# Occurrence of algae and algae-related taste and odour (T&O) compounds in the Qingcaosha Reservoir, China

Kejia Zhang, Tuqiao Zhang, Yang Deng, Naiyun Gao and Yulong Yang

## ABSTRACT

This study examined the temporal and spatial variations in the occurrence of major algae and algae-related taste and odour (T&O) compounds in Qingcaosha Reservoir, China. Water samples were collected monthly from seven sites in the reservoir and analysed using solid phase microextraction combined with gas chromatography-mass spectrometry. Results showed that green algae were dominant over the 9-month study. In addition, cyanobacteria in the summer and early autumn peaked at $3.2 \sim 9.6 \times 10^6$ cell/L. Among the four most common algae-related T&O compounds, dimethyl trisulphide was undetectable at any of the sampling sites, and 2-methylisoborneol (2-MIB), geosmin and $\beta$-cyclocitral were frequently over their respective odour threshold levels from August through October. $\beta$-cyclocitral concentration was linearly correlated with the concentration of cyanobacteria ($R^2 = 0.9831$). The peak $\beta$-cyclocitral concentrations varied from 452 to 1,293 ng/L and were observed close to the water table. In contrast, high concentrations of 2-MIB and geosmin occurred in the water overlying sediments, probably because some of the 2-MIB and geosmin came from the micro-organisms in the sediments. These results provide important information regarding algae and algae-related T&O compounds in a typical reservoir in estuary and coastal areas, and will be helpful for developing appropriate strategies to minimize undesirable T&O issues in water sources.

**Key words** | algae, reservoir water, solid phase micro extraction, taste and odour

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## INTRODUCTION

Since 2010, the Qingcaosha Reservoir in Shanghai, China has been used as a new drinking water source for China's largest city with a population of over 10 million. The reservoir with an area of 66 km$^2$ has a maximum storage capacity of $4.35 \times 10^8$ m$^3$. The designed raw water supply rate of this reservoir is $7.19 \times 10^6$ m$^3$/d (Lin *et al.* 2009) that accounts for over 50% of the current water supply in Shanghai (Zhou *et al.* 2012). Given that the Qingcaosha Reservoir is located in the estuary of the Yangtze River, the longest river in China, its retention time should be carefully selected to minimize saltwater intrusion. A too short residence time does not allow for completing of an adequate water self-purification prior to entering local water treatment plants, while a too long residence time may lead to undesirable degradation of water quality due to numerous physical, chemical and biological processes such as eutrophication. Among the common water quality issues in reservoirs, taste and odour (T&O), a key aesthetic indicator, is the most frequently complained about, as a result of extremely low odour thresholds of many T&O-induced compounds (typically at a ppt level) and ineffectiveness of traditional drinking water treatment for removal of the T&O compounds (Suffet *et al.* 1996). Apart from the potentially negative health impacts, T&O present in finished water can cause the public to lose their trust in the local water industry. Therefore, there is an urgent demand to rapidly and efficiently mitigate the T&O problems. To develop appropriate T&O mitigation strategies for a specific reservoir, a clear understanding of occurrence of T&O-induced compounds in the raw water

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is critically important. Algae-related compounds are a major T&O source. Typical algal T&O-induced compounds include geosmin, 2-methylisoborneol (2-MIB), *β*-cyclocitral and dimethyl trisulphide (DMTS). Geosmin and 2-MIB are characterized with their well-known earthy-musty flavours (Deng *et al.* 2012). *β*-cyclocitral is derived from the carotenoid degradation, and gives off grassy, wooden or tobacco odour at different levels, respectively. Its threshold concentrations in many lakes and reservoirs are within the range of 100–3,000 ng/L (Zhang *et al.* 2011a). DMTS, a very common T&O-causing compound in China, is mainly responsible for the septic/swampy odour. Recently, DMTS has been found to be produced from *Microcystis* (Zhang *et al.* 2011b), particularly during the degradation of *Microcystis* (Jüttner 1984). In analysis of these T&O-induced compounds in water sources, an accurate and sensitive analytical method of gas chromatography (GC)-mass spectrometry (MS) is applied. Prior to GC/MS analysis, a headspace solid phase microextraction (SPME) technique is often used to collect volatile organic compounds emitted from water (Morales-Valle *et al.* 2010). The absorbance values at different wavelengths are important indexes to specify the viable algae species, and cell count with the phytoplankton analyser.

