

# Carbonaceous and nitrogenous disinfection by-product formation from algal organic matter

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## Abstract

Seasonal algal blooms in drinking water sources release intracellular and extracellular algal organic matter (AOM) in significant concentrations into the water. This organic matter provides precursors for disinfection by-products (DBPs) formed when the water is subsequently chlorinated at the final disinfection stage of the potable water treatment process.

This paper presents results of AOM characterisation from five algal species (three cyanobacteria, one diatom and one green) alongside the measurement of the DBP formation potential from the AOM of six algal species (an additional diatom). The character was explored in terms of hydrophilicity, charge and protein and carbohydrate content. 18 DBPs were measured following chlorination of the AOM samples: the four trihalomethanes (THMs), nine haloacetic acids (HAAs), four haloacetonitriles (HANs) and one halonitromethane (HNM).

The AOM was found to be mainly hydrophilic (52 and 81%) in nature. Yields of up to 92.4  $\mu\text{g mg}^{-1}$  C carbonaceous DBPs were measured, with few consistent trends between DBP formation propensity and either the specific ultraviolet absorbance (SUVA) or the chemical characteristics. The AOM from diatomaceous algae formed significant amounts of nitrogenous DBPs (up to 1.7  $\mu\text{g mg}^{-1}$  C). The weak trends in DBPFP may be attributable to the hydrophilic

33 nature of AOM, which also makes it more challenging to remove by conventional water  
34 treatment processes.

35

## 36 **Keywords**

37 Algae, trihalomethanes, haloacetic acids, haloacetonitriles, characterisation

38

## 39 **Abbreviations**

40 AOM – algal organic matter	61 MBAA – monobromoacetic acid
41 BCAA – bromochloroacetic acid	62 MCAA – monochloroacetic acid
42 BDCAA – bromodichloroacetic acid	63 MXAA – monohalogenated acetic acids
43 C-DBPs – Carbonaceous DBPs	64 N-DBPs – Nitrogenous DBPs
44 DBAA – dibromoacetic acid	65 NOM – natural organic matter
45 DBCAA – dibromochloroacetic acid	66 OM – organic matter
46 DBPs – disinfection by-products	67 SUVA – specific ultraviolet absorbance
47 DCAA – dichloroacetic acid	68 TBAA – tribromoacetic acid
48 DCAN - dichloroacetonitrile	69 TC – total carbon
49 DOC – dissolved organic carbon	70 TCAA – trichloroacetic acid
50 DWI – Drinking Water Inspectorate	71 TCAN – trichloroacetonitrile
51 DXAA – dihalogenated acetic acids	72 TCM – trichloromethane
52 ECD – electron capture detection	73 TCNM – trichloronitromethane
53 EOM – extracellular organic matter	74 THMs – trihalomethanes
54 GC – gas chromatography	75 TPI – transphilic organic fraction
55 HAAs – haloacetic acids	76 TXAA – trihalogenated acetic acids
56 HANs – haloacetonitriles	77 USEPA – United States Environmental
57 HNMs – halonitromethanes	78 Protection Agency
58 HPI – hydrophilic organic fraction	79 UV <sub>254</sub> – ultraviolet absorbance at 254 nm
59 HPO – hydrophobic organic fraction	80 WHO – World Health Organisation
60 IC – inorganic carbon	

81

## 82 **1 Introduction**

83 Chlorination of drinking water is known to cause the formation of disinfection by products  
84 (DBPs) which are a health concern (Richardson, 2003). Carbonaceous DBPs (C-DBPs) such  
85 as trihalomethanes (THMs) and haloacetic acids (HAAs) are formed when the organic matter  
86 (OM) in the water reacts with chlorine. THMs are widely regulated at 80, 100, 100 and 250  $\mu\text{g}$   
87  $\text{L}^{-1}$  for the sum of four THMs in the USA, Europe, Canada and Australia respectively (USEPA,  
88 1998, Health Canada, 2012, EU, 1998, NHMRC, 2011). Nitrogenous DBPs (N-DBPs) such as  
89 haloacetonitriles (HAN) and halonitromethanes (HNM) are also of health concern and have  
90 been shown to be more cytotoxic and genotoxic than C-DBPs (Plewa, 2002). They are not  
91 regulated but some (dichloroacetonitrile and dibromoacetonitrile at 20 and 70  $\mu\text{g}$   $\text{L}^{-1}$   
92 respectively) are incorporated in the WHO drinking water guidelines (WHO, 2006). Although  
93 in the EU THMs are the only chlorinated DBPs regulated, the approach to meeting the  
94 regulation is becoming risk based; regulations make clear the duty to minimise DBPs as a  
95 whole.

96

97 The most studied type of OM is terrestrial or natural organic matter (NOM) which varies  
98 seasonally by, for example, leaching from soil (Thibodeaux and Aguilar, 2005). Advances in  
99 water treatment and an understanding of NOM behaviour have enabled sufficient and enhanced  
100 removal of organic DBP precursors to minimise DBP formation. The seasonality of the NOM  
101 quantities and character can be addressed with enhanced coagulation controlled through  $\text{UV}_{254}$   
102 (Fabris et al., 2013) and zeta potential (Sharp et al., 2006) monitoring. The yield of DBPs  
103 ( $\mu\text{g}/\text{mg}$  C or  $\mu\text{g}/\text{UV}_{254}$ ) from NOM has been shown to correlate with dissolved organic carbon  
104 (DOC) and UV absorbance at 254 nm ( $\text{UV}_{254}$ ); reported yield values for THMs and HAAs  
105 have ranged from 61 to 124  $\mu\text{g}/\text{mg}$  C across various studies (Table 2).

106

107 A less extensively studied source of OM is from algae, generating dissolved organic carbon  
108 (DOC) levels of 1-25 mg L<sup>-1</sup> (Nguyen et al., 2005) from algal organic matter (AOM)  
109 (Pivokonsky et al, 2016). Besides contributing to the organic carbon content in water, algal  
110 cells contain organic nitrogen in the form of polysaccharides, proteins, peptides, amino sugars  
111 and other trace organic acids (Huang et al., 2009). AOM arises (a) extracellularly via metabolic  
112 excretion, forming extracellular organic matter (EOM) or (b) intracellularly due to autolysis of  
113 cells, forming intracellular organic matter (IOM). AOM is known to comprise proteins, neutral  
114 and charged polysaccharides, nucleic acids, lipids and small molecules, of which  
115 polysaccharides can comprise up to 80–90% of the total release. The IOM proportion increases  
116 with increasing age of the algae system (Henderson et al., 2008). EOM and IOM are of interest  
117 when studying the DBPs formed when algae arises in source waters, since they may be  
118 recalcitrant to water treatment (Henderson et al., 2010).

