

Study of the cause of the generation of odor compounds (geosmin and 2-methylisoborneol) in the Han River system, the drinking water source, Republic of Korea

Byun Jeong Hwan ^{a,b}, Hun Nyun Kim^a, Tae Gu Kang^a, Baik-Ho Kim ^b and Myeong-Seop Byeon ^{a,*}

^a Han River Environment Research Center, National Institute of Environmental Research, Gyeonggi 427-100, Korea

^b Department of Life Science, Hanyang University, Seoul 133-791, Korea

*Corresponding author. E-mail: zacco@korea.kr

 BJH, 0000-0001-5422-2950; B-HK, 0000-0002-7144-0770; M-SB, 0000-0003-0997-2415

ABSTRACT

Paldang Lake in the Han River system is an important source for supplying drinking water. Recently, the occurrence of odor compounds geosmin and 2-methylisoborneol (2-MIB), in Lake Paldang has adversely affected the drinking water supply. The causative organisms of odor compounds have not been identified. Thus, this study analyzed the correlation between odor compounds and microorganisms and identified the odor-producing microorganisms in the Han River system. Odor compounds were primarily detected in the Bukhan River system but not in the Namhan River system. Geosmin was detected at high concentrations in summer, whereas 2-MIB was detected at high concentrations in summer and autumn. Microorganisms that appeared at the time of geosmin occurrence were actinobacteria (0.317, $p < 0.01$) and *Dolichospermum* (0.326, $p < 0.01$) among cyanobacteria, and the correlation was significant. The occurrence of 2-MIB mainly coincided with actinobacteria (0.745, $p < 0.01$) and *Pseudanabaena* (0.321, $p < 0.01$) among cyanobacteria, and the correlation was significant. Therefore, to manage the odor compounds in Lake Paldang, which is a drinking water source, it is important to control the appearance of actinobacteria and cyanobacteria in the Bukhan River system.

Key words: Actinobacteria, *Dolichospermum*, drinking water source, geosmin, 2-methylisoborneol, *Pseudanabaena*

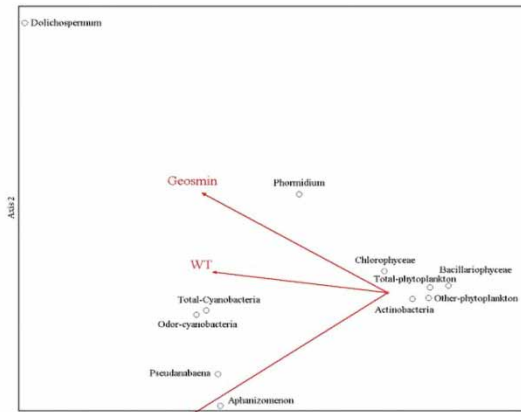
HIGHLIGHTS

- The generation of odor substances in Paldang Lake, the source of drinking water, was greatly influenced by the Bukhan River.
- Geosmin positively correlated with *Dolichospermum* and *Actinobacteria*.
- 2-MIB positively correlated with *Pseudanabaena* and *Actinobacteria*.
- For the management of odor compounds in Lake Paldang, the drinking water source, it is important to identify the appearance of cyanobacteria and actinomycetes.

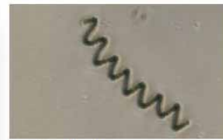
GRAPHICAL ABSTRACT



25 million people use drinking water sources of Paldang lake (PD)



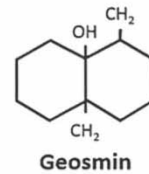
The CCA analysis between microorganisms and odor compounds



<*Dolichospermum*>



<*Phormidium*>



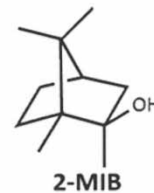
Dolichospermum and *Phormidium* produces geosmin



<*Pseudanabaena*>



<*Aphanizomenon*>



Pseudanabaena and *Aphanizomenon* produces 2-MIB

INTRODUCTION

Paldang Lake in the Han River system is the largest drinking water source in the Republic of Korea, supplying drinking water to 26 million people in Seoul; therefore, its water quality is extremely important. It is a large lake where the Bukhan and Namhan River systems merge and is greatly affected by water flowing in from the upstream. The upstream Bukhan and Namhan River systems have many artificial lake structures because of the construction of dams and weirs. However, artificial lakes with dams and weirs increase the residence time of the river water body, which leads to abnormal growth of microorganisms, resulting in serious problems in the aquatic ecosystem and water resources (Hayes & Burch 1989; Lawton & Codd 1991; Park & Jheong 2003). In particular, if actinobacteria and cyanobacteria are present at high concentrations, odor compounds produced by metabolites lead to bad odor of soils and molds in water, and unless advanced water purification is performed, the drinking water supply becomes problematic (Watson *et al.* 2008).

Representative substances that cause odor in water are geosmin and 2-Methylisoborneol (2-MIB). Geosmin was discovered in a substance separated from actinobacteria by Gerber & Lechevalier (1965), and 2-MIB was also reported as a water odor compound, originating from microorganisms such as actinobacteria and cyanobacteria (Bentley & Maganathan 1981). Besides actinobacteria, *Dolichospermum* (= *Anabaena*), *Aphanizomenon*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Planktonthrix*, and *Pseudanabaena* have been reported as cyanobacteria that produce odor compounds in water (Peterson *et al.* 1995; Sugiura *et al.* 1997; Izaguirre & Taylor 1998; Sugiura & Nakano 2000; Saadoun *et al.* 2001; Zimba *et al.* 2001). Odor

compounds in water are substances that cause esthetic discomfort, although they are not toxic (Mochida 2009). Humans can detect them in the concentration range of 0.004–0.010 $\mu\text{g L}^{-1}$. In Japan, the drinking water criterion for odor compounds is below 0.010 $\mu\text{g L}^{-1}$ (Suffet *et al.* 1995; Whelton & Dietrich 2004). In Republic of Korea, although there are no regulatory standards, the presence of odor compounds is designated as a property determining the quality of drinking water and these compounds are being maintained at the recommended level of 0.020 $\mu\text{g L}^{-1}$ or lower (MOE 2016).

Recently, odor compounds in water have attracted domestic as well as international attention as a serious environmental problem. In Republic of Korea, an abnormal proliferation of cyanobacteria occurred in the Bukhan River water system in 2011, and as a result, a high concentration of geosmin was detected among the odor compounds in Paldang Lake, which is a drinking water source. In 2014, a high concentration of 2-MIB among odor compounds was detected in the Bukhan River water system (You *et al.* 2013; Byun *et al.* 2015). After that, the occurrence of odor compounds has been continuously reported in Paldang Lake every summer and autumn. The occurrence of odor compounds in drinking water sources such as the Paldang Lake affects the supply of drinking water and requires advanced water purification technology, which is expensive (Watson *et al.* 2008). Cyanobacteria are the suspected causative microorganisms that generate odor compounds in Lake Paldang; however, odor compounds are often generated even when no cyanobacteria are present, and the cause is not clearly known.

Therefore, this study aimed to determine the cause of the occurrence of odor compounds in Lake Paldang. Odor compounds were monitored in the Bukhan and Namhan River systems, and the water system in which the substances mainly appeared was selected and studied.

MATERIALS AND METHODS

Odor compounds

Odor compounds monitoring

For odor monitoring, field items and odor were analyzed once a week from April 2017 to November 2018 at six points, including Uiam Dam (UA), Cheongpyeong Dam (CP), and Sambong-ri (SB) in the Bukhan River system, and Ipo Weir (IP), Buyongsa (BY), and Paldang Dam (PD) in the Namhan River system (Figure 1).

