

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 ₂₅₄ – ultraviolet absorbance at 254 nm
59 HPO – hydrophobic organic fraction	80 WHO – World Health Organisation
60 IC – inorganic carbon	

81

1 Introduction

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

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

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

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

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

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

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

- a) The most abundant data relate to THMs, and trichloromethane (TCM) specifically;
- b) The reported TCM yield value for a single species (*Microcystis aeruginosa*) varies by more than a factor of two across the five studies;
- c) Most studies have been based on one or two species, rather than a wider range;
- d) The chlorination conditions adopted vary between the studies with respect to the $\text{Cl}_2:\text{C}$ ratio and exposure time;
- e) The limited data available suggests that the phase of the growth cycle may also influence both the amount and the yield of the DBP generated.

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

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

Table 2: Summary of selected published chlorinated DBP yield data

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

Cl₂:C chlorine:carbon mass ratio; t chlorination time; ¹Exponential growth phase; ²Stationary growth phase; ³HPO fraction; ⁴>0.5 mg/L residual; ⁵20 mg/L; ^a – AOM, ^b – algal cells

2 Materials and methods

2.1 Algal cultivation

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

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>	1.8×10^6	14	20	16/8	120 rpm	Jaworski
<i>Aphanizomenon flos-aquae</i>	1.8×10^6	28	20	16/8	120 rpm	Jaworski
<i>Anabaena flos-aquae</i>	8.8×10^5	30	20	16/8	120 rpm	Blue/green (no N ₂)
<i>Microcystis aeruginosa</i>	1.5×10^7	32	20	16/8	120 rpm	Jaworski
<i>Asterionella Formosa</i>	2.9×10^5	24	15	14/10	By hand	Diatom
<i>Melosira sp.</i>	1.9×10^4	8	15	14/10	By hand	Diatom

2.2 AOM extraction and characterisation

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

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

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

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

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

2.3 DBP formation and quantification

Chlorination employed a method adapted from standard methods (APHA, 1992). This involved 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 seven days at 20°C . Chlorine residuals (measured in the range $0.5\text{-}1.2 \text{ mg/L}$) were quenched using 100 mg L^{-1} ammonium chloride for HAA_9 and HAN_4 and TCNM analysis and 100 mg L^{-1} sodium sulphite for THM_4 analysis. Additionally THM_4 , HAN_4 and TCNM samples were buffered at pH 4.5-5.5.

THM_4 (trichloromethane, dichlorobromomethane, dibromochloromethane, tribromomethane) HAN_4 (bromochloroacetonitrile, dibromoacetonitrile, dichloroacetonitrile, trichloroacetonitrile) and TCNM were extracted using a modified form of USEPA Method 551.1. This method involved salted liquid/liquid extraction with solvent extracts analysed by gas chromatography (GC) with microelectron capture detection (μECD) (Agilent 6890). HAA_9 (monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA), dibromoacetic acid

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

3 Results

3.1 AOM characteristics

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

Table 4: Algal organic matter characteristics from this study

Algal species	SUVA	HPO %	HPI %	Pr/AOM	Ca/AOM	Pr/Ca
<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