Most of the previous studies in the Qingcaosha Reservoir focussed solely upon the relationship of water quality and local climatic and hydrological conditions (Zhang *et al.* 2009; Zhou *et al.* 2009; Lu *et al.* 2011). Data on the occurrence of algae and major algal T&O-induced compounds in the reservoir are highly limited. The objective of this study was to determine the temporal and spatial patterns of occurrence in the predominant algae species and algal-induced T&O compounds in the Qingcaosha Reservoir, and evaluate the impacts of key factors such as nutrients and organic matters upon them.

## MATERIALS AND METHODS

### Ethics statement

Although this study involved phytoplankton, algae species, or non-living materials, it did not include the use of non-human primates in research. The water samples were collected from the Qingcaosha Reservoir in 2009, when the reservoir was not put into operation as drinking water source. No specific permits were required for the described field studies. Moreover, the field studies involving phytoplankton and algae species did not involve any endangered or protected species. During the field study, the survey was known by and got the oral permission of the local authority (The People's Government of Shanghai City and Shanghai Academy of Environmental Science).

### Reagents

All chemicals were at least of analytical grade if not otherwise noted. A total of 100 μg/mL geosmin and 100 μg/mL 2-MIB were prepared in a mixed standard methanol solution (Sigma-Aldrich, USA). DMTS (98%), *β*-cyclocitral (90%) and internal standard 2-isobutyl-3-methoxypyrazine (IB, 99%) were also purchased from Sigma-Aldrich (USA). Sodium chloride (NaCl) was obtained from Sinopharm Chemical Reagent Co., China, and baked at 450 °C for 2 hours prior to use to remove organic impurities.

### Site description

Water samples were collected from seven sites (# 1–7) in the Qingcaosha Reservoir by yacht, as shown in Figure 1. The collection frequency was once a month from April to December 2009. The sampling sites were defined by a global positioning system every time. Locations, water depths, water quality, and sampling times at the seven sites are summarized in detail in Table 1.

### Sample collection and preparation

In the field sampling, water from 0.5 m below the surface was collected and stored in a 4 L glass bottle that was sealed with a teflon cap with a zero headspace. The bottles were preserved in an icebox packed with ice packs until they were delivered to the environmental analytical laboratory and then stored in a refrigerator at 4 °C.

To analyse the T&O compounds in water, a 50 mL sample without filtration was withdrawn from the sample bottles, and transferred into 80 mL screw capped vials for

