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Effect of Rainfall and pH on Musty Odor Produced in the Sanbe Reservoir

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Abstract: Harmful cyanobacterial blooms are continuously formed in water systems such as reservoirs and lakes around the world. Geosmin and 2-methylisoborneol (2-MIB) produced by some species of cyanobacteria have caused odor problems in the drinking water of the Sanbe Reservoir in Japan. Field observations were conducted for four years (2015–2019) to investigate the cause of this musty odor. It was found that geosmin was produced by *Dolichospermum crassum* and *Dolichospermum planctonicum* (cyanobacteria), and 2-MIB was due to *Pseudanabaena* sp. and *Aphanizomenon* cf. *flos-aquae* (cyanobacteria). Changes in water temperature and pH caused by rainfall were correlated with changes in the concentration of geosmin and 2-MIB. In particular, geosmin and 2-MIB tended to occur under low rainfall conditions. When there was low rainfall, the reservoir changed to an alkaline state because the phytoplankton consumed CO_2 for photosynthesis. In an alkaline reservoir, dissolved inorganic carbon mainly existed in the form of bicarbonate (HCO_3^-). Thus, the results suggest that under such conditions in reservoirs, cyanobacteria grew easily because they could use both CO_2 and HCO_3^- for photosynthesis. Specifically, our study suggests that in order for the musty odor problem in the reservoir to be solved, it is important that the pH of the reservoir be controlled.

Keywords: musty odor; reservoir; geosmin; 2-MIB; pH

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1. Introduction

Serious damage to water resources, marine products, living environment, and tourism resources can occur because of harmful cyanobacterial blooms (HCBs) in reservoirs around the world [1–7]. When HCBs appear in a reservoir, appearance and musty odor problems can make humans uncomfortable. The musty odor (earth odor and ink odor) that is continuously generated in water systems such as reservoirs and lakes around the world has become an extremely serious problem in drinking water source ponds [8–14]. If a large amount of musty odor is produced, it requires activated carbon treatment, which causes economic loss. In freshwater systems, there are many cases in which the musty odor can be attributed to geosmin and 2-methylisoborneol (2-MIB). The geosmin, an earthy-smelling substance, was isolated in 1965 [15]. 2-MIB, a musty- or camphorous-smelling compound, was reported in 1969 [16] and independently in 1970 [17].

In Japan, the continuous generation of musty odor has become a problem in Lake Shinji, which is a brackish water area, and in Sanbe Reservoir located in Shimane Prefecture [18,19]. In 2007, when a high concentration of geosmin occurred at Lake Shinji, producers and wholesalers who harvest and sell of the brackish water bivalve (*Corbicula Japonica*) had difficulty because of the musty odor smell on the bivalve [19,20]. It was verified that the moldy odor was formed by the cyanobacteria *Coelosphaerium* sp. [19,20]. However, the cause of cyanobacterial occurrence was unknown.

In Japan, in order to use a reservoir as a water source, when geosmin or 2-MIB is present at a concentration of 10 ng L^{-1} or more, they must be actively removed in accordance with water quality standards (Drinking Water Quality Standards in Japan, Ordinance of Ministry of Health, Labor and Welfare No. 101, 2003). Moreover, for removal of the geosmin and 2-MIB, activated carbon has been generally used [21,22]. In Sanbe Reservoir, partly used as a water supply, located in the mountainous area of Oda City, Shimane Prefecture (Figure 1), a musty odor due to geosmin and 2-MIB was produced in recent years. Furthermore, an additional cost of approximately JPY 10 million annually is required to remove the musty odor. Therefore, it is important to investigate the mechanism of musty odor production; however, this has not yet been conducted. Because of the ecological characteristics of cyanobacteria, their interrelationships with the surrounding biota, their physical and chemical actions, and the environment are intricately linked [9]. Moreover, the reservoir has a residence time of several days to several weeks compared with natural lakes, and temporal changes in the water environment often occur within a short period of time. For these reasons, there are few unified views on the relationship between the occurrence of cyanobacteria and environmental factors around the reservoir. Therefore, further data accumulation is required to clarify the series of mechanisms for musty odor production.