Analysis of water odor compounds

The water odor compounds geosmin and 2-MIB were analyzed by applying the headspace-solid phase micro extraction (HS-SPME) method using gas chromatography/mass spectrometer (GC/MS) (Varian, US/CP3800) in accordance with the

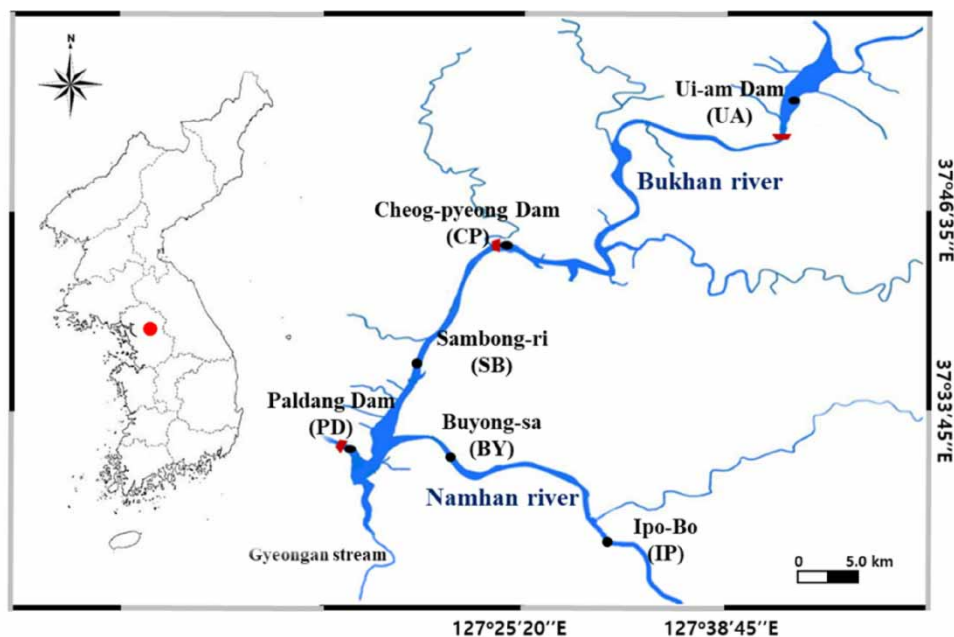


Figure 1 | Water sampling locations in Republic of Korea.

Guideline to Drinking Water Quality Monitoring Items (MOE 2016). The detection limit of odor compounds was set at $0.001 \mu\text{g L}^{-1}$. For preprocessing before analysis, the fibers were activated by flowing helium gas at a flow rate of 1 mL min^{-1} for more than 1 h at 270°C . Then, 10 mL of the sample and 3 g of purified NaCl were added to a 20 mL vial, and the sample was adsorbed to the SPME fibers for 30 min while being stirred at 70°C at 400 rpm. The adsorbed sample was desorbed at 270°C for 4 min and analyzed by GC/MS. The area was determined by obtaining the position of each analyte, and the concentration of the odor compounds in water was determined by applying the absolute test curve method. The absolute test curve was drawn by adding a standard mixture solution (47525-U, SUPELCO) to 10 mL of purified water step by step, from 0.005 up to $0.2 \mu\text{g L}^{-1}$, and was analyzed by HS-SPME GC/MS. Setting in SIM mode for quantitative analysis monitored molecular ions, base peaks and additional fragment ion at m/z 112, 125, and 97 for geosmin and m/z 95, 108, and 135 for 2-MIB. In addition, the detection limit was set to $0.002 \mu\text{g L}^{-1}$.

Microorganisms

Microorganism monitoring

Microorganisms (bacteria, cyanobacteria) were analyzed twice a month from June 2017 to October 2018 at four points, including UA, CP, and SB in the Bukhan River system, and PD.

Actinobacteria

Water was sampled at each study point from the surface layer (50 cm depth) using a Van Dorn water sampler (Niskin water sampler, General Oceanics, USA). The water samples were kept in a cool and dark place before being moved to the laboratory. To identify the bacteria, 200 mL water sample was filtered through a membrane filter (pore size $0.2 \mu\text{m}$, diam 47 mm) (Whatman, England). Then, the metagenomic DNA was extracted according to the protocol of the PowerWater DNA isolation kit (MOBIO, Germany). The bacteria were analyzed with the extracted DNA using the next-generation sequencing (NGS) method, which was commissioned by the LSW Analysis Center (www.bccio.16mb.com, Hong Kong). The operational taxonomic units of the bacteria were distinguished in accordance with 95% sequence similarity. They were identified up to the class level for accuracy in identification.

For the bacteria standing crop, the total number of cells was observed using the DAPI staining method. After staining with a Cell DAPI Detection kit (GenScript, China) using a Nucleopore black polycarbonate membrane filter (pore size $0.2 \mu\text{m}$, diam 25 mm) (Whatman, England), the cells were counted three times under an optical microscope (Nikon Eclipse 80i, Japan) using fluorescence and the average value was substituted in the NGS relative frequency result.

Phytoplankton

To identify and count phytoplanktons, the water sample was added to a 150 mL vial and fixed with Lugol solution (final concentration 2%, v/v). The phytoplanktons were identified after concentrating the fixed sample twice by settling the fixed sample for more than 48 h, and removing the supernatant using siphon according to the concentration of algae. They were identified to the genus level under an optical microscope at $\times 200$ – 1000 (Nikon eclipse E600, Japan). Komarek *et al.* (2014) were referred for the identification of cyanobacteria, and the species list distributed by the National Institute of Biological Resources was used for genus classification.

To quantify phytoplanktons, the fixed sample was mixed well, and 1 mL of the mixed sample was put in a Sedgewick–Rafter chamber (Wildco, USA) and stabilized for more than 5 min. The number of colonies or cells was counted under an optical microscope at $\times 200$ – 400 (Nikon Eclipse E600, Japan).

Statistical analysis

The Spearman's correlations were used to describe the correlations between environmental factors, odor compounds, and microorganisms (SPSS Statistics 14.IBM). Relations were considered to be statistically significant when p was less than or equal to (\leq) 0.05. In addition, canonical correlation analysis (CCA) was performed using the PC-Ord program (McCune & Mefford 1999).

RESULTS

Odor compounds in water

Geosmin

The concentration of geosmin was in the range of 0–0.246 $\mu\text{g L}^{-1}$ during the entire study period (Figure 2) in the Bukhan River system.

At UA, the concentration of geosmin was higher than the drinking water quality contamination standard once in July (0.035 $\mu\text{g L}^{-1}$) and twice in August (0.044, 0.036 $\mu\text{g L}^{-1}$) 2017. It also exceeded once in June (0.034 $\mu\text{g L}^{-1}$) 2018. At CP, the concentration exceeded once in July (0.051 $\mu\text{g L}^{-1}$) and thrice in August (0.200, 0.100, 0.029 $\mu\text{g L}^{-1}$), 2017. It did not appear in 2018. At SB, it exceeded once in July (0.040 $\mu\text{g L}^{-1}$) and twice in August (0.246, 0.034 $\mu\text{g L}^{-1}$) 2017. It also exceeded twice in August (0.021, 0.037 $\mu\text{g L}^{-1}$) 2018.

In the Namhan River system, at BY, the concentration exceeded once in August 2017 (0.033 $\mu\text{g L}^{-1}$). However, in the rest of the study period, the concentration did not exceed the criterion.

At PD, the concentration exceeded once in July (0.027 $\mu\text{g L}^{-1}$) and twice in August (0.138, 0.100 $\mu\text{g L}^{-1}$) 2017. It also exceeded twice in August (0.024, 0.038 $\mu\text{g L}^{-1}$) 2018.

