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Identification of causative compounds and microorganisms for musty odor occurrence in the Huangpu River, China

Daolin Sun[1], Jianwei Yu[1], Wei An[1], Min Yang[1,*],
Guoguang Chen[2], Shujun Zhang[3]

1. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: yangmin@rcees.ac.cn
2. Shanghai Municipal Control and Monitor Centre of Water Supply, Shanghai 20002, China
3. The Administrative Center for China's Agenda 21, Ministry of Science and Technology, Beijing 100038, China

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Abstract

There are regular problems of musty odor in the Huangpu River, a major source of drinking water for Shanghai, China. In this study, the musty odor and its main causative compounds in the Huangpu River source water were confirmed through a yearly investigation using flavor profile analysis combined with HSPME-GC-MS analysis. The investigation showed that 2-methylisoborneol (2-MIB) with a concentration level between 28.6 and 71.0 ng/L was responsible for the musty odor in summer from July to September. Microscopic observation confirmed with the cloning results showed that *Phormidium* spp., which accounted for 80%–95% of the algal cell density, was the microorganisms responsible for the production of 2-MIB and the estimated 2-MIB yield was 0.022 pg/cell. Results from a wide-area sampling campaign in the Huangpu River watershed showed that, other than the large tributaries receiving water from Tai Lake, several small creeks close to the intake may have contributed most of the 2-MIB and the *Phormidium* spp. to the Huangpu River source water. This study provides methodology for the investigation of odor causing compounds and microorganisms in river-type source water, and the result will be useful for water quality control in both source water and drinking water.

Key words: river water source; 2-methylisoborneol; musty odor; *Phormidium*; watershed

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Introduction

Undesirable taste and odor is one of the major water quality problems encountered in the drinking water industry. The earthy/musty odor that is normally caused by trans-1, 10-dimethyl-trans-9-decalol (geosmin) or 2-methylisoboneol (2-MIB) had been frequently detected in lake and reservoir-type source waters worldwide (Lin et al., 2002; Suffet et al., 2004; Westerhoff et al., 2005; Peter et al., 2009; Zuo et al., 2010). In enclosed water bodies, such as lakes and reservoirs, these odorous compounds are generally produced by certain cyanobacteria, such as *Anabeana* for geosmin and *Phormidium* and *Oscillatoria* for either 2-MIB or geosmin (Slater and Blok, 1983; Jüttner, 1995; Izaguirre et al., 1999). As benthic cyanobacteria, *Phormidium* and *Oscillatoria* require an area of shallow water with a relatively high level of transparency (Watson, 2003; Taylor et al., 2006). The earthy/musty odors had also been detected in some river systems (Burlingame et al., 1986; Lanciotti et al., 2003; Ridal et al., 2007); however, the reasons for the occurrence of these odor events could be more complicated, since the hydrological conditions in different sections of a river system can change frequently. In some cases, allochthonous soil runoff may be important (Hrudey et al., 1992; Zaitlain et al., 2003). Biofilms can also be very significant sources of geosmin and 2-MIB, even in large, fast-flowing rivers (Watson and Ridal, 2004; Vilalta and Sabater, 2005), and alternatively, plankton may be important sources in some slow-moving turbid rivers (Hishida et al., 1988; Bowmer et al., 1992). Thus the odor events may occur either due to the discharge of odorous compounds from the lakes/reservoirs or the growth of some cyanobacteria in a nutrient-rich shallow section (Hosaka et al., 1995; Vilalta et al., 2004). Besides, some acticimyates like *Streptomyces* had also been reported to be capable of producing the 2-MIB or geosmin in rivers (Jensen et al., 1994; Zaitlin and Watson, 2006).

