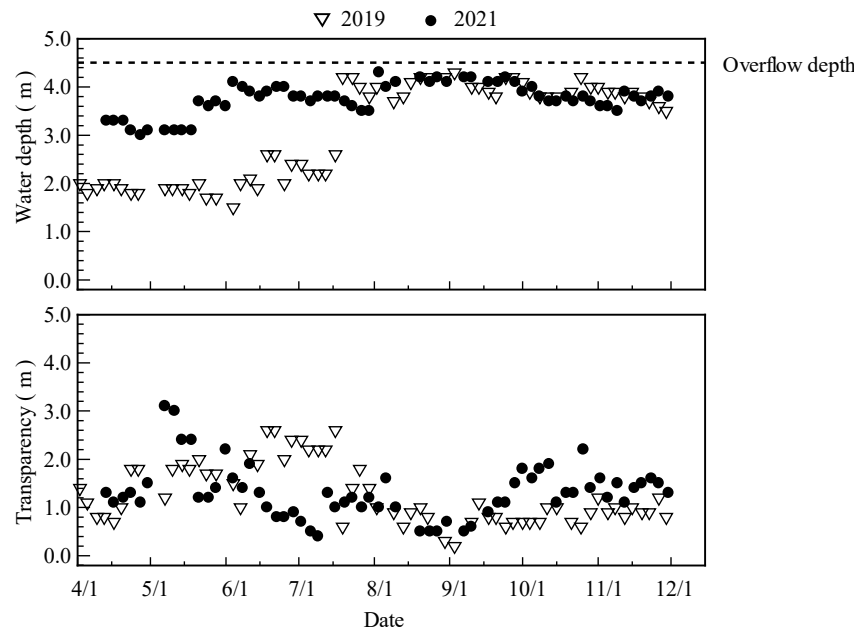


Figure 4.4 Water depth and transparency of target reservoir in 2019 and 2021

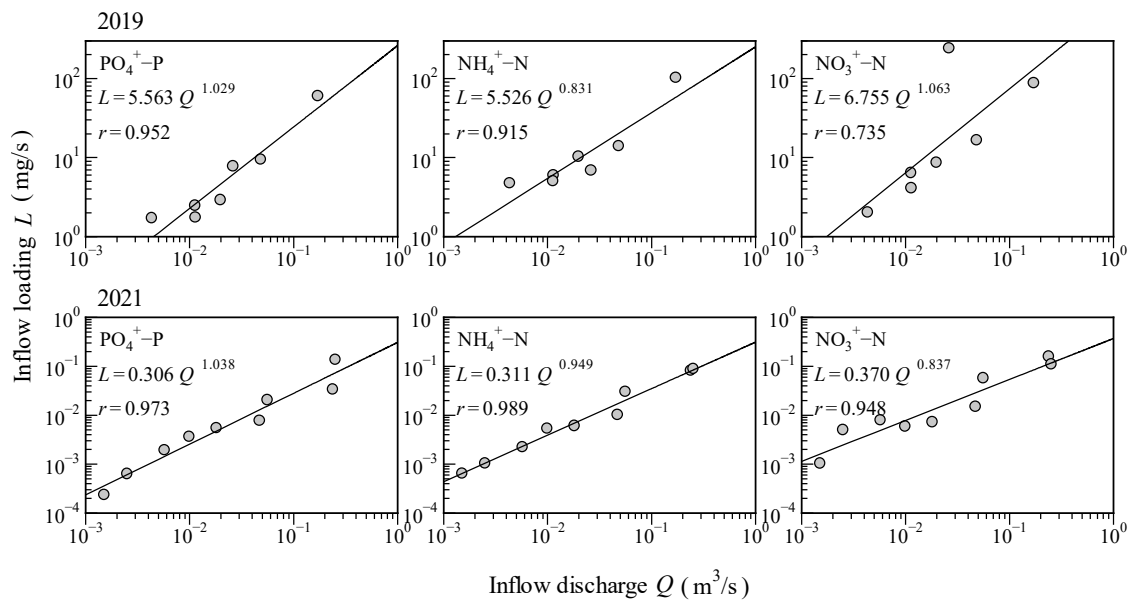


Figure 4.5 Relational expression between inflow discharge Q and nutrient inflow load L (LQ equation) in 2019 and 2021

Table 4.1 The average, maximum, and minimum of main observed water environmental parameters in 2019 and 2021

	Levels	Chl-a ($\mu\text{g/L}$)	TP (mg/L)	$\text{PO}_4\text{-P}$ (mg/L)	TN (mg/L)	$\text{NO}_3\text{-N}$ (mg/L)	$\text{NH}_4\text{-N}$ (mg/L)	N/P
2019	Ave	48.9	0.345	0.158	1.393	0.089	0.267	3.776
	Max	696.9	2.253	0.309	10.709	0.829	0.557	15.433
	Min	1.3	0.030	0.005	0.363	0.000	0.044	1.265
2021	Ave	95.3	0.595	0.120	2.415	0.248	0.057	5.613
	Max	3847.0	21.923	0.486	44.560	1.183	0.486	15.424
	Min	4.9	0.028	0.002	0.296	0.113	0.001	0.900

and the average and minimum in transparency: less than 1.5 m and less than 0.7 m). However, applying the trophic classification to the OECD standard was not necessarily adequate without using full-year data. To supplement the judgment of the severity of eutrophication, this study employed the trophic classification scheme by Forsberg and Ryding (1980). In this scheme, the trophic status could be classified by averages of TP, TN, Chl-a, and transparency in the summer of June to September. As the observation period in this research was in a heating season and TN was included as a eutrophication index, the trophic classification scheme by Forsberg and Ryding (1980) seemed suitable for evaluating the water environment in the targeted reservoir. According to this scheme, most of the water quality index in 2019 and 2021 exceeded the determination criteria of eutrophic status (TP: 0.25 to 0.1 mg/L , TN: 0.6 to 1.5 mg/L , Chl-a: 7 to 40 $\mu\text{g/L}$, and transparency: 1.0 to 2.5 m). Although the average TN in 2019 was 1.393 mg/L in the eutrophic range, its average value in the determinate period of June to September was 1.835 mg/L over the eutrophic range. Therefore, it was confirmed that the targeted reservoir was hypertrophic.

From **Figure 4.2**, the overall trend of seasonal changes in Chl-a averaged on the water surface points (north-side, center, and south-side) bore a resemblance between 2019 and 2021 in that the concentration had large fluctuation throughout the observation period in the wide order (2019: range of 10^0 to 10^3 and 2021: 10^1 to 10^3). The seasonal changes were characterized by three periods: the relatively low concentration period of April to June, in which a variation was large due to a large fluctuation, the increase period of July to mid-November, and the decrease period in late November. Chl-a increased in two steps from July to mid-November 2019 because of the temporal decrease from late August to early September. The remarkable point was that the Chl-a concentration in both years exponentially increased with a high temporal change rate. Its order ranged from 10^2 to 10^3 in summer and autumn, although it drastically lowered to 10^1 together with the temporary

decline of cyanobacteria from late August to early September. In the increase period in 2019 and 2021, a dominance of cyanobacteria with an algae class-differentiated composition ratio of 60-90 % intermittently engendered the mat-like algal bloom over a wide range in two terms of July to early August and October to mid-November as shown in **Figure 4.6**. In addition, both years had the characteristics in common in that the Chl-a averaged on water surface points exponentially increased to a peak value over the order of 10^2 in the autumn which included the maximum concentrations (2019: 696.9 $\mu\text{g/L}$ and 2021: 3847.0 $\mu\text{g/L}$) shown in **Table 4.1**, and that the increase period was prolonged until mid-November with water temperature ranging from 14 to 18 $^{\circ}\text{C}$ even though the optimal water temperature for the cyanobacteria growth would be higher than 25 $^{\circ}\text{C}$ (Paerl 2014).

