



# A systematic study on spatial and seasonal patterns of eight taste and odor compounds with relation to various biotic and abiotic parameters in Gonghu Bay of Lake Taihu, China

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## ARTICLE INFO

### Article history:

Received 28 June 2010

Received in revised form 5 October 2010

Accepted 7 October 2010

Available online 5 November 2010

### Keywords:

Taste and odor compounds

Purge-and-trap

GC-MS

Cyanobacterial blooms

Lake Taihu

## ABSTRACT

A systematic study was conducted on seasonal and spatial patterns of taste and odor (T&O) compounds with relation to biotic and abiotic parameters at fifteen sites in Gonghu Bay of Lake Taihu in 2008. We developed a sensitive and automated method to simultaneously analyze eight T&O compounds (boiling points ranging from 38 °C to 239 °C) by using Purge-and-Trap (P&T) coupled with GC/MS. Maximum particulate dimethyl trisulfide (DMTS, 69.6 ng/L) exceeded its odor threshold concentrations (OTC, 10 ng/L) and maximum dissolved DMTS was 6.1 ng/L, but still far below concentration in the drinking water pollution incident of Wuxi City in 2007 when DMTS reached 1768–11,399 ng/L. Geosmin (GEO), 2-methylisoborneol (MIB),  $\beta$ -cyclocitral,  $\beta$ -ionone and 2-isobutyl-3-methoxy-pyrazine (IBMP) occasionally or frequently exceeded their OTCs, whereas 2-isopropyl-3-methoxy-pyrazine (IPMP) and dimethyl sulfide (DMS) did not. We found for the first time significant correlations between particulate  $\beta$ -cyclocitral and  $\beta$ -ionone concentrations and intracellular and extracellular microcystin concentrations. Spatially, Nanquan Waterworks faced more risk by T&O contamination than Xidong Waterworks. High concentrations of  $\text{NO}_3\text{-N}$ , TDN and TN could be risky signs of taste and odor events by DMS, DMTS, IPMP, IBMP and GEO.

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## 1. Introduction

In the past decades, increasing eutrophication has led to frequent outbreaks of cyanobacterial blooms in many lakes around the world. Many cyanobacteria produce metabolites, such as biotoxins and/or taste and odor (T&O) compounds, which impact water supplies (Carmichael, 1997; Ibelings and Havens, 2008; Peter et al., 2009; Watson, 2003). Some of these compounds are water soluble and nonvolatile and have no odor, and are thus virtually undetectable by the consumer (Codd, 1995). Among which cyclic heptapeptide toxins microcystins (MCs) are one of the most common and well studied species (Chen et al., 2009a,b; Chorus and Bartram, 1999). On the other hand, effects of T&O compounds on human health are still unclear, research in this area might be considered less of a priority (Watson, 2003).

Recently, widespread T&O incidents in aquatic environments have caused great economic losses to the aquaculture industry, consumer complaints about drinking water and increase in cost of water treatment. Consequently increasing attention has been drawn to T&O compounds. Among the eight odor groups described in the water flavor wheel, the earthy-musty odors are of particular interest because they are

unpalatable and often encountered in water (Suffet et al., 1996). Over the past years, many studies have focused on the well-known earthy-musty algal metabolites trans-1,10-dimethyl-trans-9-decalol (geosmin, GEO) and 2-methylisoborneol (MIB) (Durrer et al., 1999; Dzialowski et al., 2009; Huang et al., 2007; Lin et al., 2002; Suffet et al., 1996; Sugiura et al., 1998; Watson et al., 2000; Westerhoff et al., 2005), whereas there are only a few studies on other cyanobacterial metabolites such as  $\beta$ -cyclocitral,  $\beta$ -ionone, 2-isopropyl-3-methoxy-pyrazine (IPMP) and 2-isobutyl-3-methoxy-pyrazine (IBMP) (Li et al., 2007; Peter et al., 2009; Xu et al., 1999). Previous studies have mainly focused on the production of T&O compounds by isolated phytoplankton species or on the natural occurrence of selected T&O compounds, while the triggers, mode and dynamics of these cyanobacterial metabolites are still unclear (Peter et al., 2009; Watson, 2003).

There are several reports on the occurrence of sulfur compounds in natural waters. Ginzburg et al. (1998) revealed the emissions of dimethyl sulfide, dimethyl disulfide and dimethyltrisulfide from *Peridinium gatunense*, and Jüttner (1984) reported high concentrations of dimethyl sulfide and dimethyl disulfide in a eutrophic pond and attributed this to the metabolism of associated bacteria during the decay of *Spirogyra* and *Oedogonium* mats. However, little is known about the possible relation between sulfur compounds and toxic cyanobacterial blooms.

Taste and odors in Taihu Lake, the third largest freshwater lake in China, have triggered great public concerns since a serious drinking

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water pollution incident happened in the summer of 2007 when a dense cyanobacterial bloom occurred (Yang et al., 2008). The offensive taste and odors were present in the source water for several days because of an intrusion of a distinct black water “agglomerate” of unknown origin into the main water intake of the Nanquan Waterworks in Wuxi City, and stopped drinking water supply of over 2 million citizens with a serious social consequence. Dimethyl trisulfide and related alkyl sulfide compounds, but not MIB and GEO, were the key odor-causing compounds in this incident (Yang et al., 2008), however, the source of these T&O compounds remains unknown. So far, little has been known about the seasonal dynamics of T&O compounds and their possible relation with biotic (e.g., bloom-forming cyanobacteria) and abiotic factors in freshwater lakes, especially of the subtropical China.

Mainly for these reasons, fifteen sampling sites (Fig. 1) were selected in Lake Taihu along the northern shore of Gonghu Bay where source water (Nanquan Waterworks and Xidong Waterworks) are located, and seasonal and spatial dynamics of T&O and MC concentrations, physicochemical parameters and biomass of major phytoplankton groups were monitored monthly during Jan to Dec 2008. The main objectives of this study were: 1) to develop a reliable, simple and sensitive method to simultaneously analyze eight volatile and semi-volatile T&O compounds (with boiling points ranging from 38 °C to 239 °C) in a relatively short time, while at the same time permitting quantitative analyses in trace concentration levels, 2) to examine seasonal dynamics of the eight T&O compounds in both dissolved and particulate forms for establishing relationships between T&O compounds and physico-chemical and biological parameters with discussion on the possible mechanisms for production of T&O compounds in the lake. These may add information for understanding the drinking water malodor incident of Wuxi City in 2007, and may also be useful for future prevention of T&O pollution incident.

## 2. Materials and methods

### 2.1. Chemicals

The standards of the eight target analytes (DMS (dimethyl sulfide), DMTS (dimethyl trisulfide), IPMP, MIB, IBMP,  $\beta$ -cyclocitral, GEO and  $\beta$ -ionone) were purchased from Sigma-Aldrich (Milwaukee, WI, USA) in the highest purity available. Stock solutions (from 10 to 100  $\mu\text{g L}^{-1}$ )

were prepared in MilliQ-water. Sodium chloride, which was applied to enhance the extraction of the T&O compounds, was reagent grade and used without further purification. MC standards (microcystin-RR, -YR, -LR) were obtained from Wako Pure Chemical Industries, Japan. Other reagents were all analytical reagent grade.

### 2.2. Study site and sample collection

Lake Taihu (30°56′–31°33′N, 119°54′–120°36′E), the third largest freshwater lake, is located in the east part of China. This area is of historical importance in trade, politics, agriculture, and culture. About 35 million people inhabit the 36,500 km<sup>2</sup> watershed of Taihu Lake. Gonghu Bay (31°31′0″N, 120°16′35″E) is located at the northeast portion of Taihu Lake, serves as the main drinking water sources of Wuxi city. It suffers a wind-driven dense surface accumulation of toxic *Microcystis* bloom in warm seasons of recent years and the toxic *Microcystis aeruginosa* was the absolute dominant species in the water column of Gonghu Bay during the bloom period (Liu et al., 2008; Xie, 2008). During January to December 2008, fifteen sampling sites were selected along the northern shore of Gonghu Bay (Fig. 1). Site 3 was nearby the Nanquan Waterworks, Site 4 was located in reed marsh, Site 13 was located near the Xidong Waterworks, and Site 15 was nearby the Wangyu River. The sampling sites were defined by a global positioning system (GPS) every month.

Water samples were collected monthly from the fifteen sites of Gonghu Bay with Tygon tubing fitted with a one-way valve during the study period. Each integrated water sample was a mixture of two subsamples: one from 0.5 m below the surface and one from 0.5 m above the bottom. Duplicate water samples were collected in 1 L narrow-necked PE bottles, leaving no headspace, stored immediately in a portable refrigerator (around 4 °C) and then transported to the laboratory. After arrival at the laboratory, all samples for off-flavor analysis were stored at –20 °C (for no more than 5 days) until analyzed.

