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Biological origins and annual variations of earthy-musty off-flavours in the Xionghe Reservoir in China

Ting Zhang, Lin Li, Yanxia Zuo, Quan Zhou and Lirong Song

ABSTRACT

The annual variations in physicochemical parameters, algal abundance and odorous compounds in the Xionghe Reservoir were investigated, in order to identify the possible odorous compounds and their origins. From May 2007 to April 2008, the algal composition, cell number and earthy-musty odorous compounds both in the water and in the flesh of fish at Sites A, B and C were determined monthly. The physicochemical parameters such as total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), pH, transparency, water temperature and chlorophyll *a* (chl *a*) were simultaneously determined. A statistical correlation was noted between the chl *a* and water temperature: 0.678 ($P < 0.05$) for Site A, 0.831 ($P < 0.01$) for Site B and 0.659 ($P < 0.05$) for Site C. Geosmin and 2-methylisoborneol (2-MIB)—in the reservoir were identified by gas chromatography-mass spectrometry (GC-MS). We observed that a large amount of *Anabaena circinalis* bloomed in surface water during the off-flavour episodes. In July 2007, we detected the highest concentration of geosmin (2.7 μg/L in the water, 0.27 μg/kg in the silver carp and 0.10 μg/kg in the crucian carp), while no 2-MIB was detected, which indicated that geosmin was mainly responsible for the off-flavour episodes in summer.

Key words | cyanobacteria, earthy-musty off-flavour, geosmin, reservoir, 2-methylisoborneol

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INTRODUCTION

Earthy-musty off-flavours in drinking water supplies and fish for human consumption are world-wide problems affecting the utilization of aquatic resources. In most cases, the development of earthy-musty off-flavours is associated with the presence of significant concentrations of geosmin or 2-methylisoborneol (2-MIB) (Tucker 2000). Both geosmin and 2-MIB are relatively stable to biological and chemical degradation, and can persist in the open water in the dissolved form for some time (Westerhoff *et al.* 2006; Jüttner & Watson 2007; Peter & von Gunten 2007). The presence of these two compounds with an earthy-musty odour in water and fish is attributed to cyanobacteria and actinomycetes (Zimba *et al.* 2001). Planktonic and periphytic cyanobacteria, as well as some actinomycetes, produce 2-MIB and geosmin in reservoirs, lakes, fish ponds, rivers, canals and within water treatment plants (WTPs) (Gerber & Lechevalier 1965; Izaguirre & Taylor 1995; Suffet *et al.* 1995; Jüttner & Watson 2007). However, subsequent studies focused on cyanobacteria, which are the dominant group in eutrophic waters, particularly in summer as the main biological origin of earthy-musty off-flavours (Kenefick *et al.* 1992; Jones & Korth 1995; Izaguirre *et al.* 1999; Vilalta *et al.* 2004).

In some regions of China, surface reservoirs are the main source of drinking water. However, rapid progress in China's economy in the past few years has greatly accelerated the eutrophication process in many reservoirs and lakes, which are used as sources of drinking water and for fisheries and agricultural purposes. This resulted in the excessive growth of cyanobacteria leading to the production of numerous secondary metabolites that are responsible for

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the unpleasant off-flavours found in water. In recent years, the presence of off-flavours has been reported in an increasing number of surface reservoirs in China (Cai *et al.* 2007; Yuan *et al.* 2007).

The Xionghe Reservoir is located in Zaoyang City, in the Hubei Province of China (see Figure 1). The surface area of the Xionghe Reservoir is 314.5 km^2 and its water capacity is 254 million m^3. It is a large-scale reservoir that is used for flood-control, irrigation, supplying water to rural areas, fisheries and recreational facilities. Episodes of strong earthy-musty off-flavour in the Xionghe Reservoir have occurred every year since 2005; this has resulted in severe off-flavour problems in drinking water and fish. In order to control and overcome this problem, it is necessary to detect the origin and dynamics of the production of off-flavours. To identify the possible odorous compounds and their origins, we sampled water and fish from the Xionghe Reservoir at three sites and investigated the annual variations in the physicochemical parameters, algal abundance and odorous compounds. The three sampling sites were Site A (31°55′45.4″N, 112°38′40.6″E), Site B (31°55′25.2″N, 112°39′17.3″E) and Site C (31°53′53.5″N, 112°40′28.9″E). Site A was located at the littoral zone of the dam; Site B close to the intake points of the waterworks; Site C in the centre of the Xionghe Reservoir.