According to **Figure 4.3A**, the N/P ratio in 2019 changed from 2 to 7 (with an average of 3.8) throughout the observation period. According to Guildford and Hecky (2000), when the N/P ratio is less than 20, the nutrient status of the reservoir is determined as nitrogen limitation of growth; this denoted that the targeted water body was in a strong nitrogen-limited status in the aquatic ecosystem, as the balance between nitrogen and phosphorus necessary for photosynthesis was not maintained. Particularly, high Chl-a concentrations were sustained from July to mid-November without alleviation of nitrogen limitation. Additionally, in **Figure 4.3B**, the N/P ratio in 2021 exhibited a nitrogen-limiting condition, similar to 2019, throughout spring to autumn. However, there were differences between the two years; the N/P ratio in 2021 varied from 1 to 14, and the nitrogen limitation was temporarily alleviated in May and July, unlike in 2019. The alleviation of nitrogen limitation in 2021 is linked to a balance between nitrogen and phosphorus available for photosynthesis.



Figure 4.6 Cyanobacteria blooms occurred on the water surface of the target reservoir in (left) October 2019 and (right) October 2021.

Nevertheless, an increase in Chl-a due to this balance was unclear, and the alleviation of nitrogen limitation did not always lead to the obvious proliferation of phytoplankton. In the following discussion, this study examined the responsiveness of Chl-a to the seasonal changes in nitrogen and phosphorus according to rainfall events as meteorological conditions based on **Figure 4.3**.

In 2019, PO₄-P exceeded 0.1 mg/L except for April and changed at a higher concentration than the DIP level in general eutrophic freshwater areas. Based on **Figure 4.3A**, it was evident that PO₄-P noticeably increased in response to rainfall events with daily precipitation exceeding 50 mm/d since mid-May. Consequently, such a rainfall event led to a significant increase in PO₄-P concentration exceeding 0.2 mg/L since July. Particularly, during heavy rainfall events associated with the stagnant rainy season in mid-July and the linear precipitation zone in late August, PO₄-P levels increased to as high as 0.3 mg/L due to exceptionally large inflow loadings from the watershed. According to the 2019 agricultural records at the experimental farm upstream of the targeted reservoir, DIP was intermittently applied as a supplementary fertilizer at multiple frequencies from April to November. Therefore, this excessive phosphorus fertilization, surpassing the adsorption ability in soil, seemed to result in a high concentration of PO₄-P in the targeted reservoir; this occurred because of a large inflow loading from agricultural fields through a box culvert. Moreover, during the increasing period of Chl-a in the summer, the reservoir received an excessive inflow loading of phosphorus. It was impossible to confirm a significant decrease in PO₄-P caused by algal uptake for photosynthesis. That is, phosphorus's temporal excessive inflow loading is linked to a strong nitrogen-limited status. On the other hand, NH₄-N in 2019 changed in the range of 0.25 to 0.4 mg/L with a slight increase due to inflow loading in mid-July and mid-August. However, there was no significant seasonal variation in the observation of NH₄-N. This result showed that the overall impact of inflow loading on NH₄-N concentration was minimal, and the decrease in NH₄-N due to uptake by algae was relatively small.

Meanwhile, NO₃-N temporally changed at a low level in the order of 10⁻² throughout the observation period except for a temporary increase over 0.1 mg/L during rainfall events in mid-July, late August, and late October. These sudden increases in NO₃-N concentration triggered by rainfall events rapidly lowered to zero level within a few days or weeks in the period when Chl-a kept over 20–30 µg/L corresponding to the maximum concentration in

the hypertrophic status. The decreases in $\text{NO}_3\text{-N}$ resulted from phytoplankton uptake as a nitrogen source for algal growth. Based on species composition at the algal class level, cyanobacteria likely dominated this uptake. In early October, when almost all the available $\text{NO}_3\text{-N}$ had been taken up by phytoplankton, Chl-a levels increased to over 102, with cyanobacteria being dominant. (**Figure 4.2**). This consideration seemed identical to the understanding that cyanobacteria would preferentially utilize $\text{NO}_3\text{-N}$ in DIN as a nitrogen source necessary for photosynthesis as mentioned in Chapter 3. Particularly the drastic and temporary increase of $\text{NO}_3\text{-N}$ exceeding 0.7 mg/L derived from a rainfall event of approximately 200 mm/d at the end of August. This event seemed to impact cyanobacteria's growth under strong nitrogen-limited conditions, resulting in the prolonged occurrence of the algal bloom until mid-November. In July, ammonium sulfate was applied as an additional fertilizer at the experimental farm. This application led to the inflow loading of $\text{NO}_3\text{-N}$ derived from the nitrification of $\text{NH}_4\text{-N}$ supplied in soil, significantly alleviating the strong nitrogen limitation for the growth of cyanobacteria.

In **Figure 4.3B**, there were very few rainfall events exceeding 20 mm/d until July 2021, and it was considered that the impact of inflow loading on $\text{PO}_4\text{-P}$ was extremely minimal during this period. Although temporary increases in $\text{PO}_4\text{-P}$ over 0.1 mg/L due to some rainfall events in the period of mid-May to early June resulted in a relatively strong nitrogen-limited state with an N/P ratio of about 5, $\text{PO}_4\text{-P}$ concentration in the reservoir changed at the low level with the order of 10^{-2} . So, the relatively high N/P ratio over 10 in this period did not result from increased nitrogen concentration through the inflow loading. Instead, it was because phosphorus remained low, unaffected by the inflow loading of nutrients associated with rainfall events. However, Chl-a from April to July 2021 seasonally changed over 20 $\mu\text{g/L}$, unlike in 2019, and frequently reached a high concentration of about 80 $\mu\text{g/L}$. Therefore, until July, there was no inhibition of photosynthesis due to low concentrations of DIN and DIP, and the impact of decreases in nutrient inflow loadings on the phytoplankton growth was very small. That is, the potential accumulation of nitrogen and phosphorus until the last year was confirmed, unlike in 2019. In addition, both $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ in the waterbody took high concentrations of approximately 0.5 mg/L during the heavy rainfall, including two events with precipitation of 150–200 mm/d in August and September. These results indicate the influence of heavy rainfall events on excess nutrient inflow loadings from fertilization in upstream agricultural fields. $\text{NO}_3\text{-N}$ decreased sharply

in late September and was kept at zero during the increase period of Chl-a exceeding 100 $\mu\text{g/L}$ with the dominance of cyanobacteria since October (**Figure 4.2**).