### 2.3. Water quality parameters, phytoplankton biomass and MC contents analysis

Values of water temperature, pH, dissolved oxygen and transparency were obtained in situ. Water temperature (WT) was measured by a WMY-01 digital thermometer (Medicinal Instrument, Shanghai,

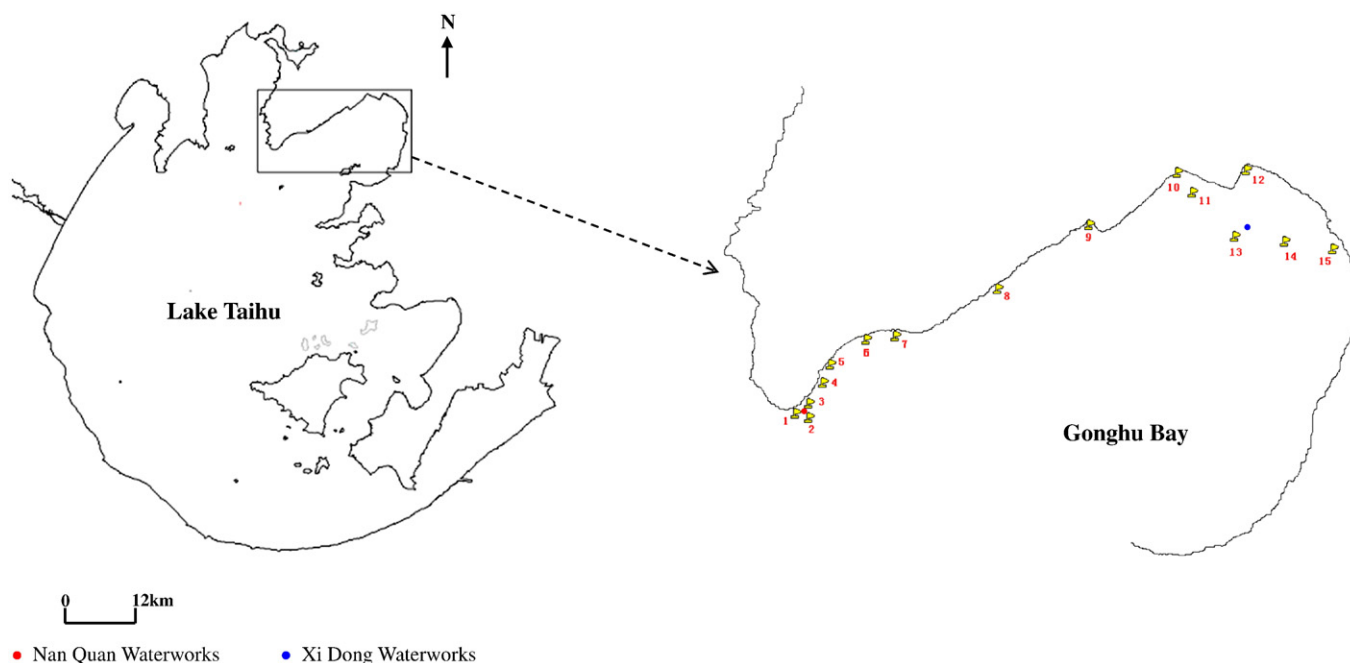


Fig. 1. Schematic diagram of the sampling sites in Gonghu Bay, Lake Taihu.

China). pH was determined with PHB-4PH metre (Leici Instrument, Shanghai, China). Dissolved oxygen (DO) was measured by a JPB-607 DO metre (Leici Instrument). Transparency was measured by a Secchi disk.

In the laboratory, orthophosphate ( $\text{PO}_4\text{-P}$ ) was analyzed by the ammonium molybdate method. Total phosphorus (TP) and total dissolved phosphorus (TDP) were measured by colourimetry after digestion with  $\text{H}_2\text{SO}_4$  (Ebina et al., 1983). Total nitrogen (TN) and total dissolved nitrogen (TDN) were determined by the Kjeldahl method. Nitrate ( $\text{NO}_3\text{-N}$ ) was analyzed using the automated Korolev/Cadmium reduction method. Ammonium ( $\text{NH}_4\text{-N}$ ) was determined by the Nessler method, and nitrite ( $\text{NO}_2\text{-N}$ ) by the  $\alpha$ -naphthylamine method (APHA, 1992). Chemical oxygen demand (COD) was measured by titrating with sodium thiosulphate (Baumann, 1974). Chlorophyll *a* (Chl-*a*) was determined spectrophotometrically (Lorenzen, 1967) after filtration on Whatman GF-C glass filters and 24 h extraction in 90% acetone. Phytoplankton samples were fixed in acetic Lugol's solution and preserved with 5% formalin after sedimentation for 48 h. For determination of phytoplankton, the supernatant was removed and approximately 50 mL residue was collected. After complete mixing, 0.1 mL of concentrated sample was counted directly in a 0.1 mL counting chamber using a Nikon microscope at a magnification of  $\times 400$ . Phytoplankton species were identified according to Hu et al. (1979). The count, including the colonial forms, was carried out by enumerating single cells. Colonial *Microcystis* cells were separated using an ultrasonic crusher, JY88-II (Scientiz, Ningbo, China). Dominant algal species were defined as those together representing at least 80% of total phytoplankton density and with a minimum individual contribution  $>5\%$  of total algal density. They were sorted into their main taxonomic groups and into the functional groups proposed by Reynolds et al. (2002).

To estimate MCs concentration in the lake water (intracellular and extracellular toxins), 1 L of the integrated water sample was filtered through a Whatman glass-fiber filter ([GF/C], Whatman, Brentford, UK). Seston onto the glass-fiber filter and the filtrate were used to detect intracellular and extracellular toxins, respectively. And then analyzed quantitatively by electrospray ionization (ESI) liquid chromatography–mass spectrometry (LC/MS) according to the method described by Chen et al. (2007).

Table 1 summarizes some characteristics of above parameters in Gonghu Bay, Lake Taihu during the study period.

#### 2.4. Sample preparation for dissolved and particulate T&O compounds

A 300 mL water sample was filtered through a Whatman GF/C glass fibre filter under a low vacuum. The filtrate was analysed for dissolved T&O compounds in the water, and the material trapped on the filter was tested for particulate T&O compounds.

The filtrate with the dissolved fraction was transferred into 40 mL standard sample bottle (O.I. Corporation, USA) with teflon-fitted caps leaving no headspace and kept sealed at 4 °C immediately (to minimize photosynthetic/microbial activity and volatilization of the analytes) and analysed within 24 h.

The filter residue with the particulate fraction was repeated freezing and thawing and then the mash was rinsed into a 250 mL round-bottomed flask with 100 mL of MilliQ-water, sodium chloride were added to give a final concentration of 20% (w/v) along with a micro stirrer. Extraction was performed with APEX Ordinary Pressure Microwave-assisted Extraction System (PreeKem Scientific Instruments Co., Ltd.). The temperature was set at 80 °C and the nitrogen (carrier gas) flow was set at 80–100 mL/min. The microwave was activated for 15 min, during which time distillates formed from the re-suspended residue migrated to the condenser and cooled by liquid nitrogen to avoid thermal decomposition and then dissolved with 50 mL MilliQ-water. The elution was also transferred to a 40 mL standard sample bottle with teflon-fitted caps, stored at 4 °C

**Table 1**

Physicochemical parameters, biomass of major phytoplankton groups and microcystin concentrations in Gonghu Bay during the study period.

Parameters	Range	Mean
Water temperature (°C)	0.49–30.81	14.83
Transparency (cm)	14.20–78.07	41.19
Water depth (m)	1.71–2.27	1.97
DO (mg/L)	0.04–11.03	7.72
pH	7.88–9.09	8.35
COD (mg/L)	3.69–7.20	5.68
Chl- <i>a</i> (mg/L)	0.003–0.06	0.03
TN (mg/L)	1.21–3.47	2.28
TDN (mg/L)	0.42–3.17	1.68
$\text{NO}_3\text{-N}$ (mg/L)	0.12–2.57	1.03
$\text{NH}_4\text{-N}$ (mg/L)	0.18–1.13	0.45
$\text{NO}_2\text{-N}$ (mg/L)	0.004–0.20	0.04
TP (mg/L)	0.06–0.20	0.15
TDP (mg/L)	0.02–0.10	0.04
$\text{PO}_4\text{-P}$ (mg/L)	0.01–0.03	0.02
Cyanophyta (mg/L)	0.00–129.65	14.15
<i>Microcystis</i> biomass (mg/L)	0.00–128.71	14.05
Bacillariophyta (mg/L)	0.00–17.71	1.20
Cryptophyta (mg/L)	0.00–2.96	0.37
Chlorophyta (mg/L)	0.00–1.53	0.13
Pyrrhophyta (mg/L)	0.00–1.92	0.08
Euglenophyta (mg/L)	0.00–0.48	0.02
Chrysophyta (mg/L)	0.00–0.85	0.01
Intracellular MC ( $\mu\text{g/L}$ )	0.00–35.42	2.59
Extracellular MC ( $\mu\text{g/L}$ )	0.00–0.46	0.04

immediately and analysed within 24 h. All the samples prepared were subjected to Purge-and-Trap (P&T) coupled with GC/MS for quantitative determination.