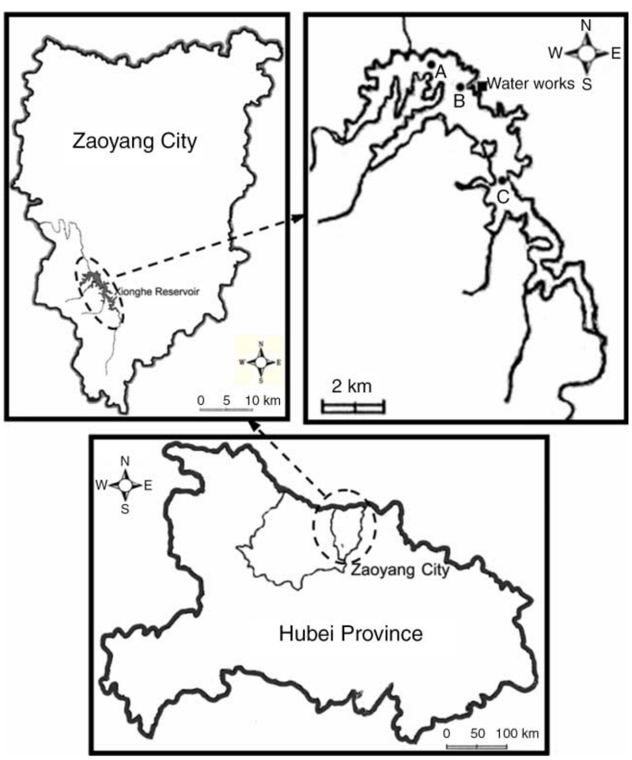

Figure 1 | Maps showing the locations of the sampling sites and Xionghe Reservoir.

MATERIALS AND METHODS

Water and phytoplankton sampling

Samples were collected from each sampling site every month (usually between 1:00 p.m. and 3:00 p.m.) from May 2007 to April 2008. In order to minimize possible diurnal variations in the water quality characteristics, the sites were always sampled in the same order, i.e. first Site A, then Site B and finally Site C. For each sampling event, a 2.5-L sampler was used to collect individual water samples at a depth of 0.5 m. We stored 500 mL of the water sample from each site in screw-capped polyethylene (PE) bottles in a refrigerator; this sample would be used for analysing the total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (chl *a*) concentrations. One litre of the water sample was mixed with 5 mL Lugol's iodine preservative and stored in PE bottles; these samples were used for algal analyses. The water sample for off-flavour analysis was collected in a narrow-necked 660-mL PE bottle and sealed with screw caps leaving no headspace and preserved in a refrigerator.

Physical and chemical analyses

The measurement of the physical and chemical parameters of the samples collected from each site was performed separately. At each sampling date, *in-situ* measurements of water temperature, pH and dissolved oxygen (DO) were concurrently measured at the depth of 0.5 m by using Hydrolab's new line of water quality multiprobe loggers (DataSonde® 4 and MiniSonde®; Hydrolab Corp., USA). A Secchi disk was used to measure the transparency of water at each site. The TN and TP concentrations were determined using the alkaline potassium persulfate digestion–UV spectrophotometric method (Ministry of Environmental Protection, The People's Republic of China 1989*a*) and the ammonium molybdate spectrophotometric method (Ministry of Environmental Protection, The People's

Republic of China 1989*b*), respectively. All the nutrient analyses were performed within 24 h of sample collection.

Chl *a* concentration analyses

The following steps were performed to extract and determine the concentration of chl *a*. First, the algae from each sample were filtered through a Whatman GF/C glass fibre filter ($D = 47$ mm). The filter was folded in quarters with forceps and placed in a small vial, which was capped and frozen so as to disrupt the cells. Chl *a* was extracted with 95% ethanol (Wintermans & de Mots 1965). At least 24 h after extraction, the concentration of chl *a* was determined by using a spectrophotometer (UV-2000, Unico Instrument Co., China).

Algal composition and abundance analyses

The 1 L of Lugol-fixed water sample was condensed to 100 mL. Of the 100 mL, 0.1 mL was placed in a phytoplankton counting chamber, and the cell number and species type were determined by observing the cells under a microscope (Olympus CX31-32C02; Olympus Corp., Japan). As to the colonial organism with trichomes twisted and folded over, first the average number of cells per circle was counted, and then the number of circles was counted. The cell number of the colonial organism with trichomes twisted and folded over was equal to the product of the average number of cells per circle multiplied by the number of circles. Every sample was counted three times, and the average value was used.

Geosmin and 2-MIB analyses

Geosmin and 2-MIB in the water samples were extracted by headspace solid-phase microextraction (HSPME), and the extracts were analysed by gas chromatography-mass spectrometry (GC-MS) according to the method described by McCallum *et al.* (1998), Li *et al.* (2005, 2007) and Zhang *et al.* (2009*b*). An 80-mL water sample was filtered through a Whatman GF/C glass fibre filter under a low vacuum. Then, the filtrate used for the determination of dissolved odorous compounds in water was placed into a 125-mL headspace vial containing a magnetic stirrer. After addition of 24 g of NaCl, the vial was sealed with a silicon-PTFE septum cap. The sealed vial was placed in a water bath with the temperature controlled at 60°C. After the syringe needle of the SPME device was pierced through the septum, the fibre was plunged out to be exposed in the headspace for adsorption of the analyte. 20 min later, the fibre was retracted back into the syringe and withdrawn from the vial, followed by immediate fit into the GC-MS inlet for desorption. Analyses were carried out with an Aglient 6890 GC system coupled with a 5973 mass spectral detector. The GC column used was HP-5MS 30 m × 0.25 mm i.d. × 0.25 μm. The carrier gas was helium with a constant pressure of 120 kPa. The injector was set at splitless mode for 2 min at 250°C. The GC oven temperature programme was as follows: hold at 60°C for 2 min; raise to 132°C (4°C/min); hold for 1 min; raise to 250°C (20°C/min); hold for 5 min. The GC-MS transfer line temperature was maintained at 280°C. The electron impact (EI) ion source of the mass spectrometer was 230°C. The EI ionization mode was used with electron energy of 70 eV.