In contrast, $\text{NH}_4\text{-N}$ has remained almost constant since September. These results suggested that the supply of $\text{NO}_3\text{-N}$ via external loading promoted the growth of cyanobacteria, which would not require $\text{NH}_4\text{-N}$ but $\text{NO}_3\text{-N}$ as a nitrogen source. $\text{PO}_4\text{-P}$ in autumn was kept at a high concentration of around 0.2 mg/L , resulting in a strong nitrogen limitation. However, it decreased from the peak value of heavy rainfall events in August and September.

It could be concluded from a common point in both 2019 and 2021 that the response of the phytoplankton, mainly including cyanobacteria, had an exceptionally sensitive response to the temporary supply of $\text{NO}_3\text{-N}$ via the inflow loadings derived from heavy rainfall events while maintaining high $\text{PO}_4\text{-P}$ concentrations exceeding 0.1 mg/L . This response led to a significant increase in Chl-a concentrations, reaching levels beyond the order of 10^3 . Therefore, the control of $\text{NO}_3\text{-N}$ elevation during the summer and autumn periods in nitrogen-limited eutrophic water bodies could link to one of the crucial countermeasures for inhibiting cyanobacterial blooms in nitrogen-limited eutrophic water bodies.

4.3.2 Water environmental features at the bottom

Figure 4.7 shows the observed DO saturation, ORP and sulfides at the bottom of the targeted reservoir in 2019 and 2021. **Figure 4.8** expresses the temporal changes in water temperature at the water surface and the bottom. There were obvious differences in the seasonal changes of DO and sulfide between both years. By focusing on such differences, this study considered what environmental factors would impact on the biochemical and hydraulic dynamics of water quality at the bottom.

In 2019, DO in the bottom water took consistently zero from late July to early October. Particularly, the anoxic water at the bottom led to the strongly reducing anaerobic condition with an ORP of -150 mV in mid-August, resulting in the generation of approximately 50 $\mu\text{g/L}$ of sulfide. In the following, the characteristics of seasonal changes in DO were discussed from viewpoint of the factors including water depth, transparency, temperature difference between the water surface and bottom, and temporal variations in sulfide concentration. From April to mid-July, the water depth remained relatively constant at around 2 m, and transparency values were approximately 2 m.

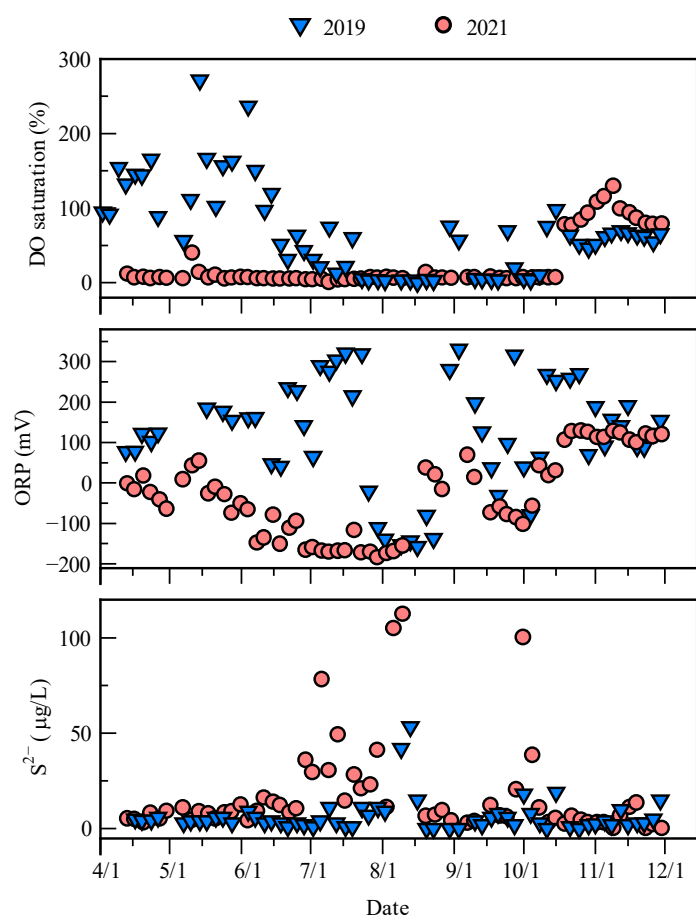


Figure 4.7 Field observation of DO, ORP and S^{2-} in 2019 and 2021

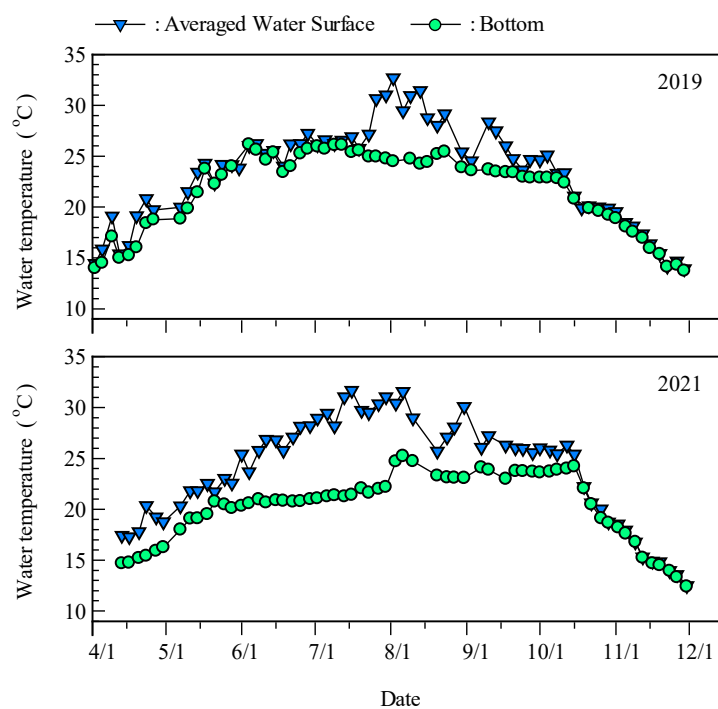


Figure 4.8 Field observation of water temperature in 2019 and 2021

In addition, the water temperature difference in this period was generally less than 1 °C even though the differences of 1.5 to 2.5 °C were observed at some days in May. That is, the relatively shallow water depth of about 2 m linked to a mixing state throughout the entire water column because of minimal attenuation of underwater light intensity in the vertical direction. As a result, DO at the bottom took a high level of saturation exceeding 100 % similarly to that at the water surface from April to mid-July. On the other hand, a large amount of inflowing water via the box culvert due to heavy rainfall of about 100 mm/day observed in mid-July rapidly increased the water depth from approximately 2 m to 4 m. Furthermore, the effects of rainfall events, including the strong precipitation rates of 150–200 mm/d in late August, contributed to maintaining water depth near full capacity until the end of November. The transparency remained at a low level since it decreased rapidly to around 1 m according to the increase in water depth at mid-July. As one of factor that the low level of transparency kept on since mid-July, a high concentration of suspended sediment was caused by not only increase of inflowing turbid water due to intermittent rainfall events exceeding 100 mm/day, but also phytoplankton proliferation with a high concentration of Chl-a ranging from 40 to 100 µg/L at even the central surface water. This decrease in transparency reduced the amount of oxygen produced by photosynthesis at the bottom, resulting in the anoxic water. Furthermore, the low level in transparency during the period from late July to September led to a significant difference in water temperature of 2 °C to 8 °C between the surface and the bottom. Particularly, the average temperature difference took a high value of approximately 5 °C in the period of sulfide generation in mid-August, indicating the presence of thermal stratification in the summer season. However, the period of thermal stratification formation due to the decrease in transparency in 2019 was limited in 2 months of August to September because the water temperature difference since October was relatively small less than 0.5 °C. In summary, the reductive anaerobic conditions observed at the bottom in the 2019 was attributed not only to a decrease in oxygen production in low-light aquatic environments, but also to the inhibition of hydraulic oxygen supply based on the water-mixing in the vertical direction due to the thermal stratification which does not necessarily have a strong intensity. In addition, the aerobic conditions kept on after the recovery from the anoxic water on October, even though the low transparency of approximately 1 m in Autumn was not recovered and continuously maintained due to the abnormal proliferation of phytoplankton. This phenomenon seemed to result from the

