Managing taste and odour metabolite production in drinking water reservoirs: The importance of ammonium as a key nutrient trigger


Keywords: Taste and odour, Reservoir, Catchment management, Drinking water

ABSTRACT

Taste and odour (T&O) compounds (most commonly 2-MIB and Geosmin) in drinking water are becoming an increasingly global problem for water management. Here, the trigger(s) for 2-MIB and Geosmin production were investigated in Plas Uchaf reservoir (North Wales, UK) with detailed water sample analysis between 2015 and 2016. Historical abstraction data from this reservoir and 4 reservoirs in Somerset (England, UK) were compared statistically using Self-Organising Map (SOM) analysis. In-reservoir measurements (2015–2016) revealed an 85% reduction in ammonium from the primary external loading source led to lower 2-MIB and Geosmin concentrations, with peak concentrations of 2-MIB declining from 60 to 21 ng l\(^{-1}\) and Geosmin declining from 140 to 18 ng l\(^{-1}\). No other measured water chemistry parameter showed a significant difference between years. The SOM results support the in-reservoir findings, revealing 2-MIB and Geosmin to be associated with high ammonium relative to nitrate for all 5 reservoirs. We conclude that ammonium is key for stimulating cyanobacterial productivity and production of T&O compounds. Whilst it is well understood that adequate availability of phosphorus is required for rapid growth in cyanobacteria, and hence should still be considered in management decisions, we suggest that monitoring sources and concentrations of ammonium is key for managing T&O outbreaks in drinking water reservoirs.

1. Introduction

Taste and odour (T&O) problems in drinking water supply associated with the secondary metabolites 2-Methylisoborneol (2-MIB) and 1,10-dimethyl-trans-9-decalol (Geosmin) are increasing in frequency and magnitude globally (Winter et al., 2011). 2-MIB and Geosmin are naturally occurring tertiary alcohols that produce strong musty and earthy odours (Rogers, 2001), and impart unfavourable tastes in naturally occurring tertiary alcohols that produce strong musty and earthy odours (Rogers, 2001), and impart unfavourable tastes in

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A wide range of organisms have been recognised as producers of 2-MIB and Geosmin, including fungi, actinomycetes, phytoplankton, and higher plants (Jüttner and Watson, 2007). In drinking supply reservoirs, 2-MIB and Geosmin are commonly reported and the primary source of these compounds are considered to be cyanobacteria (Suffet et al., 1996; Ho et al., 2007; Sun et al., 2014). T&O producing cyanobacteria can be planktonic (Jüttner and Watson, 2007), or form benthic biofilms
in shallow regions (Watson and Ridal, 2004).

Historically, water utilities have deemed high phytoplankton biomass to be a major problem in drinking supply reservoirs as they cause filter clogging and other treatment issues, which has led to a general assumption that high cyanobacterial cell counts result in high concentrations of T&O compounds in the water. A recent review paper by Watson et al. (2016) highlights how there is currently a reactive approach within the water industry for addressing T&O outbreaks in supply. Primarily confounding factors are to blame for the reactive nature, such as a lack of understanding of 2-MIB/Geosmin production; a lack of diagnostic tools; and the increasing magnitude of the problem (Harris et al., 2016; Watson et al., 2016). There is a need to understand the key trigger(s) in production of 2-MIB and Geosmin in order to inform better management practices.

2-MIB and Geosmin exist as a monoterpen and a sesquiterpen, respectively (Bentley and Meganathan, 1981). They appear to be precursors for important cellular compounds such as sterols and pigments, although the biological use of 2-MIB and Geosmin within cells is not fully understood. 2-MIB and Geosmin are produced along the metabolic pathways for isoprenoid synthesis: the 2-methylerythritol-4-phosphate isoprenoid (MEP) pathway, the mevalonate (MVA) pathway, and Leucine pathway (Jütter and Watson, 2007). The MEP pathway has been found to dominate during the rapid growth phase in actinomycetes (Seto et al., 1996), and high 2-MIB concentrations have been reported during periods of high Adenosine triphosphate (ATP) synthesis (Behr et al., 2014). The MEP pathway is considered an alternate, or concomitant pathway to the MVA and Leucine pathways, all of which are found in cyanobacteria. Periods of high productivity have been associated with high 2-MIB and Geosmin production in cyanobacteria (Zimmerman et al., 1995). The production of 2-MIB and Geosmin during periods of high productivity could explain how some studies fail to see a correlation between 2-MIB/Geosmin concentrations and phytoplankton biomass (Graham et al., 2010) and why caution needs to be taken using water quality models, for example Chong et al. (2018), for the abstracted raw water samples.