2-MIB

The concentration of 2-MIB was in the range of 0–0.280 $\mu\text{g L}^{-1}$ during the entire study period in the Bukhan River system.

At UA, the concentration of 2-MIB was higher than the drinking water quality contamination standard thrice in July (0.043, 0.055, 0.081 $\mu\text{g L}^{-1}$), four times in October (0.035, 0.048, 0.061, 0.062 $\mu\text{g L}^{-1}$), and twice in November (0.099, 0.044 $\mu\text{g L}^{-1}$) 2017. Furthermore, it exceeded four times in August (0.030, 0.037, 0.038, 0.024 $\mu\text{g L}^{-1}$), twice in September (0.022, 0.022 $\mu\text{g L}^{-1}$), and four times in October (0.025, 0.125, 0.280 $\mu\text{g L}^{-1}$) 2018. At CP, the concentration exceeded four times in July (0.025, 0.022, 0.076, 0.021 $\mu\text{g L}^{-1}$), thrice in October (0.023, 0.067, 0.064 $\mu\text{g L}^{-1}$), and four times in November (0.085, 0.080, 0.069, 0.069 $\mu\text{g L}^{-1}$) 2017. It also exceeded four times in August (0.025, 0.030, 0.041, 0.038 $\mu\text{g L}^{-1}$) 2018. At SB, concentration exceeded twice in August (0.059, 0.022 $\mu\text{g L}^{-1}$), thrice in November (0.042, 0.043, 0.046 $\mu\text{g L}^{-1}$), and twice in August (0.031, 0.034 $\mu\text{g L}^{-1}$) 2017. It also exceeded once in October (0.023 $\mu\text{g L}^{-1}$) 2018.

In the Namhan River system, at BY, the concentration exceeded once in June 2018 (0.023 $\mu\text{g L}^{-1}$). In the rest of the study period, it did not exceed the drinking water quality contamination standard.

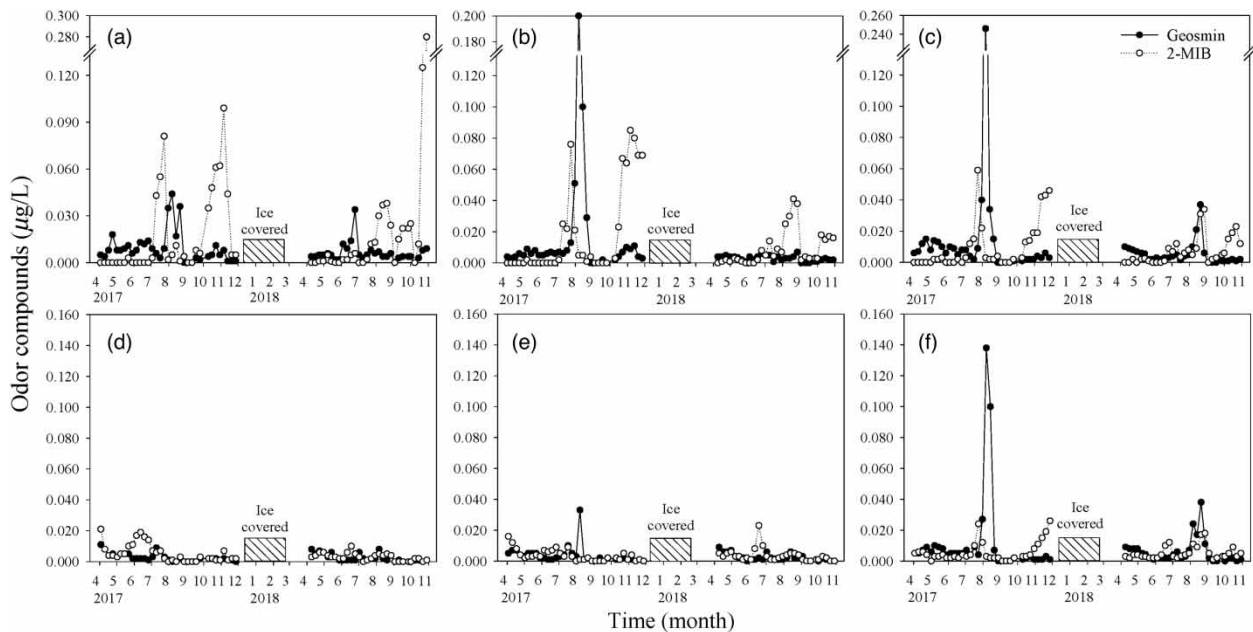


Figure 2 | Odor compounds occurring in the Namhan and Bukhan River systems and Paldang Lake. (a) UA, (b) CP, (c) SB, (d) IP, (e) BY, (f) PD.

At PD, the concentration exceeded once in July ($0.024 \mu\text{g L}^{-1}$), once in October ($0.026 \mu\text{g L}^{-1}$), and twice in August ($0.138, 0.100 \mu\text{g L}^{-1}$) 2017. It did not appear in 2018.

Microorganisms

The microorganisms were analyzed around the Bukhan River system and PD, where the water odor compounds mainly appeared from June to October 2017 and from April to October 2018.

Bacteria

During the survey period, the bacteria appeared in a total of 32 taxa in the Bukhan River system and PD in the range of $5.56\text{--}16.02 \times 10^5 \text{ cells mL}^{-1}$ (Figure 3). Among them, bacteria that appeared as dominant taxa were actinobacteria, alphaproteobacteria, and betaproteobacteria, which were in the range of $1.33\text{--}5.83 \times 10^5 \text{ cells mL}^{-1}$ (Table 1). The standing crops of the bacteria mostly showed an increasing trend from July after the Monsoon season. In the Bukhan River system, the standing crop increased during the Monsoon season, decreased slightly in September, and then increased again in October. The PD showed a similar trend of increasing standing crop in the Monsoon season, but after that, it decreased.

Cyanobacteria

During the survey period, phytoplankton appeared in a total of 71 genera and 159 species taxa in the Bukhan River system and PD in the range of $400\text{--}35,810 \text{ cells mL}^{-1}$ (Figure 4). Most of the taxa were dominated by Bacillariophyceae; whereas in summer, cyanobacteria dominated.

At the study points, the cyanobacteria abundance was $0\text{--}24,030 \text{ cells mL}^{-1}$. In both 2017 and 2018, the highest extant amount appeared in summer (July to August). During the survey period, the genera *Aphanizomenon*, *Dolichospermum*, *Merismopedia*, *Microcystis*, *Oscillatoria*, *Phormidium*, and *Pseudanabaena* appeared, of which *Dolichospermum* and *Pseudanabaena* were the most dominant among the cyanobacteria. Except for *Pseudanabaena*, other cyanobacteria mainly appeared in the summer when the water temperature was high (July to August). *Pseudanabaena* appeared for a relatively longer period than other cyanobacteria from summer to autumn (June to October).