vertical transport of oxygen via the mixing layer developed without forming the thermal stratification due to nocturnal radiative cooling at a water surface.

In 2021, the bottom was exposed to the anoxic water with a reducing state with $ORP < 0$ from April to mid-October. These long-term anaerobic conditions led to the remarkable occurrence of sulfides such as a high concentration exceeding $100\text{ }\mu\text{g/L}$ during the period from July to September. So, the impact of anoxic state at the bottom on water quality environment in 2021 differed significantly from that in 2019. In contrast to 2019, the water depth in 2021 took already approximately 3 m in April and May and varied at the around 4 m since June. Additionally, the transparency during the anaerobic periods took a relative low level of 1 m or less except for early-May. A little intensity of underwater light characterized as a low transparency would reflect a water temperature difference, which took about $2\text{ }^{\circ}\text{C}$ on average, between the surface and the bottom waters except for the period after mid-October. Especially, the temperature difference exceeded $5\text{ }^{\circ}\text{C}$ in Summer, and the maximum value over $10\text{ }^{\circ}\text{C}$ was observed with a high frequency during the period of late-July to early-August. Therefore, during the period from April to mid-October in 2021, not only the oxygen production could be significantly lower due to the inhibition of algal photosynthesis, but also of the hydraulic oxygen supply in the vertical direction could be notably suppressed due to the thermal stratification. Consequently, the water quality environment above the bottom until mid-October could be characterized not only as a simply anoxic state of $DO = 0$ but also as a strong reductive anaerobic state appropriate to sulfate reduction. Incidentally, during the period from mid-August to early September, DO environment at the bottom temporarily recovered from the strong reductive state to oxidative conditions with $ORP > 0$, leading to the disappearance of sulfide. This phenomenon seemed to result from the influence of two precipitation events exceeding 150mm/d occurred in Summer. It was inferred that the large-scale vertical mixing of water masses in the reservoir was caused by a large amount of inflowing water during heavy rainfall events as shown in **Figure 4.3B**, resulting in the occurrence of the physical supply of DO downward from the surface. A temporary recovery to aerobic condition at the bottom subsequently led to the disappearance of sulfides as an oxidation reaction of reducing substance. Subsequently, significant decreases in both DO production and supply due to reduced decline in transparency caused a strong reducing anaerobic state with $ORP < -100\text{ mV}$. Consequently, sulfides at levels around $100\text{ }\mu\text{g/L}$ generated once again on end-September. If there were no rainfall events exceeding 150mm/d

during mid-August to early September, it was assumed that the continued anaerobic state resulted in the occurrence of the sulfide at higher level significantly exceeding 100µg/L. This assumption could be regarded as one of large influences of heavy rainfall events on the water environment of the targeted reservoir.

When the anoxic state occurred at the bottom in 2021, Chl-a at the water surface regardless of locations, exhibited a relative high level with an order of 10^2 . This result suggested that the increase of phytoplankton amount was one of factors which lowered the transparency. However, during the abnormal proliferation of cyanobacteria bloom with Chl-a levels exceeding 100 µg/L since mid-October, a hypoxic or anoxic water was solved to the healthy aerobic state although the bottom was under the dark condition such that the water transparency took a low level as approximately 1.5 m in a relative deep-water depth of about 4 m. When considering that the bottom was in a supersaturated DO state similar to the water surface and that the water temperature difference took an extremely small value less than 0.5 °C during this period, it was suggested that the vertical heat convection derived from autumnal radiative cooling played a predominant role in the physical oxygen supply to the bottom, resulting in the effectively maintained aerobic conditions at the bottom. That is, the serious algal bloom in Autumn had a minimal impact on the occurrence of anoxic water at the bottom. Rather, the phytoplankton proliferation with Chl-a levels of 40 to 80 µg/L in the hot season had an extremely significant influence on the DO environment at the bottom.

It was concluded from the field observations in 2019 and 2021 that the long-term anoxic water could occur at the bottom due to the low transparency of around 1 m in the reservoir with a water depth of approximately 4 m. Particularly, the strong reducing anaerobic state which could promote a generation of sulfide was made by the of both the decrease in photosynthesis rate due to the light limitation and the inhibition of vertical transport of oxygen due to the thermal stratification. Both biochemical and hydraulic factors received the influences of the lowering of transparency stemming from the inflow loading of turbidity and the phytoplankton proliferation, which derived from heavy rainfall events. The remarkable point was that the algal bloom occurring in Autumn since October did not work on the maintenance of anoxic state as an environmental factor regardless of low transparency because the oxygen could be supplied to the bottom via the development of mixing layer. In additon, the heavy rainfall event in Summer might impact on the water quality dynamic above the bottom from view point that sulfide would temporarily disappear via the recovery from the strong reducing anaerobic state to aerobic state.

4.3.3 Scenario analyses for water quality improvement using ecosystem model.

The values of the model parameters (α_g , ξ , T_{opt} , K_{DIN} , K_{DIP} and I_{opt}) concerning the photosynthesis rate are summarized in **Table 4.2**. **Table 4.3** and **4.4** list other model parameters. Also, comparisons of the calculated and the observed results of main variables, including Chl-a, DIP, and DIN, the observed results of water temperature and rainfall events, and the limitation function of nutrients at actual simulation which will be explained later are shown in **Figure 4.9**. The observation results in the figure are the values obtained by averaging the measurement results of the water at the surface and the bottom. The results indicated the model's robustness and precision in replicating observed trends in Chl-a concentrations over time. The model successfully mirrored the growth and decline patterns in Chl-a during seasonal changes, capturing notable events such as increases from July to August, a surge from early September to the end of October, and decreases from November onwards in 2019. Similarly, in 2021, the model accurately replicated the sudden Chl-a concentration fluctuations in late October, stable periods from mid-September to mid-October, and reductions during late November.