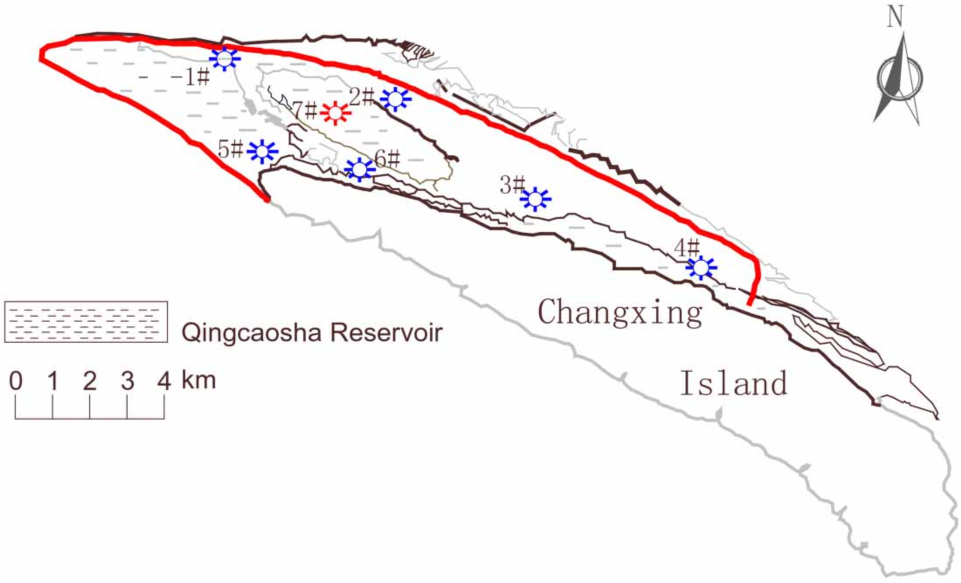

**Figure 1** | Sampling sites in Qingcaosha Reservoir (sites 1–7#).

**Table 1** | Locations, water depths, water quality, and sampling times of seven sites, 2009

| Sampling site | Location | Mean depth (m) | pH | Dissolved organic carbon (DOC, mg/L) | Sampling schedule (month/day) |
|---|---|---|---|---|---|
| **1#** | 31°29′24.8″N, 121°32′39.5″E | 1.3 | 8.04–8.64 | 2.82–5.97 | 04/15, 05/18, 06/15, 07/13, 07/28, 08/13, 08/27, 09/16, 09/28, 10/29, |
| **2#** | 31°28′59.4″N, 121°36′12.4″E | 3.9 | 8.06–8.59 | 2.52–4.72 | |
| **3#** | 31°26′19.9″N, 121°39′08.4″E | 2.4 | 8.19–8.74 | 2.52–3.37 | |
| **4#** | 31°25′05.7″N, 121°42′33.7″E | 7.9 | 8.15–8.23 | 1.93–4.34 | |
| **5#** | 31°27′17.8″N, 121°34′31.8″E | 1.5 | 8.17–8.51 | 4.22–9.99 | |
| **6#** | 31°26′57.4″N, 121°36′29.5″E | 10.2 | 8.28–8.74 | 2.73–5.61 | |
| **7#** | 31°28′20.7″N, 121°34′36.3″E | 2.1 | 8.36–8.68 | 2.80–4.53 | |

headspace extraction of dissolved and particle-bound T&O compounds. Then, IB at a concentration of 20 ng/L was added as the internal standard for the quantitative analysis. Subsequently, 15 g of dry NaCl was added and dissolved in the water sample. Thereafter, the vials were incubated in a water bath at 65 °C for 30 minutes before GC/MS analysis. To determine the algal species, 1 L samples from the sample bottles were filtered with 0.45 μm cellulose acetate membranes (50 mm i.d.). The filter residue was again dissolved in 5 mL Milli-Q water. Then, the phytoplankton species in the samples were determined and counted according to a phytoplankton analyser (PHYTO-PAM, Walz, Germany).

## Analysis

The T&O compounds (DMTS, 2-MIB, geosmin and $\beta$-cyclocitral), as well as the internal standard IB, in water were quantified using the headspace SPME (the fibre No. 57328 U) coupled with a gas chromatograph (GC, QP2010, Shimadzu, Japan) and a mass spectrometer (MS, QP2010S, Shimadzu, Japan). The detailed analysis procedure is available elsewhere (Zhang *et al.* 2012). In the selected ion monitoring mode, mass-to-charge ratio (m/z) of 126 and 79 for DMTS, m/z of 95 and 108 for 2-MIB, m/z of 112 for geosmin, m/z of 137 and 152 for $\beta$-cyclocitral, and m/z of 124 and 94 for IB were monitored, respectively.

Total nitrogen (TN) and total phosphorus (TP) were measured according to the national standard methods (GB11894-89, GB11893-89, respectively).