119  
120 The study of THM and HAA formation from AOM (Wachter and Andelman, 1984; Schmidt  
121 et al., 1998; Nguyen et al., 2005; Huang et al., 2009; Zhou et al., 2014) has generally been  
122 focused on the chlorination of water containing algal cells (Hong et al, 2008; Huang et al. 2009;  
123 Laio et al, 2015). Both algal cells and AOM can potentially generate significant amounts of  
124 THMs and HAAs. There has also been some work on the formation of nitrogenous DBPs, such  
125 as HANs, from chlorination of algal cells and/or AOM and its fractions (Oliver, 1983; Fang et  
126 al., 2010; Zhou et al., 2014). As with NOM, AOM can be fractionated according to both size  
127 and chemistry, with studies indicating the hydrophilic (HPI) chemical fraction to dominate over  
128 the transphilic (TPI) and hydrophobic (HPO) fractions regardless of the status of growth in the  
129 cell life cycle (Table 1). Studies of fraction yield, the mass of chlorinated DBP formed per unit  
130 mass of organic carbon in µg DBP per mg C, indicate similar DBP formation trends in AOM

131 as reported for NOM, the most reactive fractions being those at higher molecular weight (Lui  
132 et al, 2012) and hydrophobicity (Zhou et al, 2014).

133

134 **Table 1:** % distribution of AOM between the three chemical fractions, *Microcystis aeruginosa*

Growth phase	HPO	TPI	HPI	Reference
Exponential	27	4	69	Pivokonsky et al, 2014
Exponential	24	9	67	Zhou et al, 2014
Exponential	2	23	75	Leloup et al, 2013
Stationary	20	19	61	Leloup et al, 2013
Stationary	42	6	52	Qu et al, 2012
Stationary	24	17	59	Henderson et al, 2008

135

136 A summary (Table 2) of overall trends in yield for the C-DBPs indicate a number of key facets:

- 137 a) The most abundant data relate to THMs, and trichloromethane (TCM) specifically;
- 138 b) The reported TCM yield value for a single species (*Microcystis aeruginosa*) varies by more  
139 than a factor of two across the five studies;
- 140 c) Most studies have been based on one or two species, rather than a wider range;
- 141 d) The chlorination conditions adopted vary between the studies with respect to the to Cl<sub>2</sub>:C  
142 ratio and exposure time;
- 143 e) The limited data available suggests that the phase of the growth cycle may also influence  
144 both the amount and the yield of the DBP generated.

145

146 Interpretation of the available literature data across different studies is challenged by the  
147 different experimental conditions adopted, the differing fractions of the algal matter studied,  
148 and the limited scope of the studies in terms of the number of species investigated  
149 (predominantly one or two). It is of interest to establish whether any trends or patterns in DBP  
150 formation, and yield specifically, exist for AOM across different algal species. AOM is of  
151 practical interest since the algal solids are retained by the filtration process, the dissolved AOM  
152 component being the fraction subjected to final chlorination. Both C- and N-DBP formation is  
153 considered from AOM of six algal species at the onset of the stationary phase. Characterisation

154 encompasses hydrophilicity, charge, protein and carbohydrate content, with a view to linking  
 155 character to DBP formation potential with reference to THMs, HAAs, HANs and one HNM  
 156 (trichloronitromethane, TCNM).

157  
 158 **Table 2:** Summary of selected published chlorinated DBP yield data

Algal species	TCM	DCAA	TCAA	Cl <sub>2</sub> :C	t, h	Reference
	(µg mg <sup>-1</sup> C)					
<i>Anabaena flos-aquae</i> <sup>1 a</sup>	35	26	22	- <sup>4</sup>	168	Huang et al., 2009
<i>Anabaena flos-aquae</i> <sup>1 a</sup>	18	-	-	1.4	24	Wachter & Andelman, 1984
<i>Cyclotella meneghiniana</i> <sup>b</sup>	29	-	-	11	72, 168	Laio et al, 2015
<i>Chaetoceros mulleri</i> <sup>a</sup>	29	-	-	5	168	Nguyen et al. 2005
<i>Chlamydomonas sp.</i> <sup>b</sup>	25	213	67	20	120	Lui et al, 2012
<i>Microcystis aeruginosa</i> <sup>b</sup>	61	-	-	- <sup>4</sup>	168	Huang et al. 2009
<i>Microcystis aeruginosa</i> <sup>1 a</sup>	35	42	24	- <sup>4</sup>	168	Huang et al., 2009
<i>Microcystis aeruginosa</i> <sup>2 a</sup>	16	11	-	5	72	Fang et al., 2010
<i>Microcystis aeruginosa</i> <sup>1a</sup>	27	11	11	3	72	Qi et al, 2016
<i>Microcystis aeruginosa</i> <sup>b</sup>	21	-	-	7.1	72, 168	Laio et al, 2015
<i>Microcystis aeruginosa</i> <sup>1,3 a</sup>	33	-	-	5	72	Zhou et al, 2014
<i>Nitzschia sp.</i> <sup>b</sup>	48	25	19	10	96	Hong et al, 2008
<i>Oscillatoria sp.</i> <sup>b</sup>	26	34	39	10	96	Hong et al, 2008
<i>Oscillatoria prolifera</i> <sup>a</sup>	30	-	-	5	168	Nguyen et al. 2005
<i>Scenedesmus quadricauda</i> <sup>a</sup>	48	35	23	5	168	Nguyen et al. 2005
<i>Scenedesmus quadricauda</i> <sup>b</sup>	64	-	-	5	168	Nguyen et al. 2005

159 Cl<sub>2</sub>:C chlorine:carbon mass ratio; t chlorination time; <sup>1</sup>Exponential growth phase; <sup>2</sup>Stationary growth phase; <sup>3</sup>HPO fraction;  
 160 <sup>4</sup>>0.5 mg/L residual; <sup>5</sup>20 mg/L; <sup>a</sup> – AOM, <sup>b</sup> – algal cells  
 161

## 162 **2 Materials and methods**

### 163 **2.1 Algal cultivation**

164 Freshwater algae *Scenedesmus subspicatus* (276/20), *Aphanizomenon flos-aquae* (1401/3),  
 165 *Anabaena flos-Aquae* (1403/13B) and *Microcystis aeruginosa* (1450/3) *Asterionella Formosa*  
 166 (1005/9) (CCAP, Scotland) and *Melosira sp.* (JA386) (Sciento, UK) were cultured according  
 167 to recommended conditions (Table 3). Lighting was supplied by a *Sun-glo* and an *Aqua-glo*  
 168 30W lamp. Neutral density filters were used with the lights for all species except *Scenedesmus*  
 169 *subspicatus*. Each species grew at a different rate and reached the maximum phase of growth  
 170 with different cell concentrations (Table 3). AOM was extracted from each algal species once  
 171 exponential growth conditions had been established and at the onset of the stationary phase.  
 172 Checks were undertaken on a daily basis to ensure contamination had not occurred and to  
 173 determine cell concentrations: as with previous studies, with cultivation of algae on a similar  
 174 scale, cultures were only invaded by other organisms in the late stationary/decline phase (Lüsse  
 175 et al., 1985). Cell numbers were measured in triplicate using a light microscope and  
 176 haemocytometer.0