Geosmin and 2-MIB in fish samples were determined according to the method (microwave mediated distillation HSPME-GC-MS) described by Zhang *et al.* (2009*a*). In brief, nearly 10 g shredded fish flesh was first placed in a 250-mL cone-shaped flask equipped with a 29/42 ground glass joint and a double offset inlet adapter. The adapter was connected with two glass tubes of 6 mm inside diameter. This assembly was placed in the microwave oven, and the tubes were passed through holes at the top of the oven. Then the fish flesh was heated for 6 min by microwave while 70 ml/min nitrogen flowed into the flask from one tube, and carried the volatile away from another tube, which linked to a cold water circulatory concentrator. Then the received condensate was analysed for geosmin and 2-MIB in fish by HSPME-GC-MS.

Isolation of cyanobacteria

Isolates from the reservoir samples were cultured in liquid Casitone-salts (CT) medium. The cultures were grown at 20°C under cool-white illumination at approximately 10 μmol/m^2/s. The cultures in which geosmin or 2-MIB odour developed were plated on agar plates containing the same medium with 1% agar w/v; 0.1 or <0.1 mL of inoculums was used. *Pseudanabaena* sp. and *Anabaena circinalis* colonies were picked from the agar plates with a Pasteur-type capillary pipette, placed in BG11 medium and incubated at 25°C. The cultures were microscopically analysed in order to verify that they were unialgal.

Morphological study

The morphology of cells and filaments was studied using a Cart Zeiss Axioskop 40 light microscope with a video camera linked to computer image-analysis software (ACT-2U, version 1.1, Nikon). The following parameters were selected to describe the morphology of studied strains, the size and shape of the vegetative cells, heterocytes and akinetes: the distance between a heterocyte and the nearest akinete (counted as the number of cells); the presence or absence of terminal heterocytes and gas vesicles; and the shape of filament and its aggregation in colonies.

Statistical analyses

One-way analysis of variance (ANOVA) was used to determine significant differences among the samples collected from the three reservoir zones. Statistical analyses were performed using the STATISTICA 6.0 software for Windows. $P < 0.05$ was regarded as significant. Correlation analysis was performed using SPSS 13.0.

RESULTS AND DISCUSSION

Annual variations in physicochemical parameters

Annual variations in the TN, TP, DO, pH, transparency and water temperature of Sites A, B and C are shown in Figure 2. The highest water temperature values were observed in July, averaging 30.0°C for Site A, 30.1°C for Site B and 29.1°C for Site C, while the lowest water temperature values were observed in January, averaging