The Huangpu River, which receives water from Tai Lake and some upstream tributaries, has been used as one of the major sources of water for Shanghai (China), which

* Corresponding author. E-mail: yangmin@rcees.ac.cn

has long suffered from regular taste and odor problems in drinking water. A flavor that is characterized by musty and septic odors has frequently been identified in drinking water supplied from the Huangpu River source water (Yu et al., 2009). Previous studies have found that the musty odor was relatively strong in summer and autumn, and 2-MIB has been detected in source water with a level as high as 150 ng/L (Ma et al., 2008; Yu et al., 2009). However, the origin of the musty odor has remained unclear, which makes it difficult to take effective measures to either prevent or control the occurrence of the odor in drinking water.

In this study, the musty odor and the main causative compound for the musty odor in the Huangpu River source water was confirmed through a yearly investigation using flavor profile analysis combined with gas chromatography-mass spectrometry (GC-MS) analysis, and the potentially causative microorganisms were identified through microscopic observation combined with clone libraries analysis using a cyanobacterium specific primer. To elucidate the origins of the odor-causing compounds and microorganisms, their distributions in the complicated river network system were investigated during a wide-area sampling campaign. For the first time, this study revealed that 2-MIB produced by *Phormidium* spp. was the major causative compound for the musty odor in Huangpu River source water and some nearby small tributaries may have contributed most of the compound. This result will be useful for source water management.

1 Materials and methods

1.1 Sampling

There was a complicated river network in the Huangpu River watershed, as shown in **Fig. 1**. Among different upstream tributaries, the main three were Xietang, Yuanxiejing and Maogang, which contributed approximately 50%, 25% and 15% respectively, of the water content of the Huangpu River. Xietang comes from the Taipu River whose source is Tai Lake. Maogang receives water from the southern area of the watershed and Yuanxiejing obtains water from several creeks between Maogang upstream and Xietang downstream. There are several creeks close to the intake, which contributed approximately 10% of the total water quantity.

The sampling sites are shown in **Fig. 1**. Raw water (HPJ2) supplied to different waterworks was sampled at least once a month in 2009. A sampling campaign was performed in late September, 2010 to investigate the distributions of the odor compounds and causative microorganisms in the Huangpu River water basin.

Water samples of 500 mL for odor evaluation and analysis of odorants were collected in amber glass bottles without headspace and kept at 4°C before analysis. A one liter water sample was taken for algal analysis by adding Lugol iodine solution. The analysis for odorants was done within 3 days after sample collection and the analysis for algae was done within a week.

1.2 Chemicals and materials

The organic solvents of methanol and methylene dichloride of pesticide grade were purchased from Fisher (USA). Standard compounds including 2-MIB, geosmin, 2-isobutyl-3-methoxy pyrazine (IBMP), 2-isopropyl-3-methoxy pyrazine (IPMP) and trichloranisole (TCA) were products of Sigma-Aldrich Co., USA. Stock solution of 100 μg/L was prepared by diluting different standard solutions with ultra-pure water. NaCl and Na_2SO_4 of analytical grade were obtained from Beijing Chemicals Ltd., China and heated to 450°C for two hours before use.

1.3 Odor evaluation

Flavor profile analysis was used for odor evaluation. A detailed description of training and applications for the flavor profile analysis method can be found in the Standard Methods for Water and Wastewater (APHA et al., 1998). In this study, the panel was made up of at least four persons in each test. Seven-point scales of 1–12 were used to describe the intensity of samples.

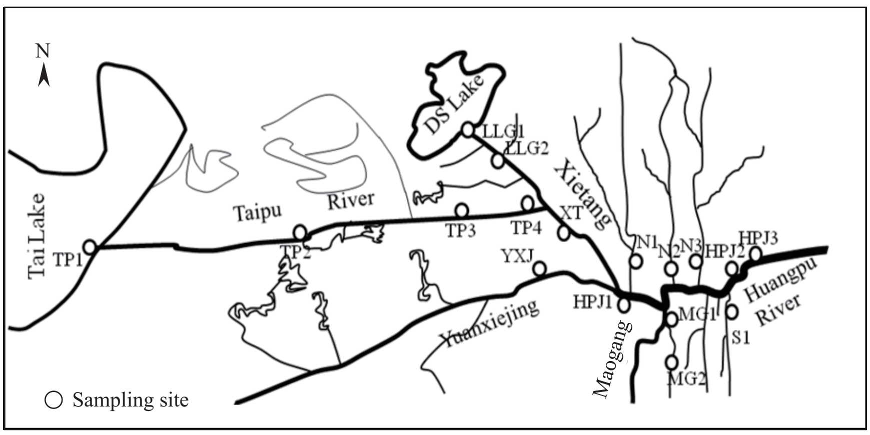

Fig. 1 Sampling sites in the Huangpu River watershed, Shanghai, China.