177

178 **Table 3:** Algae cell concentrations and time of growth

Algal species	Max. cell concentration (cells/ml)	Days taken	Cultivation temperature (°C)	Light/dark cycle (h)	Shaking regime	Growth media
<i>Scenedesmus subspicatus</i>	$1.8 \times 10^6$	14	20	16/8	120 rpm	Jaworski
<i>Aphanizomenon flos-aquae</i>	$1.8 \times 10^6$	28	20	16/8	120 rpm	Jaworski
<i>Anabaena flos-aquae</i>	$8.8 \times 10^5$	30	20	16/8	120 rpm	Blue/green (no N <sub>2</sub> )
<i>Microcystis aeruginosa</i>	$1.5 \times 10^7$	32	20	16/8	120 rpm	Jaworski
<i>Asterionella Formosa</i>	$2.9 \times 10^5$	24	15	14/10	By hand	Diatom
<i>Melosira sp.</i>	$1.9 \times 10^4$	8	15	14/10	By hand	Diatom

179

180

181 **2.2 AOM extraction and characterisation**

182 AOM was extracted by centrifuging 1 L of algal cell suspension at 4,000 rcf (relative  
183 centrifugal force) for 15-30 minutes. The supernatant was filtered with a 0.7 µm glass  
184 microfiber filter paper (Fisher Scientific, UK).

185

186 Specific ultraviolet absorbance (SUVA) in L m<sup>-1</sup> mg C<sup>-1</sup> was determined from the ratio of the  
187 254 nm UV absorbance (m<sup>-1</sup>) to the DOC concentration (mg C L<sup>-1</sup>). UV absorbance was  
188 measured using a Jenway 6505 UV/Vis spectrophotometer (Patterson Scientific, UK). The  
189 isoelectric point was determined by measuring the zeta potential (mV) over a pH range from  
190 0-10. Zeta potential was measured using a Malvern ZetaSizer 2000 (Malvern, UK).  
191 Measurements were carried out in triplicate.

192

193 Carbohydrate content was determined using the phenol–sulphuric acid method (Zhang et al.,  
194 1999; Dubois et al., 1956). Protein analysis was carried out using the modified Lowry method  
195 (Frølund et al., 1995). Glucose and bovine serum albumin were used for calibration with  
196 absorbance at 480 nm and 750 nm respectively using the Jenway spectrophotometer. Protein  
197 and carbohydrate measurements were triplicated.

198

199 The hydrophilicity and hydrophobicity of the AOM samples was determined by fractionation  
200 using XAD resins (XAD-7HP and XAD-4) in tandem according to Malcolm and MacCarthy  
201 (1992) and reported by Sharp et al. (2006). Charge density ( $\text{meq g}^{-1}$ ) was measured using a  
202 back titration adapted from Kam and Gregory (2001) and described in Sharp et al. (2006).

203

204 DOC was measured using a Shimadzu TOC-5000A analyser (Shimadzu, UK) on filtered  
205 samples. DOC was calculated by subtraction of the measured inorganic carbon (IC) from the  
206 total carbon (TC). The machine was calibrated daily. Up to five replicates were measured and  
207 an average of three reported to reduce the coefficient of variance to  $<2\%$ .

208

### 209 **2.3 DBP formation and quantification**

210 Chlorination employed a method adapted from standard methods (APHA, 1992). This involved  
211 buffering samples at pH 7, adding an excess of free chlorine at  $5 \text{ mg Cl}_2 \text{ mg}^{-1} \text{ C}$  and storing for  
212 seven days at  $20^\circ\text{C}$ . Chlorine residuals (measured in the range  $0.5\text{-}1.2 \text{ mg/L}$ ) were quenched  
213 using  $100 \text{ mg L}^{-1}$  ammonium chloride for HAA<sub>9</sub> and HAN<sub>4</sub> and TCNM analysis and  $100 \text{ mg}$   
214  $\text{L}^{-1}$  sodium sulphite for THM<sub>4</sub> analysis. Additionally THM<sub>4</sub>, HAN<sub>4</sub> and TCNM samples were  
215 buffered at pH 4.5-5.5.

216

217 THM<sub>4</sub> (trichloromethane, dichlorobromomethane, dibromochloromethane, tribromomethane)  
218 HAN<sub>4</sub> (bromochloroacetonitrile, dibromoacetonitrile, dichloroacetonitrile, trichloroaceto-  
219 nitrile) and TCNM were extracted using a modified form of USEPA Method 551.1. This  
220 method involved salted liquid/liquid extraction with solvent extracts analysed by gas  
221 chromatography (GC) with microelectron capture detection ( $\mu\text{ECD}$ ) (Agilent 6890). HAA<sub>9</sub>  
222 (monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid  
223 (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA), dibromoacetic acid



224 (DBAA), bromodichloroacetic acid (DBCAA), dibromochloroacetic acid (DBCAA), and  
 225 tribromoacetic acid (TBAA)) were analysed using a modified form of USEPA Method 552.3  
 226 (Tung et al., 2006). The derivatised HAAs (methyl esters) were measured using GC- $\mu$ ECD.  
 227 All samples were chlorinated and analysed in duplicate. The limit of quantification for all DBPs  
 228 was  $1 \mu\text{g L}^{-1}$ , except for MCAA where the quantification limit was  $2 \mu\text{g L}^{-1}$ . DBP yields were  
 229 calculated by dividing the concentration of DBP (in  $\mu\text{g L}^{-1}$ ) by the DOC concentration (in mg  
 230  $\text{L}^{-1}$ ) to give values in  $\mu\text{g mg C}^{-1}$ .

231

### 232 **3 Results**

#### 233 **3.1 AOM characteristics**

234 AOM from all algae characterised was predominantly hydrophilic, as suggested by low SUVA  
 235 values ( $0.34\text{-}1.7 \text{ m}^{-1} \text{ L mg C}^{-1}$ ) and verified by the high percentage (from 54% for *Scenedesmus*  
 236 *subspicatus* to 81% for the cyanobacteria *Anabaena flos-aquae*) of hydrophilic material (Table  
 237 4). This is in accordance with other researchers, for which HPI fractions of 52-73% have been  
 238 reported (Qu et al., 2012, Henderson et al., 2009). The charge density of all extracted AOM  
 239 was negligible except for that from the cyanobacteria *Microcystis aeruginosa*, measured at 0.2  
 240  $\text{meq g}^{-1}$  and indicating the excreted organics to be predominantly uncharged. The isoelectric  
 241 point of the AOM samples ranged from 0.9 to 3.2 with the lowest value observed for the AOM  
 242 from the diatom *Asterionella Formosa*. The protein:carbohydrate mass ratio was similar for  
 243 the AOM from *Aphanizomenon flos-aquae*, *Anabaena flos-aquae* and *Scenedesmus*  
 244 *subspicatus* ranging from 1.1-1.5. In contrast the AOM from *Microcystis aeruginosa* has been  
 245 reported as having a much lower ratio of 0.4-0.62 (Qu et al, 2012; Henderson et al, 2008).