#### 2.5. Instrumentation and determination

The Eclipse 4660 Purge-and-Trap Sample Concentrator combined with 4551-A autosampler (O.I. Corporation, USA) was coupled to gas chromatography/mass spectrometry (GC/MS) for the direct analysis of eight kinds of target T&O compounds from lake water.

Optimized experimental parameters of P&T including trap (#10, Tenax/silica gel/carbon molecular sieve), injection volume of 25 mL, purge tube temperature of 45 °C, purge time of 11 min, purge flow rate of 45 mL/min, desorb preheat temperature of 185 °C and desorb temperature of 190 °C (2 min) were chosen.

Separation and qualitative analysis was done with GCMS-QP2010 plus system (Shimadzu, Japan) using DB-17MS column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ , Agilent, USA), and helium as carrier gas (1 mL/min) and split ratio of 10:1 was chosen. The GC was programmed from 40 °C (constant temperature for 2 min) to 100 °C (30 °C/min) and to 135 °C (5 °C/min) and finally to 250 °C (30 °C/min, hold constant for 5 min). For the selected ion monitoring (SIM) mode,  $m/z$  62 and 47 for DMS,  $m/z$  126, 79 and 111 for DMTS,  $m/z$  137, 152 and 124 for IPMP,  $m/z$  95, 108 and 135 for MIB,  $m/z$  124, 94 and 151 for IBMP,  $m/z$  137, 152 and 123 for  $\beta$ -cyclocitral,  $m/z$  112, 125 and 149 for geosmin, plus  $m/z$  177, 92 and 135 for  $\beta$ -ionone were monitored (Table 2). Quantities of each analyte were determined by comparison to the corresponding standard curve respectively.

#### 2.6. Statistical analysis

Pearson correlation analysis was conducted to determine the relationship between T&O compounds (dissolved and particulate fraction) concentrations to MC contents, physicochemical parameters, and biomass of major phytoplankton group using SPSS for Windows (Ver 13.0; SPSS, Chicago, IL, USA). The relationships were considered significant at  $P < 0.05$ .

**Table 2**

Features for the identification of each analyte and precision, detection limit and linearity for each analyte achieved with developed method.

VOC name	Retention time (min)	<i>m/z</i> value selected	Relative standard deviation <sup>b</sup> (%)	Detection limit <sup>c</sup> (ng/L)	Linearity <sup>d</sup>
Methyl sulfide (DMS)	1.94	62 <sup>a</sup> , 47	3.1	0.9 (0.3–1 × 10 <sup>3</sup> )	0.9986
Dimethyl trisulfide (DMTS)	6.63	126 <sup>a</sup> , 79, 111	4.9	0.8 (10)	0.9982
2-isopropyl-3-methoxypyrazine (IPMP)	7.66	137 <sup>a</sup> , 152, 124	6.7	1.0 (20)	0.9999
2-methylisoborneol (MIB)	8.91	95 <sup>a</sup> , 108, 135	7.3	0.6 (15)	0.9996
2-isobutyl-3-methoxypyrazine (IBMP)	9.15	124 <sup>a</sup> , 94, 151	3.0	0.8 (2–16)	0.9996
β-cyclocitral	10.41	137 <sup>a</sup> , 152, 123	7.6	1.1 (0.5–19.3 × 10 <sup>3</sup> )	0.9997
Geosmin (GEO)	12.86	112 <sup>a</sup> , 125, 149	2.6	0.3 (4)	0.9996
β-ionone	13.59	177 <sup>a</sup> , 92, 135	3.2	1.0 (7)	0.9979

Note: OTC values from Malleviale and Suffet (1987), Young et al. (1996) and Watson and Ridal (2002).

<sup>a</sup> Quantitative ion.<sup>b</sup> Precision (repeatability): relative standard deviation of the six-fold analysis of 5 ng L<sup>-1</sup> samples.<sup>c</sup> Detection limit: signal to noise of 3, and aquatic odor threshold concentrations (OTC; ng/L) are included in brackets.<sup>d</sup> Linearity: eight concentration levels from 1.0 to 500 ng L<sup>-1</sup>.

### 3. Results and discussion

#### 3.1. Method validation

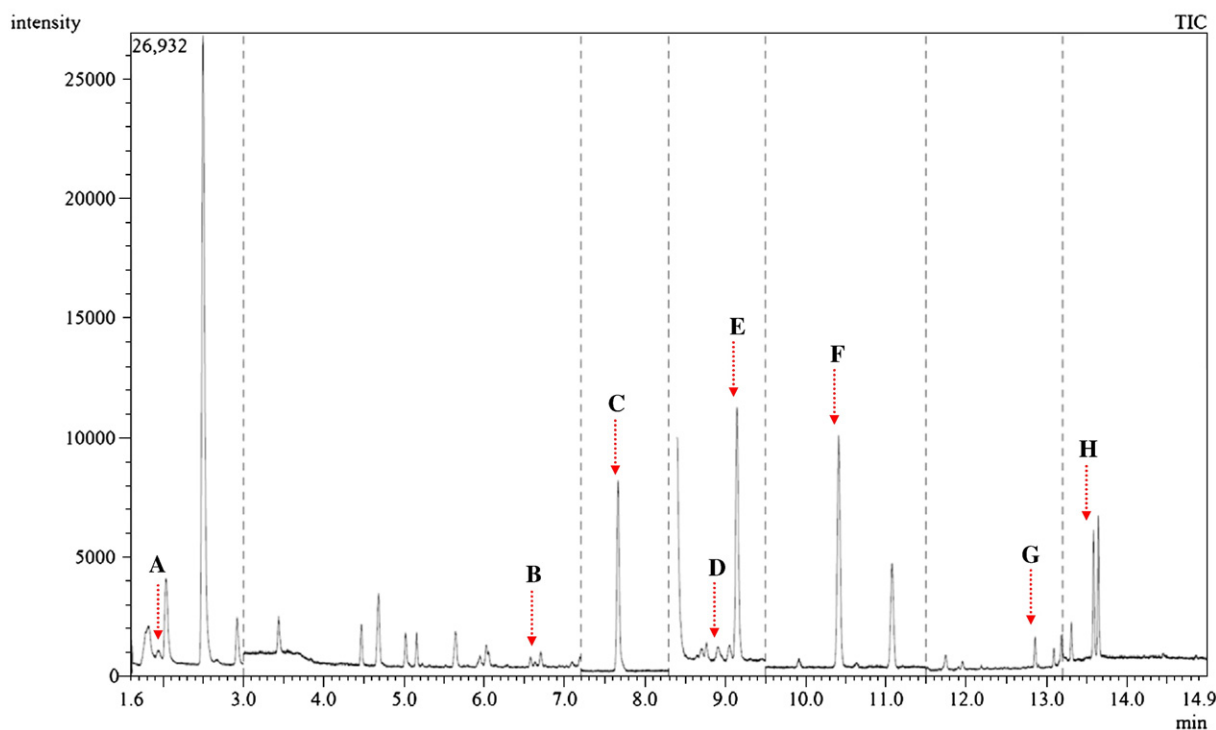
Due to the very low odor thresholds of some T&O compounds (low ppt-range), the sensitivity of routine analytical systems is not always sufficiently adequate to measure them in the raw surface water, which are likely to be missed in the analysis when their weak signals are overlapped by the background of the complicated matrix. Furthermore, currently reported sample preparations often include numerous steps and the handling of samples, while each step may introduce errors, possible interferences and loss of analytes.

In order to simultaneously analyze the eight volatile and semi-volatile T&O compounds (boiling points ranging from 38 °C to 239 °C) at their concentration levels of the water samples, P&T concentration

followed by GC/MS method was developed. The relevant analytical parameters for the quantitative P&T-GC/MS analysis were validated with external standards of the identified T&O compounds as described in Table 2. The method was applied to raw surface water collected from Gonghu Bay, Lake Taihu (Fig. 2) and was found to be rapid, highly-automatized, reproducible and sensitive enough to detect the compounds at a nanogram per liter concentration level or beneath their threshold odors, requiring only a small sample and a small amount of organic solvents.

