Correlating 2-MIB and microcystin concentrations with environmental parameters in two reservoirs in south Taiwan

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Abstract Cyanobacteria are present in many drinking water reservoirs in Taiwan, and some of them may produce off-flavour compounds and natural toxins. To investigate the correlation among two groups of cyanobacterial metabolites, microcystins and 2-methylisoborneol (2-MIB), and other environmental parameters, approximately 22 water quality and meteorological parameters were monitored for two source waters (Moo-Tan and Tseng-Wen reservoirs) in south Taiwan from August 2003 to April 2005. Monitoring results showed that the two groups of cyanobacterial metabolites were present in the source waters. Concentrations of 2–30 ng/L of 2-MIB was observed for the two reservoirs, while that of the total concentrations of the five microcystin congeners measured were between 30 and 340 ng/L. The concentration of both 2-MIB and microcystins showed higher concentrations in warmer seasons. A stepwise regression technique was employed to correlate 2-MIB and microcystins concentrations with all the corresponding water quality and meteorological parameters. Correlations among 2-MIB concentration, microcystin concentration, water temperature and air temperature were found in the water samples collected from both reservoirs. The correlations may provide a simple means for the water utility to anticipate the two groups of cyanobacterial metabolites in the two source waters.

Keywords Drinking water; 2-methylisoborneol; microcystins; temperature

Introduction

The presence of Cyanobacteria which produce toxic and off-flavour compounds in drinking water sources and recreational waters has received increasing attention worldwide. This Cyanobacteria–related problem commonly occurs in the more eutrophic reservoirs and lakes (Haider et al., 2003). The Carlson’s Trophic State Index (CTSI) (Carlson, 1977) is often used as an indication of trophic state of a waterbody. It is calculated from chlorophyll-a, Secchi Disk depth or total phosphorus, based on empirical equations. An index value of 0 to 100 is possible for a lake, and a lake with CTSI > 50 is characterised as eutrophic. According to the monitoring results of 2000 to 2004 from Taiwan Environmental Protection Administration (TWEPA, 2005), approximately half of Taiwan’s 20 major reservoirs were categorised as eutrophic based on the CTSI. In Cyanobacteria-laden drinking water reservoirs, two major groups of cyanobacterial metabolites, off-flavour compounds and natural toxins, are commonly observed. Among the cyanobacterial metabolites, the most prevalent odourant in the Taiwanese drinking water reservoirs is 2-methylisoborneol (2-MIB), while the most abundant cyanotoxins are microcystins (Lin and Tseng, 2005).

The growth of cyanobacteria (in particular, the genus Microcystis) has been linked to several water quality and meteorological parameters, including nutrient content,
irradiance and water temperature (Falconer, 2005). These factors may influence the cell numbers of cyanobacteria, and the generation of their metabolites in the cells and water. In addition, the concentrations of several odour compounds, such as geosmin, β-ionone, and β-cyclocitral, have been linked to the presence of *Microcystis* and *Anabaena* (Jones and Korth, 1995). These compounds may be used as biomarkers for the possible presence of potentially toxic cyanobacteria species; however, it should be noted that many non-toxic cyanobacteria produce these odour compounds, and conversely, toxin-producing strains are often odourless. The two most commonly observed musty/earthy odour compounds, geosmin and 2-MIB, can sometimes co-occur with microcystins in bloom samples (Carmichael, 2001). Therefore, there is some potential to examine patterns in cyanobacterial toxins, some off-flavour compounds, and environmental parameters. In this study, the two major groups of cyanobacterial metabolites, microcystins and 2-MIB, and 22 other water quality and meteorological parameters were collected for two source waters (Moo-Tan and Tseng-Wen reservoirs) in south Taiwan for nearly 2 years. These parameters were then analysed for correlations. The results of this study may help predict the presence of cyanotoxins and off-flavour compounds in the source waters.