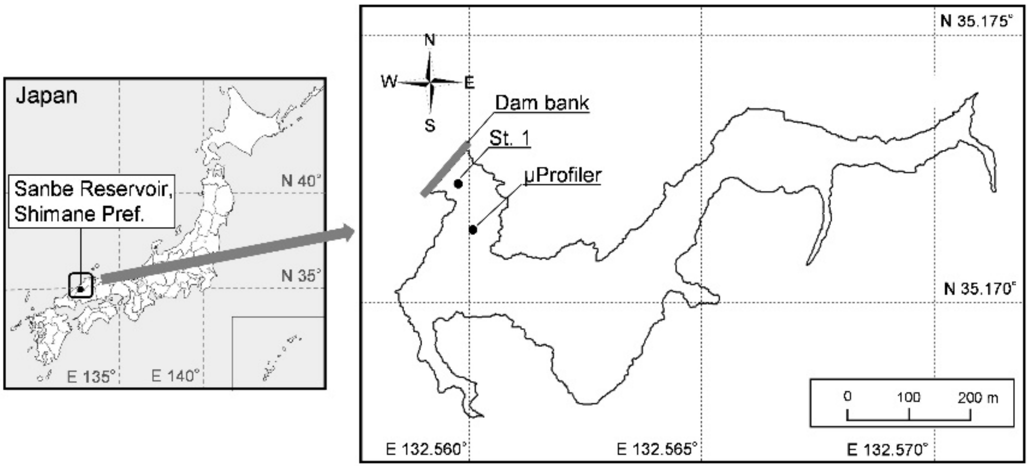

Figure 1. Study area in the Sanbe Reservoir, Japan, with field observation station (St.1), μ profiler. The map of Japan was generated by data from Geospatial Information Authority of Japan (https://www.gsi.go.jp/tizu-kutyu.html) (accessed on 1 January 2021).

To clarify the relationship between environmental factors, such as water quality and weather conditions, as well as musty odor, we needed to accumulate sufficient data, but such research has not yet been conducted. In this study, we discuss the relationship between environmental changes, such as rainfall and water pH, in the reservoir, as well as the generation of musty odor using data collected from a musty odor survey conducted at the Sanbe Reservoir for the past four years by our research group. We believe that this study provides invaluable observation data and useful reports for the management of musty odor in other reservoirs.

2. Materials and Methods

2.1. Study Site

Sanbe Reservoir (35°10′11.5″ N; 132°33′45.2″ E, Figure 1) is in the mountainous area of Oda City, Shimane Prefecture, Japan. The reservoir has a surface area of 0.23 km^2, a maximum depth of 31.0 m, a mean depth of 10.7 m, and a volume of 2.45×10^6 m^3. It was built in 1996 to supply water for Oda City, flood control for the Sanbe River, drinking water, agricultural irrigation, and power generation. The main inflow comes from the east, and there are residential areas in the watershed. In the water purification plant located in the south of the reservoir, drinking water is constantly taken from a 10 m depth (elevation 117 m) and delivered to the purification plant through a tunnel.

2.2. Sampling and Analysis of Geosmin and 2-Methylisoborneol (2-MIB)

A field survey was performed at St.1 (water depth 28 m) of the Sanbe Reservoir (Figure 1) from October 2015 to December 2019. Water was sampled from depths of 0.5 m, 9 m (elevation 118 m), 17 m (elevation 110 m), and 27 m (elevation 100 m, 1 m above the bottom), the distance from the water surface, at St.1 using a water sampler (Kitahara 2-L type Rigo Co., Ltd., Bunkyo-ku, Japan). The geosmin and 2-MIB concentrations in the water were measured using a gas chromatograph (GC-7890, Agilent, Santa Clara, CA, USA) equipped with a quadrupole mass spectrometer (MS) (5977 B, Agilent) and a purge and trap autosampler (Aotmx, Teledyne Tekmar, Sauzend Oaks, CA, USA), as described by the Ordinance of the Ministry of Health, Labour and Welfare No. 261, 2003. Gas chromatography/mass spectrometry (GC/MS) analysis was performed in the selected ion monitoring mode (*m/z* 112). As an internal standard, 2,4,6-trichloroanisole-d3 (*m/z* 115) was spiked into the sample. The detection limit was 1 ng L^{-1}. We used the geosmin, 2-MIB, inflow freshwater, water temperature taken from regular inspections in the Shimane Prefecture.

2.3. Vertical Distribution of Water Quality

Continuous observations were conducted from April 2018 to March 2019 using an automatic elevating water quality meter (μ plofiler, Hydrolab, model DS5X) installed near St.1 (Figure 1). Vertical measurements of water temperature, electrical conductivity (EC), turbidity (Turb.), phytoplankton index (Chl-*a*), pH, and cyanobacteria index (PCY) from the lake surface to the lake bottom were measured every hour at each 0.5 m depth.

2.4. Observations of Morphological Characteristics and Counting of Cell Numbers for Phytoplankton

Water for observation of phytoplankton was sampled from water surfaces at depths of 0.5 m or 1.0 m at St.1 (Figure 1) using a water sampler (Kitahara 2-L type Rigo Co., Ltd., Bunkyo-ku Japan) in June and September in 2017 and 2019 when the odor was heavily being produced. The samples were transferred to the laboratory in a cooled and shaded environment. The experimental procedures in the laboratory were performed on the basis of the work of Godo et al. [19]. The field sample was condensed 100-fold as follows. Raw sample water (198 mL) was filtered through a membrane filter with 0.45 μm pore size (Millipore Corp., Burlington, MA, USA) under low vacuum conditions. The deposits on the filter were carefully scraped using a small spatula and diluted to 2 mL of sample water. Next, 2 mL of 2.5% glutaraldehyde was added to the 100-fold condensed sample. Once the sample had settled, the supernatant liquid was removed as possible with a pipette, and the deposits were resuspended in 2 mL of 5% formalin. This process means that the sample was concentrated 100-fold. The shapes of colonies and cells of phytoplankton, including cyanobacteria, diatom, dinoflagellate, euglenoid, and green algae, were observed under an optical microscope with a fluorescent attachment (Olympus BX60, Olympus Corp., Tokyo, Japan) at a magnification of 100× or 400×. Moreover, they were randomly measured with a micrometer at a magnification of 1000×. The number of colonies and cells was counted, and the relative abundance frequency was divided into five stages: high abundance (cc, 45–80% of observed colonies and cells), abundant (c, 30–45%), common (+, 15–30%), rare (r, 8–15%), and very rare (rr, 2–8%) [23].