Correlation between microorganisms and odor compounds in water

Spearman correlation analysis was performed to examine the correlation between odor compounds in water and microorganisms, which appeared during the study period (Table 2). Results showed that geosmin had a positive correlation with total

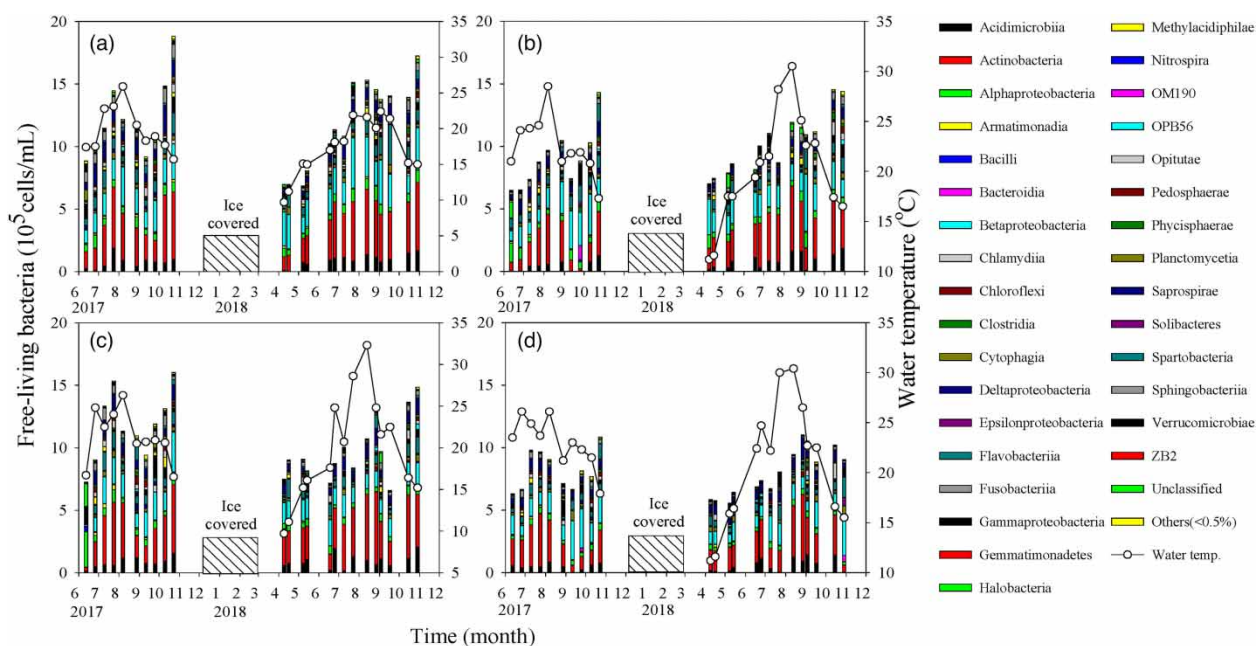


Figure 3 | Monthly fluctuation of relative abundance of bacteria in Bukhan River system and Paldang Lake. (a) UA, (b) CP, (c) SB, (d) PD.

Table 1 | Dominant taxa of bacteria and phytoplankton in Bukhan River system and Paldang Lake

	Uiam (UA)		Chungpyeong (CP)		Sambong (SB)		Paldang (PD)	
	Bacteria ^a	Algae ^b	Bacteria	Algae	Bacteria	Algae	Bacteria	Algae
2017-06-12	Acti.	<i>Rhod.</i>	Alpa.	<i>Rhod.</i>	Alpa.	<i>Aula.</i>	Acti.	<i>Aula.</i>
2017-06-26		<i>Frag.</i>	Beta.	<i>Frag.</i>	Beta.	<i>Pseu.</i>		
2017-07-10		<i>Rhod.</i>	Acti.	<i>Rhod.</i>	Acti.	<i>Aula.</i>		
2017-07-24		<i>Micr.</i>		<i>Pseu.</i>		<i>Pseu.</i>		
2017-08-07		<i>Doli.</i>		<i>Doli.</i>				<i>Rhod.</i>
2017-08-28		<i>Aula.</i>		<i>Syne.</i>		<i>Phor.</i>		<i>Scen.</i>
2017-09-11		<i>Cycl.</i>	Beta.	<i>Rhod.</i>	Beta.	<i>Apha.</i>	Beta.	<i>Cycl.</i>
2017-09-25	Beta.	<i>Rhod.</i>				<i>Dino.</i>		<i>Aula.</i>
2017-10-10	Acti.	<i>Rhiz.</i>				<i>Frag.</i>		<i>Rhod.</i>
2017-10-23		<i>Rhod.</i>	Acti.		Acti.		Acti.	<i>Cycl.</i>
2018-04-09	Beta.	<i>Syne.</i>	Beta.	<i>Syne.</i>		<i>Syne.</i>		<i>Syne.</i>
2018-04-16			Acti.					<i>Cycl.</i>
2018-05-08	Acti.							<i>Syne.</i>
2018-05-14								<i>Cycl.</i>
2018-06-18				<i>Dino.</i>	Beta.	<i>Dino.</i>		
2018-06-25				<i>Syne.</i>	Acti.	<i>Frag.</i>		<i>Aula.</i>
2018-07-09		<i>Rhod.</i>		<i>Aula.</i>		<i>Aula.</i>		<i>Cycl.</i>
2018-07-23				<i>Rhod.</i>				
2018-08-13		<i>Syne.</i>		<i>Syne.</i>		<i>Micr.</i>		<i>Micr.</i>
2018-08-27				<i>Pseu.</i>		<i>Syne.</i>		<i>Meri.</i>
2018-09-03			Beta.	<i>Rhod.</i>				
2018-09-17		<i>Volv.</i>	Acti.	<i>Pseu.</i>		<i>Volv.</i>		<i>Volv.</i>
2018-10-15		<i>Aula.</i>		<i>Aula.</i>		<i>Aula.</i>		<i>Rhod.</i>
2018-10-22		<i>Pseu.</i>		<i>Acan.</i>		<i>Rhod.</i>	Beta.	<i>Aula.</i>

^aBacteria group – Acti: Actinobacteria, Alpa: Alapaproteobacteria Beta: Betaproteobacteria.

^bAlgae group – *Aula*: *Aulacoseira*, *Cycl*: *Cyclotella*, *Dino*: *Dinobryon*, *Doli*: *Dolichospermum*, *Frag*: *Fragilaria*, *Meri*: *Merismopedia*, *Micr*: *Microcystis*, *Pseu*: *Pseudanabaena*, *Rhod*: *Rhodomonas*, *Syne*: *Synedra*, *Volv*: *Volvox*.

phytoplankton ($r = 0.357$, $p < 0.01$), Bacillariophyceae ($r = 0.225$, $p < 0.05$), cyanobacteria ($r = 0.314$, $p < 0.01$), *Dolichospermum* ($r = 0.442$, $p < 0.01$), *Pseudanabaena* ($r = 0.205$, $p < 0.05$), and actinobacteria ($r = 0.317$, $p < 0.01$). 2-MIB showed positive correlation with cyanobacteria ($r = 0.412$, $p < 0.01$), *Pseudanabaena* ($r = 0.321$, $p < 0.01$), total bacteria ($r = 0.608$, $p < 0.01$), and actinobacteria ($r = 0.745$, $p < 0.01$).

The CCA result of odor compounds in water and microorganisms showed that among the odor cyanobacteria, *Aphanizomenon* and *Dolichospermum* were closely correlated with geosmin, whereas *Pseudanabaena*, total odor cyanobacteria, and total cyanobacteria were closely correlated with 2-MIB (Figure 5).

The total phytoplanktons, other phytoplanktons, and actinobacteria taxa showed insignificant correlations with water odor compounds.