1.4 Extraction of odorants

The analytical scheme of solid phase microextraction (SPME) was applied as described in a previous study (Lin et al., 2002). A fiber coated with a 30/50-μm divinylbenzene/carboxen/polydimethylsiloxene film (No.57348, Supelco, USA) was chosen for SPME extraction. The fiber was injected through a silicon-Teflon coated septum and placed into the headspace of a 75 mL extraction vial containing 50 mL water sample. After adding 12.5 g NaCl to the samples, the vials were controlled at 65°C using a water-jacketed system. Extraction was performed for 30 min under mixing.

1.5 GC-MS analysis

The GC-MS analysis was performed on a GC-MS (HP 6890/5975, Agilent, USA) equipped with a 30 m long capillary column (HP-5, Agilent, USA) according to a previous study (Yu et al., 2009). The SPME fiber was introduced directly into injector for 3 min exposure time. The GC temperature program was as follow: 40°C for 3 min, raised to 240°C at 8°C/min and kept for 5 min. For qualitative analysis, the full scan mode from 50 to 400 m/z was used for the primary identification of odorous compounds according to NIST database, further confirmed by odorous compound standards. The SIM mode was used for the quantification of 2-MIB and geosmin, quantitative ions of which were 95 and 112 m/z, respectively. The detection limits were 2 ng/L for 2-MIB and 1.5 ng/L for geosmin.

1.6 Algal enumeration

Algal cell numbers were counted with a microscope (BX 51 Olympus, Japan) as described in a previous study (Li et al., 2010). A 1-mL aliquot was placed on the slide and algae were counted at 500 times magnification. The magnification of 1000 times with an oil immersion objective was used to identify algae to genera or to species according to common primary taxonomic reference (Palmer et al., 1977).

1.7 Cloning and phylogenetic analysis

All gene manipulation was done following the procedures described before (Oikawa and Ishibashi, 2004; Li et al., 2010). Here is the concise description of the procedures used by this article. Algae from 500 mL of water sample were harvested by membrane filtration with 0.22-μm-pore-size filters (GSWP, Millipore). DNA extraction included lysozyme, SDS-proteinase K and phenol-chloroform treatments. The standard 50 μL PCR mixture (Takara, Dalian, China) included 10 pmol primer; 100 ng total DNA; 1×Ex-Tap buffer; 1.5 mmol/L $MgCl_2$; 200 μmol/L dNTP; 1.25 units Takara rTap DNA polymerase. The PCR condition for the amplification of the cyanobacterial 16S rRNA gene using cyanobacteria specific primer (cya f-GGGGAATYTTCCGCAATGGG) was as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 sec and a final extension at 72°C for 15 min. The PCR product was purified with a QIAquick PCR cleanup kit (Qiagen, Inc., Chatsworth, USA) and cloned into the TOPO TA cloning vector PCR2.1, with TOP10 *Escherichia coli* transformants according to the manufacturer's instructions (Invitrogen, Shanghai, China). Cloned inserts were amplified from lysed colonies by PCR with plasmid vector-specific primers M13F and M13R under the same conditions as for the 16S rRNA gene listed above. Base sequences were determined using an ABI 3730 automated sequencer (Invitrogen, Shanghai, China). The sequences were submitted to Genbank. The sequences were subjected to BLAST analysis to obtain similar sequences with NCBI database, and then the phylogenetic tree was constructed using the MEGA 4. The 16S rRNA nucleotide sequence data from this study were deposited in the GenBank database under the accession numbers JQ814722-JQ814748.