2.5. Establishment of Laboratory Culture

Single-colony isolation was conducted using the pipet-washing method [24] for all species under a stereo microscope. Isolated specimens of the cyanobacterial trichome were maintained in glass tubes containing 10 mL CA medium [25] under the following growth conditions: 20 °C temperature, approximately 25 μmol photons m^{-2} s^{-1} light (provided by cool white fluorescent tubes), and 12 h/12 h light and dark cycles. After culturing for 2 weeks, the geosmin and 2-MIB concentrations in the CA medium were measured using the same method used for lake water. The isolated cyanobacterial cultures were observed by fluorescence microscopy (Olympus BX51, Olympus Corp., Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku, 163-0914, Japan) with ethidium bromide staining to confirm axenic strain [26,27]. After the growth of cyanobacterial strains, 100–150 µL of cyanobacterial culture was stained. When no bacterial cell was observed, a cyanobacterial strain was axenic.

2.6. Analysis of Nucleotide Sequences

DNA was extracted from the cultures of 2-MIB-or geosmin-producing strains using ISOPLANT (Nippon Gene Co., Ltd., Toyama, Japan), according to the manufacturer's instructions. The 2-MIB and geosmin biosynthetic genes were PCR-amplified using KOD FX neo polymerase (Toyobo Co., Ltd., Osaka, Japan) with primer sets MIBF2 (5-CTGCCCTAATAAAGGGATAAGAGC-3) and MIBR2 (5-GGTTGAACTATGTGCGCTCCTA TAA-3) [28], and geo78F (5-GCATTCCAAAGCCTGGGCTTA-3) and geo982R (5-ATCGCAT GTGCCACTCGTGAC-3) [12]. The PCR cycle consisted of a pre-run at 94 °C for 2 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 61 °C (*mib*) or 56 °C (*geoA*) for 30 s, and extension at 68 °C for 30 s, followed by a final extension at 68 °C for 5 min. Primers MIBR2 and geo78F were used to determine the nucleotide sequences. The nucleotide sequences were compared with those obtained from the National Center for Biotechnology Information (NCBI) using the BLAST algorithm [29].

3. Results and Discussion

3.1. Variation Characteristics of Geosmin and 2-MIB in Sanbe Reservoir

Figures 2 and 3 show the changes of geosmin and 2-MIB concentration in time, respectively, at depths of 0.5 m, 9 m, 17 m, and 27 m below the surface of the water at St.1. The geosmin at the epilimnion (0.5 m depth from the water surface) was detected in values exceeding 100 ng L^{-1} in June 2016, June 2017, and June and July 2019, and also a high concentration of geosmin tended to occur in early summer (June to July). In contrast, 2-MIB at the epilimnion was detected in values exceeding 100 ng L^{-1} in November 2015, August and September 2017, and November 2019; moreover, a high concentration of 2-MIB tended to occur from summer to late autumn (August to November). However, in 2018, both geosmin and 2-MIB showed low concentrations below 10 ng L^{-1} (the Japanese standard for water quality). The geosmin was measured not only in the epilimnion but also at concentrations above 10 ng L^{-1} at the hypolimnion (1 m above the bottom) in the Sanbe Reservoir (Figure 2). In contrast, 2-MIB increased in the hypolimnion with a slight delay, specifically when the epilimnion increased (Figure 3). Moreover, the geosmin and 2-MIB concentrations decreased from the upper layer to the lower layer. However, as shown in the figure, the geosmin on 11 July 2016, as well as 2-MIB on 15 September 2017 and 12 November 2017, were confirmed near the bottom layer, although it was relatively low.

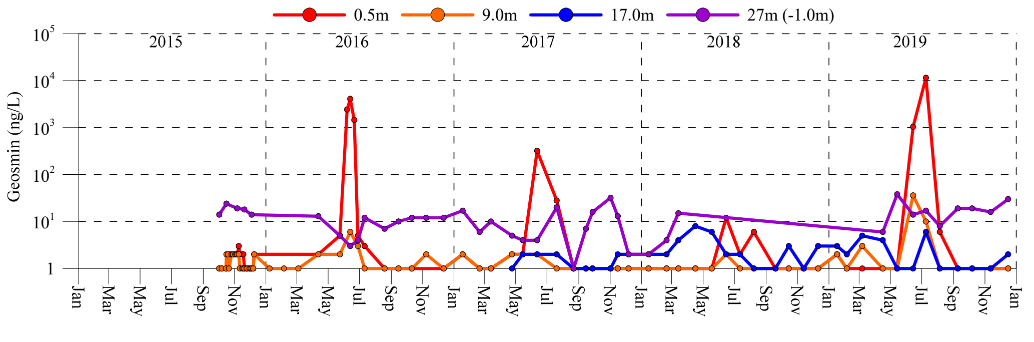

Figure 2. Time-series change of geosmin at depths of 0.5, 9, 17, and 27 m (1 m above the bottom) below the surface of the water in the Sanbe Reservoir.