DISCUSSION

Microorganisms observed in Bukhan River water system and Paldang Lake

When the Bukhan River water system and PD were investigated, where odor compounds in water above the drinking water quality monitoring criteria were often observed, actinobacteria, alphaproteobacteria, and betaproteobacteria were found to be the dominant taxa. These taxa have been reported as species distributed in a variety of environments, including freshwater,

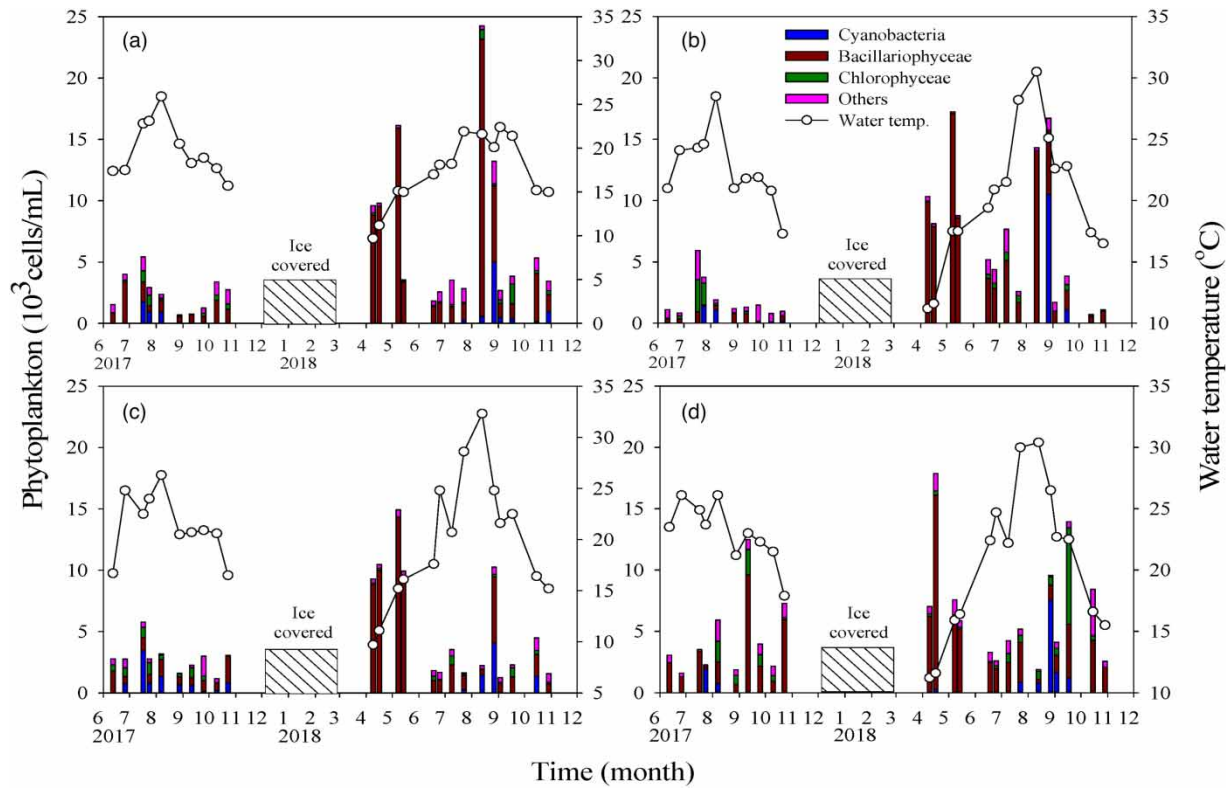


Figure 4 | Monthly fluctuation of relative abundance of phytoplankton in Bukhan River system and Paldang Lake. (a) UA, (b) CP, (c) SB, (d) PD * other: taxa that are not cyanobacteria, Chlorophyceae, and Bacillariophyceae.

Table 2 | Spearman correlation coefficient between microorganisms and odor compounds

	Bacillariophyceae	Cyanobacteria	T-Phytoplankton	Dolichospermum	Pseudanabaena	T-Bacteria	Actinobacteria
Geosmin	0.225*	0.314**	0.357***	0.442**	0.205*	0.072	0.317**
2-MIB	0.027	0.412**	0.186	0.156	0.321**	0.608**	0.745**

* $p < 0.05$, ** $p < 0.01$, ***T-phytoplankton, total phytoplankton; T-Bacteria, total bacteria.

seawater, and soils (Betnardet *et al.* 1996; Newton *et al.* 2011; Parveen *et al.* 2011; Kang *et al.* 2013; Bai *et al.* 2017). This is consistent with the findings of previous studies that actinobacteria and betaproteobacteria, which were mostly dominant during the study period, have high dominance in freshwater ecosystems (Klausen *et al.* 2005; Newton *et al.* 2011). The bacteria standing crop tended to increase after the Monsoon season (June–July). Because of rainfalls, large amounts of bacteria present in soils flow into the water systems, which directly increases the bacteria standing crop. At the same time, high concentrations of nutrients also flow into water systems, creating a beneficial environment for the proliferation of bacteria (Jensen *et al.* 1994).

The phytoplankton that appeared during the investigation period changed from the dominant diatoms and silver flagellates in spring to cyanobacteria in the summer when the water temperature became high, and then back to diatoms and silver flagellates in the autumn when the water temperature dropped. These community changes were identical to the succession characteristics of the Bukhan River and Paldang Lake (Byun *et al.* 2015). In summer, cyanobacteria taxa *Anabaena*, *Microcystis*, *Phormidium*, and *Pseudanabaena* were dominant, and these species were consistent with the species known to dominate the waters of the Bukhan River and Lake Paldang (Youn *et al.* 2020). *Dolichospermum*, *Microcystis*, and *Phormidium* appeared and disappeared in summer when the water temperature was high. However, *Pseudanabaena*

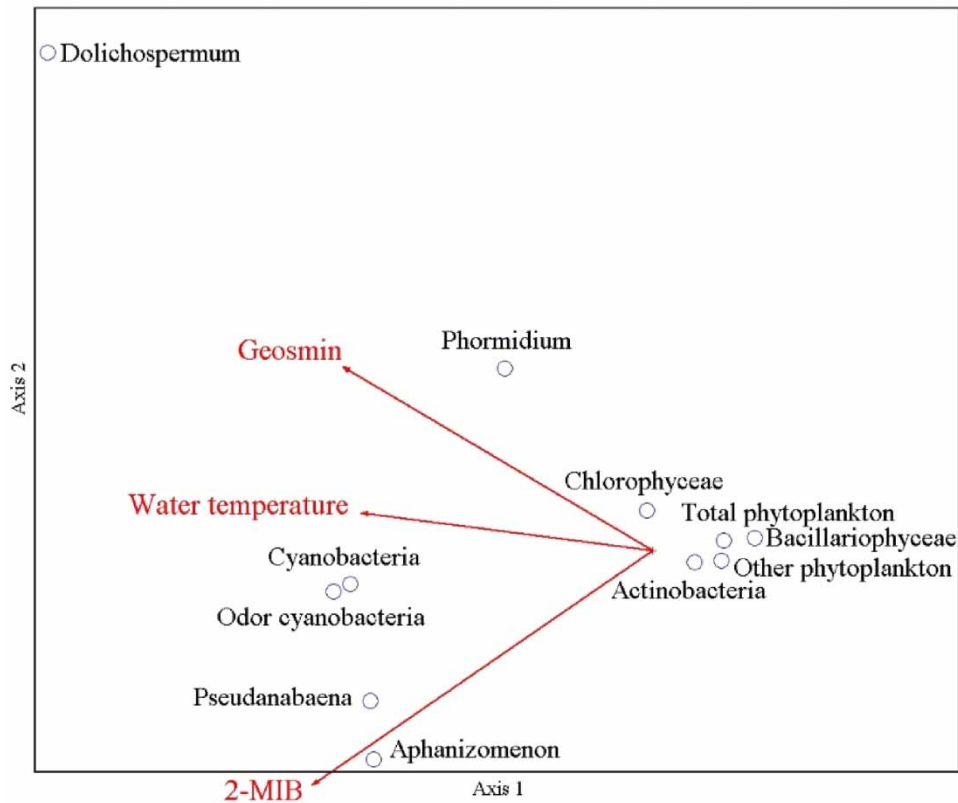


Figure 5 | Canonical correlation analysis (CCA) between odor compounds, water temperature, and microorganisms Other phytoplankton: taxa that are not cyanobacteria, Chlorophyceae, and Bacillariophyceae.

appeared even in spring and autumn, when the water temperature was relatively low. *Pseudanabaena* can grow in a wider temperature range than other cyanobacteria, and thus, appeared for a relatively long term (Gao *et al.* 2018).