Here, we investigated at small spatial and temporal scales, variation in 2-MIB and Geosmin in a well-studied water supply reservoir, Plas Uchaf, in North Wales, UK, (53°13′46.0446″N, 3°32′48.9546″W; Fig. 1a). In 2015, 2016 a detailed sampling programme of 5 sample points within Plas Uchaf was initiated. Sampling included a single sample point at a feeder site, Dolwen Reservoir, situated approximately 1 km to the south of Plas Uchaf. Historical abstraction data were also collected from Plas Uchaf prior to 2015, which included the measurement of 2-MIB and Geosmin. These data were used to analyse potential trigger(s) for 2-MIB and Geosmin production that had been attributed to cyanobacteria frequently observed in the reservoirs, namely Oscillatoria spp. and Dolichospermum spp. (formerly Anabaena). The same analysis was applied to similar datasets from Chew, Cheddar, Blagdon, and Dolurch reservoirs, all located in Somerset, UK (Fig. 1b), using Self-organising map (SOM) analysis. The aim of the study was to determine the trigger(s) of 2-MIB and Geosmin production in water supply reservoirs and understand nutrient dynamics and sources that affect the production of these metabolites in cyanobacteria in order to better advise the Water Industry on monitoring and management to mitigate T&O events.

2. Methods

2.1. Study sites

Plas Uchaf is managed by Dŵr Cymru Welsh Water (DCWW). It is fed by Dolwen reservoir (Fig. 1a) and both sites are in an upland catchment comprising low density habitation and livestock farming. Dolwen reservoir is fed by groundwater supply and three small tributaries. There are no other point source inflows to Plas Uchaf, other than the primary pumped input abstracted from the River Aled, 1.5 km to the west. Five locations within Plas Uchaf and one site in Dolwen Reservoir (Fig. 1a) were sampled regularly between 2015 and 2016, enabling spatial and temporal analysis of T&O production in the reservoir.

All study sites have separate, primarily agricultural catchments. Chew Valley (51°21′3.67″N, 2°37′57.68″W) and Blagdon (51°20′15.82″N, 2°42′45.40″W) are both lowland reservoirs, managed by Bristol Water. Both are river fed, Chew from multiple small rivers, and Blagdon mainly from the River Yeo with multiple smaller inflows. Cheddar reservoir (51°17′0.58″N, 2°48′30.66″W) is pumped with water taken from the Cheddar Yeo river and is managed by Bristol Water. Durleigh is a lowland reservoir (managed by Wessex Water) fed by a small stream and occasionally pumped with water from the Bridgwater and Taunton Canal, which enters the reservoir via a culvert on the southern bank. All sites included in this study cover a range of trophic states (mesotrophic to hypereutrophic) and different locations to provide evidence for T&O metabolite production more generally than at specific sites.

2.2. Sampling methods

Raw water abstracted from Plas Uchaf was sampled weekly for analysis of biological, physical and chemical variables. Historical data were analysed for 2-MIB, Geosmin, nitrate, Total Nitrogen (TN), and Total Phosphorus (TP) for the period 2012 to the end of 2016. For the other four reservoirs, raw water abstracted from the intake was sampled weekly from 2014 to the end of 2017 (instead of TP, Orthophosphate (OP) was analysed in Bristol Water reservoirs).

In 2015, routine monthly water sampling within Plas Uchaf and Dolwen reservoirs was initiated and data are presented comparing 2015 and 2016. Sampling consisted of obtaining surface water samples (in triplicate, 1 L volume), collected in acid washed polyethylene plastic bottles. These samples were analysed for the same suite of variables as for the abstracted raw water samples.