It should be noted, however, that as the eight target T&O compounds simultaneously determined here have quite different volatility and polarity, our efforts of optimizing experimental parameters mentioned above were to yield chromatograms with “balanced” peak areas when all eight analytes were present at a similar concentration level.

#### a) MS-chromatogram (total ion current of the MS in the single ion mode) of the water sample

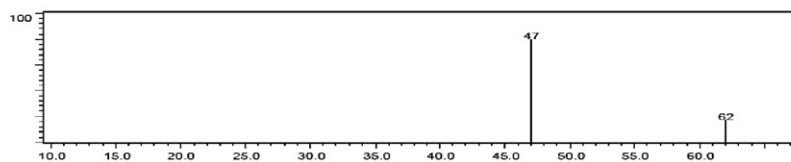


**Fig. 2.** MS-chromatogram (total ion current of the MS in the single ion mode) and mass spectra of the eight targets compounds of a water sample analyzed with P&T-GC/MS (collected from Gonghu Bay, Lake Taihu, May 2008, site 3, particle-bound fraction). Shown are: A) methyl sulfide, B) dimethyl trisulfide, C) 2-isopropyl-3-methoxypyrazine, D) 2-methylisoborneol, E) 2-isobutyl-3-methoxypyrazine, F) β-cyclocitral, G) geosmin, H) β-ionone.

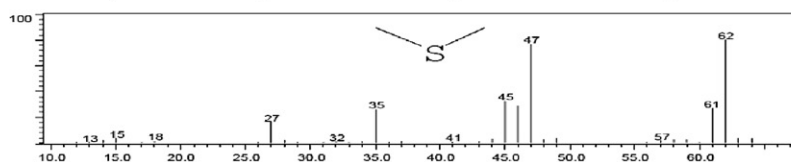


## b) Mass spectra of the eight targets compounds of the water sample.

Line#:1 R.Time:1.941(Scan#:410) MassPeaks:2 RawMode:Averaged 1.940-1.942(409-411) BasePeak:47.00(223) BG Mode:Calc. from Peak Group 1 - Event 1

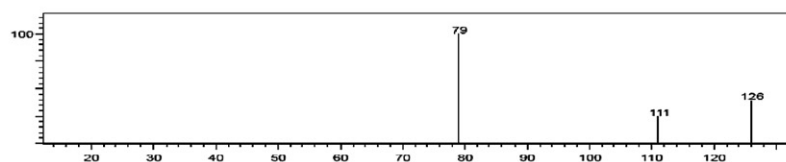


Hit#:8 Entry:158 Library:NIST05.LIB SI:75 Formula:C2H6S CAS:75-18-3 MolWeight:62 RefIndex:471 CompName:Dimethyl sulfide \$\$ Methane, thiois- \$\$ Methyl sulfide \$\$ Dimethyl monosulfide \$\$ Dimethyl thioether \$\$ DMS \$\$ Methyl Monosulfid

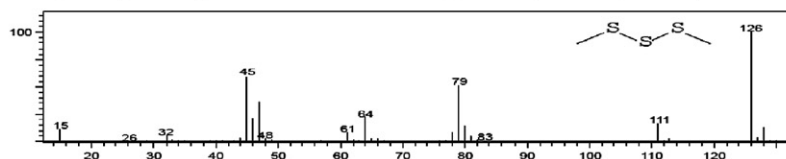


A)

Line#:2 R.Time:6.627(Scan#:6033) MassPeaks:2 RawMode:Averaged 6.626-6.628(6032-6034) BasePeak:79.00(95) BG Mode:Calc. from Peak Group 2 - Event 1

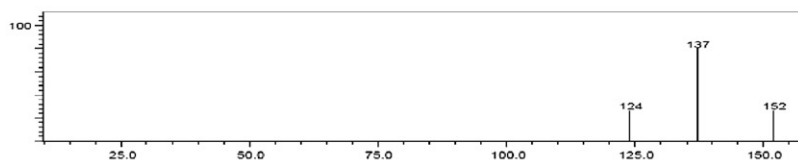


Hit#:1 Entry:6138 Library:NIST05.LIB SI:95 Formula:C2H6S3 CAS:3658-80-8 MolWeight:126 RefIndex:972 CompName:Dimethyl trisulfide \$\$ Trisulfide, dimethyl \$\$ Methyl trisulfide \$\$ CH3SSSCH3 \$\$ 2,3,4-Trithiapentane \$\$ 1,3-Dimethyltrisulfane # \$\$

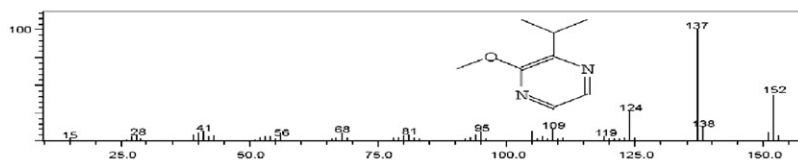


B)

Line#:3 R.Time:7.664(Scan#:7278) MassPeaks:3 RawMode:Averaged 7.663-7.665(7277-7279) BasePeak:137.00(4775) BG Mode:Calc. from Peak Group 3 - Event 1

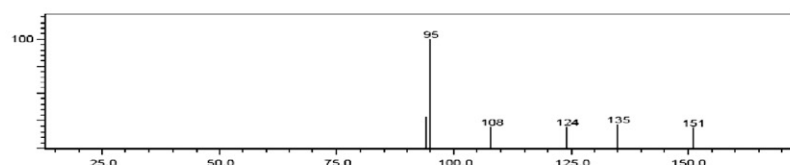


Hit#:2 Entry:15598 Library:NIST05.LIB SI:91 Formula:C8H12N2O CAS:25773-40-4 MolWeight:152 RefIndex:1105 CompName:Pyrazine, 2-methoxy-3-(1-methylethyl)- \$\$ 2-Isopropyl-3-methoxy pyrazine \$\$ 2-Methoxy-3-isopropylpyrazine \$\$

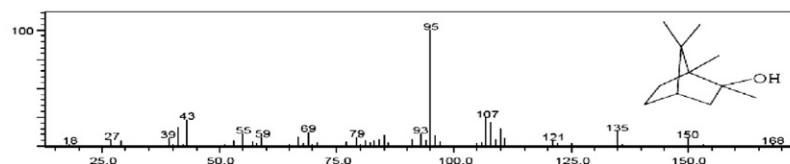


C)

Line#:4 R.Time:8.905(Scan#:8344) MassPeaks:4 RawMode:Averaged 8.903-8.907(8343-8345) BasePeak:95.00(517) BG Mode:Calc. from Peak Group 4 - Event 1



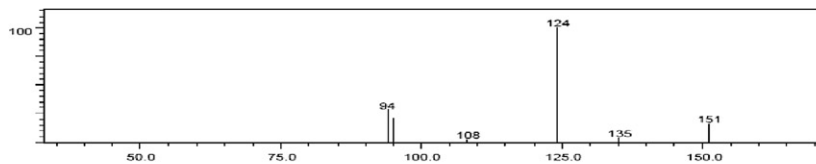
Hit#:1 Entry:23768 Library:NIST05.LIB SI:77 Formula:C11H20O CAS:2371-42-8 MolWeight:168 RefIndex:1161 CompName:2-Methylisborneol



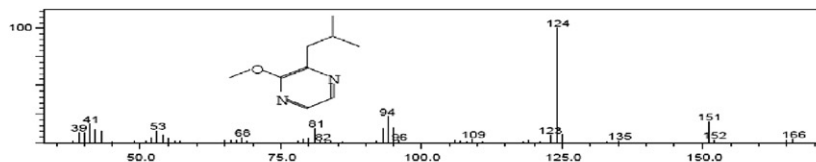
D)

Fig. 2 (continued).