Occurrence of odor compounds in Paldang Lake

The pattern of occurrence of odor compounds at each investigation point was similar to that of UA, CP, and SB, which are the water systems of the Bukhan River, and that of PD. Odor compounds in the Namhan River water system rarely showed concentrations higher than the drinking water monitoring standards. Therefore, it is considered that the odor compounds generated in the Paldang Lake are greatly affected by the Bukhan River water system.

The occurrence pattern of geosmin and 2-MIB was similar to the appearance pattern of cyanobacteria, and to the pattern of change in the amount of actinobacteria (Figure 6). As a result, cyanobacteria (*Aphanizomenon*, *Dolichospermum*, *Phormidium*, and *Pseudanabaena*) that appeared during the investigation period were the taxa reported to produce odors (Peterson *et al.* 1995; Sugiura *et al.* 1997; Izaguirre & Taylor 1998; Sugiura & Nakano 2000; Saadoun *et al.* 2001; Zimba *et al.* 2001), along with actinobacteria, which appeared mainly as the dominant group in the bacterial community. Therefore, the pattern of occurrence of odor compounds and the appearance of microorganisms are similar (Klausen *et al.* 2005; Tung *et al.* 2006; Jiang *et al.* 2007; Wang & Cane 2008).

Geosmin showed a concentration higher than the drinking water monitoring standard, mainly in summer. This coincides with the appearance of *Dolichospermum circinale* and *Dolichospermum planctonicum* cyanobacteria. Thus, it can be considered that cyanobacteria affected geosmin generation (Tsao *et al.* 2014; Kim *et al.* 2018; Youn *et al.* 2020). 2-MIB concentration was found to be higher than the drinking water monitoring standard, mainly in summer and autumn, which is thought to have been influenced by the appearance of *Pseudanabaena* sp. (Wang *et al.* 2011; Chui *et al.* 2016; Izaguirre & Taylor 1998).

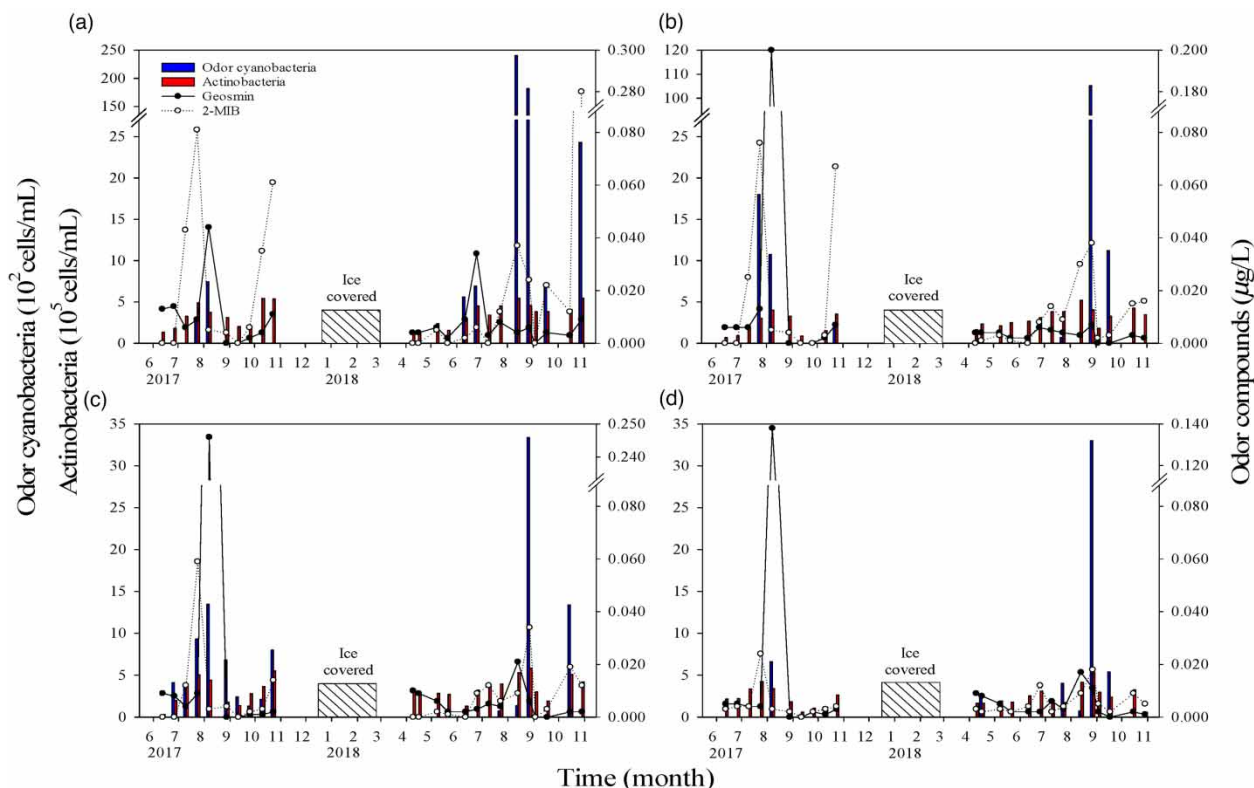


Figure 6 | Odor compounds and microorganisms occurring in Bukhan River system and Paldang Lake. (a) UA, (b) CP, (c) SB, (d) PD.

Therefore, the odor compounds generated in Lake Paldang (PD) were greatly affected by cyanobacteria and odor compounds that appeared in the waters of the Bukhan River.

Correlation between microorganisms and odor compounds in water

The Spearman correlation analysis and CCA both showed a close correlation between geosmin and genus *Dolichospermum*; and between 2-MIB and genus *Pseudanabaena*. The presence of geosmin and 2-MIB coincides with the appearance time of *Dolichospermum* and *Pseudanabaena*, respectively. This was because cyanobacteria appeared in high abundance during the summer months of July–August when the water temperature was high, and most of them produced odor compounds. In particular, 2-MIB had a high positive correlation with *Pseudanabaena*. This result is consistent with previous studies, which showed that *Pseudanabaena* appearing in the Bukhan River water system has a positive correlation with 2-MIB (Byun *et al.* 2015; Pimolrat *et al.* 2015; Zhang *et al.* 2016). These results suggest that cyanobacteria of the genera *Dolichospermum* and *Pseudanabaena* that appeared in the water system of the Bukhan River and Paldang Lake have a great influence on the generation of odor compounds (geosmin, 2-MIB).

In the case of actinobacteria, the Spearman correlation analysis showed a positive correlation with geosmin and 2-MIB, whereas the CCA result showed that the relationship with geosmin and 2-MIB was insignificant. During the investigation period, the amount of actinobacteria present appeared evenly at all points, showing that the range of change in actinobacteria abundance was lower than that of cyanobacteria. Since the occurrence of odor compounds is less dynamic in actinobacteria than in cyanobacteria, there is less relationship with odor compounds in the CCA results.