246

247 **Table 4:** Algal organic matter characteristics from this study

<i>Algal species</i>	<i>SUVA</i>	<i>HPO</i> %	<i>HPI</i> %	<i>Pr/AOM</i>	<i>Ca/AOM</i>	<i>Pr/Ca</i>
<i>Aphanizomenon flos-aquae</i>	0.79	18	63	0.99	0.9	1.1
<i>Anabaena flos-aquae</i>	0.34	8	81	0.52	0.34	1.5
<i>Scenedesmus subspicatus</i>	1.18	26	54	1.5	1.2	1.2

248 Pr Protein concentration, Ca carbohydrate concentration, nm not measured  
249

250 The low charge density values indicate diminished quantities of the charged hydrophilic  
251 polysaccharides, and the presence of uncharged polysaccharides such as acetylamino sugars,  
252 sulphated sugars and carboxylated sugars (Leppard, 1995). These charged hydrophilic  
253 polysaccharides have been detected in AOM extracted from the stationary but not the  
254 exponential phase (Henderson et al., 2008). The organics excreted from AOM thus comprise  
255 low-SUVA organics such as hydrophobic proteins and uncharged hydrophilic polysaccharides  
256 (Edzwald, 1993), as well as proteins, peptides, carbohydrates and possibly amino acids (Bond  
257 et al., 2009, Pivokonsky et al., 2014).

258

### 259 **3.2 DBP formation**

#### 260 **3.2.1 Trihalomethanes**

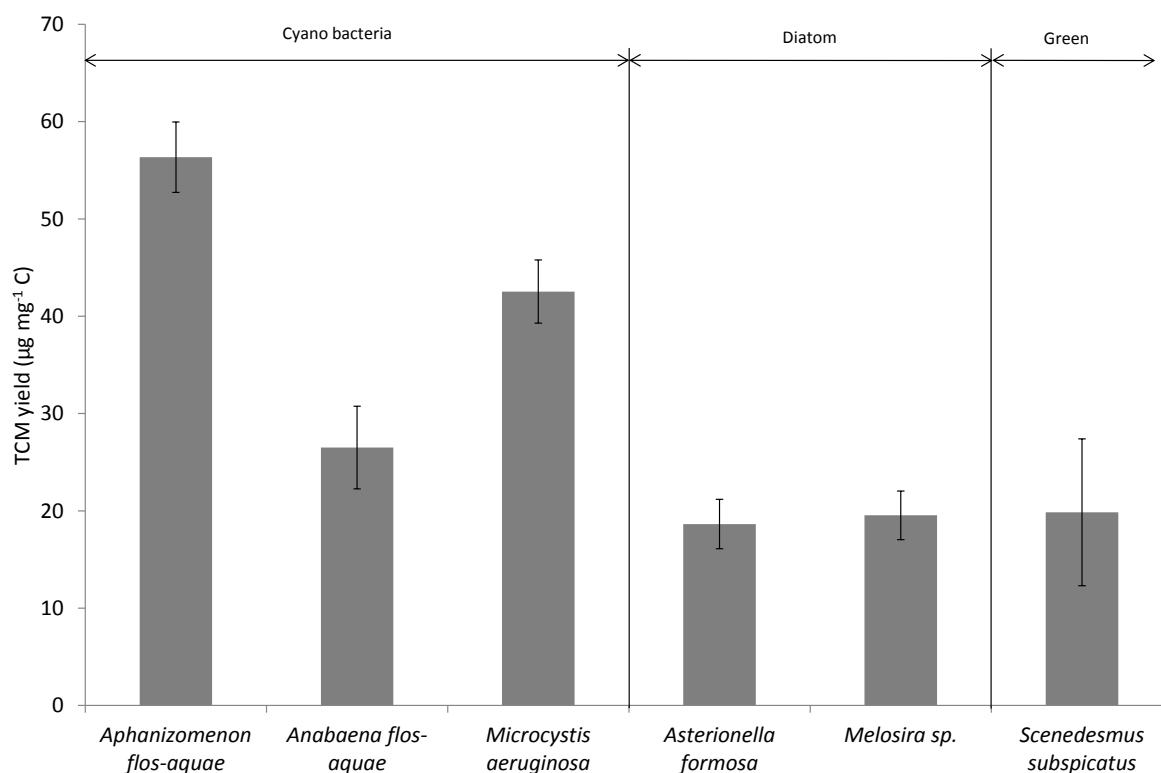
261 Under the chlorination conditions adopted, and specifically the absence of bromide, TCM  
262 accounted for more than 99% by mass of the THMs formed from the AOM for all six algal  
263 species studied (Figure 1). *Aphanizomenon flos-aquae*-AOM followed by *Microcystis*  
264 *aeruginosa*-AOM formed the most TCM of all the species measured at  $56.6 \pm 3.6 \mu\text{g mg}^{-1} \text{C}$   
265 and  $42.6 \pm 3.3 \mu\text{g mg}^{-1} \text{C}$  respectively. The remaining four AOM samples formed similar levels  
266 of THMs between  $18.7 \pm 2.5 \mu\text{g mg}^{-1} \text{C}$  and  $26.6 \pm 4.3 \mu\text{g mg}^{-1} \text{C}$ . The data complements that  
267 from previous studies (Table 1), with similar levels for *Microcystis aeruginosa*-AOM (Huang  
268 et al., 2009, Fang et al., 2010) and *Anabaena flos-aquae*-AOM (Huang et al., 2009; Wachter  
269 and Andelman, 1984). In contrast, THM formation reported for *Scenedesmus quadricauda*-  
270 AOM by Nguyen et al. (2005), referring to AOM extracted during stationary phase, varied  
271 depending on the algal growth tank size from  $48 \pm 12$  to  $64 \pm 14 \mu\text{g mg}^{-1} \text{C}$ , significantly higher  
272 than the  $19.9 \pm 7.5 \mu\text{g mg}^{-1} \text{C}$  measured in the current study. Algae grown under the same  
273 conditions and from the same tank have been shown to exhibit different behaviour depending  
274 on the algal type. The THM yield can vary with growth phase (*Anabaena flos-aquae*-AOM,

275 Huang et al., 2008) but has also been shown not to vary significantly with growth phase when  
276 normalised with respect to DOC (*Scenedesmus quadricauda*-AOM, Nguyen et al., 2005;  
277 *Microcystis aeruginosa*-AOM, Huang et al., 2009).

278 Comparison with alternative OM sources reveals that AOM exerts a moderate to low reactivity  
279 with chlorine. For instance THM yield concentrations generated from NOM formation  
280 potential tests have been reported to range from 20-281  $\mu\text{g mg}^{-1} \text{C}$  with a median of 63  $\mu\text{g mg}^{-1}$   
281  $\text{C}$  for a range of 35 water sources (Allgeier and Summers 1995, Afcharian et al., 1997, Collins  
282 et al., 1986, Nokes et al., 1999, Singer et al., 1995, Teksoy et al., 2008, Yang et al., 2015, Pifer  
283 and Fairey 2014). This indicates that although AOM may not be the biggest contributor to the  
284 formation of THMs compared to NOM, it could still make a significant contribution to the  
285 THMs formed.