2 Results and discussion

2.1 Characterization of odor and causative compounds in source water

Musty odor, together with septic/sewer, swimming pool, decaying vegetation and grassy odors, were identified during the flavor profile analysis evaluation, showing that there is a very complex mixture of flavor in the Huangpu River source water. **Figure 2** shows the variations of the musty odor strength and 2-MIB concentrations for the duration of a year. Both the musty odor and 2-MIB exhibited a similar variation trend, and a high musty odor strength (3.5–5.3) and 2-MIB concentration (28.6–71.0 ng/L) were observed in summer from July to September when the water temperature was high (25°C in average). During GC-MS analysis, only 2-MIB and geosmin were detected among the 5 common musty/earthy odor compounds including IPMP, IBMP and TCA. However, geosmin was constantly lower than 6 ng/L. Previous studies have also shown that relatively high levels of 2-MIB existed in the Huangpu River source water (Ma et al., 2008; Yu et al., 2009). Consequently we concluded that the main musty odor compound must be 2-MIB.

Dissolved and total 2-MIB were analyzed for some selected samples during the investigation. In the Huangpu River source water, 93.9% of the total 2-MIB was dissolved, suggesting that it will be difficult to remove 2-MIB through the removal of algae. Thus some special measures including powdered activated carbon dosing or ozonation are required to remove the odor from source water.

2.2 Characterization of 2-MIB producing cyanobacterium in source water

Diversified algal cellular aggregates (including 3 cyanobacteria, 11 diatoms, 6 chlorophytes, etc.) were observed under the microscope with the *Phormidium*/*Oscillatoria*-like filamentous one being dominant. The trichome of this filamentous cyanobacterium was 3–5 μm in diameter and was not constricted at the cross-walls. The cells were 1.0–2.5 μm long with granular contents and the apical cells were bluntly rounded, slightly tapered or conical. Some sheaths were very fine. The morphology of this cyanobacterium was similar to that of *Phormidium* Cal. Aq.0100 (Taylor et al., 2006).

16S rRNA gene analysis was performed to assist taxonomic identification as shown in **Fig. 3**. A total of 27 sequences amplified from a sample were obtained and the majority of sequences were affiliated with *Phormidium tergestinum* in the cyanobacteria phylum (16 of 27), followed by *Nitzsdria* in the diatom phylum (4 of 27), and some uncultured bacterium clones (6 of 27). Consequently, it is clear that *Phormidium* spp., which has been known for its ability to produce 2-MIB (Taylor et al., 2006), was the dominant cyanobacterium in the Huangpu River.

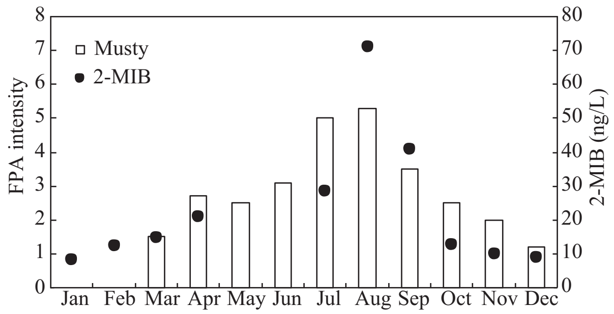

Fig. 2 Variations of musty odor and 2-MIB in source water in 2009.

Table 1 summarizes the cell counting results over a period from July to October, 2009. The cell density of algae was not so high, varying from < 400 cells/mL in October to a maximum of 5980 cells/mL in August, with *Phormidium* spp. as the dominant type (84%– 93%).