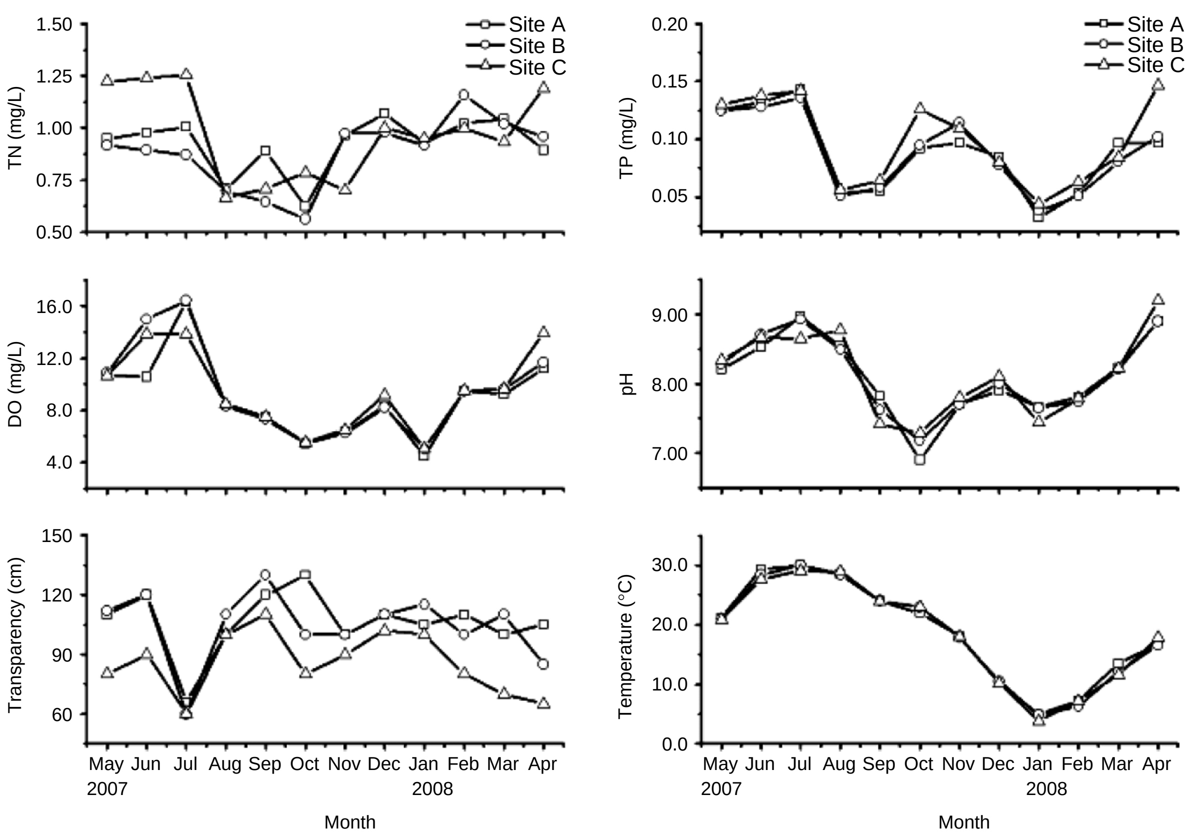

Figure 2 | Annual variations in the physicochemical parameters (TN, TP, DO, pH, transparency and temperature) in the Xionghe Reservoir.

4.9°C for Site A, 4.8°C for Site B and 3.8°C for Site C. The lowest Secchi depth values for the three sampling sites were observed in July, which coincided with the time of *A*. *circinalis* bloom, averaging 66, 61 and 60 cm for Sites A, B and C, respectively. The Secchi depth values of Site C were significantly lower than those of Sites A and B (ANOVA, $P < 0.05$ for both), which may be because frequent fertilization occurred at Site C.

The reservoir water tended to be alkaline, and the pH values of the three sampling sites ranged between 7.0 and 9.2. Annual variations in DO values were similar for all the three sampling sites. The lowest TP concentrations were also recorded in January; the average values for Sites A, B and C were 0.032, 0.038 and 0.044 mg/L, respectively. The annual average values of TP were 0.088 mg/L for Sites A and B, and 0.099 mg/L for Site C. The TN concentrations generally ranged between 0.563 and 1.255 mg/L. The annual average values of TN for Sites A, B and C were 0.922, 0.882 and 0.970 mg/L, respectively. These data indicate that water from the Xionghe Reservoir was eutrophic, with Site C being more heavily polluted than Sites A and B.

Annual variations in chl *a* and algal abundance

The concentrations of chl *a* at Sites A, B and C are shown in Figure 3. The annual average concentration of chl *a* was 27.93 μg/L for Site A, 27.76 μg/L for Site B and 33.48 μg/L for Site C. The annual variation in chl *a* concentration

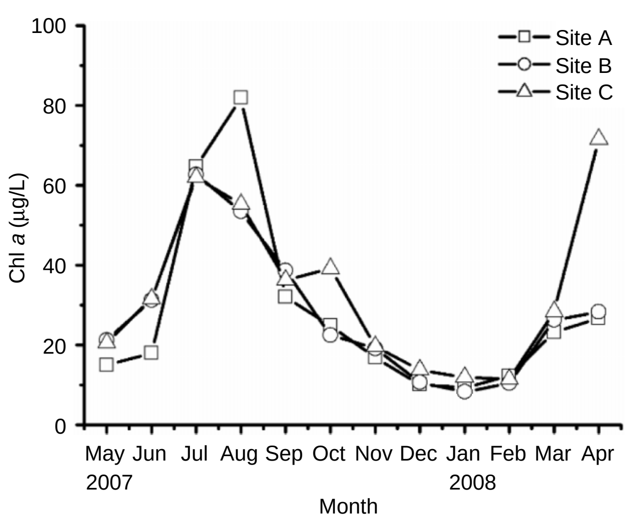

Figure 3 | Annual variations in chl *a* concentration in the Xionghe Reservoir.

at Site A was similar to that at Site B. The lowest chl *a* concentrations for Sites A and B were observed in January when the water temperature was the coldest, it increased as the water temperature increased, and reached a peak value in August (81.91 μg/L for Site A) and July (62.76 μg/L for Site B). For Site C, two peaks were observed–one in July (61.99 μg/L) and the other in April (71.56 μg/L). The lowest chl *a* value was observed in February (11.48 μg/L). A statistically significant correlation was noted between the chl *a* concentration and water temperature: 0.678 ($P < 0.05$) for Site A, 0.831 ($P < 0.01$) for Site B and 0.659 ($P < 0.05$) for Site C.

The composition and abundance of phytoplankton showed seasonal variation (see Figure 4). Both total algal and cyanobacterial abundance (cell number) at the three sampling sites showed a peak value in July. Cyanobacteria were observed frequently in summer and not very frequently in winter. The abundance of cyanobacteria at the three sampling sites accounted for approximately 98.9%, 98.9% and 97.4% of the total algal abundance in July, while it accounted for only 9.4%, 10.6% and 10.2% of the total algal abundance in February. In July, algal communities were dominated by *A. circinalis* (Cyanophyta), the abundance of which accounted for 81.7%, 63.1% and 48.3% of the abundance of cyanobacteria at Sites A, B and C, respectively. *A. circinalis* is known to be a common planktonic bloom-forming cyanobacterium (Beltran & Neilan 2000; Mitrovic *et al.* 2001), and Henley (1970) first reported that it produces geosmin. In the Xionghe Reservoir, the average cell number of *A. circinalis* reached a maximum (5.12×10^7 cells/L) in July, which coincided with the highest geosmin level in water and fish. Therefore, the results indicated that *A. circinalis* might be the biological origin of geosmin in the Xionghe Reservoir.