286 Further to this, microbially derived OM has been shown to exhibit a yield of 23-43  $\mu\text{g THMs}$   
287  $\text{mg}^{-1} \text{C}$  (Sirivedhin and Gray, 2005), with the yield reported to vary little across the three  
288 chemical fractions (Zhou et al, 2014). AOM is known to most resemble hydrophilic NOM and  
289 microbially derived OM and consists of hydrophilic polysaccharides and hydrophobic proteins  
290 (Henderson et al., 2008), so a comparison can therefore be made with the yield of proteins and  
291 carbohydrates. The THM yield has been reported to range from 41 to 51  $\mu\text{g THM mg}^{-1} \text{C}$  for  
292 four proteins (Scully et al., 1988), and carbohydrates have been observed to form similar levels  
293 of THMs (42 to 65  $\mu\text{g THM mg}^{-1} \text{C}$ ) for 10 carbohydrates (Navalon et al., 2008), broadly  
294 consistent with the trends shown in Figure 1.

295

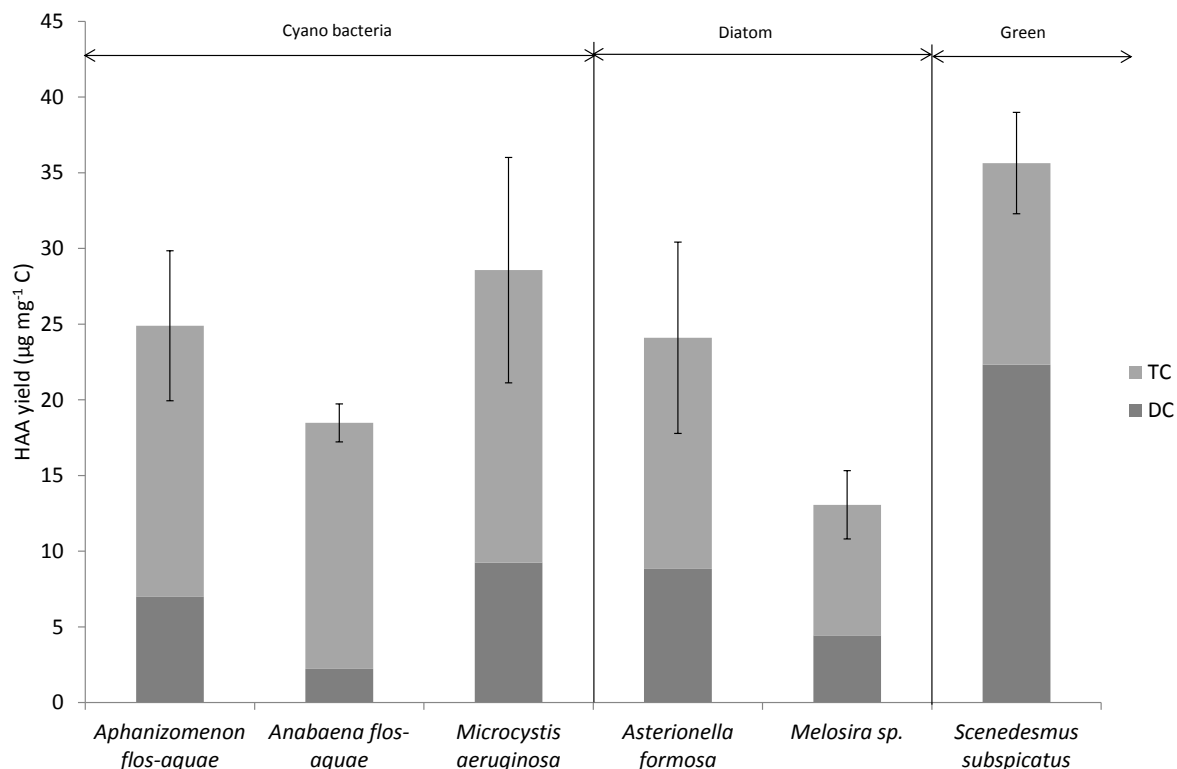


**Figure 1:** TCM concentrations produced by the AOM from each algal species

### 3.2.2 Haloacetic acids

As with the THM data, brominated species did not feature amongst the HAAs assayed. DCAA and TCAA comprised more than 99% of the total HAAs formed on a mass basis for the AOM of all 6 species of algae (Figure 2), consistent with Nguyen et al. (2005). *Scenedesmus subspicatus*-AOM formed the most HAAs of all the species at a yield of  $35.8 \pm 2.3 \mu\text{g mg}^{-1} \text{C}$  followed by *Microcystis aeruginosa*-AOM with yield of  $28.7 \pm 7.5 \mu\text{g mg}^{-1} \text{C}$ . AOM from *Aphanizomenon flos-aquae* and *Asterionella formosa* was comparable in HAA yield with values of  $24\text{--}25 \mu\text{g mg}^{-1} \text{C} \pm \sim 20\%$ . The second-lowest HAA yield was observed for *Anabaena flos-aquae*-AOM at  $18.7 \pm 1.3 \mu\text{g mg}^{-1} \text{C}$  with the lowest value of  $13.2 \pm 2.3 \mu\text{g mg}^{-1} \text{C}$  recorded for *Melosira sp.*-AOM. As with the THM yield values, those for HAAs measured by Nguyen et al. (2005) from AOM from the stationary phase were higher than those observed in the current study for *Scenedesmus*-AOM ( $60 \pm 7.7$  compared to  $35.8 \pm 3.4 \mu\text{g mg}^{-1} \text{C}$ ) when the AOM was taken at the onset of the stationary phase. This was also the case with values reported from

313 the stationary phase by Huang et al. (2009) compared to those observed in the current study  
 314 (66 compared to 29  $\mu\text{g mg}^{-1}\text{C}$  for *Microcystis aeruginosa*-AOM, and 48 compared to 19  $\mu\text{g}$   
 315  $\text{mg}^{-1}\text{C}$  for *Anabaena flos-aquae*-AOM). The higher yield from *Microcystis aeruginosa*-AOM  
 316 compared to *Anabaena flos-aquae*-AOM (Figure 2) corroborates the findings of Huang et al  
 317 (2009), attributable to the difference in HPO content (Table 4).



318  
 319 **Figure 2:** HAA concentrations produced by the AOM from each algal species

320  
 321 The TCAA:DCAA ratios observed in the current were comparable to those reported in the  
 322 literature for *Scenedesmus*-AOM: 0.60 compared to 0.33-0.69 reported by Nguyen et al. (2005)  
 323 over a number of days of stationary growth. However the same ratios reported by Huang et al.  
 324 (2009) for AOM from *Microcystis aeruginosa* and *Anabaena flos-aqua* (0.57 and 0.85  
 325 respectively) extracted during the stationary phase were significantly lower than those from the  
 326 current study (2.1 and 7.3 respectively) for samples taken at the onset of the stationary phase.  
 327 The difference may be attributable to the varying amino acid content, which can have wide

328 ranging HAA yield values - insignificant to  $106 \mu\text{g mg}^{-1} \text{C}$  according to Hong et al., 2009 - and  
329 may consist largely of aromatic/cyclic amino acids (Bond et al., 2009).