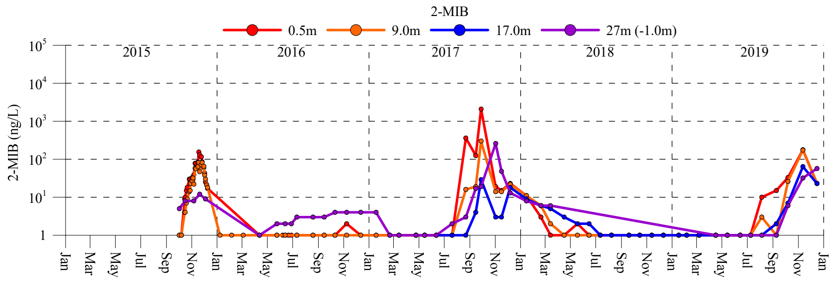

Figure 3. Time-series change of 2-MIB at depths of 0.5, 9, 17, and 27 m (1 m above the bottom) below the surface of the water in the Sanbe Reservoir.

3.2. Identification of Phytoplankton and Musty Odor-Producing Species

Using single axenic algal culture strains, the ability or inability to generate musty odor was determined by measuring the generated geosmin and 2-MIB and genetic analysis of the single algae. *Dolichospermum planctonicum* and *Dolichospermum crassum* were geosmin-producing species, whereas *Pseudanabaena* sp. and *Aphanizomenon* cf. *flos-aquae* were 2-MIB-producing species (Figure 4). geoA and mib genes in the isolated *Dolichospermum* and *Pseudanabaena* strains were PCR-amplified, respectively. Their nucleotide sequences showed high similarities with the sequences of geosmin synthase gene in *Dolichospermum* and 2-MIB synthase in *Pseudanabaena*. The production of geosmin and 2-MIB by these species was also reported by Watson et al. [13]. The cyanobacteria cf. *Geitlerinema* sp. and cf. *Cuspidothrix* sp., which were sometimes abundant in Sanbe Reservoir, could not produce musty odors.

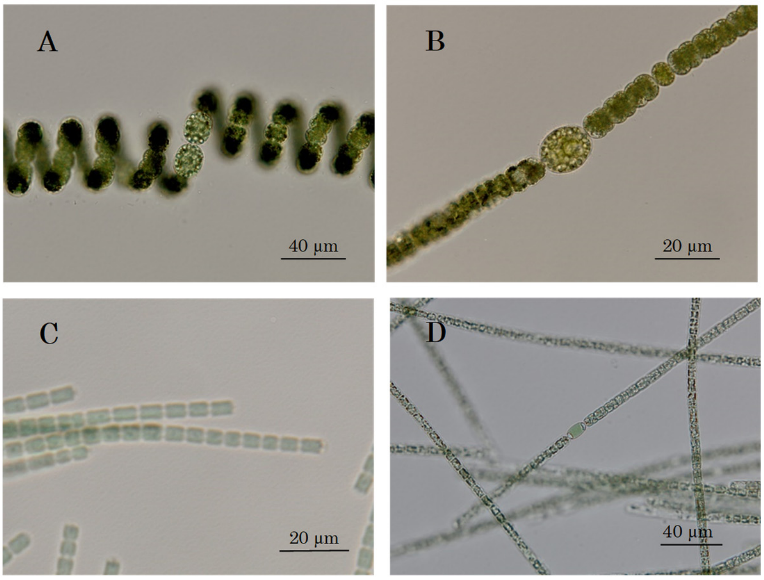

Figure 4. Morphological characteristics of musty-odor-producing cyanobacteria isolated from Sanbe Reservoir. (**A**) *Dolichospermum crussum* (geosmin-producing species): cell diameter of 8.5–11 µm. (**B**) *Dolichospermum planctonicum* (geosmin-producing species): cells diameter of 16.8–17.5 µm. (**C**) *Pseudanabaena* sp (2-MIB producing species): cells diameter of 1.5–1.7 µm. (**D**) *Aphanizomenon* cf. *flos-aquae* (2-MIB producing species): cells diameter of 4.6–5.0 µm.