## RESULTS AND DISCUSSION

### Algae species and TN/TP

Figure 2 presents data on the temporal variations of different algae species in the study period. Over the 9 months of this study, the average monthly algal cell count was approximately $10^7$ cell/L, but the cell counts were highly time specific. The monthly cell counts during July–October 2009 were higher than those observed in other months. Particularly, the monthly cell count at $1.8 \times 10^7$ cell/L peaked in August 2009. At any specific month, diatoms, green algae and cyanobacteria were three dominant algal species. When the temperature was low, such as in April, November and December 2009, diatoms accounted for 53–62% of overall algal cells, probably because diatoms have a low requirement in solar irradiation and temperature compared with other species (Hakanson *et al.* 2003). In contrast, during August–October 2009, cyanobacteria gradually prevailed with a fraction of 32–53% in the overall algal cells. The finding suggests that a warm climatic condition favours the growth of cyanobacteria. In any month of this study, green algae were popular in the Qingcaosha Reservoir, with a fraction of >20% in the overall algae.

Nitrogen (N) and phosphorus (P) are two principal limiting nutrients for aquatic algal production due to their short supply compared to demands of cellular growth (Lv *et al.* 2011). TN at most of the seven sites peaked in April (1.38–1.89 mg/L) and August (1.54–2.08 mg/L), as a result of the nitrogen fixation and denitrification in the ecosystems (Gao *et al.* 2009). In August, the algae blooming maximized the nitrogen fixation dominant. In April, phosphorus became the limiting major nutrient for the algae growth, and dissolved phosphate could be absorbed by algae (Chen *et al.* 2009). The concentrations of TP in all the sampling sites were below 0.2 mg/L, and thus the water quality met with the Class III of Chinese surface water environmental quality standards.

The mean ratios of TN to TP (TN/TP) at all the seven sampling sites at different months are shown in Figure 3. Over the study period, TN/TP varied between 11.3 and 40.9. During August–November when the overall algae were abundant, TN/TP was approximately 15, lower than TN/TP in other months. The observation was in agreement with findings in many other studies that a high concentration of phosphorus, along with a low N/P supply ratio, favoured algae blooming (Liu *et al.* 2011). Sakamoto (1966)