330

331 HAA formation was positively correlated HPO ( $R^2 = 0.94$ ) which was attributed mainly to  
332 DCAA formation. Conversely the hydrophilic content was negatively correlated to HAA  
333 formation ( $R^2 = 0.87$ ), again closely linked to DCAA formation.

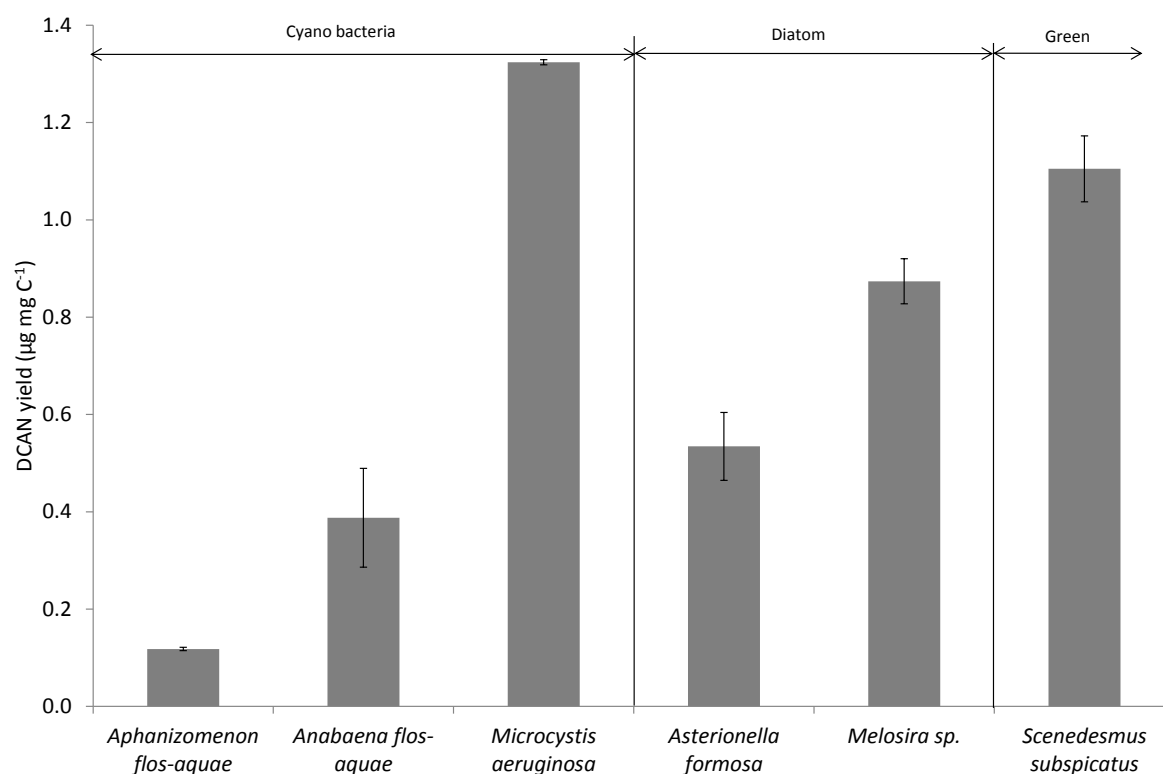
334

### 335 **3.2.3 Haloacetonitriles**

336 Dichloroacetonitrile (DCAN) comprised >99% of the total HANs formed on a mass basis for  
337 the AOM for all 6 algal species (Figure 3). Trichloroacetonitrile (TCAN) was not detected in  
338 any samples likely due to base-catalysed hydrolysis at  $\text{pH} > 5.5$  (Croué and Reckhow, 1989).  
339 *Microcystis aeruginosa*-AOM, *Scenedesmus subspicatus*-AOM and *Melosira sp.*-AOM  
340 generated the highest HAN yields of all the species measured at  $1.32 \pm 0.01$ ,  $1.10 \pm 0.07$  and  
341  $0.87 \pm 0.05 \mu\text{g mg}^{-1} \text{C}$  respectively. *Aphanizomenon flos-aquae*-AOM produced the lowest  
342 yields ( $0.12 \pm 0.003 \mu\text{g mg}^{-1} \text{C}$ ), with *Asterionella formosa*-AOM and *Anabaena flos-aquae*-  
343 AOM exhibiting similar values of  $0.53 \pm 0.07$  and  $0.39 \pm 0.10 \mu\text{g mg}^{-1} \text{C}$  respectively. The  
344 formation potential for HANs from AOM has been studied by Fang et al. (2010) and for  
345 fractionated AOM (Zhou et al., 2014). These authors reported slightly higher values of  $\sim 1.5 \mu\text{g}$   
346  $\text{mg}^{-1} \text{C}$  DCAN from chlorination of *Microcystis aeruginosa*-AOM compared to the current  
347 study, perhaps because the AOM was extracted during the stationary phase. For fractionated  
348 samples, values of total HANs from chlorination of *Microcystis aeruginosa*-AOM over 3 days  
349 ranged from  $1.5\text{--}2.6 \mu\text{g mg}^{-1} \text{C}$ , with the HPO fraction having the greatest formation potential  
350 (Zhou et al., 2014). This equates to a value of  $1.8 \mu\text{g mg}^{-1} \text{C}$ , based on the relative amount of  
351 each fraction, which is slightly higher than the values found in the current study for *Microcystis*  
352 *aeruginosa*-AOM but does not take into account the synergistic effects encountered when  
353 chlorinating non-fractionated samples (Kent et al., 2011). HAN yields from algal cells have

354 been reported under similar chlorination conditions, albeit with a 3-day exposure, of 0.76  $\mu\text{g}$   
 355  $\text{mg}^{-1}$  C DCAN and 0.05  $\mu\text{g}$   $\text{mg}^{-1}$  C TCAN from algal cell suspensions of *Microcystis*  
 356 *aeruginosa* (Fang et al., 2010). Under the same chlorination conditions as reported here, at  
 357 double the chlorine dose, Oliver (1983) reported DCAN formation of 2.3  $\mu\text{g}$   $\text{mg}^{-1}$  C and 0.5  $\mu\text{g}$   
 358  $\text{mg}^{-1}$  C for cyanobacterial (*Anabaena* Texas 1447) and green (*Scenedesmus basiliensis*) algal  
 359 suspensions respectively.