Table 1 and Table S1 list the identification of phytoplankton results for 2017 and 2019. At that time, cyanobacteria, diatoms, dinoflagellates, euglenoids, and green algae appeared, but only cyanobacteria that may produce musty odors are noted. Geosmin-producing *D. crissum* was observed in June 2017 and 2019. *D. planctonicum* appeared in June 2019. They were not detected in September 2017 and 2019. When 2-MIB occurred in September 2017 and 2019, 2-MIB-producing *Pseudanabena* sp. was observed. *A.* cf. *flos-aquae* appeared in September 2019.

Table 1. Relative abundance of cyanobacteria at epilimnion in Sanbe Reservoir.

Sampling Date Sampling Depth Odor in the Field	22 June 2017 0.5 m Geosmin	1 September 2017 0.5 m 2-MIB	27 June 2019 1 m Geosmin	25 September 2019 1 m 2-MIB
Aphanocapsa sp.	−	rr	−	r
Aphanothece sp.	−	r	−	−
Microcystis novacekii	−	−	−	rr
Microcystis sp.	−	−	r	r
Woronichinia naegeliana	−	−	rr	−
Pseudanabena sp. *[1]	−	r	−	+
cf. *Geitlerinema* sp.*[3]	−	c	−	c
cf. *Cuspidothrix* sp.*[3]	+	−	rr	−
Aphanizomenon cf. *flos−aquae* *[1]	−	−	−	c
Dolichospermum crassum *[2]	r	−	+	−
Dolichospermum planctonicum *[2]	−	−	c	−

cc: high abundance, c: abundant, +: common, r: rare, rr: very rare, −: absent. *[1]: 2-MIB-producer, *[2]: geosmin-producer, *[3]: non-producer.

On the basis of the observation and genetic analysis, we concluded that geosmin in the Sanbe reservoir from June to July was caused by *D. planctonicum* and *D. crassum*, while 2-MIB was caused by *Pseudanabaena* sp. and *A.* cf. *flos-aquae* from August to November Therefore, to reduce the damage caused by the musty odor of the surface water of the Sanbe Reservoir, we needed to pay attention to the growth behavior of *D. planctonicum*, *D. crassum*, *Pseudanabaena* sp., and *A.* cf. *flos-aquae*.

3.3. Effect of Rainfall on Geosmin and 2-MIB

Figure 5 shows the average monthly freshwater inflows to the Sanbe Reservoir in deviations from the long-term average from 2016 to 2019. There were three different types of the inflow in the annual course. In the years 2016–2017, two periods were quite clearly visible: wet—when the monthly average of inflow was greater than the average in the given months from the 4-year period, and dry—when, conversely, the monthly averages were lower. This wet season was from September/October to February and the dry season from March to August/September. The next two years showed a completely different type of inflow. In 2018, from March to September, monthly averages were most often significantly higher than the four-year averages, while 2019 was exceptionally dry. In 2018, when both geosmin and 2-MIB concentrations were low (as shown in Figures 2 and 3), the annual water inflow to the reservoir was the highest in four years. On the other hand, in 2019, when the average monthly water supply was higher than average, both geosmin and 2-MIB concentrations were high.

Figure 6 shows a comparison of inflow with geosmin in the epilimnion from 2016 to 2018. Geosmin concentrations beyond 10 ng L^{-1} were observed from June to July. However, the maximum concentration (14 ng L^{-1}) in 2018 was very low, several times lower than in 2016 and 2017 (over 100 ng L^{-1}). In 2018, there were about three peaks of inflow over 3 m^3 s^{-1} day^{-1}, compared with the inflow in May 2016 and 2017, when high concentrations of geosmin were confirmed. It was necessary to further investigate whether an inflow of 3 m^3 s^{-1} day^{-1} (and bigger) affected the geosmin concentration.

Figure 7 shows a comparison of the surface 2-MIB on the epilimnion and the amount of inflow from 2017 to 2019. 2-MIB concentrations were observed above 10 ng L^{-1} from August to December only in 2017 and 2019. They were not detected in 2018, when as many as four tributary peaks above 7 m^3 s^{-1} day^{-1} were observed from July to October. On the basis of the results of this analysis on the relationship between precipitation and odorous compounds, we assumed that the change in the musty smell was influenced by fluctuations in water quality under the influence of the inflow. It was necessary to further investigate whether an inflow of 7 m^3 s^{-1} day^{-1} (and bigger) affected the 2-MIB concentration.