Previous study results show that cyanobacteria affect the occurrence of odor compounds more than actinobacteria (Xu *et al.* 2010). However, there are reports that some organisms other than cyanobacteria may be the cause of odor compounds being generated when cyanobacteria do not appear (Parinet *et al.* 2010; Cole & Williams 2011; Lee *et al.* 2011). Recently, actinobacteria have been considered to be the causative organism of odor-producing compounds (Anuar *et al.* 2017; Whangchai *et al.* 2017). Since the pattern of increase and decrease in the amount of actinobacteria present in this study and the pattern of occurrence of odor compounds appeared very similar, it can be considered that actinobacteria

may have had some influence on the occurrence of odor compounds in the Bukhan River and Paldang Lake. There is also a previous study that shows that actinomycetes present in the sediments produce a lot of odor compounds. In addition to these, actinobacteria in the aquatic free-living bacterial community are also considered to influence the occurrence of odor compounds. This can be the basis for explaining the odor compounds that occur in the water body when cyanobacteria do not appear (Tung *et al.* 2006; Zuo *et al.* 2009; Lee *et al.* 2011; Asquith *et al.* 2013; Whangchai *et al.* 2017).

At the PD point of Lake Paldang, the presence of odor compounds, and cyanobacteria were relatively lower than those of the upper Bukhan River (UA, CP, SB) points, which is thought to be due to the inflow from the Namhan River as well as the Bukhan River (Han *et al.* 1995; Kim 1996).

Nevertheless, the occurrence of odor compounds in Paldang Lake showed a pattern similar to that of the Bukhan River system, and the occurrence is considered to be the effect of Uiam lake (UA), located upstream among the serial dams in the Bukhan River system. UA is the only point in the Bukhan River system that passes through a large city and also has a source of pollution, such as a sewage treatment plant (Park *et al.* 2003). Harmful cyanobacteria and odor compounds have frequently appeared here in the past. Therefore, it is necessary to carefully observe the appearance of cyanobacteria *Dolichospermum* and *Pseudanabaena* in the Bukhan River system to effectively manage the odor compounds in Lake Paldang, which is a drinking water source.

Moreover, when the abundance of actinobacteria was high, the concentration of odor compounds was also high. In particular, actinobacteria is highly likely to be the causative organism for odor compounds that occur during times when cyanobacteria do not appear. This is an important result for the management of odor compounds in drinking water sources, and additional studies on actinobacteria in water are required.

CONCLUSIONS

The causes of odor compounds generated in the Paldang Lake, the largest drinking water source in the Republic of Korea, are as follows:

- Odor compounds from Paldang Lake are more affected by the Bukhan River system than the Namhan River system.
- Geosmin is generated by the influence of the genus *Dolichospermum* of cyanobacteria and actinobacteria in summer, and 2-MIB is generated by the influence of genus *Pseudanabaena* and Actinobacteria in summer and autumn.
- During the period of study, the pattern of occurrence of cyanobacteria, actinobacteria, and odor compounds in UA and that of CP, SB, and PD, and the downstream were similar. Therefore, odor compounds generated in UA have a great influence on the generation of odor compounds downstream.
- In conclusion, for the management of odor compounds in Paldang Lake, it is necessary to check the occurrence of odor compounds in the Bukhan River system. In particular, it is important to control the growth of cyanobacteria and actinobacteria in UA.

ACKNOWLEDGEMENT

This work was conducted as part of an R&D project (NIER-2021-01-01-085) of the National Institute of Environmental Research of the Ministry of Environment

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Anuar, N. S. S., Kassim, A. A., Utsumi, M., Iwamoto, K., Goto, M., Shimizu, K., Othman, N., Zakaria, Z., Sugiura, N. & Hara, H. 2017 Characterization of musty odor-producing actinomycetes from tropics and effects of temperature on the production of musty odor compounds. *Microbes and Environments* **32** (4), 352–357.
- Asquith, E. A., Evans, C. A., Geary, P. M., Dunstan, R. H. & Cole, B. 2013 The role of actinobacteria in taste and odour episodes involving geosmin and 2-methylisoborneol in aquatic environments. *Journal of Water Supply: Research and Technology* **62** (7), 452–469.

- Bai, L., Cao, C., Wang, C., Xu, H., Zhang, H., Slaveykova, V. H. & Jiang, H. 2017 Toward quantitative understanding of the bioavailability of dissolved organic matter in freshwater Lake during cyanobacteria blooming. *Environmental Science & Technology* **51**, 6018–6026.
- Bentley, R. & Maganathan, R. 1981 Geosmin and methylisoborneol biosynthesis in *Streptomyces* evidence for an isoprenoid pathway and its absence in non-differentiating isolates. *FEBS Letters* **125**, 220–222.
- Betnardet, J. F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. 1996 Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *International Journal of Systematic and Evolutionary Microbiology* **46**, 128–148.
- Byun, J. H., Hwang, S. J., Kim, B. H., Park, J. R., Lee, J. K. & Lim, B. J. 2015 Relationship between a dense population of cyanobacteria and odorous compounds in the North Han River System in 2014 and 2015. *Korean Journal of Ecology and Environment* **48** (4), 263–271. (in Republic of Korea).
- Chui, Y. T., Yen, H. K. & Lin, T. F. 2016 An alternative method to quantify 2-MIB producing cyanobacteria in drinking water reservoirs: method development and field applications. *Environmental Research* **151**, 618–627.
- Cole, B. & Williams, B. 2011 Grahamstown Dam Water Quality & Aquatic Ecological Functioning. A Compilation of Scientific and Adaptive Management Studies. Hunter Water Corporation Newcastle, NSW.
- Gao, J., Zhu, J., Wang, M. & Dong, W. 2018 Dominance and growth factors of *Pseudanabaena* sp. in drinking water source reservoirs, Southern China. *Sustainability* **10**, 3936.
- Gerber, N. N. & Lechevalier, H. A. 1965 Geosmin and earth-smelling substances isolated from actinomycetes. *Applied Microbiology* **13**, 935–938.
- Han, M. S., Auh, Y. Y., Ryu, J. K., Yoo, K. I. & Choi, Y. K. 1995 Ecological studies on Pal'tang river-reservoir system in Korea 2. Changes in phytoplankton community structure. *Korean Journal of Limnological Society* **28** (3), 335–344. (in Republic of Korea).
- Hayes, K. P. & Burch, M. D. 1989 Odorous compounds associated with algal blooms in South Australian waters. *Water Research* **23**, 115–121.
- Izaguirre, G. & Taylor, W. D. 1998 A *Pseudanabaena* species from Castaic Lake, California, that produces, 2-methylisoborneol. *Water Research* **32**(5), 1673–1677.
- Jensen, S. E., Anders, C. L., Goatcher, L. J., Perley, T., Kenefick, S. & Hrudehy, S. E. 1994 Actinomycetes as a factor in odour problems affecting drinking water from the North Saskatchewan river. *Water Research* **28** (6), 1393–1401.
- Jiang, J., He, X. & Cane, D. E. 2007 Biosynthesis of the earthy odorant geosmin by a biofunctional *Streptomyces coelicolor* enzyme. *Nature Chemical Biology* **3** (11), 711–715.
- Kang, J. Y., Chun, J. & Ripka, R. 2013 *Flavobacterium aciduliphilum* sp. nov., isolated from freshwater, and emended description of the genus *Flavobacterium*. *International Journal of Systematic and Evolutionary Microbiology* **63**, 1633–1638.
- Kim, Y. J. 1996 Ecological study of phytoplankton community and trophic states using indicators in Lake Paltang. *Korean Journal of Limnological Society* **19** (4), 323–345. (in Republic of Korea).
- Kim, K., Park, C., Yoon, Y. & Hwang, S. J. 2018 Harmful cyanobacterial material production in the North Han River (South Korea): genetic potential and temperature-dependent properties. *International Journal of Environmental Research and Public Health* **15**, 444.
- Klausen, C., Nicolaisen, M. H., Strobel, B. W., Warnecke, F., Nielsen, J. L. & Jørgensen, N. O. G. 2005 Abundance of actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds. *FEMS Microbiol Ecology* **52**, 265–278.
- Komarek, J., Kastovsky, J., Mares, J. & Johansen, J. R. 2014 Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* **86**, 295–335.
- Lawton, L. A. & Codd, G. A. 1991 Cyanobacteria (blue green algal) toxins and their significance in U.K. and European waters. *Journal of the Institution of Water & Environmental Management* **5**, 460–465.
- Lee, G. C., Kim, Y. S., Kim, M. J., Oh, S. A., Choi, I., Choi, J., Park, J. G., Chong, C. K., Kim, Y. Y., Lee, K. & Lee, C. H. 2011 Presence, molecular characteristics and geosmin producing ability of actinomycetes isolated from South Korean terrestrial and aquatic environments. *Water Science and Technology* **63** (11), 2745–2751.
- McMune, B. & Mefford, M. J. 1999 *PC-ORD Multivariate Analysis of Ecological Data*. Version 4. MjM Software Design, Gleneden Brach, Oregon, USA.
- Ministry of Environment (MOE) 2016 *Drinking Water Quality Monitoring Guideline*. Ministry of Environment, Sejong-si,, Republic of Korea. (in Republic of Korea).
- Mochida, K. 2009 Evaluation of the cytotoxicity of geosmin and 2-methylisoborneol using cultured human, monkey, and dog cells. *Biocontrol Science* **14** (1), 35–38. (in Japan).
- Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D. & Bertilsson, S. 2011 A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews* **75** (1), 14–49.
- Parinet, J., Rodriguez, M. J. & Serodes, J. J. 2010 Influence of water quality on the presence of off-flavour compounds (geosmin and 2-methylisoborneol). *Water Research* **20**, 5847–5856.
- Park, H. K. & Jheong, W. H. 2003 Long-term changes of algal growth in Lake Paldang. *Journal of Korean Society on Water Quality* **19** (6), 673–684. (in Korean).
- Park, H. K., Byeon, M. S., Kim, E. K., Lee, H. J., Chun, M. J. & Jung, D. I. 2004 Water quality and phytoplankton distribution pattern in upper inflow rivers of Lake Paldang. *Journal of Korea Society on Water Quality* **20** (6), 615–624. (in Korean)