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

3.2 DBP formation

3.2.1 Trihalomethanes

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

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

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

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

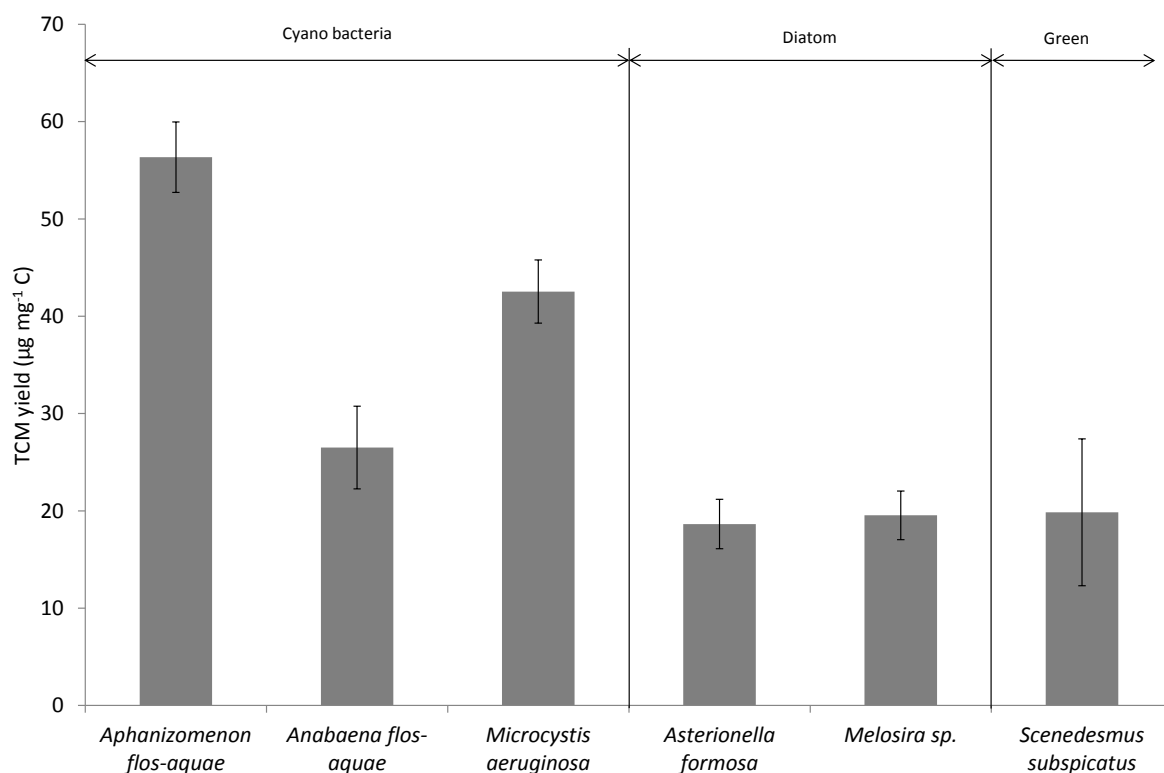


Figure 1: TCM concentrations produced by the AOM from each algal species

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 ± 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