All water samples for Plas Uchaf were processed at the DCWW Glaslyn laboratories, and Chew, Cheddar, and Blagdon samples were analysed by ALS environmental (ALS). Raw water samples abstracted from Durleigh Reservoir were processed at the Wessex Water (WW) Scientific Centre. All laboratories and all methods listed in Table 1 are UKAS (United Kingdom Accreditation Service) accredited, and all laboratories follow quality assurance procedures, so comparability of data between laboratories is considered reliable. All measured parameters, and the methods and equipment used in analysis are listed in Table 1.

2.3. Statistical analysis

Plas Uchaf data were analysed using the freely available statistical software package PAST (Hammer et al., 2001). Nutrient data were normal (Shapiro Wilks test) and of equal variance (Levene's test) and were analysed for significant difference between sample points (factor 1) sites and year (factor 2) using two-factor Analysis of Variance (ANOVA). Regression analysis was performed to investigate the spatial and temporal relationships between 2-MIB and Geosmin with ammonium concentration after determination of a significant difference in these variables between sample points.

The historical abstraction data (2014–2017) from all reservoirs were cleaned by removing data points for measurements at the limit of detection (LOD) and normalised to values between 0 and 1. For each reservoir and each site within Plas Uchaf, a Self-Organising Map (SOM) was used for dimensionality reduction to identify the principal variables in T&O production. This comparatively recent addition to large neural network models that employ unsupervised, competitive learning to transform high-dimensional data into a discrete lattice of neurons/nodes (Uriarte and Martin, 2005; Skupin and Agarwal, 2008). Within this neural network of spatial
mapping, clusters are defined by inputs that share common features. In mathematical terms, networks learn to form clusters from the training data without help, and the process is henceforth referred to as unsupervised learning. Competitive learning refers to output neurons competing against each other to be activated, as only one neuron can be activated at a time. The best matching unit (BMU), or winning neuron, is the neuron whose weight vector most closely matches the input vector based on the Euclidean distance between them (Bullinaria, 2004). The BMU weighting is iteratively updated and hence neurons surrounding the BMU are updated respectively, but to a lesser extent as the competitive learning process proceeds. Self-organisation is completed once the updated weights for neurons stabilise with respect to

Table 1
List of parameters, methods, and equipment used. Reference for different laboratories: ALS Environmental (ALS), Welsh Water (DCWW), and Wessex Water (WW).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Equipment</th>
</tr>
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<tbody>
<tr>
<td>Total Oxidised Nitrogen (TON)</td>
<td>Colorimetry</td>
<td>Thermo Scientific Aquakem 600 spectrophotometer</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Colorimetry then TON minus nitrate</td>
<td></td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>Extracted using solid phase extraction (SPE) and eluted using Dichloromethane. Analysed by Gas chromatography and mass spectrophotometry (GC/MS/MS)</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
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</tr>
<tr>
<td>Nitrate</td>
<td></td>
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<tr>
<td>2-MIB</td>
<td>Extracted using solid phase extraction (SPE) and eluted using Dichloromethane. Analysed by Gas chromatography and mass spectrophotometry (GC/MS/MS)</td>
<td></td>
</tr>
<tr>
<td>Geosmin</td>
<td>Extracted using methanol and analysed spectrophotometrically (DWCC and ALS)</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-α</td>
<td>Extracted using methanol and analysed spectrophotometrically (DWCC and ALS)</td>
<td></td>
</tr>
<tr>
<td>Total Phosphorus (TP)</td>
<td>Inductively coupled plasma mass spectrometry (ICP-MS)</td>
<td></td>
</tr>
<tr>
<td>Algal cell counts</td>
<td>Fixed with Lugol's and placed in sedimentation chamber. Identification and enumeration using an inverted microscope (DWCC and ALS)</td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>Tubidimeter</td>
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Fig. 1. (a) Map of Plas Uchaf and Dolwen reservoirs, North Wales, UK, showing sample points (SPT) 1–6 used in this study and the two main inflows into Plas Uchaf (Dolwen surface flow and River Aled pumped input). (b) Orientation of all reservoirs in SW England and Wales, UK. Plas Uchaf (green) is located in North Wales and the other 4 reservoirs are located in Somerset, UK. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
the input vector, creating the topologically ordered map where the spatial location of a neuron in the output space corresponds to a particular feature of the input space (Bullinaria, 2004). The process is repeated for randomly selected inputs over many training epochs.