Line#:5 R. Time:9.145(Scan#:8488) MassPeaks:6 RawMode:Averaged 9.143-9.147(8487-8489) BasePeak:124.00(6431) BG Mode:Calc. from Peak Group 4 - Event 1

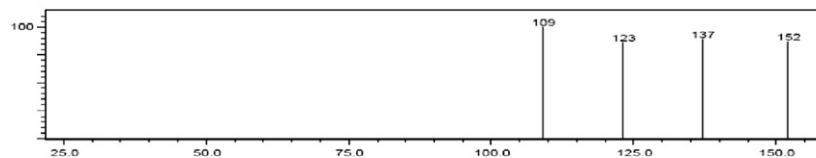


Hit#:1 Entry:22410 Library:NIST05.LIB SI:89 Formula:C9H14N2O CAS:24683-00-9 MolWeight:166 RetIndex:1204 CompName:Pyrazine, 2-methoxy-3-(2-methylpropyl)- \$\$ 2-Isobutyl-3-methoxypyrazine \$\$ 2-Methoxy-3-isobutylpyrazine \$\$

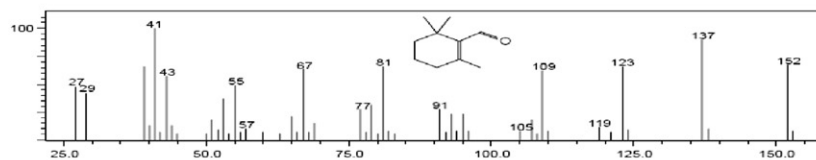


E)

Line#:6 R. Time:10.413(Scan#:9796) MassPeaks:4 RawMode:Averaged 10.412-10.413(9795-9797) BasePeak:109.00(2681) BG Mode:Calc. from Peak Group 5 - Event 1

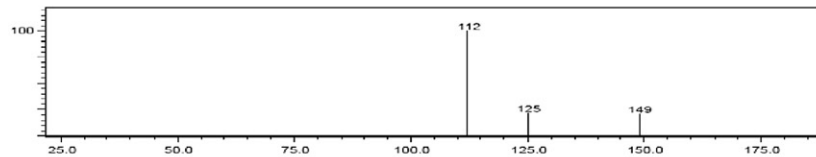


Hit#:1 Entry:15787 Library:NIST05.LIB SI:88 Formula:C10H16O CAS:432-25-7 MolWeight:152 RetIndex:1204 CompName:1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl- \$\$ .beta.-Cyclocitral \$\$ 1-Formyl-2,6,6-trimethyl-1-cyclohexene \$\$ 2,6,6-Trimethyl-1

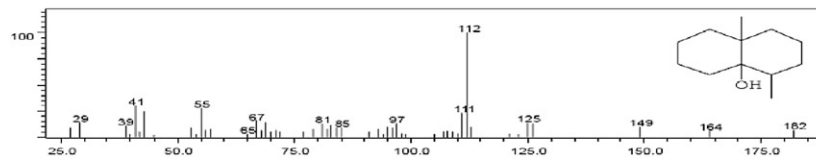


F)

Line#:7 R. Time:12.860(Scan#:1690) MassPeaks:90 RawMode:Averaged 15.667-15.683(1689-1691) BasePeak:112.10(5030) BG Mode:Calc. from Peak Group 1 - Event 1

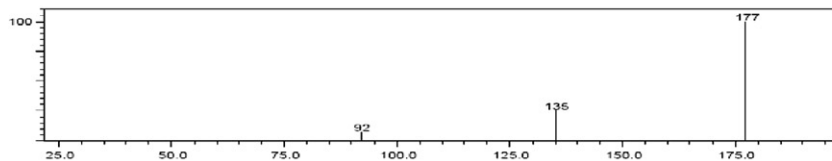


Hit#:1 Entry:31289 Library:NIST05.LIB SI:87 Formula:C12H22O CAS:19700-21-1 MolWeight:182 RetIndex:1386 CompName:4a(2H)-Naphthalenol, octahydro-4,8a-dimethyl-,(4.alpha.,4a.alpha.,8a.beta.)- \$\$ 4a(2H)-Naphthalenol, octahydro-4,8a-dimethyl-, [4S-(4a

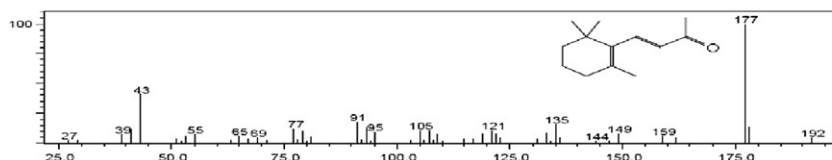


G)

Line#:8 R. Time:13.587(Scan#:1837) MassPeaks:109 RawMode:Averaged 16.892-16.908(1836-1838) BasePeak:177.10(47045) BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:36566 Library:NIST05.LIB SI:95 Formula:C13H20O CAS:14901-07-6 MolWeight:192 RetIndex:1457 CompName:3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- \$\$ .beta.-Ionone \$\$ 4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one \$\$ .beta.-Cy



H)

Fig. 2 (continued).

### 3.2. Temporal and spatial dynamics of the dissolved and particulate T&O compounds

#### 3.2.1. DMS and DMSTs

The monthly variations of DMS and DMST concentrations in the dissolved and particulate samples are shown in supplementary data file (Fig. S1A). Measurable levels of DMS were detected in dissolved and particulate fractions during most sampling period (except January, July and October). A sudden increase of dissolved and particulate DMS was observed from March to April, and then gradually decreased, whereas the maximum particulate DMS concentration was recorded in September. However, the peak concentrations (42.47 ng/L in dissolved and 201.18 ng/L in particulate fractions) and annual mean concentrations (6.46 ng/L in dissolved and 25.71 ng/L in particulate fractions) were both far below its odor threshold concentrations (OTC,  $0.3\text{--}1.0 \times 10^3$  ng/L). DMSTs were found infrequently. In fact, this compound was observed only in March, September and December with very low concentration. There were two distinct peaks of particulate DMST concentrations (16.87 ng/L in March and 69.55 ng/L in September, respectively), both exceeding its OTC (10 ng/L), whereas dissolved DMST concentrations reached only 6.13 ng/L (in March) and 0.20 ng/L (in December), both below its OTC value. Annual mean concentration of DMSTs was 0.53 ng/L in dissolved fraction, and 7.20 ng/L in particulate fraction (Table 3).

During the taste and odor event of Lake Taihu in 2007, ammonium concentration increased suddenly from 0.23 to 0.97 mg/L on 28 May to 4.0 mg/L on 31 May, and then decreased gradually. Samples collected on 4 June 2007 from both the drinking-water intake and the water agglomerate were detected, and DMST concentrations in the two samples reached as high as 11,399 and 1768 ng/L, respectively (Yang et al., 2008). The author deemed that dimethyl trisulfide and related alkyl sulfide compounds mainly accounted for the malodor in the event. In the present study,  $\text{NH}_4\text{-N}$  concentration ranged between 0.18 and 1.13 mg/L, with a mean value of 0.45 mg/L. Furthermore, DMS and DMST concentrations were far below the values detected in the black water “agglomerate” event, although the particulate DMST concentrations in March and September 2008 exceeded its OTC value. It appears that under normal circumstances, concentrations of DMS and DMSTs are probably too low to generate black water “agglomerate” event, although higher risk were present in March and September in the present study.

#### 3.2.2. MIB and GEO

MIB and GEO are secondary metabolites produced by cyanobacteria, actinomycetes, fungi and myxobacteria, and responsible for most reports of source water odors (Dzialowski et al., 2009; Smith et al., 2008; Suffet et al., 1996; Watson et al., 2000; Westerhoff et al., 2005). In the present study, total concentrations of GEO in the water ranged from 0 to 11.29 ng/L, and the annual mean concentrations in dissolved and particulate fractions were 1.96 and 1.37 ng/L, respectively. Total concentrations of MIB in the water ranged from 0 to 18.69 ng/L, and the annual mean concentrations in dissolved and particulate fractions were 1.91 and 2.59 ng/L, respectively (Table 3). Although MIB concentrations were always higher than geosmin concentrations, both followed similar seasonal trends. MIB concentrations showed four distinct peaks (see Fig. S1B in Supplementary Information, SI, for more data) in January, April, July to September, and December, respectively, and the two highest values that exceeded its OTC were recorded in July (18.69 ng/L) and September (18.14 ng/L), respectively. Four distinct GEO peaks presented in February (5.60 ng/L), May (4.65 ng/L), July (11.29 ng/L), and November (8.07 ng/L), respectively (Fig. S1B), all exceeding its OTC.

Seasonal variations in MIB concentrations seem quite variable among different waters of the world. Xu et al. (1999) reported that in Lake Donghu of China, MIB showed three distinct peaks in January, April and September, respectively. Westerhoff et al. (2005) reported

that in three reservoirs in Phoenix of USA, MIB concentrations generally increased from April to September, and then declined for 2–3 months to near-zero levels. Peter et al. (2009) examined dissolved and particle-bound taste and odor compounds in three Swiss lake waters with different nutrient levels from eutrophic to oligotrophic, but did not observe any seasonal or spatial trend, probably due to rather low concentrations of MIB (not exceeding 3 ng/L).