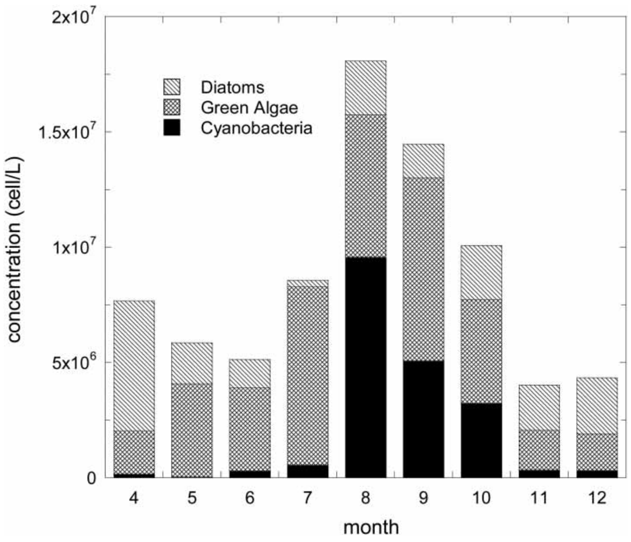

**Figure 2** | Variations in algae populations in Qingcaosha Reservoir from April to December 2009.

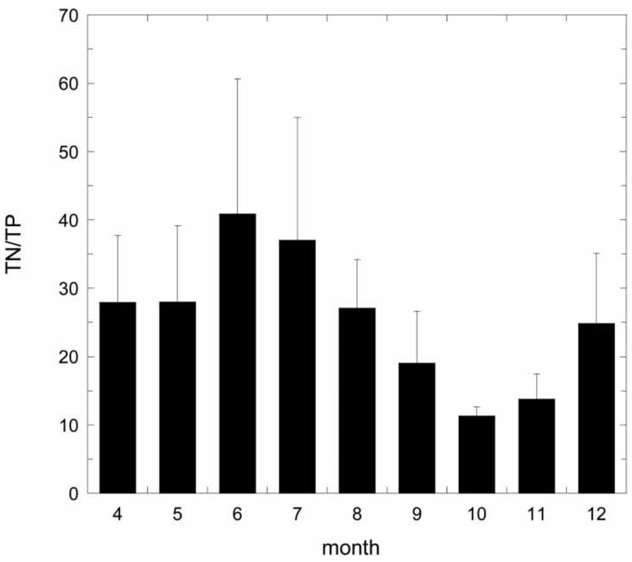

**Figure 3** | Changes in TN/TP ratios in Qingcaosha Reservoir, 2009.

proposed that the phytoplankton biomass relied upon TP at TN/TP > 17, upon TN at TN/TP < 10, and upon both TN and TP at TN/TP = 10–17. Based on the criteria, the limiting nutrients for the phytoplankton production were both TN and TP in September, October, and November, while only TP was the control nutrient in other months.

## Temporal variation of T&O compounds

Quantitative analysis of the selected T&O compounds, including DMTS, 2-MIB, geosmin and *β*-cyclocitral, was performed using SPME combined with GC/MS. Over the study period, DMTS was not detectable in all the sites, thereby indicating that algae rarely decayed because dissolved oxygen (DO) was always maintained at a level as high as 6–9 mg/L.

Total 2-MIB, geosmin and *β*-cyclocitral concentrations over time in the seven sampling sites are shown in Figure 4(a)–4(c), respectively. Generally, high concentrations of 2-MIB, geosmin and *β*-cyclocitral typically occurred in summer and early autumn. In particular, the maximum concentrations of 2-MIB (277.7 ng/L) and geosmin (29.2 ng/L) were observed in site #5 in September. These findings were significantly greater than their respective odour threshold levels of 6–42 ng/L (2-MIB) and 4–20 ng/L (geosmin) (Jones & Korth 1995). As shown in Figure 1, site #5 near the bank is located in a downwind area. 2-MIB and geosmin are produced primarily by cyanobacteria, and incidentally by others such as actinomycete (Watson 2003; Zaitlin & Watson 2006). In this study, it was noted that the concentrations of 2-MIB and geosmin were positively correlated with the concentrations of organic matters (e.g. DOC = 4.22–9.99 mg/L at site #5) and algae 5# ($7.5 \times 10^7$ cell/L at site #5).

In contrast, the maximum concentrations of *β*-cyclocitral (244.9–1293.3 ng/L) were observed in August 2009. Among the seven sampling sites, the highest *β*-cyclocitral level occurred at site #6 where the fraction of cyanobacteria reached 53% of the overall algae though the total algae concentration was not the highest. The relationship between the three T&O compounds and cell densities of different algae is shown in Table 2. As seen, the concentrations of 2-MIB, geosmin and *β*-cyclocitral were linearly correlated with the