Using the SomToolbox MATLAB package from the Helsinki University of Technology (Vestano and Alhoniemi, 2000), each SOM was trained with the batch-training algorithm som.make. In the SOM topology, patterns of the input variables are displayed on component planes of a 5x2 hexagonal-node grid, which was selected as the optimal size when considering the topographic and quantization errors (Uriarte and Martín, 2005). SOM component planes can be regarded as an analogue of PCA loading plots. Each node represents a certain position in the map space and is consistent across all component plots. Colours in the component plots show the values that variables have in the map structure, with yellow indicating higher values and blue lower. The similarity between neighbouring nodes is highlighted in the U-matrix, which is weighted according to Euclidean distance and indicated by colours on the map (Park et al., 2004), where blues designate smaller distances (similarity) and yellows larger (difference). The som.varplane function with ‘varwise’ scaling, was used to visualise the distribution of parameters for each weight vector in the output map. Bar plane plots rank the variables according to their perceived relative importance for each node. Clustering was performed using the k-means algorithm for each SOM topology (kmeans_clusters function). K-means is a standard partitioning clustering approach that minimises the sum-of-squared-error between points in the clusters and their corresponding cluster centroids (Selim and Ismail, 1984). Significant differences between clusters was assessed using single-factor ANOVA and post hoc Tukey’s test in PAST.

3. Results

At the Plas Uchaf intake, 2-MIB concentrations peaked in late summer of 2014 and July 2015 and Geosmin concentrations were highest in summer 2015 (Supplementary Data Fig. S1). Lower peaks in both compounds were observed in 2016. The highest concentrations of 2-MIB and Geosmin were 59 and 140 ng l\(^{-1}\) respectively at sample point 5 (SPT5) in 2015, compared to 21 and 17 ng l\(^{-1}\) in 2016 at the same site.

Analysis of water samples from the five sites within the reservoir showed clear seasonal patterns in 2015 and 2016, with an inverse relationship between 2-MIB (Fig. 2a) and Geosmin (Fig. 2b) concentrations and TN:TP ratio. Changes in TN:TP were largely driven by decreases in TN caused by a summer decline in nitrate concentration (data not shown), which was attributed to denitrification and biological uptake by planktonic and benthic algae and cyanobacteria. Nitrite concentrations at all reservoirs studied were frequently (~60% of data) below the level of detection, with values when recorded always less than 5% of total oxidised nitrogen (nitrate and nitrite) and were hence not considered further. The same relationship between TN:TP and 2-MIB/Geosmin concentrations was observed in Durleigh from 2013 to 2017 (Supplementary Fig. S2), although the inverse relationship is much clearer for 2-MIB concentrations compared to Geosmin. The changes in TN:TP at Durleigh appear to be driven by both summer decline in nitrate and concomitant increase in TP.

In Plas Uchaf, concentrations of 2-MIB and Geosmin showed clear spatial and temporal variation at the five sites within the reservoir. The variation is driven by a strong positive correlation with the spatial pattern of ammonium concentration (Supplementary Fig. S3). Both 2-MIB and Geosmin were highest at sites with greatest ammonium concentration in 2015 (Supplementary Fig. S3) but were lower in 2016 when ammonium concentrations were also low (< 0.1 mg l\(^{-1}\)). There was a significant decrease in ammonium concentration at all five sample points within the reservoir between 2015 and 2016 (Fig. 3). The largest decrease (85%) in the average ammonium concentration was observed in Dolwen reservoir adjacent to the compensation flow to Plas Uchaf (Fig. 3). No other measured water chemistry variable showed significant differences between the five sample points or between 2015 and 2016 (Fig. 4). TP showed variation at sites 1 and 2 in 2016 (Fig. 4B) due to higher concentrations in late summer, but these were not significantly different to 2015 concentrations or between sample points.