In the present study, three distinct peaks of total GEO concentrations were respectively observed in February, May and July, when dissolved fraction comprised 92.9%, 64.5% and 72.0% of the total GEO, respectively. And the fourth peak was present in early winter with particulate fraction comprised 100% of the total GEO. Peter et al. (2009) reported increase in dissolved GEO concentrations in the epilimnion from March to July in the Swiss lakes, whereas GEO was absent in the particle-bound fraction. Durrerl et al. (1999) reported that in a stratified mesotrophic prealpine lake, in the autumn and early winter, surface films contributed essentially to the particle-bound fraction of GEO exhibiting a maximum concentration of 21 ng/L, while in the clear-water period (May), dissolved fraction of GEO increased dramatically (up to 93% of the total) whereas particle-bound GEO showed only minor changes. Dzialowski et al. (2009) developed predictive models for geosmin-related taste and odor in drinking water reservoirs of Kansas, USA, but failed to find out consistent spatial patterns in GEO production either between or within the reservoirs. The great variation in GEO pattern between different waters might be explained by the fact that the dynamics of GEO production can vary among different species and sometimes even among strains of the same species. Furthermore, some cyanobacteria excrete most of the geosmin during growth whereas other species retain a large intracellular pool (Mohren and Jüttner, 1983; Watson, 2003). It seems that the great variation in seasonality of MIB and GEO is also related to the complexity in their production by different organisms with different physiological status.

#### 3.2.3. IPMP and IBMP

IBMP and IPMP are two kinds of pyrazines compounds that produce vegetable odor and vegetable to decaying odor, respectively. IBMP was described to be formed during microbial degradation of grass under anaerobic conditions, while IPMP was known to be a product of soil organisms (actinomycetes) and has been found in many food and water samples (Khiari et al., 1997).

In the current study, equally low concentrations of dissolved and particle-bound IBMP were found throughout the year. The concentrations never exceeded 2.40 ng/L (Fig. S1C), and the annual mean concentrations in dissolved and particulate fractions were 0.31 and 0.37 ng/L, respectively (Table 3). This may explain why IBMP did not show seasonal or spatial trend in this study, and it is certain that IBMP concentration was too low to produce odor during the study period.

IPMP was present at detectable levels throughout the year, and IPMP were much higher in the particle-bound (0.03–6.80 ng/L) than in the dissolved (0.13–2.13 ng/L) fractions. On average, more than 83% of the IPMP existed in particulate form. After a gradual increase from January to February, a substantial increase of IPMP was observed from March to April, and then gently decreased in the following two months, whereas suddenly dropped to a rather low concentration in July, and maintained at the low levels until a little increase in December. The peak concentration recorded in April was 8.33 ng/L, lower than its OTC value. Peter et al. (2009) observed in three Swiss lakes that IPMP was present in all particle-bound samples (3–16 ng/L), but not in the dissolved fraction, and that IPMP decreased during the clear water phase period, but increased with the re-growth of phytoplankton.

#### 3.2.4. $\beta$ -cyclocitral and $\beta$ -ionone

Due to potent odors (tobacco, floral or fruity) and extremely low OTCs, many nor-carotenoids often contribute towards aquatic odor,

**Table 3**  
Seasonal changes of total T&O compounds concentrations, percentage of the dissolved and particulate fractions of each analyte, and occurrence of T&O compounds (dissolved, particulate) that exceeded their OTC values at the 15 sampling sites in Gonghu Bay, Lake Taihu during the study period.

	DMS			DMTS			IPMP			MIB			IBMP			β-cyclocitral			GEO			β-ionone		
	TC	D	P	TC	D	P	TC	D	P	TC	D	P	TC	D	P	TC	D	P	TC	D	P	TC	D	P
	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)	(ng/l)	(%)	(%)
Jan	0.00	--	--	0.00	--	--	3.68	0.0	100.0	2.12	23.1	76.9	0.00	--	--	0.81	0.0	100.0	3.52	100.0	0.0	0.00	--	--
Feb	0.13	100.0	0.0	0.00	--	--	4.13	0.0	100.0	1.67	28.0	72.0	3.00	20.0	80.0	3.13	0.0	100.0	5.60	92.9	7.1	0.00	--	--
Mar	64.67	27.3	72.7	23.00	26.7	73.3	8.27	17.7	82.3	0.53	0.0	100.0	0.87	100.0	0.0	2.07	32.4	67.6	3.40	52.9	47.1	0.00	--	--
Apr	84.87	50.0	50.0	0.00	--	--	8.33	25.6	74.4	3.07	21.7	78.3	0.00	--	--	1.33	0.0	100.0	0.60	0.0	100.0	0.00	--	--
May	16.11	17.6	82.4	0.00	--	--	5.42	24.6	75.4	1.60	100.0	0.0	3.41	52.8	47.2	25.74	32.6	67.4	4.65	64.5	35.5	12.28	0.0	100.0
Jun	13.80	100.0	0.0	0.00	--	--	4.87	0.0	100.0	0.00	--	--	0.33	0.0	100.0	26.27	55.8	44.2	0.96	0.0	100.0	14.33	45.6	54.4
Jul	0.00	--	--	0.00	--	--	0.42	0.0	100.0	18.69	0.0	100.0	0.05	0.0	100.0	537.61	42.9	57.1	11.29	72.0	28.0	50.44	16.7	83.3
Aug	0.47	100.0	0.0	0.00	--	--	0.16	84.4	15.6	5.64	20.1	79.9	0.00	--	--	538.12	43.4	56.6	0.53	100.0	0.0	45.52	9.9	90.1
Sep	201.18	0.0	100.0	69.55	0.0	100.0	0.42	0.0	100.0	18.14	99.2	0.8	0.00	--	--	124.84	2.1	97.9	0.40	100.0	0.0	40.80	3.7	96.3
Oct	0.00	--	--	0.00	--	--	0.87	100.0	0.0	0.28	0.0	100.0	0.40	100.0	0.0	260.64	0.7	99.3	0.93	100.0	0.0	46.60	0.0	100.0
Nov	3.87	0.0	100.0	0.00	--	--	0.00	--	--	0.00	--	--	0.00	--	--	77.06	15.7	84.3	8.07	0.0	100.0	1.47	0.0	100.0
Dec	0.93	14.3	85.7	0.20	100.0	0.0	1.60	29.2	70.8	2.27	23.5	76.5	0.00	--	--	0.00	--	--	0.00	--	--	0.00	--	--
AMC	32.17	6.46	25.71	7.73	0.53	7.20	3.18	0.53	2.65	4.50	1.91	2.59	0.67	0.31	0.37	133.13	42.06	91.08	3.33	1.96	1.37	17.62	1.75	15.87
OTC	0.3-1×10 <sup>3</sup>			10			20			15			2-16			0.5-19.3×10 <sup>3</sup>			4			7		

D-T&O	site 1	site 2	site 3	site 4	site 5	site 6	site 7	site 8	site 9	site 10	site 11	site 12	site 13	Site 14	Site 15
Jan															
Feb	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Mar	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Apr	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
May	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Jun	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Jul	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Aug	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Sep	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Oct	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Nov	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Dec	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●

P-T&O	site 1	site 2	site 3	site 4	site 5	site 6	site 7	site 8	site 9	site 10	site 11	site 12	site 13	Site 14	Site 15
Jan	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Feb	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Mar	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Apr	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
May	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Jun	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Jul	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Aug	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Sep	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Oct	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Nov	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●
Dec	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●	●●●●●

TC: total concentrations (dissolved + particulate) of the T&O compounds, D (%): percentage of the dissolved T&O compounds, P (%): percentage of the particulate T&O compounds, AMC (ng/L): annual mean concentration, OTC (ng/L): odor threshold concentration.

Note: values for each month are the mean concentration of fifteen sites, values exceed OTC are highlighted with red color.

GEO: ● (≥1 fold) ● (≥2 fold), MIB: ● (≥1 fold) ● (≥2 fold), IBMP: ● (≥1 fold) ● (≥2 fold), IPMP: ● (≥1 fold) ● (≥2 fold), DMDS: ● (≥1 fold) ● (≥2 fold), DMS: ● (≥1 fold) ● (≥2 fold), β-ionone: ● (≥1 fold) ● (≥2 fold), β-cyclocitral: ● (≥1 fold) ● (≥2 fold).

Note: site 3 was nearby the Nanquan Waterworks, site 13 was nearby the Xidong Waterworks, D: dissolved, P: particulate.



although not with the same widespread impact as geosmin and MIB (Watson, 2003). Like GEO and MIB,  $\beta$ -cyclocitral and  $\beta$ -ionone also belong to terpenoids, surprisingly few studies have investigated their seasonal dynamics.

In the present study,  $\beta$ -cyclocitral was present at detectable levels throughout the year, whereas  $\beta$ -ionone occurred only from May to November (see Fig. S1D in Supplementary Information, SI, for more data). Substantially high concentrations of  $\beta$ -cyclocitral and  $\beta$ -ionone appeared from July and maintained until October. High  $\beta$ -cyclocitral concentrations (537.6 ng/L in total with 42.9% in dissolved fraction, 538.1 ng/L in total with 43.4% in dissolved fraction) were recorded in July and August, respectively, both slightly exceeding its OTC (500 ng/L). Whereas, high  $\beta$ -ionone concentrations that exceeded its OTC (7 ng/L) were recorded from May to October, mostly in particulate fraction. This indicates that  $\beta$ -ionone was more important for the production and release of violet odor than  $\beta$ -cyclocitral during our study period.