- Parveen, B., Reveilliez, J. P., Mary, I., Ravet, V., Bronner, G., Mangot, J. F., Domaizon, I. & Debroas, D. 2011 Diversity and dynamics of free-living and particle-associated Betaproteobacteria and Actinobacteria in relation to phytoplankton and zooplankton communities. *FEMS Microbiology Ecology* **77**, 461–476.
- Peterson, H. G., Hrudey, S. E., Cantin, I. A., Perley, T. R. & Kenefick, S. L. 1995 Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals. *Water Research* **29** (6), 1515–1523.
- Pimolrat, P., Whangchai, N. & Chitmanat, C. 2015 Off-flavor characterization in high-nutrient-load tilapia ponds in northern Thailand. *Turkish Journal of Fisheries and Aquatic Sciences* **15**, 273–281.
- Saadoun, I. M. K., Schrader, K. K. & Blevins, W. T. 2001 Environmental and nutritional factors affecting geosmin synthesis by *Anabaena* sp. *Water Research* **35**, 1209–1218.
- Suffet, I. H., Malleyialle, J. & Kawczynski, E. 1995 *Advances in Taste-And-Odor Treatment and Control*. American Water Works Association, Denver, USA.
- Sugiura, N. & Nakano, K. 2000 Causative microorganisms for musty odor occurrence in the Eutrophic Lake Kasumigaura. *Hydrobiologia* **434** (1–3), 145–150.
- Sugiura, N., Nishimura, O., Inamor, Y., Ouchiyama, T. & Sudo, R. 1997 Grazing characteristics of musty-odor-compound-producing *Phormidium tenue* by a microflagellate, *Monas guttula*. *Water Research* **31** (11), 2792–2796.
- Tsao, H. W., Michinaka, A., Yen, H. K., Giglio, S. & Hobson, P. 2014 Monitoring of geosmin producing *Anabaena circinalis* using quantitative PCR. *Water Research* **49**, 416–425.
- Tung, S. C., Lin, T. F. & Lin, G. M. 2006 Identification of 2-MIB and geosmin producers in Feng-Shen Reservoir in South Taiwan. *Water Science and Technology: Water Supply* **6** (2), 55–61.
- Wang, C. M. & Cane, D. E. 2008 Biochemistry and molecular genetics of the biosynthesis of the earthy odorant methylisoborneol in *Streptomyces coelicolor*. *Journal of the American Chemical Society* **130** (28), 8908–8909.
- Wang, Z., Xu, Y., Shao, J., Wang, J. & Li, R. 2011 Genes associated with 2-methylisoborneol biosynthesis in cyanobacteria: isolation, characterization, and expression in response to light. *PLoS One* **6** (4), e18665.
- Watson, S. B., Ridal, J. & Boyer, G. L. 2008 Taste and odour and cyanobacterial toxins: impairment, prediction, and management in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **65**, 1779–1802.
- Whangchai, N., Pimpimon, T., Sompong, U., Suwanpakdee, S., Gutierrez, R. & Itayama, T. 2017 Study of geosmin and 2-MIB producers in Phayao Lake, Thailand. *International Journal of Bioscience* **7** (3), 177–185.
- Whelton, A. J. & Dietrich, A. M. 2004 Relationship between intensity concentration and temperature for drinking water odorants. *Water Research* **38**, 1604–1614.
- Xu, L., Xiong, B., Wang, J., Cao, H. & Zhao, W. 2010 Relationship between concentrations of odorous compounds and biomass of phytoplankton and actinomycetes in freshwater ponds of Beijing, China. *Aquaculture International*. **18**, 245–254.
- You, K. A., Byeon, M. S., Youn, S. J., Hwang, S. J. & Rhew, D. H. 2013 Growth characteristics of blue-green algae (*Anabaena spiroides*) causing tastes and odors in the North-Han River, Korea. *Korean Journal of Ecology and Environment* **46**, 135–144. (in Republic of Korea).
- Youn, S. J., Kim, H. N., Yu, S. J. & Byeon, M. S. 2020 Cyanobacterial occurrence and geosmin dynamics in Paldang Lake Watershed, South Korea. *Water and Environment Journal* **34**, 634–643.
- Zhang, T., Zheng, L., Li, L., Wan, C. & Li, R. 2016 2-Methylisoborneol production characteristics of *Pseudanabaena* sp. FACHB 1277 isolated from Xionghu Reservoir, China. *Journal of Applied Phycology* **28**, 3353–3362.
- Zimba, P. V., Grimm, C. C., Dionigiand, C. P. & Weirich, C. R. 2001 Phytoplankton community structure, biomass, and off-flavor: pond size relationships in Louisiana catfish ponds. *Journal of the World Aquaculture Society* **32** (1), 96–104.
- Zuo, Y., Li, L., Wu, Z. & Song, L. 2009 Isolation, identification and odour-producing abilities of geosmin/2-MIB in actinomycetes from sediments in Lake Lotus, China. *Journal of Water Supply: Research and Technology* **58** (8), 552–561.

First received 4 September 2022; accepted in revised form 27 January 2023. Available online 14 February 2023