Cell counts for planktonic cyanobacteria showed a dominance in Dolichospermum spp. (formerly Anabaena spp.), with low counts of Oscillatoria spp. and Microcystis spp. as the only other cyanobacteria taxa recorded. Cyanobacteria cell counts accounted for over 95% of the total algal cell counts (data not shown) in 2015 and 2016. Anabaena accounted for over 80% of these cell counts and was more abundant in 2015 than 2016. It was noted that Oscillatoria sp. was observed to form extensive benthic mats at sample point 5 (a shallow area < 2 m deep), adjacent to the inflow from Dolwen (Andrade and Parslow pers. obs.), however no benthic samples were counted. Dolichospermum were the dominant cyanobacterial taxa observed in Blagdon (132 observations between 2014 and 7). In Durleigh Planktothrix was the most frequently observed taxa, but many other known T&O producing taxa have been observed including, but not limited to: Dolichospermum, Anphanizomenon, and Aphanocapsa. Counts of Planktothrix in Durleigh have increased since 2015. In 2017, Planktothrix accounted for on average 75% of the total algal cell counts in Durleigh (data not shown).

Here, we focus on the Self-Organising Map (SOM) results for sample points 1 and 5 (SPT1 and 5) in Plas Uchaf. SPT5 was selected as it is the site closest to the Dolwen inflow and the site most influenced by the drop in ammonium concentrations between 2015 and 2016. SPT1 is the site closest to the outflow in Plas Uchaf and therefore the site of interest for water quality managers. SOM component plots can be regarded as an analogue of PCA loading plots and the bar plane plots rank the variables according to their perceived relative importance for each node (hexagon). The component plot for SPT5 shows that nitrate correlates against all other variables (Fig. 5a) as the high weightings (yellow) occur mainly in the first 2 nodes in the plot, opposite of the high weightings for all other variables. Visual analysis of the bar planes reveals that as the weighting of nitrate decreases, the weighting of ammonium, 2-MIB and Geosmin increases (Fig. 5b). Clustering by k-means and single factor ANOVA showed that nitrate concentrations were significantly higher (p < 0.01) in C2 compared to C1 and C3 (Fig. 5c). Clusters 1 and 3 are associated with lower concentrations of ammonium, although not significant. Concentrations of 2-MIB and Geosmin are significantly higher in C3 compared to C1 (p < 0.01) and C2 (p < 0.05). No significant differences are observed in other variables. Similarly, the component plots for SPT1 show nitrate correlates against all other variables and Geosmin, total phosphorus, and ammonium correlate together (Fig. 6a). The bar plane plots reveal nitrate weightings are high when weightings of all other variables, except Geosmin, are low (Fig. 6b). The weighting of 2-MIB and ammonium increase when the weighting of nitrate decreases (Fig. 6b). Clustering and ANOVA indicated nitrate concentrations are significantly higher in C1 (p < 0.01) than C2 and C3 (Fig. 6c). Chlorophyll-a concentrations, cyanobacteria, and pH are all significantly higher in C3 compared to other clusters (chlorophyll-a and pH: p < 0.01; Cyanobacteria: p < 0.05). Whereas, concentrations of 2-MIB and Geosmin are significantly higher (p < 0.01) in C2 compared to C1 and C3 (Fig. 6c). Although higher in C2, no significant differences are observed between clusters for ammonium and TP concentrations.