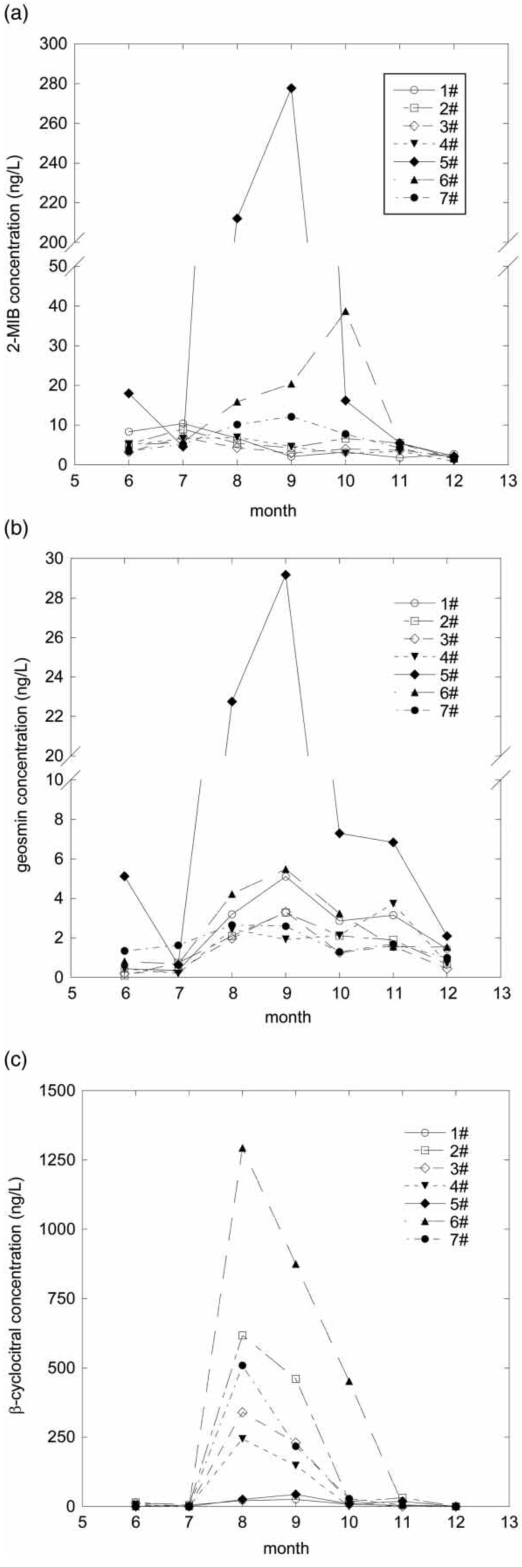

**Figure 4** | Variations of T&O compound concentrations in sampling sites of reservoir, 2009 ((a) 2-MIB, (b) geosmin, (c) *β*-cyclocitral).

**Table 2** | Significant regression relationships between the T&O compounds and different algae

| Related coefficient ($R^2$) | 2-MIB | Geosmin | $\beta$-cyclocitral |
|---|---|---|---|
| Diatoms | 0.0006 | 0.0232 | 0.0138 |
| Green algae | 0.4753 | 0.2244 | 0.2941 |
| Cyanobacteria | 0.7447 | 0.6378 | 0.9831 |

densities of cyanobacteria with $R^2 = 0.7447$, 0.6378 and 0.9831, respectively. The production quota of 2-MIB, geosmin and $\beta$-cyclocitral were approximately 4, 0.6 fg/cell and 50 fg/cell, respectively, in the Qingcaosha Reservoir. The strongest correlation between cyanobacteria and $\beta$-cyclocitral further demonstrated that $\beta$-cyclocitral was produced principally by cyanobacteria, particularly *Microcystis*. Jüttner & Hoflacher (1985) revealed that $\beta$-cyclocitral was the product of an oxidative cleavage reaction of $\beta$-carotene bound on *Microcystis*. In our Taihu Lake samples, the strong correlation was also observed between the *Microcystis* cells and $\beta$-cyclocitral concentrations ($R^2 = 0.95$) (data not shown here). Therefore, under field conditions, a $\beta$-cyclocitral concentration may be an excellent indicator to estimate the cell concentrations of *Microcystis* that are not conveniently and rapidly identified and analysed, when the cells are roughly in the same growth phase.