360



361  
 362  
 363

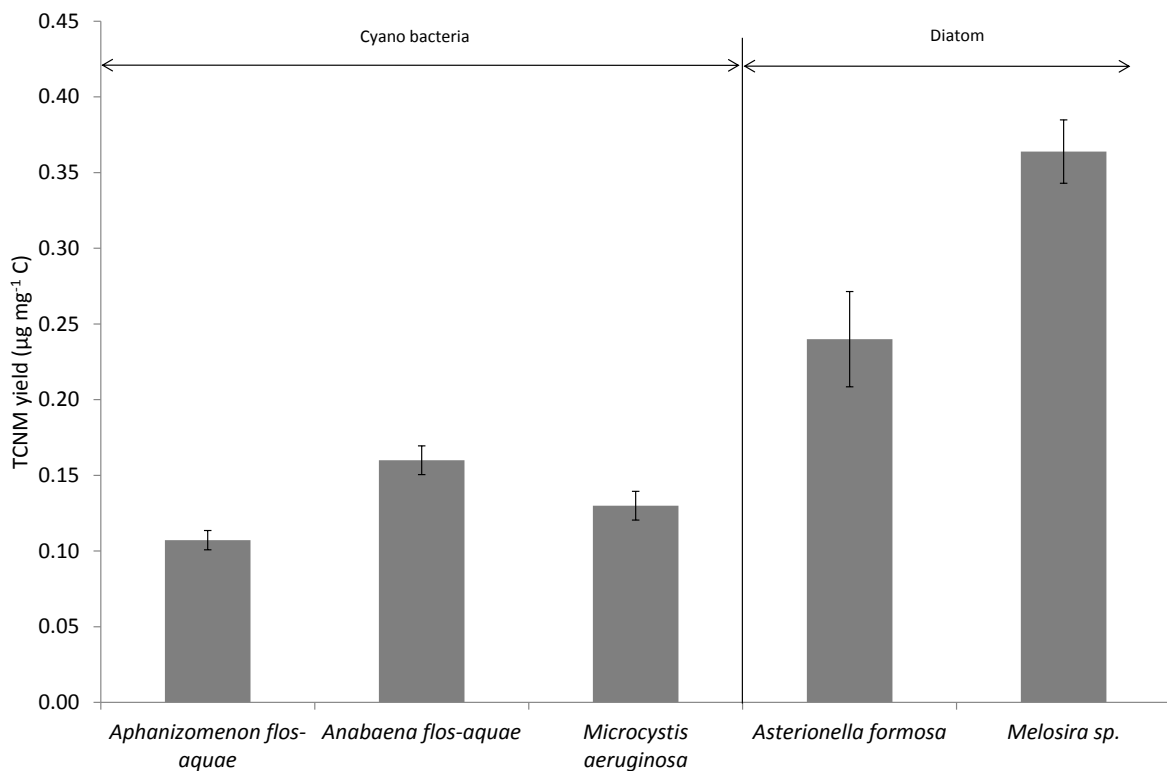
**Figure 3:** DCAN concentrations produced by the AOM from each algal species

364 In terms of THM and HAA formation, algal cells have been observed to produce similar or  
 365 greater amounts than the corresponding AOM (Huang et al., 2009). Differences in algal  
 366 species, organic fractions (dissolved matter vs. whole cells), and chlorination conditions make  
 367 comparison with published challenging. However, the values for AOM reported in the current  
 368 study are of the same magnitude as those reported in the literature for algal cell suspensions  
 369 and less than the yield of DCAN from isolated fulvic acid (4.3  $\mu\text{g}$   $\text{mg}^{-1}$  C) (Oliver, 1983).

370 Under identical chlorination conditions, Lee et al. (2007) reported the chlorination of isolated  
 371 NOM fractions to produce DCAN levels ranging from 1.65 to 2.31  $\mu\text{g mg}^{-1}$  C from TPI neutral  
 372 and colloidal fractions largely consisting of amino sugars, polysaccharides and proteins.  
 373 Contrary to Oliver (1983), HPO fractions (including fulvic acid) produced DCAN levels of  
 374 0.33-0.77  $\mu\text{g mg}^{-1}$  C (Lee et al., 2007) which could be related to the differing chlorine doses.  
 375

### 376 3.2.4 Halonitromethane

377 TCNM was the only HNM measured in the current study (Figure 4). *Melosira sp.*-AOM formed  
 378 the most TCNM ( $0.36 \pm 0.02 \mu\text{g mg}^{-1}$  C) of all the species measured at followed by *Asterionella*  
 379 *formosa*-AOM ( $0.24 \pm 0.03 \mu\text{g mg}^{-1}$  C). Similar values were observed for *Anabaena flos-aquae*-  
 380 AOM, *Microcystis aeruginosa*-AOM and *Aphanizomenon flos-aquae*-AOM forming  
 381  $0.16 \pm 0.01 \mu\text{g mg}^{-1}$  C and  $0.13 \pm 0.01 \mu\text{g mg}^{-1}$  C and  $0.11 \pm 0.01 \mu\text{g mg}^{-1}$  C TCNM respectively.  
 382 TCNM formation by *Scenedesmus subspicatus*-AOM was below the limit of detection.  
 383



384  
 385 **Figure 4:** TCNM concentrations produced by the AOM from each algal species compared to literature values

386



387

388 To the authors' knowledge the formation potential for HNMs from AOM or algal cells has only  
389 been studied for *Microcystis aeruginosa*-AOM (Fang et al., 2010) with slightly higher values  
390 than observed here perhaps expected due to the difference in growth phase as described for the  
391 other DBPs measured. Values of TCNM reported from chlorination of isolated NOM fractions  
392 average at  $0.33 \mu\text{g mg}^{-1} \text{C}$  (Lee et al., 2007) similar to the values reported here.

393

#### 394 **4 Discussion**

395 AOM is mainly hydrophilic in character and on chlorination has the potential to form  
396 significant amounts of C- and N-DBPs. An unsuccessful attempt was made to link the  
397 characteristics to the DBPs formed as this has been shown to be applicable for NOM and DBPs  
398 (e.g. with NOM, THM formation can correlate positively with SUVA for a range of water  
399 samples for high SUVA (>3) waters (Ates et al., 2007, Reckhow et al., 1990). No relationship  
400 was observed between SUVA and the DBPs measured in the current study and, apart from the  
401 close correlation of HAA with the HPO fraction (Section 3.2.2), there was no correlation  
402 evident between DBPFP and any chemical fraction.

403

404 No pattern in DBP formation with algal taxonomic group was evident. For instance, AOM  
405 could form significant amounts of C-DBPs (illustrated by the specific cyanobacterial species  
406 AOM and green algal AOM) or less significant amounts of C-DBPs (illustrated by the AOM  
407 from diatomaceous species). Cyanobacterial AOM may be expected to produce significant  
408 amounts of nitrogenous DBPs compared to green and diatomaceous AOM since cyanobacterial  
409 algae are nitrogen fixers and liberate up to 45% of their fixed nitrogen as organic-N (Huang et  
410 al., 2009, Westerhoff and Mash, 2002). Indeed, when looking at formation of HANs, one  
411 particular cyanobacteria is more reactive (*Microcystis aeruginosa*-AOM) but significant

412 amounts of HAN are also formed by green and diatomaceous AOM. The AOM from the  
413 diatoms (*Melosira sp.* and *Asterionella formosa*) forms the most TCNM followed by the  
414 cyanobacterial AOM, with no formation of TCNM by the green AOM (*Scenedesmus*  
415 *subspicatus*). Therefore when considering the risk of DBPs formed by a particular algal species,  
416 it is important that the AOM produced from diatomaceous algae is considered as it can be a  
417 significant precursor to HAN and HNM formation.