Self-Organising Map (SOM) results for all other reservoirs and sites in Plas Uchaf are provided in the supplementary data (Supplementary Fig. S4-S11). The SOM component plots for multiple sites in Plas Uchaf, the Durleigh intake, and Cheddar intake show that nitrate correlates against all other variables (Supplementary Data) as the component plane pattern for nitrate is inverse to the plane patterns of other variables. For all reservoirs, the bar plane plots reveal that as the weighting of nitrate decreases, the weightings of ammonium, 2-MIB and Geosmin increase (Fig. S4-S11). However, Geosmin in Chew load highly with orthophosphate and cyanobacteria (Fig. S11), and in Dolwen, Geosmin
4. Discussion

The rising frequency and magnitude of T&O outbreaks in drinking water reservoirs is an ever-increasing issue for the water industry. Determining the triggers of T&O production in cyanobacteria is essential for management of raw water quality. This study has identified ammonium as a key trigger for production of 2-MIB and Geosmin in cyanobacteria from the analysis of in-reservoir measurements at Plas Uchaf reservoir (Figs. 2–4, Supplementary Fig. S4-S7), North Wales, UK, and these findings are supported by SOM analysis of historical intake data from 4 other reservoirs in Somerset UK (Supplementary Fig. S8-S11). Furthermore, the findings presented in this study grant the theory that ammonium trigger(s) stimulates cyanobacterial productivity, which in turn stimulates the synthesis of 2-MIB and Geosmin along isoprenoid pathways in cyanobacteria. Whilst it is well understood that adequate availability of phosphorus is required for rapid growth in cyanobacteria, and hence should still be considered in management decisions, we suggest that monitoring sources and concentrations of ammonium is key for managing T&O outbreaks in drinking water reservoirs.

The peaks in 2-MIB and Geosmin concentrations at Plas Uchaf coincided with higher cyanobacteria cell counts in summer 2015 and the SOM results for all reservoirs (Figs. 5 and 6, Fig. S4-S11) show cyanobacteria are associated with 2-MIB and Geosmin. Frequently, 2-MIB and Geosmin concentrations in aquatic systems have been associated with high cyanobacteria biomass, where light and temperature permit primary productivity (Jüttner, 1984). The cyanobacteria community observed in Plas Uchaf in 2015 and 2016 indicate that there are likely 2 main taxa responsible for the production of 2-MIB and Geosmin. The known Geosmin producer Dolichospermum (Smith et al., 2008) dominated the planktonic cyanobacteria community in the deeper northern end of Plas Uchaf, and the presence of Oscillatoria as a benthic biofilm in
The shallow region of Plas Uchaf is a likely source of 2-MIB and Geosmin (Su et al., 2015). A recent study by Xuwei et al. (2019) found that Oscillatoria were responsible for T&O production in the ‘non-blooming’ area of Lake Taihu, which indicates that high biomass is not always associated with 2-MIB/Geosmin concentrations. There is potential that 2-MIB and Geosmin are produced by other sources, such as actinomycetes (Park et al., 2014). However, Jüttner and Watson (2007) state that cyanobacteria are the primary source of T&O compounds in drinking water reservoirs and the presence of T&O producing cyanobacteria at all study sites suggests they are an important source of T&O compound production.

Thermal stratification is a common feature of reservoirs during summer and can result in lower dissolved oxygen concentrations near the sediments, promoting the reduction of iron (Fe$^{3+}$ to Fe$^{2+}$) and release of iron bound phosphorus into the labile pool (Wang et al., 2019). Labile phosphate can be released into the water column through diffusion or turbulent mixing, so changes in TN:TP could be a result of increased phosphorus supply. Increased phosphorus availability has been frequently reported as a cause of cyanobacteria blooms in water bodies (O’Neil et al., 2012; Paerl and Otten, 2013; Bormans et al., 2016) and Harris et al. (2016) reported higher 2-MIB and Geosmin concentrations are associated with lower TN:TP ratios. However, water samples from the five sites in Plas Uchaf showed no increase in TP over the summer (Fig. 2), meaning that the decrease in nitrate concentration due to denitrification, phytoplankton assimilation, and reduced external loading drove the observed change in TN:TP ratio, and likely contributed to Geosmin and 2-MIB production in Plas Uchaf. Both respiration and decomposition of cyanobacteria during a bloom can contribute to anoxic conditions and provide a carbon source for denitrifying bacteria (Chen et al., 2012). In Durleigh the changes in TN:TP are attributed to both a decline in nitrate and an increase in TP in the summer (Fig. S2). Reduced pumping from the canal in low flow in Durleigh combined with decreased run-off results in lower external loading of nitrate during the summer. Warmer temperatures and increased residence times are linked to increased denitrification rates (Hill, 1988; Ke et al., 2008), which are typical conditions of reservoirs in summer. It is likely a combination of these factors contributed to the decline in nitrate observed in summer in all reservoirs studied. TP in Durleigh is attributed to both internal loading due to lower dissolved oxygen concentrations near the sediments and reduced external loading in the summer. SOM results for Durleigh, Cheddar, Chew, and most sites in Plas Uchaf revealed TP/OP to correlate with 2-MIB and Geosmin. It is evident that phosphorus is required for cyanobacterial productivity and needs to be considered in management, although it does not appear to be the key trigger for 2-MIB and Geosmin production.