**Materials and methods**

**Site description**

Both Tseng-Wen Reservoir (TWR) and Moo-Tan Reservoir (MTR) are located in south Taiwan, and are important source waters in south Taiwan. TWR is the largest reservoir in Taiwan, with a storage capacity of $7.0 \times 10^9$ m$^3$ and an area of $1.7 \times 10^6$ m$^2$. For MTR, the storage capacity and area are $3.1 \times 10^7$ m$^3$ and $1.4 \times 10^6$ m$^2$, respectively. The mean residence time of the water is approximately 3 years for TWR, and approximately 0.25 year for MTR.

The reservoirs were monitored for major water quality parameters seasonally by Taiwan Environmental Protection Administration (TWEPA). Based on the monitoring data of TWEPA (2005), the average (and standard deviation) of the major water quality measured at 0.5–1 m below water surface for MTR between 2000 and 2004 are 20.6 ($\pm$ 8.4) µg/l for total phosphorus (TP), 3.4 ($\pm$ 4.8) NTU for turbidity, 1.9 ($\pm$ 0.6) m for Secchi Disk depth (transparency; SD), and 7.5 ($\pm$ 6.0) µg/l for chlorophyll-a (chl$\alpha$). Those for TWR are 30.9 ($\pm$ 37.5) µg/l TP, 4.5 ($\pm$ 5.9) NTU, 1.9 ($\pm$ 0.8) m SD, and 4.8 ($\pm$ 3.2) µg/l chl$\alpha$. Based on the monitored data of TP, transparency and chl$\alpha$, the average Carlson Trophic State Index (CTSI) may be calculated. The CTSI from 2000 to 2004 is between 45 and 50 for TWR, and is between 45 and 52 for MTR. Based on this, both reservoirs may be categorised as meso-eutrophic. The sampling locations for both reservoirs were near the water surface (0.5 m below the surface) above their water intakes. The water samples were collected approximately once a month from August 2003 to April 2005, normally between 10–11 am. Amber glass bottles of 0.25 L were used for the collection of water samples for 2-MIB and geosmin, and 2-L plastic bottles were used for microcystins. All the samples were collected without headspace and were kept at 4°C before analysis. The samples were pre-treated using ultrasound and followed by solid phase extraction within 48 hours for toxin determination, and were analysed for 2-MIB and geosmin mostly within 1–3 days and in 7 days for only a few cases.

**Chemicals**

Standards of microcystin-LR were purchased from Supelco (analytical grade, USA) and other microcystins (YR, RR, LF, and LW) from End Biosciences (analytical grade, USA). The analytical standard for 2-MIB was purchased from Sigma (analytical grade, Germany) at a concentration of 2 mg/l, while that for geosmin was obtained from Wako
(analytical grade, Japan) at 0.1 mg/l. To prepare calibration standards, the toxins were dissolved into methanol, and then diluted with the reagent water from a Mili-Q water purification system (Millipore Corp., Bedford, MA, US) to pre-determined concentrations. For 2-MIB calibration standards, Milli-Q water was used to dilute the analytical standards to pre-determined concentrations.

Extraction of microcystins
For the extraction of microcystins, water samples (1.5 L) were first ultrasound (Digital Sonifier, Branson, US) at 4°C for the breakage of cells. The samples were then filtered through a 47-mm, 0.2 μm (mixed cellulose ester) membrane filter (Advantec MFS, USA). The filtrate was then passed through a solid phase extraction (SPE) cartridge (Supelco, USA) packed with 1 gram of C18 for the concentration of toxins. Five mL of methanol (90%, Chromatography Grade, Merck, US) were then used to desorb the trapped microcystins on the SPE cartridge. The microcystin-methanol mixture was then purged with high purity nitrogen until a volume of 200 μl was reached. The mixed solution was used for analysis.