That is, it was possible to satisfactorily simulate the Chl-a dynamic by determining model parameters α_g , ξ , T_{opt} , and I_{opt} without adjusting values of K_{DIN} and K_{DIP} according to temporal changes in water temperature. Setting parameters of equation (2) in each water temperature zone led to reproduce, to some extent, sharp fluctuations in the concentration of Chl-a together with a large transformation in the existence ratio of cyanobacteria. Such results were never reproduced by determining the model parameter as a constant throughout the calculation period. However, there was a need to examine whether the division of temperature zones and setting parameter values was appropriate from the viewpoint of algology. Moreover, the model effectively accounted for the influence of external factors such as heavy rainfall events, accurately predicting DIP and DIN concentration spikes in response to these occurrences in 2019 and 2021. While there were minor discrepancies in the simulation of DIP and DIN, attributed to challenges in assuming perfectly mixed water due to changing weather conditions, the model's overall performance remained strong. In the actual phenomenon, the inflow loading of DIP and DIN came in from the box-culvert, spreading out over a small area. However, in the model, this study considered the inflow loading to mix suddenly throughout the entire water body. That is why there was a period when the reproducibility of DIN/DIP was low due to the complete mixture model.

Table 4.2 Setting values of model parameters concerning the photosynthesis rate

Water temperature zones	ξ	T_{opt} (°C)	α_g (1/d)	I_{opt} (W/m ²)	K_{DIP} (mg/L)	K_{DIN} (mg/L)
$T \geq 25.0$	5.0	32.0	8.5	20.0	0.0075	0.012
$21.0 \leq T < 25.0$	4.0	30.0	8.0	20.0		
$19.0 \leq T < 21.0$	2.0	25.0	3.5	35.0		
$12.0 \leq T < 19.0$	3.0	25.0	3.0	35.0		
$T < 12.0$	2.0	21.0	2.0	35.0		

Table 4.3 Stoichiometric values

Model parameters	Symbols	Value
Phytoplankton phosphorus/carbon ratio	P:C _{PP}	1.10E-03
Zooplankton phosphorus/carbon ratio	P:C _{ZP}	1.28E-02
POM phosphorus/carbon ratio	P:C _{POM}	0.80E-02
DOM phosphorus/carbon ratio	P:C _{DOM}	3.30E-03
Phytoplankton nitrogen/carbon ratio	N:C _{PP}	3.30E-03
Zooplankton nitrogen/carbon ratio	N:C _{ZP}	5.00E-02
POM nitrogen/carbon ratio	N:C _{POM}	1.00E-02
DOM nitrogen/carbon ratio	N:C _{DOM}	1.00E-02

Table 4.4 Parameters of the state variables

Model parameters	Symbols	Value	Unit
Phytoplankton			
Respiratory rate	α_{rp}	0.065	day ⁻¹
Temperature effect constant for respiration	β_{rp}	0.0634	
Nonpredatory mortality rate	α_{npm}	45.0*0.0001	day ⁻¹
Temperature effect constant for mortality	β_{npm}	0.0693	
Excretion rate	v_e	0.12	mg/m ³
Settling velocity	w_{pp}	0.15*0.1	m/day
Zooplankton			
Growth rate	α_{gz}	0.04	day ⁻¹
Temperature effect constant for growth	β_{gz}	0.0588	
Total mortality rate	α_{zm}	5.0E-0.4	day ⁻¹
Temperature effect constant for mortality	β_{zm}	0.0693	
Assimilation efficiency	e	0.7	
Gross growth efficiency	g	0.16	
Ivlef constant	λ	0.0063	
Algae threshold concentration for zooplankton predation	C_{th}	0.05	mg/m ³
POM			
Mineralization rate	α_{mip}	0.004	day ⁻¹
Effect of temperature on POM mineralization	β_{mip}	0.0693	°C ⁻¹
DO half-saturation constant for mineralization	K_{DO1}	1.5	mg/L
Settling velocity	w_{POM}	0.432	m/day
DOM			
Mineralization rate	α_{mid}	0.0004	day ⁻¹
Effect of temperature on DOM mineralization	β_{mid}	0.0693	°C ⁻¹
DO half-saturation constant for mineralization	K_{DO2}	1.5	mg/L
Sediment-water exchange flux of DIP	α_{fp}	0.2	day ⁻¹
Effect of temperature on exchange flux of DIP	β_{fp}	0.0677	°C ⁻¹
Sediment-water exchange flux of DIN	α_{fn}	4.00	day ⁻¹
Effect of temperature on exchange flux of DIN	β_{fn}	0.0392	°C ⁻¹

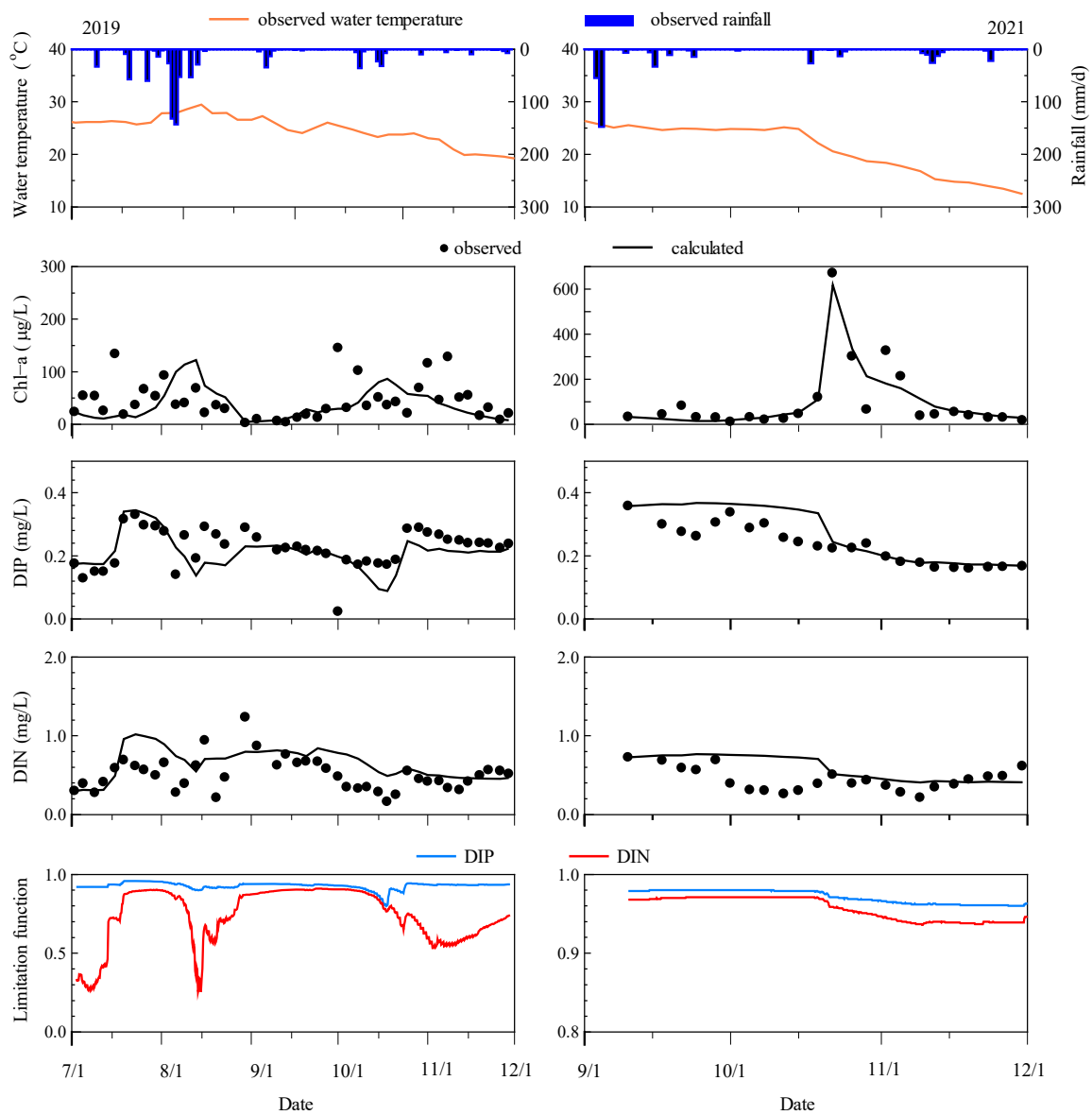


Figure 4.9 Observed results of water temperature and rainfall, the comparison of calculated and observed changes of Chl-a, DIP, DIN, and the limitation function in actual simulation in 2019 and 2021