### 3.2.5. Spatial patterns of eight T&O compounds

The fifteen sites could be divided into three groups: the first group included sites 1 to 5, located in the connecting area between Gonghu Bay and Meiliang Bay (the most eutrophic part of Lake Taihu); the second group included sites 6 to 10, located in the littoral zone with reed marsh; and the third group included sites 11 to 15, located in the north-east corner of the Gonghu Bay. Sites 11 to 15 were near the inlet of a water diversion facility from the Yangtze River to Lake Taihu, and thus had a high water exchange rate (Xie, 2008). High concentrations of dissolved and particulate T&O compounds that exceeded respective OTC frequently presented at sites 1–5, the area where Nanquan Waterworks is located nearby. While, levels of T&O compounds were relatively low at sites 11–15, indicating lower risk faced by Xidong Waterworks due to faster water exchange (Table 3).

## 3.3. Correlations between T&O compounds and physico-chemical parameters, microcystin, and algal biomass

### 3.3.1. Nitrogen and phosphorus

Neither  $\text{NH}_4\text{-N}$  nor TP showed correlations with the dissolved concentrations of the eight T&O compounds. However, dissolved concentrations of DMS, DMTS, IPMP, IBMP and GEO showed significant correlations with  $\text{NO}_3\text{-N}$ , TDN and TN, and DMS and DMTS also showed significant correlations with  $\text{NO}_2\text{-N}$ . Significant correlations were also found between TDP and dissolved concentrations of DMS, DMTS, IPMP and IBMP, and positive correlation was found between  $\text{PO}_4\text{-P}$  and dissolved concentrations of IPMP ( $r = 0.194$ ,  $P < 0.05$ ) (Table 4).

Neither  $\text{NH}_4\text{-N}$  nor TP nor  $\text{PO}_4\text{-P}$  showed correlations with the particulate concentrations of the eight T&O compounds. In fact, only IPMP showed significant correlations with  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , TDN, TN and TDP, while IBMP showed positive correlation with  $\text{NO}_3\text{-N}$  ( $r = 0.181$ ,  $P < 0.05$ ).

Yang et al. (2008) suggested that the taste and odor incident caused by the black water “agglomerate” in Lake Taihu was associated with the unusually high concentrations of ammonium levels. Different from that extreme event, neither dissolved nor particulate concentrations of all the eight T&O compounds showed correlations with the concentration of  $\text{NH}_4\text{-N}$  in our study.

Dzialowski et al. (2009) observed negative relationships between GEO (only dissolved geosmin was detected in their study) and dissolved inorganic phosphate ( $\text{PO}_4\text{-P}$ ) concentrations in drinking water reservoirs, and speculated that possibly increased phytoplankton depleted available  $\text{PO}_4\text{-P}$ , resulting in subsequent blooms and increases in geosmin concentrations. However, the present study found no relationship between  $\text{PO}_4\text{-P}$  and dissolved or particulate concentrations of GEO, whereas dissolved concentrations of GEO

showed significant correlations with  $\text{NO}_3\text{-N}$  ( $r = 0.201$ ,  $P < 0.01$ ), TDN ( $r = 0.165$ ,  $P < 0.05$ ) and TN ( $r = 0.304$ ,  $P < 0.01$ ), respectively (Table 4).

The present results indicate that in Lake Taihu, the production of T&O compounds was more closely associated with nitrogen. Regardless of the mechanisms involved, high concentrations of  $\text{NO}_3\text{-N}$ , TDN and TN could be risky signs for the occurrence of taste and odor events, especially caused by DMS, DMTS, IPMP, IBMP and GEO. Of course, anaerobic decomposition of cyanobacterial blooms (with high  $\text{NH}_4\text{-N}$  concentration) may trigger massive production of these T&O compounds, causing serious water pollution incident.

### 3.3.2. Other physicochemical parameters

There were strong negative correlations between the two forms of  $\beta$ -cyclocitral and  $\beta$ -ionone and DO, SD and pH. For DO, shown are  $\beta$ -cyclocitral ( $r = -0.182$ ,  $P < 0.05$  for dissolved form,  $r = -0.225$ ,  $P < 0.01$  for particulate form) and  $\beta$ -ionone ( $r = -0.235$ ,  $P < 0.01$  for dissolved form,  $r = -0.160$ ,  $P < 0.05$  for particulate form). For SD, shown are  $\beta$ -cyclocitral ( $r = -0.174$ ,  $P < 0.05$  for dissolved form,  $r = -0.218$ ,  $P < 0.01$  for particulate form) and  $\beta$ -ionone ( $r = -0.209$ ,  $P < 0.01$  for dissolved form). For pH, shown is  $\beta$ -cyclocitral ( $r = -0.170$ ,  $P < 0.05$  for particulate form). Strong positive correlations were also found between particulate form of these two compounds and COD, Chl-a, and WT. For COD, shown are  $\beta$ -cyclocitral ( $r = 0.193$ ,  $P < 0.05$ ) and  $\beta$ -ionone ( $r = 0.178$ ,  $P < 0.05$ ). For Chl-a, shown are  $\beta$ -cyclocitral ( $r = 0.212$ ,  $P < 0.01$ ) and  $\beta$ -ionone ( $r = 0.175$ ,  $P < 0.05$ ). For WT, shown is  $\beta$ -ionone ( $r = 0.286$ ,  $P < 0.01$ ) (Table 4). The positive correlation with COD and Chl-a and the negative correlation with DO and SD implies that trophic status is a good predictor of  $\beta$ -cyclocitral and  $\beta$ -ionone concentrations.

In Arizona reservoirs, temperature is a critical factor in production and distribution of MIB and geosmin (Westerhoff et al., 2005). In contrast, no relationship could be found between water temperature and MIB or GEO in our study. Dissolved concentration of GEO was closely correlated with COD ( $r = 0.221$ ,  $P < 0.01$ ), and GEO was the secondly important T&O compounds with relatively high risk to drinking water supply in terms of concentration exceeding OTC value and the associated duration.

There were significant negative correlations between the particulate forms of IPMP and IBMP and COD and WT. This is generally in agreement with the seasonal dynamics of IPMP and IBMP, i.e., higher concentrations of IPMP and IBMP occurred in March and April (Fig. S1C) with lower water temperature and better water quality than in summer.

In the present study, particulate concentration of DMS showed negative correlation with Chl-a which declined when cyanobacterial population began to decompose with color gradually changing from green to the characteristic blue. Negative correlation was also found between DO and particulate concentration of DMTS. As expected, positive correlations were found between water temperature and particulate concentration of DMS ( $r = 0.178$ ,  $P < 0.05$ ) and DMTS ( $r = 0.217$ ,  $P < 0.01$ ). It seems that degradation of cyanobacteria might have produced these T&O compounds.

### 3.3.3. Algal biomass and MC concentration

In the present study, phytoplankton communities were dominated by Bacillariophyta from January to April, accompanied by considerable amounts of Cryptophyta in March and April. From May to November, Cyanophyta (mainly *Microcystis aeruginosa*) dominated absolutely. Both intracellular and extracellular MC concentrations were positively correlated with *Microcystis* biomass ( $r = 0.465$ ,  $P < 0.01$  and  $r = 0.448$ ,  $P < 0.01$ , respectively) and Cyanophyta biomass ( $r = 0.513$ ,  $P < 0.01$  and  $r = 0.501$ ,  $P < 0.01$ , respectively) during the study period.