418

419 The chlorination of AOM involves the reaction between chlorine and molecules including  
420 uncharged hydrophilic polysaccharides, proteins, peptides and carbohydrates. The amino acids  
421 present in freshwater algae (as free amino acids and proteins or peptides) comprise at least 17  
422 of the 20 standard amino acids (Fowden, 1951, Lewis and Gonzalves, 1962) with different  
423 species containing different amino acids. For example, some cyanobacterial algal species  
424 contain no cysteine whereas some green algal species contain cysteine but no lysine. All the  
425 amino acids present in algae can potentially be present in AOM. It is known that acid  
426 polysaccharides such as uronic acids can be excreted by algae in response to low nutrient stress  
427 (Costerton, 1984). The polysaccharides present in algae and extracellularly comprise the  
428 carbohydrates rhamnose, galactose, arabinose, fucose, mannose, glucuronic acid, uronic acid,  
429 glucose (Rezanka and Sigler, 2007).

430

431 Amino acids, carbohydrates and carboxylic acids have been studied with respect to their DBP  
432 formation (Chu et al., 2012, Shan et al., 2011, Bond et al., 2009, Navalon et al., 2008, Trehy et  
433 al., 1986). The study of carbohydrates (Navalon et al., 2008) showed that they were reactive  
434 with respect to THM (40-65  $\mu\text{g mg}^{-1} \text{C}$ ). In studies of amino acids, the key finding was that the  
435 compounds can have similar physicochemical properties but divergent DBP formation. For  
436 example, glutamic and aspartic acid have very similar log  $K_{ow}$ , pKa, and molecular weight.

437 However, on chlorination aspartic acid forms DCAA, trichloroacetaldehyde, and DCAN at  
 438 0.26, 0.02, and 0.06 mol THM/mol compound (mol/mol) respectively, whereas none of these  
 439 species are formed from glutamic acid chlorination (Bond et al., 2009). On the other hand, little  
 440 difference was observed between formation of HNM (Shan et al., 2011) and DCAN (Wang et  
 441 al., 2013) from glutamic and aspartic acids, emphasising the different pathways of formation  
 442 for each group of DBPs. Mechanisms of formation have been proposed for these pathways  
 443 (Table 5). A study on carboxylic acids (Bond et al., 2009) showed that  $\beta$ -dicarbonyl 3-  
 444 oxopentanedioic is reactive with respect to THM and trichloropropane formation but not HAA  
 445 formation. This corroborated a previous report stating that the reactivity of carbohydrates and  
 446 carboxylic acids towards chlorine to be low (WHO, 2000) with reference to the chlorine  
 447 demand of the carbohydrates, though this report did not consider that significant amounts of  
 448 some DBPs could still be formed.

449

450 **Table 5:** Proposed pathways for DBP formation from amino acid precursors

DBP	Precursors	Intermediate	Substitution location	Reference
HNMs	Chemical structure of precursors not considered to be important			Wang et al., 2013, Shan et al., 2011
HANs	Aspartic acid, asparagine	dichlorocyanoacetic acid	nr	Wang et al., 2013
	Tyrosine	benzyl cyanide	$\alpha$ -carbon	
	Histidine	2-(1-chloro-1H-imidazol-4-yl)-acetonitrile	$\alpha$ -carbon	Li and Blatchley, 2007
THMs	Tyrosine	4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol	nr	Chu et al., 2012
HAAs	Aspartic acid and glutamic acid	$\beta$ -keto acid such as 3-oxopentanedioic acid or cyanoacetic acid	Variable	Bond et al., 2009

451 nr – not reported

452

453

454 While AOM is present at lower concentrations than other DBP precursors such as NOM, its  
 455 nature means that it is recalcitrant to treatment by methods such as coagulation. Whilst  
 456 optimised coagulation has been shown to remove the algae *C. Vulgaris*, *M. aeruginosa* and *A.*

457 *Formosa* by 71, 55 and 46 % respectively (Henderson et al., 2010), the removal of dissolved  
458 AOM is more challenging due to its uncharged hydrophilic nature; enhanced techniques such  
459 as pre-ozonation demonstrating only partial success (Widrig et al., 1996). Given the escalation  
460 of eutrophication of water sources in recent years due to anthropogenic effects, increasing the  
461 levels of phosphorus and nitrogen entering water sources (Ward and Wetzel, 1980, Burrini et  
462 al., 2000) AOM is likely to be a significant contributor to DBP formation in treated drinking  
463 waters.

464 Another important consideration is the toxicity of the DBPs formed, particularly the  
465 nitrogenous DBPs. A recent study (Zeng et al., 2016) on potable water reuse investigated a  
466 range of DBPs throughout the treatment train and looked at the contribution of each DBP to  
467 the toxicity of the water. The toxicity was determined as a function of concentration and toxic  
468 potencies of each DBP. The toxicity in this case for unregulated halogenated DBPs was based  
469 on in vitro chromic cell cytotoxicity which has some limitations and the authors stressed that  
470 they were determining relative rather than absolute risk. Nonetheless they found that HANs,  
471 haloacetamides and to a lesser degree haloacetaldehydes dominated the additive toxicity in  
472 membrane filtrate. Thus it is important, when considering whether to use an algal impacted  
473 source, that the concentration of nitrogenous DBPs (particularly HANs and haloacetamides) in  
474 the treated water may be elevated compared to a source that is not algal impacted.

475

## 476 **5 Conclusions**

477 A study of the characteristics of formation of chlorinated disinfection by products from algal  
478 organic matter (AOM) has revealed the following:

479

- 480 • AOM is mainly hydrophilic in character, with between 52 and 81% being made up of HPI  
481 fraction, and on chlorination has the potential to form up to 92.4 µg carbonaceous and 1.7  
482 µg nitrogenous DBPs per mg organic carbon;
- 483 • No pattern in DBP formation with algal taxonomic group was evident;
- 484 • Few consistent trends between DBP formation propensity and either the specific ultraviolet  
485 absorbance (SUVA) or the AOM chemical characteristics were evident, such that  
486 characterisation of the AOM may be of limited use in determining DBP formation;
- 487 • Although little studied, the AOM from diatomaceous algae forms significant amounts of  
488 nitrogenous DBPs (up to 1.7 µg mg<sup>-1</sup> C).

489

490 The hydrophilic nature of AOM, which is autochthonous in nature, makes it more difficult to  
491 remove effectively using conventional water treatment processes than allochthonous natural  
492 organic matter (NOM), which is also more hydrophobic in nature. This offers an explanation  
493 for the generally observed trend of seasonally high chlorinated DBP levels associated with  
494 higher temperatures and thus commensurately greater microbial production rates with  
495 accompanying AOM generation.

496

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