In late September, 2009, a sampling campaign was conducted over a wider range (a total of 5 sampling points including HPJ2, MG1, YXJ, LLG2 and TP4) to confirm the relationship between 2-MIB and *Phormidium* spp. cell density. These data, together with the data in **Table 1**, are shown in **Fig. 4**. The linear relationship between 2-MIB and *Phormidium* spp. cell density was observed, with a R^2 of 0.7189, further proving that 2-MIB was mainly produced by *Phormidium* spp. According to this relation-

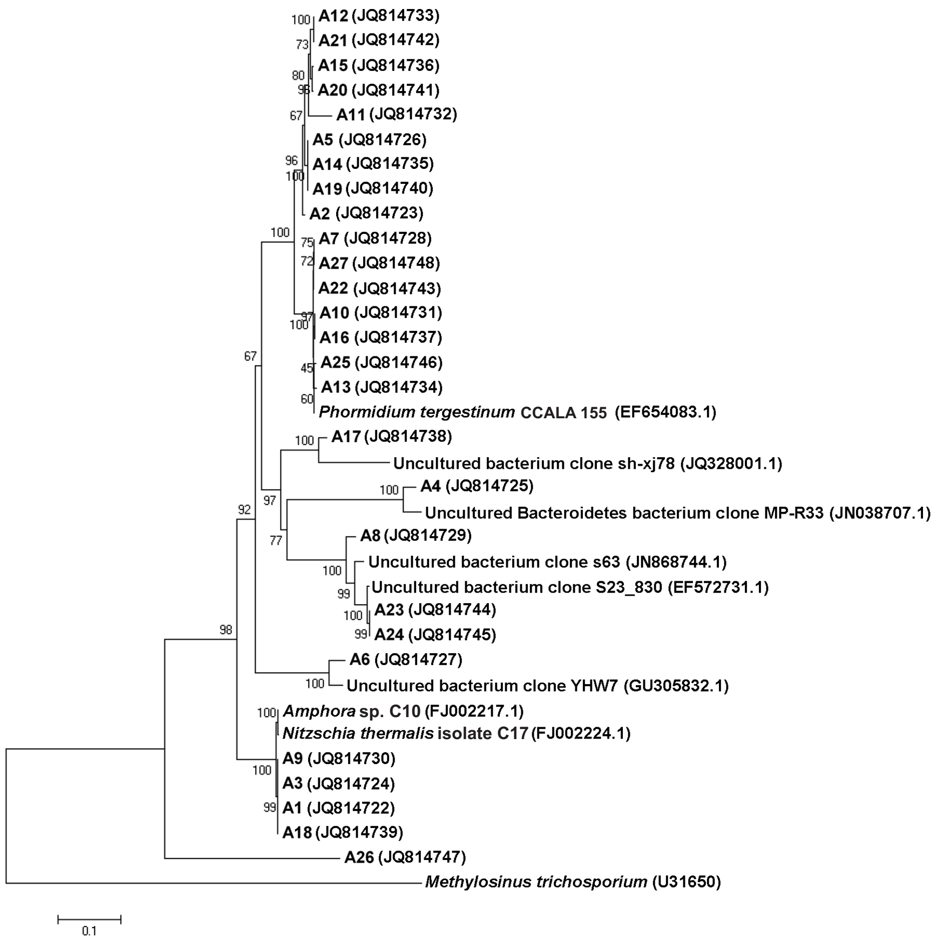

Fig. 3 Phylogenetic relationship between sample clones and related sequences based on cyanobacterium 16S rRNA gene sequences (neighbor-joining method).

Table 1 Abundance of algae in source water during the high temperature period

Sampling date	Abundance (cells/mL)		Percent of *Phormidium*
	Phormidium	Total algae	
09 Jul	1470	1590	92%
22 Jul	968	1150	84%
06 Aug	5470	5980	91%
08 Sep	843	909	93%
24 Sep	1034	1108	93%
13 Oct	320	378	85%

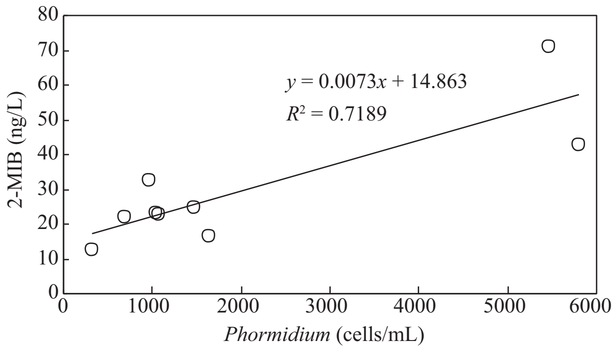

Fig. 4 Relationship between 2-MIB and *Phormidium* cell density.

ship, the 2-MIB yield was approximately 0.022 pg/cell, which was in accordance with previous studies (0.0025–0.01 pg/cell by *Phormidium* tenue and 0.25 pg/cell by *Phormidium* spp.) (Negoro et al., 1988; Li, 2009).