The SOM results for all studied reservoirs showed that 2-MIB and Geosmin loaded highly with higher ammonium relative to nitrate (Figs. 5 and 6, Fig. S2-S9). Ammonium is the most reduced form of
nitrogen in aquatic systems that can be assimilated by phytoplankton (Flores and Herrero, 2005; Gilbert et al., 2016). Cyanobacteria have been found to assimilate ammonium more efficiently than nitrate (Hampel et al., 2018). For example, Saadoun et al. (2001) reported that Geosmin production in *Dolichospermum* (then *Anabena*) is inhibited by nitrate and promoted by ammonium. Together with the findings of this study, Saadoun et al. (2001) found a significant correlation between increasing ammonium concentrations and Geosmin production, and Harris et al. (2016) reported increased 2-MIB and Geosmin concentrations during periods of high ammonium concentrations relative to nitrate. Therefore, 2-MIB and Geosmin production is likely triggered when ammonium concentrations are high relative to nitrate, as it stimulates more rapid growth by cyanobacteria.

Other factors such as pH, temperature (Somerset reservoirs - supplementary data) and chlorophyll-$a$ were associated with 2-MIB and Geosmin in the SOM results. High photosynthetic activity reduces dissolved CO$_2$ concentrations in the water and leads to increased pH (Visser et al., 2016). Furthermore, cyanobacteria are known to remain productive in alkaline environments below pH 10 (da Silva Brito et al., 2018). Here, higher pH values are likely caused by increased photosynthesis and not a direct factor in 2-MIB and Geosmin production. Cyanobacterial productivity is affected by water temperature. For example, Van der Ploeg et al. (1995) found 2-MIB production and maximal growth rates in *Oscillatoria cf. chalybea* to be greatest at 28°C. However, Saadoun et al. (2001) found no correlation between Geosmin concentration in *Dolichospermum* and temperature. Similarly, Alghanmi et al. (2018) found no relationship between light intensity and temperature for 2-MIB and Geosmin production in two isolated cyanobacteria. A recent study by Kim et al. (2018) showed that maximal secondary metabolite productivity (amount of secondary metabolite per unit biomass) occurred when biomass was lowest, at 15°C. SOM results for the Somerset reservoirs revealed 2-MIB and Geosmin correlated with temperature (Fig. S8-S11), but this could be a relic of the summer decline in nitrate. Nevertheless, in UK reservoirs, warmer temperatures are likely to promote higher productivity in cyanobacteria. Although temperature may influence the production of 2-MIB and Geosmin in cyanobacteria, our results suggest that it is not the most important factor in production.