Annual variations in the geosmin and 2-MIB levels

We identified two odorous compounds (geosmin and 2-MIB) by GC-MS at the three sampling sites of the Xionghe Reservoir. The annual variations in the geosmin and 2-MIB levels in Sites A, B and C are shown in Figure 5 (in water) and Figure 6 (in fish). Off-flavour episodes in the surface water of the Xionghe Reservoir occurred from late spring through early autumn. Geosmin was observed from

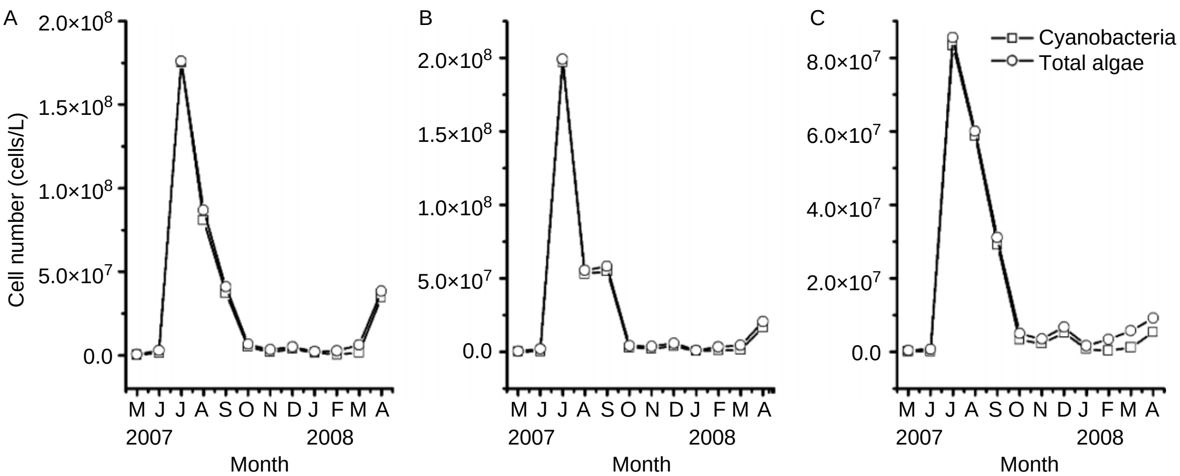

Figure 4 | Annual variations in the cyanobacterial and total algal abundance in the Xionghe Reservoir: A, Site A; B, Site B; C, Site C.

May to September 2007, and 2-MIB occurred in May 2007 and April 2008. The highest concentration of geosmin was observed in July and that of 2-MIB was observed in April: the average values for geosmin were 2,711.5, 2,592.7 and 441.1 ng/L and those for 2-MIB were 287.2, 378.4 and 188.9 ng/L for Sites A, B and C, respectively. The concentrations of both geosmin and 2-MIB at Site C were much lower than those at Sites A and B, which may be associated with the geographical location of the sampling sites. Site C was located relatively upstream, while Sites A and B were located downstream. Since water flows from the upstream to the downstream zone, both off-flavour producing cyanobacteria and odorous compounds may be transported to the downstream zone. In the other months for which odorous compounds could be detected in the water, the concentration of geosmin ranged between 10.0 and 60.5 ng/L, while that of 2-MIB ranged between 65.7 and 169.0 ng/L. Lake Castaic, a large reservoir in Southern California, experienced a two-month episode of 2-MIB in the autumn of 1993, and the 2-MIB levels in the water column reached or exceeded 150 ng/L (Izaguirre & Taylor 1998). Izaguirre *et al.* (1999) also demonstrated that the concentration of 2-MIB was 23 ng/L and that of geosmin was 4 ng/L in the San Vicente Reservoir, while an off-flavour episode due to 2-MIB began in Lake Skinner with 2-MIB levels of 5–10 ng/L. Moreover, *Pseudanabaena*