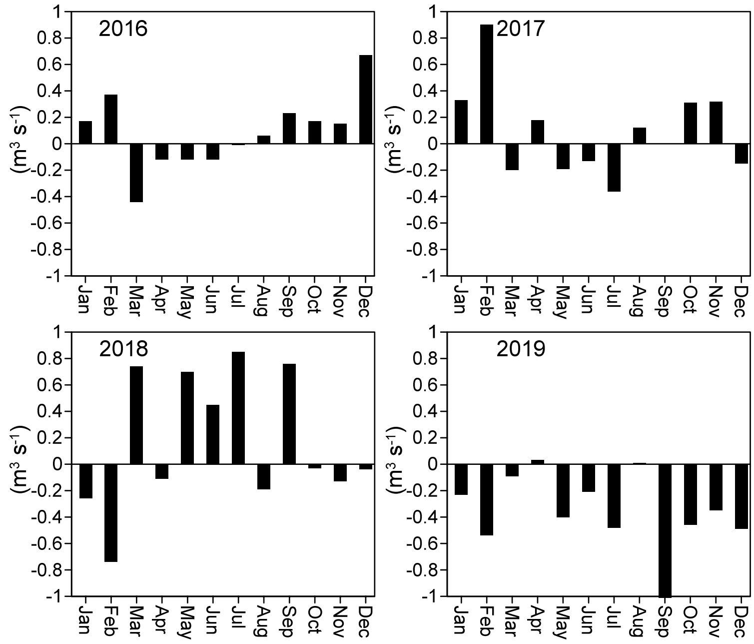

Figure 5. Average monthly freshwater inflows ($m^3\ s^{-1}$) to the Sanbe Reservoir in deviations from the long-term average (2016–2019).

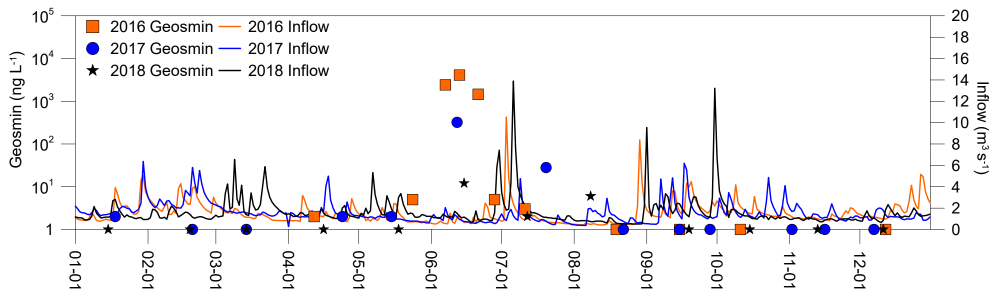

Figure 6. Comparison of the amount of inflow with Geosmin on the epilimnion from 2016 to 2018.

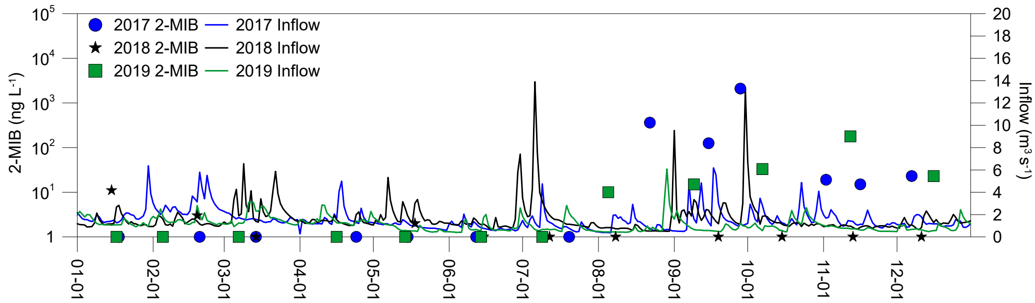

Figure 7. Comparison of the amount of inflow with 2-MIB on the epilimnion from 2017 to 2019.

3.4. Effect of pH and Water Temperature on the Cyanobacteria

Figure 8 shows the relationship between geosmin, 2-MIB, and water temperature of surface water (0.5 m) in Sanbe Reservoir from October 2015 to December 2019. The presence of geosmin at a concentration of 10 ng L^{-1} or more was observed in a relatively narrow range of water temperature—from 20° to 25 °C. 2-MIB in this concentration was recorded in a much wider range of water temperatures—from 10° to 28 °C.

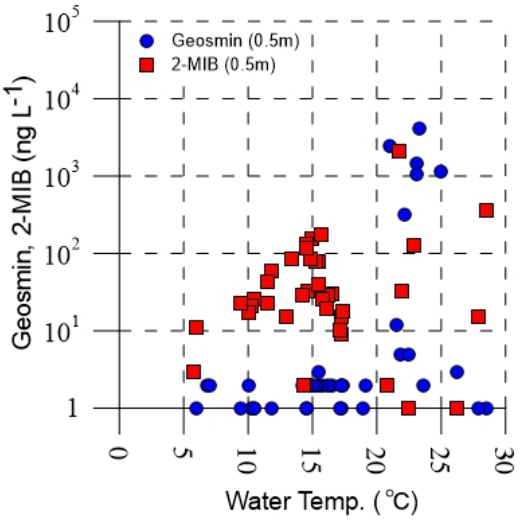

Figure 8. Relationship between geosmin, 2-MIB, and water temperature on the epilimnion in Sanbe Reservoir from October 2015 to December 2019.