Analysis of microcystins
Analysis of microcystins was conducted with a liquid chromatography (LC) (model LC-10ADvp, Shimadzu, Japan) equipped with a photodiode array detector (PDA) (model SPD-M10Avp, Shimadzu, Japan) and electrospray ionisation mass spectrometer (ESI-MS) (model LCMS-2010EV, Shimadzu, Japan). A reverse C18 column (TSK-GEL ODS-100S) (250 × 4.6 mm, Tosoh, Tokyo, Japan) was selected to separate microcystins using a gradient formula. Acetonitrile (eluent 1) and 20 mM of ammonium formate (eluent 2) were used as the elution solution. Both eluents contain 50 mM of formic acid. The flow rate was set at 0.7 ml/min, and the gradient was generated using eluent 1 and eluent 2. Starting with 10% eluent 1 and 90% eluent 2, the ratio of eluent 1 was increased linearly to 50% within 35 min, maintained for 10 min, and decreased linearly to 10% within 5 min. After 50 min, the LC system was ready for the next injection. The toxins were quantified and confirmed using MS with selected ion monitoring (SIM) mode and PDA in the analysis. The ion spectra can be found in Lin and Tseng (2005). For all the analysis, the oven temperature (Model CTO-10Avp, Shimadzu, Japan) was maintained at 40°C. The method detect limit for the five MCS (-LR, -RR, -YR, -LW and –LF) for MS (under SIM mode) were approximately 2–10 ng/L. In the analysis, quality control (QC) samples were also prepared and analysed for each batch of samples. The QC results show that duplicates were all <30%, laboratory control standards were within 91–109%, and laboratory fortified matrix standards were within 83–120%.

Analysis of 2-MIB and geosmin
The concentrations of 2-MIB and geosmin were analysed using head-space solid-phase microextraction (HSPME) coupled with a gas chromatograph (GC, HP-6890) and a mass spectrometer (MS, HP-5973). The procedure for the analysis of 2-MIB and geosmin was the same as described in Lin et al. (2002, 2003). Quality control (QC) samples were also prepared and analysed. The QC results show that duplicates (<12%), laboratory control standards (80–120%), and laboratory fortified matrix standards (83–120%) were all within reasonable ranges.

Analysis of water quality and collection of meteorological parameters
Water quality parameters, including ammonium, nitrate, nitrite, phosphate, pH, suspended solid (SS), hardness, sulphate, turbidity, biological oxygen demand (BOD), chemical
oxygen demand (COD), total organic carbon (TOC), chloride, iron, magnesium, pH, chlorophyll a, conductivity, transparency and water temperature, were also monitored for the two reservoirs. The analytical methods were either according to the corresponding standard methods suggested by National Institute of Environmental Analysis (NIEA) of Taiwan EPA or APHA et al. 1995. In addition, three meteorological parameters, namely average daily (air) temperature, solar radiation and precipitation, were also collected from two local meteorological stations. The station for TWR is right beside the reservoir, and that for MTR is approximately 10 km from the reservoir.

Results and discussion

Long-term monitoring results

The concentration changes of the two groups of cyanobacterial metabolites, along with water temperature at the sampling time, are illustrated in Figure 1. The total microcystin concentration (LR, YR, RR, LF and LW) was between 10 and 250 ng/l for MTR, and between 10 and 340 ng/l for TWR. These were well below the World Health Organisation guidelines for drinking water of 1.0 μg/l. For 2-MIB, concentrations of 2–30 ng/l were observed for TWR, while much lower concentrations, often below 5 ng/l, were measured in Moo-Tan Reservoir. Another earthy odorant, geosmin, was mostly below detection limit (approximately 0.5 ng/l) in these two reservoirs. As shown in the figure, the concentration of both 2-MIB and microcystins followed the trend that higher concentrations were present in warmer seasons for both reservoirs. This may reflect that temperature may be an important factor governing the production of these two metabolites.

To examine the relationships among the microcystins, 2-MIB, geosmin and other water quality and meteorological parameters collected, a stepwise regression technique was employed (Garcia-Villavova et al., 1997). In this analysis, microcystins and 2-MIB concentrations were assumed to be dependent variables, and other parameters were assumed to be independent variables. To perform the regression, both the analysis of variance (ANOVA) and t test were employed using the Statistical Products and Service Solution (SPSS) software (Chicago, IL, US). Note that air temperature was not included in the analysis, as it is highly correlated with water temperature (see next section). The regression coefficients (R²) for the regression models selected are listed in Table 1. Most of the parameters were not selected into the regression models, except water temperature, conductivity, pH, sulphate, DO, nitrate, TOC and transparency. In fact, as shown in the table, water temperature was found to be the strongest predictor of the presence of both microcystins and 2-MIB concentrations in TWR (MC-1, MIB-1; Table 1), although it accounted for only ~50% of the variance. Water temperature was also the parameter most strongly correlated to microcystins concentrations in MTR. The addition of 2-MIB