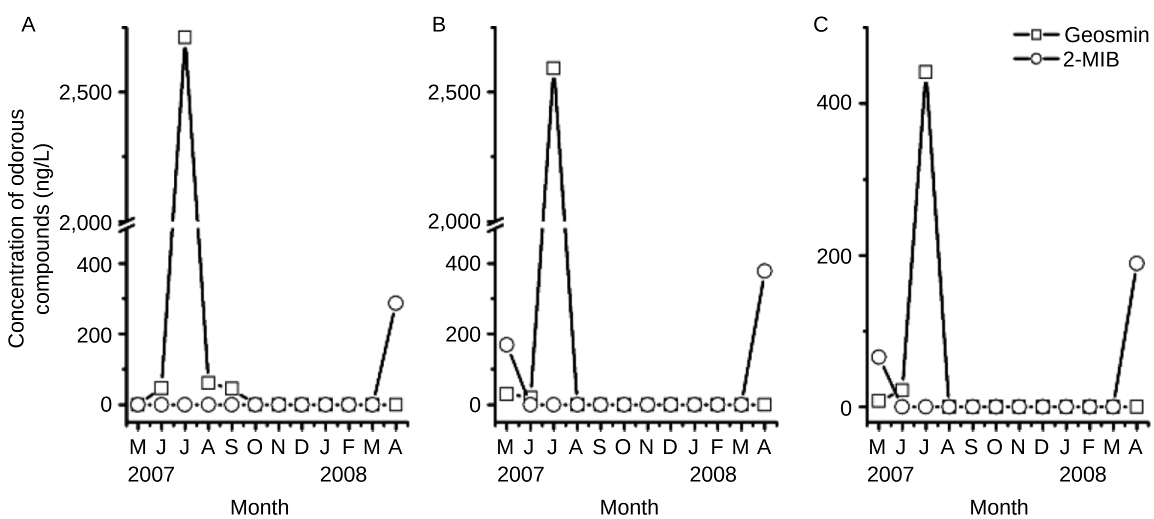

Figure 5 | Annual variations in the concentration of odorous compounds in the Xionghe Reservoir: A, Site A; B, Site B; C, Site C.

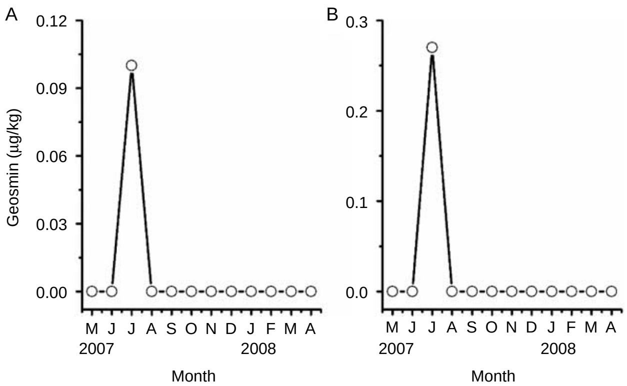

Figure 6 | Annual variations in the concentration of odorous compounds in the flesh of fish in the Xionghe Reservoir: A, Crucian carp; B, Grass carp.

had been implicated in 2-MIB problems in five other reservoirs (two of them outside of California) (Taylor *et al.* 2006) and the Arizona Canal (Baker *et al.* 2006). *Pseudanabaena*, which was known in Japan as the different name *Phormidium tenue* (first mentioned in Izaguirre & Taylor 1998), was also long implicated in 2-MIB problems in Lake Biwa and Lake Kasumigaura (Yagi *et al.* 1983; Sugiura *et al.* 1986). As to geosmin, Wang *et al.* (2005) reported that the highest concentration of geosmin recorded in Clinton Lake was 175 ng/L. Odour threshold concentrations (OTCs) as low as 4–20 and 6–42 ng/L in water have been reported for geosmin and 2-MIB, respectively (Krasner *et al.* 1983; Persson 1983; Mallevialle & Suffet 1987; Suffet & Mallevialle 1993; Young *et al.* 1996; Rashash *et al.* 1997; Ridal *et al.* 1999; Young & Suffet 1999; Davies *et al.* 2004). Therefore, it was concluded that the concentrations of geosmin and 2-MIB in the Xionghe Reservoir significantly exceeded the OTC values of water considered fit for human consumption and those of some other water bodies in the world; this finding indicated that the Xionghe Reservoir could be used for carrying out research on off-flavour problems in China.

Both geosmin and 2-MIB are accumulated in the food web and thus concentrated in the flesh of fish (Jüttner 1984). Therefore, these two odorous compounds were also detected in the flesh of omnivorous crucian carp and phytophagous silver carp inhabiting Xionghe Reservoir at the same time. It was observed that the concentration of geosmin in these fish was detected in July, at the time of *A. circinalis* blooming in the reservoir; the concentration of geosmin in the silver carp (0.27 μg/kg) was higher than that in the crucian carp (0.10 μg/kg). It has been indicated that the feeding behaviour of fish plays an important role in the uptake of compounds with earthy-musty odours. Geosmin and 2-MIB in the water could enter the fish via ingestion of cyanobacterial cells, absorption through the skin and most easily across the gills of fish (Johnsen & Lloyd 1992; Dionigi *et al.* 1998). In our study, we found that the concentrations of geosmin in both water and fish simultaneously reached a peak value in July. Various papers and reports included in a comprehensive review (Tucker 2000) had shown that off-flavour problems in freshwater fish occurred world-wide, and most of them were related to the earthy/muddy taint of farmed channel catfish (*Ictalurus punctatus*) and rainbow trout (*Onchorhynchus mykiss*). The bioaccumulation of earthy-musty taints in fish have been shown to be dependent on many factors, such as the exposure concentration of geosmin/MIB, water temperature, fat content, species and size of fish (Johnsen & Lloyd 1992; Johnsen *et al.* 1996; Howgate 2004). Robertson *et al.* (2005) had conducted the uptake experiment in 0.5 m^3 square glass fibre tanks, after exposure to a geosmin concentration (100 ng/L) under static conditions for 24 h at 14.5°C, rainbow trouts rapidly accumulated geosmin and reached the maximum level of 2.94 ± 0.13 μg/kg at 6 h. An investigation was also made on the occurrence of geosmin in water and rainbow trout in UK farms (Robertson *et al.* 2006) found the highest geosmin concentration in water was about 25 ng/L and that the maximum geosmin level of 7.2 μg/kg was observed in trout. Compared with the above data obtained in a tank or a farm, the distribution ratios for geosmin between fish flesh and water in the Xionghe Reservoir were relatively low. One reason why this phenomenon occurred might be that the horizontal and vertical distributions of geosmin concentration in the water column of the large reservoir varied very widely compared to a small tank or fishpond, and fish in a reservoir could swim freely from a place with a high level of geosmin to one with a low level, so that geosmin contents in fish were quite low.