## Vertical profiles of algae and T&O compounds

The vertical profiles of algae and their T&O compounds over the water depth are shown in Figure 5(a)–5(d) (site #6, July–December 2009). As seen in Figure 5(a), the cell concentrations of algae decreased with the increasing depth in the reservoir over all of the study period. The finding is likely because the growth of algal cells was limited by low water temperature and reduced light intensities in deep water. In Figure 5(b), the vertical patterns of $\beta$-cyclocitral concentrations were synchronized with the profiles of algal densities, cyanobacteria in particular. The highest concentration was observed at a depth of 0.5 m, and varied within 452–1293 ng/L from August to October. The vertical profiles of 2-MIB concentrations in this study are shown in Figure 5(c). Of note, the concentrations of 2-MIB at 0.5 m were extremely high from the July to the November relative to the concentrations observed at the other two depths, with a peak at 39 ng/L in the November. The trend was correlated with the patterns of algae blooms in surface water. The 2-MIB concentrations at 5.5 and 10 m depths were very close at any specific month, all below 10 ng/L. Finally, geosmin exhibited a different vertical distribution over the water depth, as shown Figure 5(d). The highest geosmin concentrations were observed at 10 m (0.7–5.7 ng/L), followed by geosmin at 0.5 m (0.7–5.5 ng/L). The lowest concentrations occurred at the depth of 5.5 m (0.2–2.3 ng/L). Different from $\beta$-cyclocitral, the sediments closest to the 10 m depth could contribute 2-MIB and geosmin into water. Previous studies showed that actinomycetes were mainly found in terrestrial environments and could produce 2-MIB and geosmin that might enter the overlying water near sediments (Zaitlin & Watson 2006). Another study reported that the concentrations of 2-MIB and geosmin near to sediments were greater than those in surface water in the Xionghe Reservoir, China (Zuo *et al.* 2010). As seen from Table 2, less of a correlation between 2-MIB ($R^2 = 0.7447$)/geosmin ($R^2 = 0.6378$) and cyanobacteria was observed than that between $\beta$-cyclocitral and cyanobacteria ($R^2 = 0.9831$). The observation reflected that 2-MIB and geosmin might derive from a diversity of micro-organisms, fungi and phytoplankton. Similarly, Dzialowski *et al.* found that cyanobacterial biovolume is not a consistent predictor of dissolved geosmin (Dzialowski *et al.* 2009). Zuo *et al.* (2010) found cyanobacteria, actinomycetes, and other odour-producers were not responsible for 2-MIB occurring in one reservoir. Therefore, the sources of geosmin and 2-MIB continue to remain more complicated than $\beta$-cyclocitral.

## CONCLUSIONS

This study provided key information regarding the temporal and spatial variations in the occurrence of major algal species and algae-induced T&O compounds in Qingcaosha Reservoir, China. Three principal algae species, including diatoms, green algae and cyanobacteria, were observed in the 9-month study. Over all of the study period, green algae were present. From the August to the October of 2009, cyanobacteria gradually became dominant, with a