Figure 9 shows the time-series changes at a depth of 1 m (μ profiler data) at the Sanbe Reservoir station (Figure 1) and the inflow water temperature and rainfall from May to October in 2017, 2018, and 2019 (Japan Meteorological Agency Database [30]). The fluctuation patterns of the water temperature of the epilimnion and the inflow water temperature were very similar. Furthermore, there was a high correlation between the water temperature of the epilimnion and the inflow water temperature for all periods ($p < 0.001$, $R^2 = 0.989$). The significant decrease in water temperature from June to July in 2018 was confirmed to be due to the rapid increase in total rainfall (268 mm day^{-1}) over the 9 days from 28 June to 6 July.

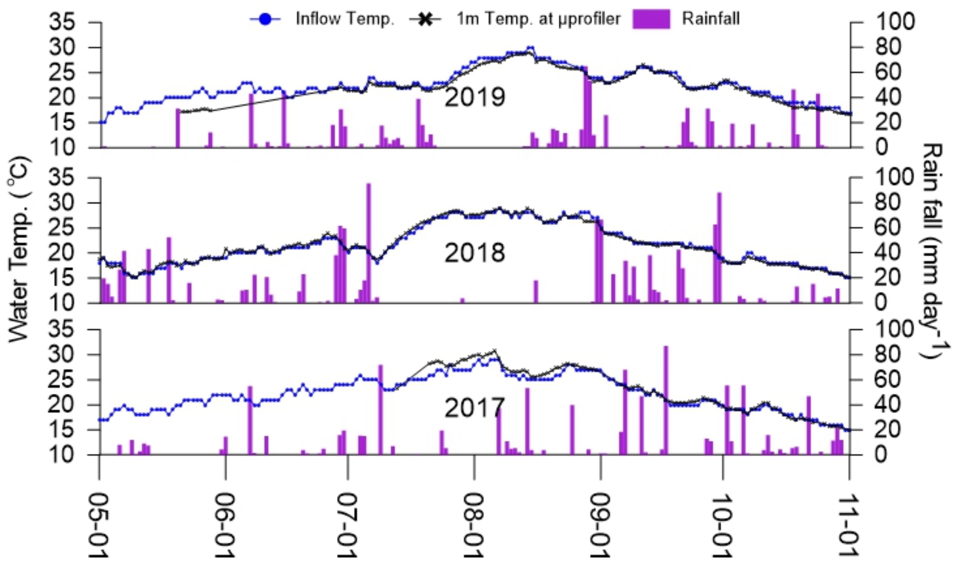

Figure 9. Time-series changes at a depth of 1 m (μ profiler data) at the Sanbe Reservoir station, the inflow water temperature, and rainfall from May to October in 2017, 2018, and 2019.

Figure 10 shows the vertical distribution of the electrical conductivity (EC), turbidity (Turb.), phytoplankton index (Chl-*a*), pH, water temperature, and cyanobacteria index (PCY) in the Sanbe Reservoir from 22 June to 21 July 2018. It can be inferred that the decrease in EC concentration on 30 June and 7 July was due to the invasion of two inflows of freshwater to the epilimnion. This means that the inflow of freshwater was muddy because the turbidity in the epilimnion also increased at that time. Furthermore, the water temperature, pH, and Chl-*a* decreased simultaneously. After July 12, Chl-*a* and pH increased slowly with increasing water temperature over 25 °C, whereas PCY was only slightly detected near the water surface.

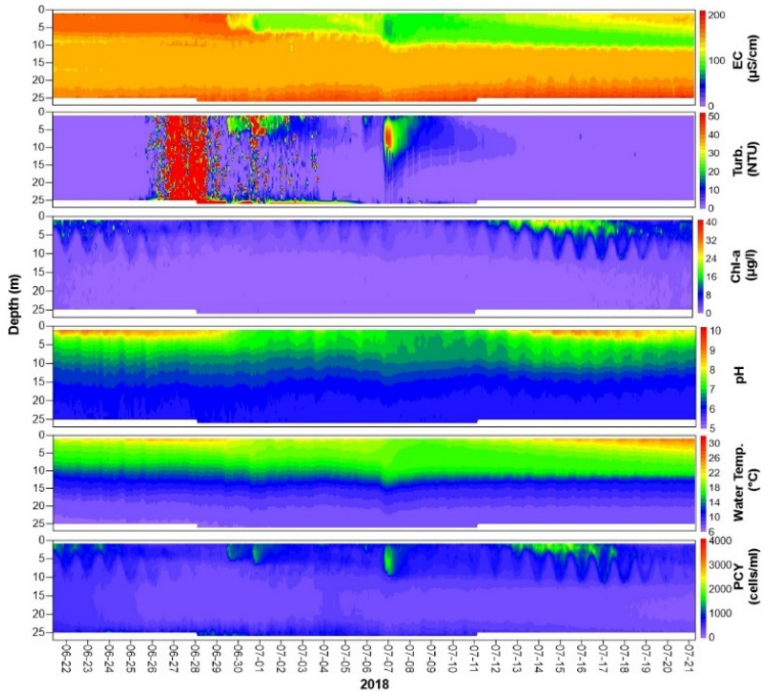

Figure 10. Vertical distribution of electrical conductivity (EC), turbidity (Turb.), phytoplankton content (Chl-*a*), pH, water temperature, and cyanobacteria index (PCY) in the Sanbe Reservoir from 22 June to 20 July 2018.