![Figure 1](https://iwaponline.com/wst/article-pdf/55/5/33/439486/33.pdf)
### Table 1 Stepwise regression models for the microcystins and 2-MIB concentration, and water quality and meteorological parameters

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Number of samples</th>
<th>Correlation number</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moo-Tan</td>
<td>17</td>
<td>MC-1: MCs = -258.2 + 14.7 WT</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-2: MCs = -475.3 + 13.3 WT + 1.0 Con.</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-3: MCs = -728.9 + 11.5 WT + 0.8 Con. + 44.3 pH</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-4: MCs = -819.0 + 10.8 WT + 0.5 Con. + 49.3 pH + 10.2 Sul.</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-1: MIB = 1.5 + 2.2 N1</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-2: MIB = -1.8 + 2.1 N1 + 209.2 TP</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-3: MIB = -48.7 + 1.9 N1 + 324.7 TP + 5.5 pH</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-4: MIB = -52.8 + 1.9 N1 + 236.4 TP + 6.1 pH + 0.1 Tur.</td>
<td>0.95</td>
</tr>
<tr>
<td>Tseng-Wen</td>
<td>19</td>
<td>MC-1: MCs = -193.0 + 11.9 WT</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-2: MCs = -356.9 + 19.9 WT + 5.8 MIB</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-3: MCs = -481.0 + 13.6 WT + 3.7 MIB + 35.4 DO</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-4: MCs = -670.2 + 12.8 WT + 3.5 MIB + 54.5 DO + 85.7 N1</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MC-5: MCs = -467.9 + 7.6 WT + 3.9 MIB + 34.2 DO + 78.4 N1 + 61.5 TOC</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-1: MIB = -28.3 + 1.38 WT</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-2: MIB = -34.5 + 1.77 WT + 0.032 MCs</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIB-3: MIB = -24.5 + 0.9 WT + 0.6 MCs + 3.2 Tra.</td>
<td>0.81</td>
</tr>
</tbody>
</table>

MCs = total microcystin (ng/l), MIB = 2-MIB (ng/l), WT = water temperature (°C), Con. = conductivity (μmho/cm), DO = dissolved oxygen (mg/l), N1 = nitrate (mg N/l), sulphate = sulfate (mg/l), TOC = total organic carbon (mg/l), Tur. = turbidity (NTU), Tra. = Secchi depth transparency
into the model for TWR improved the regression marginally (R² of 0.48 and 0.56 respectively; MC-2, Table 1). Although the cyanobacteria that produce microcystins and 2-MIB may not be the same, the production of these two metabolites both increased with higher temperatures in Tseng-Wen Reservoir. On the other hand, 2-MIB concentrations in MTR were mostly very low (often <5 ng/l), and weakly correlated to nitrate, TP, turbidity and pH. It is difficult to further analyse the 2-MIB data for MTR. Therefore, 2-MIB data were only analysed for TWR, while microcystins data were analysed for both reservoirs.

Figure 2 shows the observed concentrations versus the estimated concentrations from the simple correlations for microcystins and 2-MIB for TWR. In each of the correlations, only one or two independent parameters are considered, as MC-1, MC-2, MIB-1 and MIB-2 listed in Table 1. Figure 2(a) and (c) suggested that the general concentration trends for both microcystins and 2-MIB were able to be captured by the simple correlations. With one more parameter added into the correlations, Figure 2(b) and (d) suggested that the discrepancy between observed and estimated concentrations became smaller.