Cyanobacteria blooms develop quickly and unpredictably in freshwater, resulting in the rapid uptake and accumulation of odorous compounds in fish (Shelby *et al.* 2004). The presence of off-flavour compounds in fish has been reported to be a serious setback to aquaculture (Yamprayoon & Noomhorm 2003). For example, off-flavour in cultured catfish results in the largest economic losses to catfish producers in the south-eastern United States of \$20–30 million annually (Schrader *et al.* 2005). Hence, developing a method to eliminate off-flavours from fish is currently a necessity. On the basis of our results, we suggest that the first step in controlling off-flavours in fish is reducing the concentration of odorous compounds in water. Furthermore, it is preferable to reduce the growth of geosmin- or MIB-producing cyanobacteria in water bodies.

Isolation and identification of odour-producing strains in the Xionghe Reservoir

Two cyanobacterial strains were isolated from the Xionghe Reservoir. Planktonic *A. circinalis* (see Figure 7A), which produced a high concentration of geosmin–as detected by GC-MS–was isolated from the surface water during its bloom. Vegetative cells are 8–13 μm in diameter, spherical or oval, usually with gas vesicles. Heterocytes are 8–11 μm in diameter, spherical or nearly so, usually smaller than vegetative cells. Akinetes are cylindrical or sometimes curved when mature, 21–27 μm long, 15–20 μm wide, solitary and distant from heterocytes. Trichomes are free-floating and irregularly coiled. The shape of a terminal cell is rounded. We also isolated planktonic *Pseudanabaena* sp. (see Figure 7B) from the littoral zone water in May 2007; it was confirmed by GC-MS that this species produced 2-MIB. This isolate is filamentous. The filaments (trichomes) are solitary, straight or slightly arcuated, not very long, without any branch and sheath, and deeply constricted at cross walls. Cells are cylindrical, 5–7.5 μm long, 2.5 μm wide, sometimes with gas vesicles at the cross walls. The apical cell is cylindrical, with a cupola-like polar aerotope. The cell content is blue-green and homogeneous. Cyanobacteria, both planktonic and benthic forms, are known to produce geosmin or 2-MIB. Planktonic forms are known to be significantly involved in polluting water because they were already present in the water column (Izaguirre & Taylor 1998). Therefore, the above-mentioned isolates played considerably important roles in the off-flavour episodes of the Xionghe Reservoir.

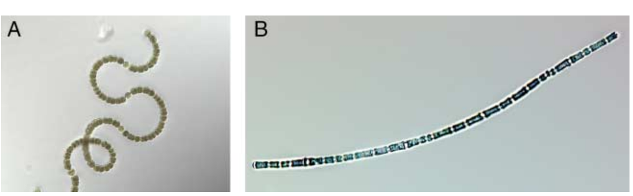

Figure 7 | Photomicrograph of *Anabaena circinalis* (×64, A) and *Pseudanabaena* sp. (×100, B).

Correlation between odorous compounds, algae and physicochemical parameters

Since the Spearman correlation coefficient, as a rank correlation coefficient, can reflect the direction and close degree of correlation between two variables which do not accord with the bivariate normal distribution, Spearman correlation analysis between the odorous compounds, algae and physicochemical parameters was made by SPSS 13.0 as shown in Table 1. The geosmin concentration was strongly correlated with the amount of *A. circinalis*; the correlation coefficients were 1.000 for Sites A, B and C ($P < 0.01$ for all). The presence of 2-MIB was also statistically correlated with the appearance of *Pseudanabaena* sp.; their correlation coefficients were 1.000 ($P < 0.01$ for all) for Sites A, B and C. On the basis of this strong relationship between *A. circinalis* and geosmin intensity as well as those between *Pseudanabaena* sp. and 2-MIB, we suggested that the cyanobacteria *A. circinalis* and *Pseudanabaena* sp. were the most likely and significant sources of geosmin and 2-MIB in the Xionghe Reservoir. Geosmin and 2-MIB were first isolated from actinomycetes (Gerber & Lechevalier 1965) and later evidenced in cyanobacteria (Safferman *et al.* 1967; Medsker *et al.* 1968; Kikuchi *et al.* 1973); however, cyanobacteria are known to be the main contributor to off-flavour episodes (Yagi 1988). Actinomycetes may contribute to off-flavour episodes in lakes, reservoirs and aquaculture ponds following cyanobacterial bloom die-offs (Schrader & Blevins 1999). In this study, off-flavour episodes occurred only from late spring through early autumn, which further demonstrates that cyanobacteria may be mainly responsible for the off-flavour episodes in the Xionghe Reservoir. Some other studies have also shown that off-flavour in surface water is caused by cyanobacteria. Vakkuri (1980) indicated an association between *Anabeana* sp. and the off-flavour in lake water. Persson (1982) found a correlation between earthy-musty odour and the amount of *Oscillatoria agardhii*. The cyanobacterium *O.* cf. *chalybea* has been found to contribute to 2-MIB production in