3.5. Relationship between Cyanobacteria and Rainfall

HCBs are continuously formed in water systems such as reservoirs and lakes around the world. They adversely affect water resources, marine products, living environments, and tourism resources. In particular, when geosmin and 2-MIB derived from cyanobacteria occur in reservoirs, their musty odor can cause serious problems in drinking water reservoirs. In this study, we analyzed observation data including cyanobacteria, water quality, and rainfall for four years (2015–2019) in the Sanbe Reservoir. As a result, it has been verified that the growth of cyanobacteria is strongly influenced by changes in water temperature and pH due to the inflow of rainfall. In particular, it was found that cyanobacterial geosmin and 2-MIB tend to occur under conditions of low rainfall. Since rainwater has a low pH, the reservoir keeps subacidity state when the rainwater inflow to the reservoir increased. On the other hand, when the inflow of rainwater is small, the consumption of carbon dioxide (CO_2) by photosynthesis of phytoplankton raises the pH.

When the reservoir exhibits a high pH condition, CO_2 exists primarily in the form of bicarbonate (HCO_3^-) [31]. Under such conditions, cyanobacteria can proliferate predominantly because it can use directly not only CO_2 but also HCO_3^- for photosynthesis. The environmental factors required for phytoplankton growth are affected not only by water temperature but also by insolation, sufficient nutrients, and the concentration of CO_2 or HCO_3^-. Inorganic carbon acquisition by marine phytoplankton has been studied in laboratory cultures of green algae [32,33] cyanobacteria [33,34], and diatom [34,35]. These studies have shown that these organisms utilize CO_2 as well as HCO_3^- as a photosynthetic C source. The uptake of HCO_3^- by cyanobacteria [33,34] appears to occur through a direct transport system, while indirect mechanisms of HCO_3^- uptake have been documented in green algae [32] and diatom [34]. Further, Tortell and Morel [34] reported that phytoplankton assemblages grown under low-CO_2 conditions (150 ppm) possess external CA activity and take up CO_2 through dehydration of HCO_3^- to CO_2, whereas the assemblages cultured under high-CO_2 (750 ppm) appeared to lack external CA activity and rely solely on CO_2 as an exogenous source of carbon for photosynthesis. Nakajima et al. [35] reported that in a marine diatom, the SLC4 family protein localized to the plasma membrane facilitates HCO_3^- uptake, which is highly dependent on Na^+ ions at concentrations over 100 mM.

In seawater (pH 8.3), the clear majority of dissolved inorganic C (DIC) exists as HCO_3^- [36], with <1% of free CO_2 as the substrate for C fixation [31]. However, in freshwater lakes (pH 6–9), generally, pH decreases to 6–7 after rainfall; thus, free CO_2 is approximately 70 –20% (high-CO_2 state). Therefore, according to the results of Tortell and Morel [34], it is inferred that the growth of green algae and diatoms at low pH (i.e., high-CO_2) in the Sanbe Reservoir is mainly CO_2-dependent. In addition, it has been suggested that the dominance of cyanobacteria in high-pH freshwater (i.e., low-CO_2) can be attributed to the ability to directly utilize the abundant HCO_3^- ions as a source of inorganic C for photosynthesis [37]. Thus, species that possess direct HCO_3^- transport mechanisms (such as cyanobacteria) may gain a competitive advantage over those that rely solely on CO_2 uptake or indirect HCO_3^- uptake via external CA-catalyzed dehydration. As shown in Figure 10, in years when rainfall was higher than normal, the pH in the reservoir decreased as the inflow water (low pH) to the reservoir increased. Therefore, since diatoms and green algae other than cyanobacteria were able to grow sufficiently, the competitive relationship between the three parties was reset, and therefore it could be inferred that the growth of cyanobacteria was suppressed. In contrast, the occurrence of 2-MIB was also inferred to have been controlled by the inflow from August to September 2018 (Figures 5 and 7). Thus, in the management of the odor in the reservoir, it is important to control the pH of the euphotic zone in the reservoir. Moreover, it is more important to pay attention to the years when rainfall was less than normal.

4. Conclusions

Geosmin and 2-MIB occurred primarily during low annual rainfall in the Sanbe Reservoir. Therefore, it is important to control the reservoir's pH to reduce the musty odor in the reservoir. In managing the odor in the reservoir, it is important to control the reservoir's pH. Moreover, it is more important to pay attention to the years when the rainfall is less than normal. In this study, only the effects of cyanobacterial geosmin and 2-MIB, which occur in a large amount in epilimnion, were considered. It is also necessary to consider the effect of geosmin and 2-MIB formed by actinomycetes in the hypolimnion, although it occurs in small amounts. The related analysis is now underway.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13243600/s1, Table S1: Relative abundance of diatom, dinoflagellate, euglenoid, and green algae at epilimnion in Sanbe Reservoir.

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