The growth limitation function of nutrients is determined by lower values of the Michaelis–Menten equation of DIN and DIP, as shown in Equation (5). **Figure 4.9** illustrates each growth limitation function value of DIN and DIP. In both 2019 and 2021, the limitation function of DIN consistently remained lower than DIP throughout the experimental period, indicating that the reservoir was experiencing nitrogen limitation, consistent with field observation findings. Therefore, the ecosystem model is deemed suitable to effectively predict the seasonal prevalence of phytoplankton and nutrients in this hypertrophic reservoir. Based on the results of the inflow loadings, the study could be conducted successfully in different scenarios.

Figure 4.10 illustrates the simulated condition based on 2019 results, examining the rate of change in the average concentration of Chl-a when altering nutrient inflow loadings in three scenarios: DIP only (**Figure 4.10A**), DIN only (**Figure 4.10B**), and DIP and DIN together (**Figure 4.10C**). In **Figure 4.10A**, a 30% reduction in DIP concentration has no immediate impact on the average Chl-a concentration, indicating stability. However, a discernible decrease in Chl-a concentration occurs when the DIP reduction is over 30%, signaling a shift from nitrogen to phosphorus limitation. The Chl-a concentration reaches the eutrophic level, as defined by OECD standards (Ave: 8–25 $\mu\text{g/L}$), with a 60% reduction in DIP loading. In the second scenario (**Figure 4.10B**), when only DIN changes, there is a direct relationship between increasing DIN concentrations and a corresponding growth in Chl-a concentration, indicative of nitrogen limitation. Especially when DIN rises by 60% of the initial concentration, Chl-a reaches 60 $\mu\text{g/L}$, emphasizing the critical need to address nitrogen loading for effective algae control. Achieving an eutrophic Chl-a level (Ave: 8–25 $\mu\text{g/L}$) necessitates a significant 40% reduction in DIN loading. The third scenario, when changing both DIP and DIN, is shown in **Figure 4.10C**; the effect of reducing the DIN load varies depending on the change rate in the DIP load. For example, when the DIP reduction rate is 40%, the decrease of DIN loading by 40% is necessary to reduce the average concentration of Chl-a to the eutrophic level. In addition, it is expected that Chl-a could reduce to mesotrophic level (Ave: 2.5–8 $\mu\text{g/L}$) only by reducing 80% of DIN load.

In the second condition in 2021, shown in **Figure 4.11**, the change rate in the Chl-a average concentration during the calculation period in each scenario was examined when the initial loadings of nutrients changed in three different scenarios: DIP only (**Figure 4.11A**), DIN only (**Figure 4.11B**), and DIP and DIN together (**Figure 4.11C**). In **Figure 4.11A**,

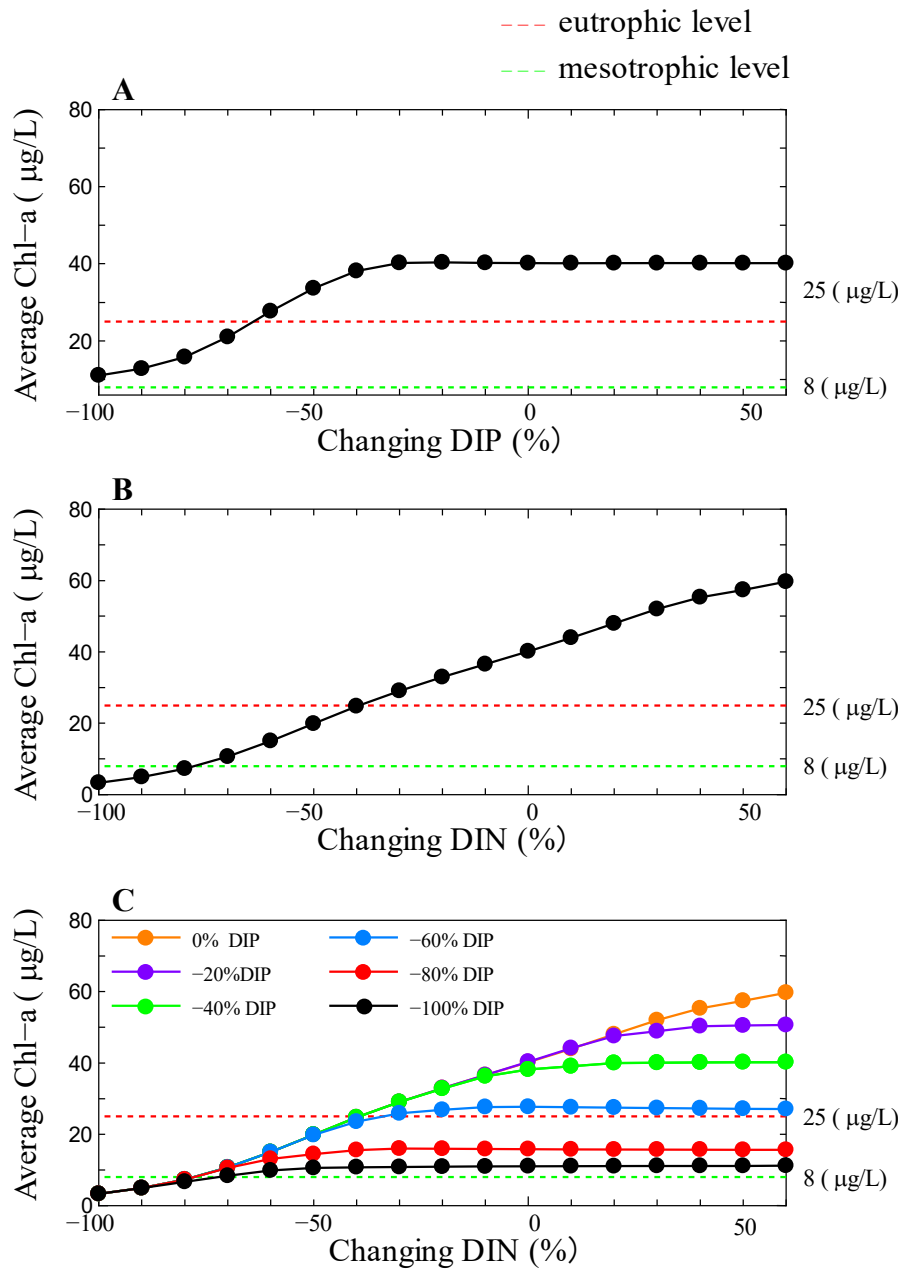


Figure 4.10 Average Chl-a concentration changes with decreasing inflow loading of (A) DIP only, (B) DIN only and (C) both DIP and DIN in 2019