Geosmin and MIB are known to be typical algal secondary metabolites and account for most reported source-water odors around the world. In the present study, neither dissolved nor particulate concentrations of GEO and MIB showed correlation to

**Table 4**

Correlations between concentrations of T&O compounds (dissolved, particulate) and physicochemical parameters, biomass of major phytoplankton groups and microcystin concentrations of water samples.<sup>a</sup>

	DMS	DMTS	IPMP	MIB	IBMP	β-cyclocitral	GEO	β-ionone
<i>D-T&amp;O</i>								
NH <sub>4</sub> -N								
NO <sub>2</sub> -N	.236**	.237**						
NO <sub>3</sub> -N	.328**	.237**	.215**		.189*		.201**	
TDN	.277**	.269**	.230**		.217**		.165*	
TN	.208**	.243**	.213**		.233**		.304**	
PO <sub>4</sub> -P			.194*					
TDP	.271**	.370**	.313**		.177*			
TP								
COD	-.292**		-.220**				.221**	
DO						-.182*		-.235**
Chl-a								
pH	-.179*	-.159*						
WT								
WD								
SD						-.174*		-.209**
Ex-MC								
In-MC								
<i>Microcystis</i>								
Cyanophyta	-.165*							
Chlorophyta								
Bacillariophyta								
Cryptophyta	.235**	.185*	.268**					
Pyrrophyta								
Euglenophyta								
Chrysophyta								
DMS		.207**	.395**					
DMTS	.207**		.629**		.346**			
IPMP	.395**	.629**			.470**			
MIB								
IBMP		.346**	.470**				.327**	
β-cyclocitral								.177*
GEO					.327**			
β-ionone						.177*		
<i>P-T&amp;O</i>								
NH <sub>4</sub> -N								
NO <sub>2</sub> -N			.234**					
NO <sub>3</sub> -N			.436**		.181*			
TDN			.384**					
TN			.291**					
PO <sub>4</sub> -P								
TDP			.295**					
TP								
COD			-.293**		-.169*	.193*		.178*
DO		-.178*				-.225**		-.160*
Chl-a	-.154*		-.172*			.212**		.175*
pH						-.170*		
WT	.178*	.217**	-.256**		-.218**			.286**
WD								
SD						-.218**		
Ex-MC						.165*		.159*
In-MC						.184*		.208**
<i>Microcystis</i>								
Cyanophyta						.275**		.187*
Chlorophyta						.317**		.229**
Bacillariophyta			.175*					
Cryptophyta								
Pyrrophyta								
Euglenophyta			.160*					
Chrysophyta								
DMS		.833**						
DMTS	.833**							
IPMP								
MIB							.301**	
IBMP								
β-cyclocitral								.848**
GEO				.301**				
β-ionone						.848**		

<sup>a</sup> Significant at the \*\* $P < 0.01$  and \* $P < 0.05$  levels. D-T&O: dissolved taste and odor compounds, P-T&O: particulate taste and odor compounds, Ex-MC: extracellular microcystin, In-MC: intracellular microcystin, DMS: methyl sulfide, DMTS: dimethyl trisulfide, IPMP: 2-isopropyl-3-methoxypyrazine, MIB: 2-methylisoborneol, IBMP: 2-isobutyl-3-methoxypyrazine, GEO: geosmin.

intracellular or extracellular MC concentrations or biomass of any phytoplankton phylum. Similarly, Dzialowski et al. (2009) found that cyanobacterial biovolume is not a consistent predictor of dissolved geosmin, and Durrerl et al. (1999) indicated that correlations between a particular taxon and particle-bound geosmin cannot be expected to occur in any instance in natural waters. On the contrary, a few researchers observed positive correlation between geosmin and cyanobacterial biovolume (Jüttner and Watson, 2007; Sugiura et al., 2004). Li et al. (2007) suggested that dissolved odorous compounds are affected by various factors, such as rate of odor production by algal cells, biodegradation by microbes, photolysis by sunlight, absorption by particles, disturbance by wave and volatilisation of odorous compounds themselves.

It is known that GEO and MIB show little habitat-related pattern in synthesis among cyanobacteria. In fact, the number of cyanobacterium species known to date to produce these compounds is small (~50 species, and *Microcystis aeruginosa* produces neither GEO nor MIB), comparatively more *Oscillatoriales* are known to produce geosmin or MIB (~30) (Watson, 2003). The lack of potent GEO and MIB producers such as several species of *Oscillatoria* and the absolute predominance of *Microcystis aeruginosa* might explain the absence of correlations between GEO and MIB and algal biomass or MC concentrations in our study lake.

In the present study, particulate concentrations of  $\beta$ -cyclocitral and  $\beta$ -ionone were correlated significantly with Chl-a and biomass of total cyanobacteria or *Microcystis*. This is in agreement to the results of Lake Dianchi by Li et al. (2007). Significant correlations were also present between particulate  $\beta$ -cyclocitral and  $\beta$ -ionone concentrations and intracellular and extracellular microcystin concentrations in the present study (Table 4). Such correlations have not been reported for surface water before.  $\beta$ -cyclocitral and  $\beta$ -ionone are products of oxidative decomposition of  $\beta$ -carotene in *Microcystis* sp. (Jüttner, 1988; Watson, 2003). These two terpenoids and MCs are intrinsic metabolites of cyanobacteria, though produced by different metabolic ways (Watson, 2003). Together these studies with the fact that *Microcystis* occupied more than 93% of total cyanobacteria biomass during our study period, satisfactorily explain the good relationships between  $\beta$ -cyclocitral and  $\beta$ -ionone and cyanobacterial biomass or cyanotoxin contents.

It is interesting to note that negative correlation was found between dissolved DMS and total cyanobacterial biomass ( $r = -0.165$ ,  $P < 0.05$ ), suggesting that degradation of cyanobacteria might have produced this T&O compound. Positive correlations were also found between Cryptophyta biomass and dissolved concentrations of DMS ( $r = 0.235$ ,  $P < 0.01$ ), DMTS ( $r = 0.185$ ,  $P < 0.05$ ) and IPMP ( $r = 0.268$ ,  $P < 0.01$ ), between particulate IPMP concentration and Bacillariophyta biomass ( $r = 0.175$ ,  $P < 0.05$ ) or Euglenophyta biomass ( $r = 0.160$ ,  $P < 0.05$ ) in the present study.

### 3.3.4. Correlations between T&O compounds

There were close relations among the eight target T&O compounds in our study. For particulate T&O compounds, significant correlations were found between DMS and DMTS ( $r = 0.833$ ,  $P < 0.01$ ), between  $\beta$ -cyclocitral and  $\beta$ -ionone ( $r = 0.848$ ,  $P < 0.01$ ), and also between MIB and GEO ( $r = 0.301$ ,  $P < 0.01$ ). For dissolved T&O compounds, significant correlations were found among DMS, DMTS and IPMP. Furthermore, dissolved IBMP was closely correlated with IPMP ( $r = 0.470$ ,  $P < 0.01$ ) and GEO ( $r = 0.327$ ,  $P < 0.01$ ), while  $\beta$ -cyclocitral coincided with  $\beta$ -ionone ( $r = 0.177$ ,  $P < 0.01$ ) (Table 4). These correlations suggest that some T&O compounds might have derived from the same source (like DMS and DMTS, GEO and MIB) or produced from the same metabolic pathway (like  $\beta$ -cyclocitral and  $\beta$ -ionone). These features might be helpful for better understanding the behaviors of these T&O compounds further with rational countermeasures in source water in the future.

It should be noted, however, that during the study period, we did not observe concentrations of T&O compounds as high as the drinking water malodor incident of Wuxi City in 2007, although we conducted monthly monitoring at 15 sites around the two Waterworks. In our future works, efforts should be made on more intense monitoring (e.g., at an interval of 2–3 days) on T&O compounds in places (e.g. small bays) where cyanobacterial scums accumulate densely and anaerobic decomposition occur actively in the summer, so as to establish more accurate relationship between T&O compounds and relevant factors for future better incident prediction, warning and prevention.

## 4. Conclusions

1. A sensitive and automated method was developed to simultaneously analyze eight T&O compounds (boiling points ranging from 38 °C to 239 °C) by using P&T coupled with GC/MS working in SIM mode with detection limits of about 0.3–1.1 ng/L.
2. The maximum DMTS (69.6 ng/L in particulate fractions) exceeded its OTC (10 ng/L), and the maximum dissolved DMTS was 6.1 ng/L, but these figures were still far below those in the drinking water pollution accident of Wuxi City in 2007 when DMTS reached 1768–11,399 ng/L. GEO, MIB, IBMP and  $\beta$ -cyclocitral sometimes exceeded their OTC. High  $\beta$ -ionone exceeded its OTC (7 ng/L) from May to October. Spatially, Nanquan Waterworks faced more risk by T&O contamination than Xidong Waterworks.
3. It is the first time to report significant correlations between particulate  $\beta$ -cyclocitral and  $\beta$ -ionone concentrations and intracellular and extracellular microcystin concentrations. The lack of potent GEO and MIB producers such as *Oscillatoria* and predominance of *M. aeruginosa* might explain the absence of correlations between GEO, MIB and algal biomass or MC concentration. Nitrogen was more closely associated with the production of T&O compounds, and high concentrations of  $\text{NO}_3\text{-N}$ , TDN and TN could be risky signs of taste and odor events by DMS, DMTS, IPMP, IBMP and GEO.

## Acknowledgements

We wish to give our heartfelt thanks to Drs. Zhang DW, Guo LG and Zhang M for providing fieldwork support. Thanks are also given to both the anonymous reviewer and Dr. MacKenzie AB for their spending time in handling and improving the manuscript. This study was jointly supported by the Knowledge Innovation Project of the Chinese Academy of Sciences (KZCX1-YW-14-6-1) and National Basic Research Program of China (973 Program) (2008CB418101).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2010.10.010.

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