Concentrations of 2-MIB and Geosmin correlated with ammonium concentrations spatially at the five sites within Plas Uchaf (Fig. S3). The reduction in ammonium supply from Dolwen in 2016 compared to 2015 correlated with a reduction in 2-MIB and Geosmin concentrations over the same years. No other measured water chemistry variable changed significantly between the two years (Fig. 4), so the 85% reduction in ammonium supply from Dolwen (Fig. 3) is the likely cause of reduced 2-MIB and Geosmin concentrations in 2016. Whilst these data do not provide mechanistic proof, we suggest that ammonium supply, relative to other nutrient concentrations is the key trigger for 2-MIB and Geosmin production in cyanobacteria. A likely hypothesis would be that enhanced ammonium stimulates a rapid increase in productivity which stimulates synthesis of metabolites such as 2-MIB and Geosmin. If these metabolites are synthesised in excess, then during less favourable periods, their release into the water column likely causes the T&O episodes reported by the Water Industry. This mechanism needs further study, but evidence from 5 different reservoirs appears strong enough to advise the management and monitoring of water supply reservoirs. We suggest that mitigating T&O problems in water supply reservoirs requires the identification of the internal/external source(s) of ammonium. External sources of ammonium and nitrate can be from river/stream inflows, surface flows, and groundwater, particularly within agricultural catchments with livestock or arable crops where N-rich fertilisers are used (Jeppesen et al., 2011). Anaerobic conditions in

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Fig. 5. (a) SOM component planes for each input variable and U-matrix for SPT5 in Plas Uchaf – near the Dolwen inflow. Each map unit (hexagon) represents a certain position in the map space, which is consistent across all component plots. Colours in the component plots show the values that variables have in the map structure, with yellow indicating higher values and blue lower. The U-matrix indicates similar map units (hexagons) based on Euclidean distance, with blue = shorter distance, and yellow = larger distance. (b) Bar planes show the weights for each input variable (see key) in each map unit (hexagon). Minimum and maximum values are based on weight scales in the component plots (a). (c) The three defined clusters from k-means cluster analysis. Clustering groups these data points according to similarity based on Euclidean distance between points in the clusters, and their corresponding cluster centroids. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
hypolimnetic waters can suppress nitrification and lead to accumulation of ammonium in the benthic sediments (Beutel, 2006). Subsequent internal loading of ammonium into the overlying water column can occur through diffusion or turbulent mixing.

5. Conclusions

This study has shown that 2-MIB and Geosmin production correlated with ammonium concentration in Plas Uchaf reservoir both spatially (within the reservoir), and temporally (between 2015 and 2016) correlating with external loading of ammonium. Findings from the SOMs for this site and an additional four lowland reservoirs strongly indicated that 2-MIB and Geosmin production is greatest when ammonium concentrations are high relative to nitrate, and phosphorus is bioavailable (lower TN:TP ratios). The production of 2-MIB and Geosmin in Plas Uchaf has been linked with planktonic Dolichospermum (Geosmin-producer) and benthic Oscillatoria (2-MIB- and Geosmin-producer). We suggest that high ammonium concentrations relative to nitrate triggers an increase in productivity of these cyanobacteria and hence an increase in production of 2-MIB and Geosmin as intermediate metabolites. In order to manage 2-MIB and Geosmin production in drinking water supply reservoirs, we advise that sources of ammonium (internal and external) are identified alongside the traditional approach of managing/monitoring phosphorus supply. A reduction in ammonium concentrations from all sources is likely essential for reservoir and catchment management in order to reduce the growing issues associated with T&O compounds in drinking water.

Author contributions

RGP and ES wrote the manuscript and carried out data analysis. TMCA contributed to field work, data analysis and manuscript preparation; JP and GG contributed to field work; DW and CB supervised ES in the analysis and contributed to manuscript preparation; TF and PP contributed to manuscript preparation. RL and SH contributed to data collection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.04.123.

References


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Fig. 6. (a) SOM component planes and U-matrix for SPT1 in Plas Uchaf – near the intake. Each map unit (hexagon) represents a certain position in the map space, which is consistent across all component plots. Colours in the component plots show the values that variables have in the map structure, with yellow indicating higher values and blue lower. The U-matrix indicates similar map units (hexagons) based on Euclidean distance, with blue = shorter distance, and yellow = larger distance. (b) Bar planes show the weights for each input variable (see key) in each map unit (hexagon). Minimum and maximum values are based on weight scales in the component plots (a). (c) The three defined clusters from k-means cluster analysis. Clustering groups these data points according to similarity based on Euclidean distance between points in the clusters, and their corresponding cluster centroids. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)