Water temperature and air temperature
Water temperature was shown to be the most important factor relevant to 2-MIB concentration in TWR, and to microcystins concentrations in the two reservoirs monitored. However, water temperature is not necessarily measured for the reservoirs all the time, and the data are not as accessible as those of air temperature. Mean daily (air) temperature is a standard measured item in every meteorological station and the data can be easily downloaded from the website of the meteorological agency in Taiwan. Therefore, it would be easier if air temperature can replace the water temperature as the input parameter for the regression models. To evaluate if water temperature data collected during...
sampling can be linked to the air temperature collected from the corresponding meteorological stations for both TSR and MTR, a linear regression was performed. Very good correlation was observed for both reservoirs, $R^2 = 0.77$ for TWR and $R^2 = 0.94$ for MTR. These high correlation coefficients indicate that the mean daily temperature may be related to the appearance of toxins and 2-MIB.

Correlation among 2-MIB, microcystins and mean air temperature

The concentration of cyanobacterial metabolites may be considered to reflect the growth history of cyanobacteria. Therefore, a change of environmental condition may cause the change of metabolite concentrations. As water temperature and air temperature may have good correlation with cyanobacterial metabolites concentrations, an analysis of temperature history with the concentration of microcystins was conducted. In the analysis, the data of mean daily (air) temperature from 1 to 20 days before the sampling time were selected to represent the temperature history in the reservoirs. For the correlation with cyanobacterial metabolites, the average of 1 to 20 days of mean daily temperature in the data was used.

Figure 3 illustrates the regression coefficient ($R^2$) of the correlation between different average mean daily temperatures with microcystins in TWR and MTR. In the figure, the first day (horizontal or X axis) and the last day (vertical or Y axis) represent the time span of mean daily temperature used in the analysis. For example, for the case of $X = 5$ days and $Y = 10$ days, it represents an average of 6 days of mean daily temperature, from 5 to 10 days before sampling. The numbers shown in the contours are the $R^2$ values.

As illustrated in Figure 3(a) for TWR, the regression coefficient ($R^2$) for the case of microcystins was found to be highest in the upper left corner of the plot, implying that the starting time should be close to the sampling time and the average of temperature history should be long. In fact, the highest regression coefficient was found for an average of 18-day temperature history starting from one day before sampling, i.e. $(X = 1, Y = 18)$. A similar pattern was also found for 2-MIB in TWR (data not shown). For MTR (as in Figure 3(b)), a different time span was found for the highest $R^2$ value, at around $X = 1$ and $Y = 14$. This may imply that different reservoirs may have different metabolite production characteristics.

The extracted average mean daily air temperature (with highest $R^2$) for both reservoirs was then plotted with the two groups of cyanobacterial metabolites. For TWR, an average
temperature taken over the period 18 days before sampling was used, and for MTR, an average was taken over 14 days before sampling was used. Figure 4 demonstrates the correlations for air temperature and the two groups of cyanobacterial metabolites. Although other environmental conditions may affect microcystins and 2-MIB concentrations in the water, it is shown in the figure that 2-MIB and microcystins concentrations in the two water sources were correlated with the history of air temperature during the time period of this study. These correlations may have a potential to provide a simple means for the water utility to roughly estimate the two cyanobacterial metabolites in the two source waters.

Summary and conclusions
Microcystins and 2-methylisoborneol (2-MIB), and 22 other water quality and meteorological parameters were monitored for Tseng-Wen and Moo-Tan Reservoirs in south Taiwan from August 2003 to April 2005. Both microcystins and 2-MIB were mostly observed at low concentrations in the two reservoirs. A concentration of 2 to 30 ng/l of 2-MIB was observed for the two reservoirs, while that of the summation of the five microcystins was between 30 and 340 ng/l. A stepwise regression technique was employed to correlate 2-MIB and microcystins concentrations with all the water quality and meteorological parameters. The water temperature was correlated to microcystins concentrations for both reservoirs, and to 2-MIB concentration in Tseng-Wen Reservoir only. The relationship between the concentrations of the two cyanobacteria metabolites and the length of the preceding period over which the average mean air temperature was calculated was examined for both reservoirs. It was found that microcystins and 2-MIB concentrations in TWR and MTR were best predicted by periods of 18 and 14 days,
respectively, likely reflecting the differences in size and residence time of the two systems. These correlations may have a potential to be used to roughly estimate the two groups of cyanobacterial metabolites in the two source waters.

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