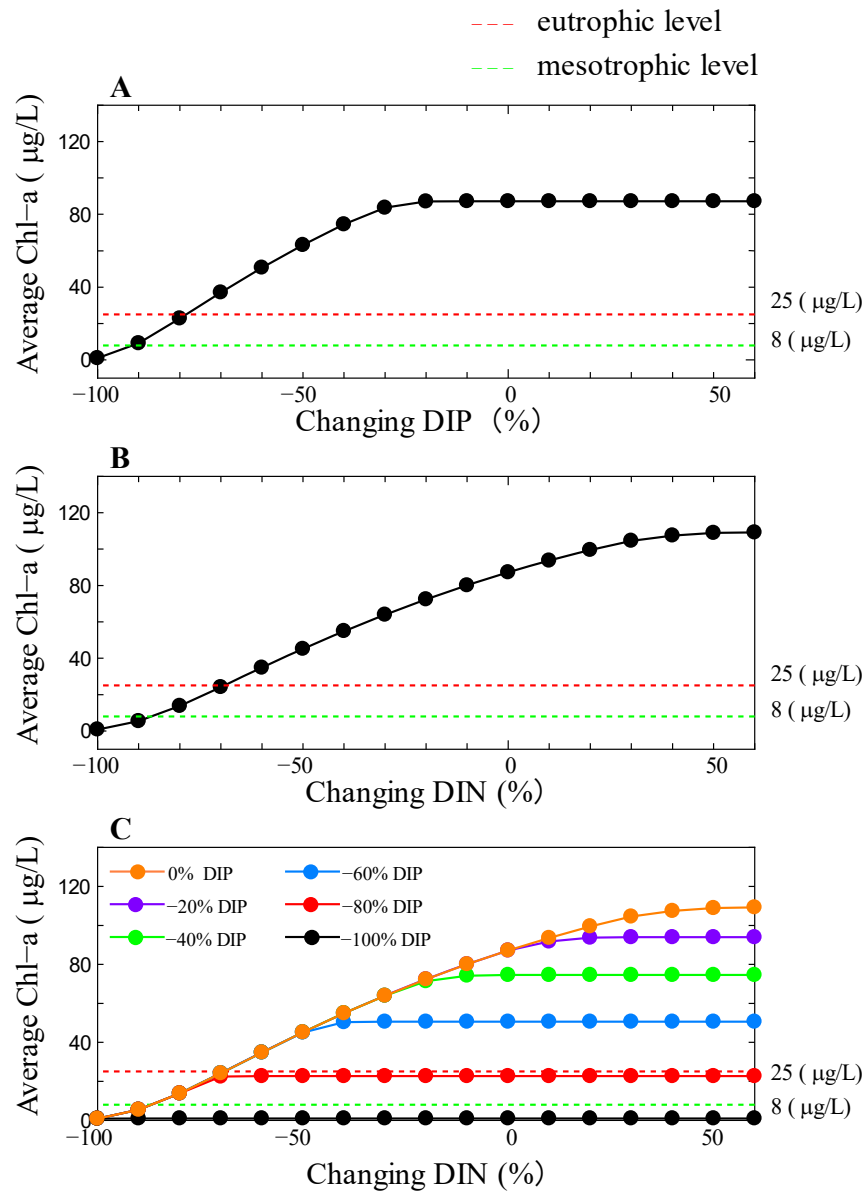


Figure 4.11 Average Chl-a concentration changes with decreasing initial loading of (A) DIP only, (B) DIN only and (C) both DIP and DIN in 2021

the Chl-a concentration remains steady with a 20% reduction in DIP from the initial concentration. However, a substantial decrease in Chl-a becomes noticeable when the DIP reduction exceeds 20%. If the DIP concentration drops by over 80% from the initial level, the reservoir's water quality could reach the eutrophic state (Ave Chl-a: 8-25 $\mu\text{g/L}$). Moving on to **Figure 4.11B**, the Chl-a concentration could decrease to the eutrophic level when the DIN reduction surpasses 70% of the initial concentration. These findings underscore the nitrogen-limited nature of the reservoir. In **Figure 4.11C**, when both the initial concentrations of DIP and DIN decrease simultaneously, maintaining Chl-a at the eutrophic level necessitates reductions of over 70% or 80% in DIP. Moreover, to bring Chl-a down to the mesotrophic level (Ave: 2.5-8 $\mu\text{g/L}$), it's imperative to reduce DIN concentrations by at least 90%.

Drawing insights from both scenarios, it becomes evident that in this reservoir with a limitation in nitrogen, diminishing nitrogen levels has a more pronounced effect in curbing phytoplankton growth than phosphorus reduction. The study's results highlight the importance of accurately identifying the limiting factor, whether nitrogen or phosphorus, within the reservoir's ecosystem. This understanding is key to implementing effective measures for preventing algal blooms.

4.4 Conclusions

This study provided novel evidence concerning the influence of nitrogen and phosphorous inflow loadings on the abnormal proliferation of phytoplankton through periodic observations and the actual status of aquatic environmental issues in the hypertrophic nitrogen-limited reservoir. In particular, the seasonal change in phytoplankton, algae species dominance, nitrogen, and phosphorus levels were studied through water quality monitoring for two years in the reservoir where the long-term cyanobacteria blooms had occurred at the water surface during autumn. The water quality prediction model was constructed by considering the results obtained from the periodic water quality observations. This model provided scenario analyses to evaluate how to inhibit the occurrence of cyanobacterial blooms. The main outcomes obtained by this study can be summarized as follows:

From the observation results, the targeted reservoir was confirmed to be hypertrophic under nitrogen-limited conditions. Additionally, the temporal nitrogen loadings were the

main cause of algal abnormalities. Severe hypertrophication was confirmed with mat-like cyanobacteria blooms over a wide area in summer 2019 and autumn 2019 and 2021. Cyanobacteria was the dominant species during summer and autumn in 2019 and during autumn in 2021. The research observed a consistent trend in two years: phytoplankton, particularly cyanobacteria, displayed a remarkable sensitivity to temporary increases in $\text{NO}_3\text{-N}$ caused by heavy rainfall. This sensitivity, combined with consistently high $\text{PO}_4\text{-P}$ concentrations exceeding 0.1 mg/L, significantly elevated Chl-a levels, surpassing the order of 10^3 .

Field observations in 2019 and 2021 also concluded that long-term anoxic conditions could develop at the bottom of a reservoir with a water depth of approximately 4 meters and low transparency of around 1 meter. This anoxic state, which promoted sulfide generation, resulted from reduced photosynthesis due to light limitation and inhibited vertical oxygen transport caused by thermal stratification. The reduction in transparency was influenced by turbidity inflow and phytoplankton proliferation, often triggered by heavy rainfall events. Notably, autumn algal blooms since October did not maintain the anoxic state because the mixing layer supplies oxygen to the bottom. Additionally, heavy summer rainfall events could temporarily shift water quality from anoxic to aerobic, causing sulfide to disappear.