2.3 Distribution of *Phormidium* spp. and 2-MIB in the Huangpu River watershed

In order to identify the potential sources of *Phormidium* spp. and 2-MIB in the Huangpu River watershed, a sampling campaign was conducted across various tributaries in a wide area in September, 2010.

Figure 5 shows the concentration profiles of 2-MIB and *Phormidium* spp. in the watershed of the river. Although the sample from the TP1 section, which was close to Tai Lake (**Fig. 1**), exhibited a relatively high level of 2-MIB (53.2 ng/L) and *Phormidium* spp. (3590 cells/mL), their levels above the HPJ1 section (including the HPJ1 section) were quite low (6.5–25.0 ng/L and 65–1302 cells/mL, respectively). Consequently the tributaries above the HPJ1 section contributed a negligible amount of 2-MIB and *Phormidium* spp. to the Huangpu River source water. On the other hand, S1 belonging to the creek that was closest to the HPJ2 intake exhibited the highest 2-MIB and *Phormidium* spp. levels (92.5 ng/L and 14608 cells/mL), followed by MG2 (85.0 ng/L and 7257 cells/mL), N2 (73.9 ng/L and 5737 cells/mL), N3 (71.7 ng/L and 2623 cells/mL) and MG1 (62.7 ng/L and 6301 cells/mL). All of the 5 samples exhibited a 2-MIB level significantly higher than that detected at HPJ2, suggesting that these creeks may contribute most of the 2-MIB to the Huangpu River source water. Compared to other tributaries, the four creeks

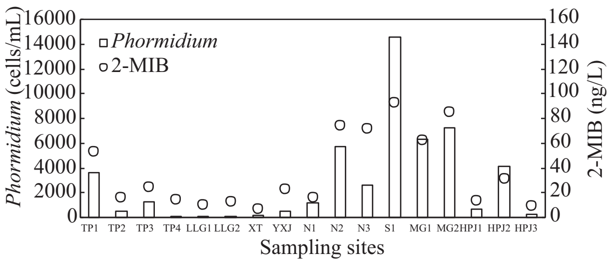

Fig. 5 2-MIB and *Phormidium* profiles in the Huangpu River watershed. HPJ2- intake; MG1, G2-Maogang section; N2, N3, S1-three creeks close to the intake.

were usually very shallow (water depth in 1.5–3.0 metre) and the flow rate was quite low. Meanwhile, they flowed through rapidly developing region and were influenced by anthropic activities, such as animal breeding, agriculture and urban effluent discharges (TOC in 5.6–7.5 mg/L). Such hydrological and trophic conditions may have favored the growth of odor-causing algae in these creeks. Though further confirmation is necessary, this study showed for the first time that 2-MIB produced mainly by *Phormidium* spp. may largely be contributed by water from the 4 creeks close to the intake. Consequently it will be possible to control the musty odor problems effectively either by reducing the 2-MIB level in these creeks or by limiting the flow of water from them during the high temperature seasons.

3 Conclusions

For the first time, this study revealed that 2-MIB, mainly produced by *Phormidium* spp. in some shallow tributaries close to the intake of source water, is the major causative compound contributing to the musty odor in Huangpu River. The levels of 2-MIB were higher during the summer season with a peak in August. *Phormidium* spp. in the Huangpu River has a 2-MIB yield 0.022 pg/cell. It may be possible to control the musty odor problems effectively either by reducing the 2-MIB level in these creeks or by limiting the flow of water from them during the high temperature seasons.

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