Table 1 | Correlation coefficients between odorous compounds, algae and physico-chemical parameters at Sites A, B and C ($n = 36$)

Spearman coefficient (*r*)	Cyanobacteria (cells/L)	*A. circinalis* (cells/L)	*Pseudanabaena* sp. (cells/L)
TN (mg/L)			
Site A	−0.364	−0.191	−0.441
Site B	−0.392	−0.449	−0.378
Site C	−0.252	0.491	0.105
TP (mg/L)			
Site A	0.046	0.208	0.592*
Site B	−0.028	0.546	0.098
Site C	0.028	0.399	0.410
DO (mg/L)			
Site A	0.021	0.295	0.573
Site B	0.035	0.508	0.154
Site C	−0.070	0.383	0.280
pH			
Site A	0.378	0.558	0.643*
Site B	0.133	0.316	0.294
Site C	0.140	0.129	0.389
Transparency (cm)			
Site A	−0.320	−0.203	−0.082
Site B	−0.390	0.257	−0.234
Site C	0.007	−0.057	−0.262
Temperature (°C)			
Site A	0.497	0.815†	0.664*
Site B	0.399	0.703*	0.217
Site C	0.441	0.657*	0.389
Geosmin (ng/L)			
Site A	0.800	1.000†	0.800
Site B	0.500	1.000†	0.500
Site C	0.500	1.000†	−0.500
2-MIB (ng/L)			
Site A	−0.500	−0.886	1.000†
Site B	−0.500	−0.926	1.000†
Site C	−0.500	−0.932	1.000†

*Significant correlation at the level of $P < 0.05$.
†Significant correlation ($P < 0.01$).

Mississippi Delta aquaculture ponds (van der Ploeg *et al.* 1995). Understanding the biological origins of odorous compounds is important for the prediction, control and management of off-flavour problems in the water body. Both *A. circinalis* and *Pseudanabaena* sp. are planktonic odour producers and thus readily detectable in water samples. In this special case that both *A. circinalis* and *Pseudanabaena* sp. are planktonic odour producers and strongly correlated with the concentration of geosmin and 2-MIB in the Xionghe Reservoir, it may be feasible to predict and control off-flavour problems by monitoring the variations of the doubted odour producers and their abundance. However, this method should be used carefully, since 2-MIB production by *Pseudanabaena* seems to have a strain-specific property (Izaguirre & Taylor 1998, Izaguirre *et al.* 1999).

Compared to rivers and natural lakes, reservoirs could be influenced by many artificial factors. The Xionghe Reservoir is also a key water-control project that intercepts the Xiong River. During mid July and early August 2007, it rained continuously for more than 10 days. In order to ensure safety, the sluice gate of the Xionghe Reservoir was opened, and water was released continuously for 14 days. The flow rate of the discharging water was approximately 800 m^3/s, and the total volume of the water released was approximately $5.0 \times 10^7\, m^3$, which was nearly 20% of the maximum storage capacity of the reservoir (this information was provided by the administrative office of the Xionghe Reservoir). Meanwhile, the equivalent amount of rainfall flowed into the reservoir. Therefore, the values of TN, TP, DO and algal abundance (particularly, cyanobacterial abundance) significantly decreased after the large-scale exchange of water, and the geosmin-induced off-flavour episode that occurred in July 2007 had abated. The reason why the off-flavour episode rapidly disappeared may be related to the following three aspects. Firstly, most of the algal abundance (including planktonic odour producers, *A. circinalis* and *Pseudanabaena* sp.) and odorous compounds in the surface water were discharged downstream. Secondly, the remaining small part of planktonic odour producers and their odorous compounds were further diluted by the large amount of rainfall. Lastly, as was shown in Figure 2, the sudden change of environmental factors (TN, TP, DO, pH, transparency and water temperature) between July 2007 and August 2007 after the large-scale exchange of water might inhibit the growth of odour-producing cyanobacteria and affect the seasonal variation of algae composition. Previous studies (Bowmer *et al.* 1992; Blevins *et al.* 1995; Saadoun *et al.* 2001; Zhang *et al.* 2009*b*) had found light intensities, temperatures and nutrients significantly influenced the growth and geosmin production of filamentous cyanobacteria, such as *Anabaena circinalis*, *Anabaena* sp. and *Lyngbya kuetzingii*. It seemed that 'exchanging water' may act as an effective method to mitigate and solve off-flavour problems in reservoirs.

CONCLUSIONS

This study clearly demonstrated that both geosmin and 2-MIB are present in the Xionghe Reservoir, as indicated by GC-MS analysis. Spearman correlation analysis revealed that the concentration of geosmin was significantly associated with the abundance of *A. circinalis*, while that of 2-MIB was significantly associated with the amount of *Pseudanabaena* sp. Hence, we believe that the biological origins of geosmin and 2-MIB in the Xionghe Reservoir surface water may be *A. circinalis* and *Pseudanabaena* sp., respectively. The concentration of geosmin in phytophagous silver carp was higher than that in omnivorous crucian carp; this finding indicated that the bioaccumulation of odorous compounds in fish was significantly associated with the feeding behaviour of fish.

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