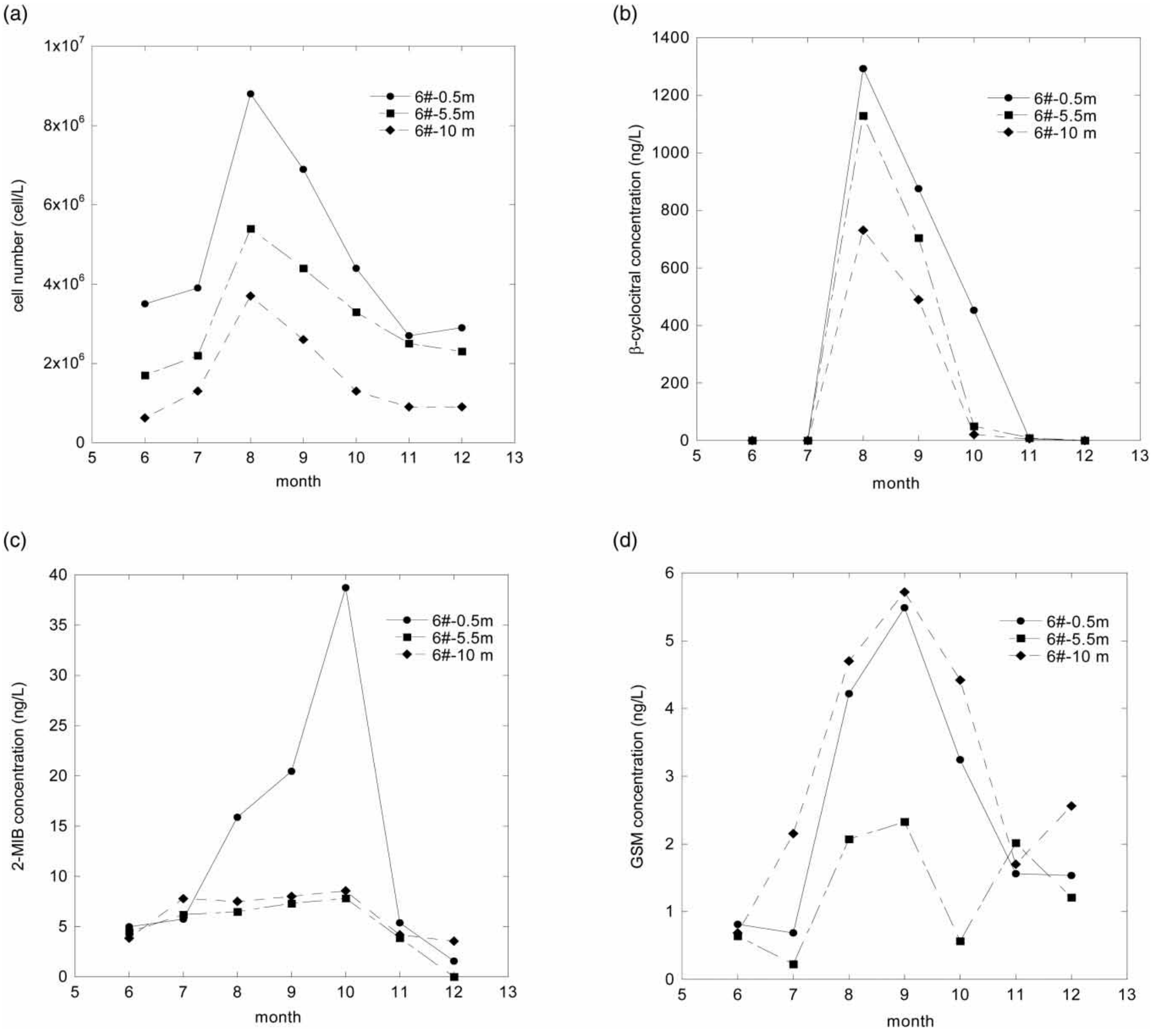

**Figure 5** | Concentration profiles of algae and T&O compounds in reservoir, 2009 ((a) algae, (b) *β*-cyclocitral, (c) 2-MIB, (d) geosmin).

32–53% fraction in the overall algae. Among the common algae-induced T&O compounds, DMTS was not detected at any of the sampling sites due to a high DO in the water, but three other T&O compounds were observed, including *β*-cyclocitral, 2-MIB, and geosmin. *β*-cyclocitral concentrations were strongly correlated with the cell number of cyanobacteria ($R^2 = 0.9831$). Therefore, a *β*-cyclocitral concentration may be a good indicator to estimate the cell number of cyanobacteria in a reservoir. High concentrations of 2-MIB and geosmin were found near sediments of the reservoir, thereby indicating that 2-MIB and geosmin might originate from not only phytoplankton, but also from micro-organisms and fungi in the sediments. The results added to our knowledge of the occurrence and fate of major algae and algal-related T&O compounds in the reservoirs located in estuary and coastal areas, and provided a foundation to develop appropriate strategies to minimize the algae-caused T&O issues in the raw water.

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