The validity of the ecosystem model was confirmed through two different cases. The first case was set based on the observation results in 2019 when the overgrowth of phytoplankton occurred mainly by the nutrient inflow loading due to rainfall in summer. The second one was established using observation data in 2021 when nutrients from autumn rain events promoted abnormal phytoplankton growth. The Chl-a, DIP, and DIN simulation results could reproduce both cases' tendencies and seasonal change successfully. Therefore, the ecosystem model has high validity in effectively predicting the seasonal prevalence of phytoplankton growth in this hypertrophic reservoir. The model successfully replicated certain sharp fluctuations in Chl-a levels, a phenomenon previously unattainable when employing a constant model parameter throughout the simulation period. However, further investigation is necessary to ascertain the suitability of segmenting temperature zones and assigning parameter values from an algological perspective. Thus, a future imperative lies in refining the categorization of water temperatures and aligning model parameters accordingly.

The trend of phytoplankton growth was predicted when the nutrient inflow loadings were changed in different scenarios. The research outcomes demonstrate that in reservoirs characterized by nitrogen limitation, the reduction of nitrogen has a substantially more pronounced effect in curbing phytoplankton growth than reducing phosphorus levels. These findings emphasize the critical necessity of accurately identifying the limiting factor, whether nitrogen or phosphorus, within the reservoir's ecological system. This precise understanding is paramount in implementing targeted strategies to effectively prevent the occurrence of algal blooms.

Chapter 5 General conclusions

Many agricultural reservoirs have recently been subjected to excessive inflows of DIN and DIP due to fertilization practices on farmlands. This has led to severe eutrophic conditions, notably characterized by the abnormal growth of phytoplankton. A particular concern is the formation of mat-like cyanobacterial blooms on the water's surface, which cause significant environmental issues. These blooms deplete oxygen levels, damage aquatic ecosystems and necessitate immediate measures to ensure sustainable agricultural development. This situation underscores the critical need for effective management of nutrient inflows and the implementation of strategies to counteract the adverse effects of eutrophication on water quality and biodiversity. Tackling these challenges is crucial for maintaining the long-term health and sustainability of both agricultural and aquatic ecosystems.

In the first study, the laboratory experiments provided critical insights into water environmental engineering, particularly focusing on quantitatively assessing the bactericidal effect of Cu^{2+} in inhibiting algal overgrowth under high nutrient concentrations. The water quality experiments were conducted on a water tank scale, allowing for controlled conditions that closely mimic natural environments. The study focused on several key aspects: the reduction effect of high cyanobacterial concentrations, the suppression of Chl-a increase over the long term, and the elimination of mat-like algal blooms. One significant finding from the study was the ability of Cu^{2+} to inhibit the overgrowth of cyanobacteria and effectively eliminate mat-like algal blooms. Cyanobacteria, often responsible for harmful algal blooms, were shown to be particularly susceptible to the bactericidal effects of Cu^{2+} . However, it was found that under high nutrient concentrations, the long-term suppression of Chl-a increase could not be expected. A nutrient-rich environment tends to favor the proliferation of Chlorophyceae (green algae), which appeared to be less susceptible to the bactericidal effect of Cu^{2+} . This implies that while Cu^{2+} can effectively manage cyanobacterial blooms, it may not be as effective against other types of algae that thrive in nutrient-rich conditions. The study suggests that a hybrid countermeasure, combining the bactericidal effects of Cu^{2+} with strategies to reduce nutrient concentrations, is necessary to effectively control algal blooms. The implications of this study are significant for water management and environmental protection. By understanding the specific conditions under

which Cu^{2+} is most effective, more targeted and efficient strategies can be developed to combat algal blooms. This approach not only addresses the immediate problem of harmful algal blooms but also contributes to the long-term sustainability of water resources by encouraging practices that reduce nutrient pollution.

In the second study, significant insights were presented on the impact of nitrogen and phosphorus inflow loadings from agricultural activities on phytoplankton proliferation in a hypertrophic reservoir. This comprehensive research spanned over two years, meticulously examining the seasonal variations in phytoplankton populations, the dominance of various algae species, and the fluctuating levels of nitrogen and phosphorus in a reservoir prone to cyanobacterial blooms. By employing a detailed and systematic approach, the study sought to uncover the underlying mechanisms driving algal blooms and to propose effective strategies for their mitigation. The findings of the study revealed that the reservoir was hypertrophic, characterized by excessive nutrient enrichment, specifically under conditions of nitrogen limitation. This means that despite the overall high nutrient levels, the growth of phytoplankton was primarily limited by the availability of nitrogen. Temporal nitrogen loadings, particularly those associated with agricultural runoff, were identified as the primary cause of algal abnormalities. These loadings varied with agricultural practices and weather patterns, with heavy rainfall events triggering significant increases in nitrate-nitrogen concentrations. Such increases were found to be particularly influential in promoting the dominance of cyanobacteria during the study period. Cyanobacteria was shown to be particularly sensitive to these temporary spikes in nitrate-nitrogen. The study documented that cyanobacterial blooms often followed these spikes, leading to periods of intense algal proliferation that disrupted the ecological balance of the reservoir. These blooms were not only a symptom of nutrient imbalance but also contributed to further water quality degradation, as cyanobacteria can produce toxins. To understand and predict these patterns, a robust ecosystem model was developed, which was meticulously validated through observational data collected over the study period. The model demonstrated good reproducibility of the observed results, successfully anticipating shifts in phytoplankton growth patterns and accurately replicating the abnormal proliferation of several algae species under different nutrient loading scenarios. This model proved to be a powerful tool for simulating the impact of various nutrient management strategies and for predicting the outcomes of potential interventions. By conducting different nutrient loading scenarios

within the model, the research highlighted the crucial role of nitrogen reduction in curbing phytoplankton growth, especially in nitrogen-limited reservoirs. It became evident that even in environments with high overall nutrient levels, managing nitrogen inputs was key to controlling algal blooms. By accurately identifying the limiting factors - primarily nitrogen in this case - the model allowed for the development of targeted strategies that could be implemented to reduce nutrient inputs and manage water quality more effectively.

Combining these findings, the study suggests that managing algal blooms in nitrogen-limited reservoirs involves not only controlling nutrient inflows but also adjusting Cu^{2+} concentrations based on the specific conditions of the water body. An effective approach to eutrophication must consider these varying factors to mitigate algal blooms while minimizing environmental impact. These results can be used to develop a technique for suppressing phytoplankton overgrowth and to integrate existing methods into a hybrid approach for more effective algal control in eutrophic agricultural reservoirs affected by excessive inflows of phosphorus and nitrogen. Therefore, they will contribute to the environmental remediation and conservation of regional water resources, ensuring sustainable agricultural development in rural areas.

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This study was conducted at the Laboratory of Water Environmental Engineering, Graduate School of Bio-resource and Bio-environmental Sciences, Kyushu University, Japan. The success of this study lies in providing fundamental knowledge in water environmental engineering, specifically in quantitatively assessing the bactericidal effect of Cu^{2+} on inhibiting algal overgrowth under high nutrient concentrations, as well as in developing an innovative water production model applicable to hypertrophic water bodies. I extend my sincere gratitude to the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) for providing a full scholarship.

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