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

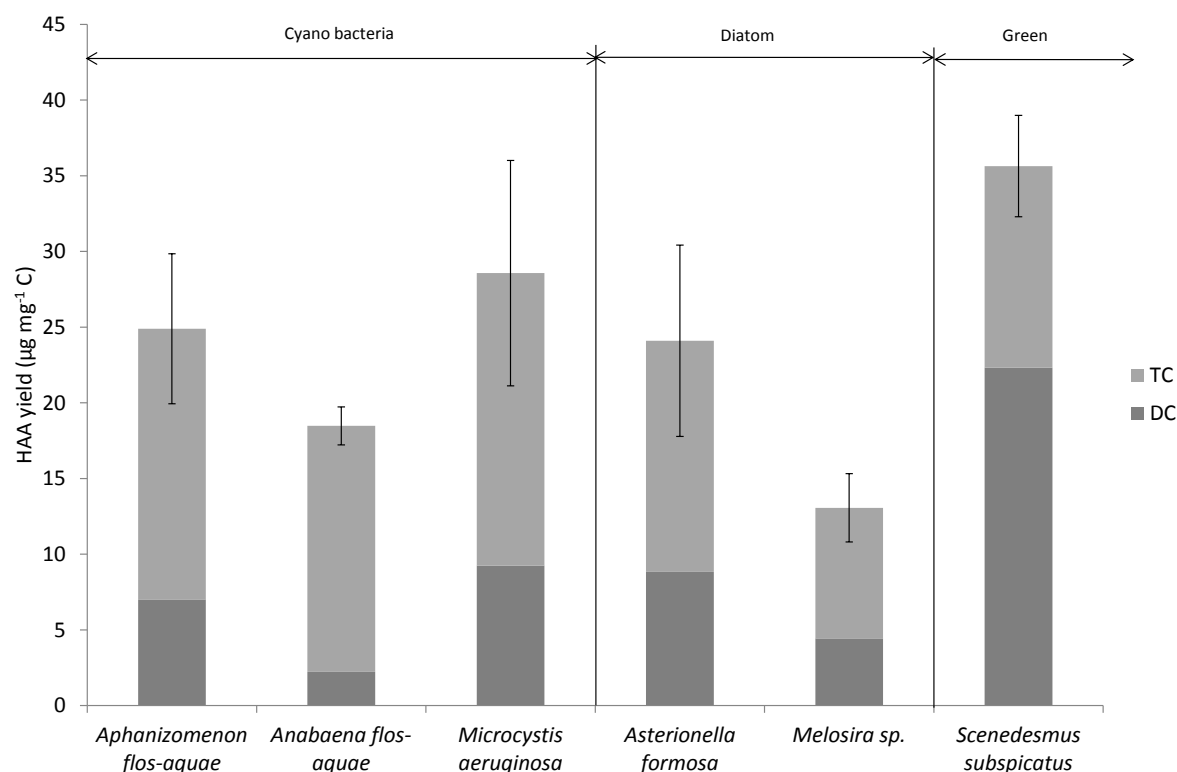


Figure 2: HAA concentrations produced by the AOM from each algal species

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

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

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

3.2.3 Haloacetonitriles

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

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

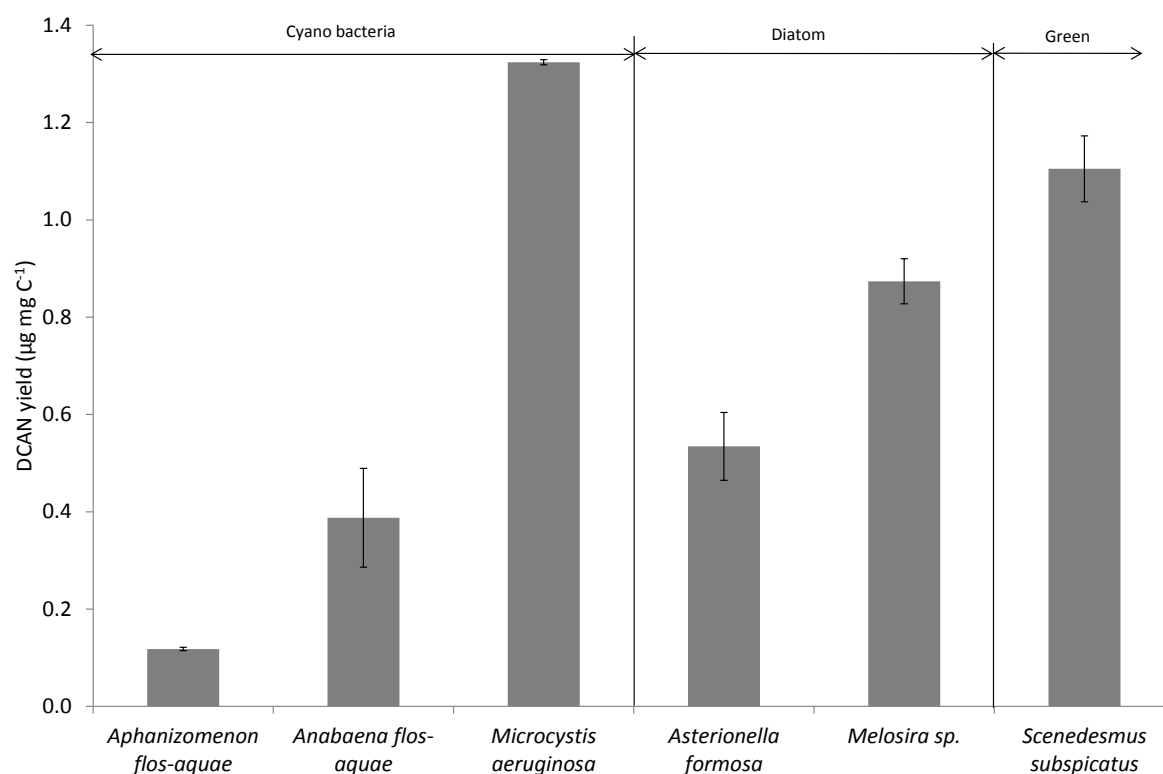


Figure 3: DCAN concentrations produced by the AOM from each algal species

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

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

3.2.4 Halonitromethane

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

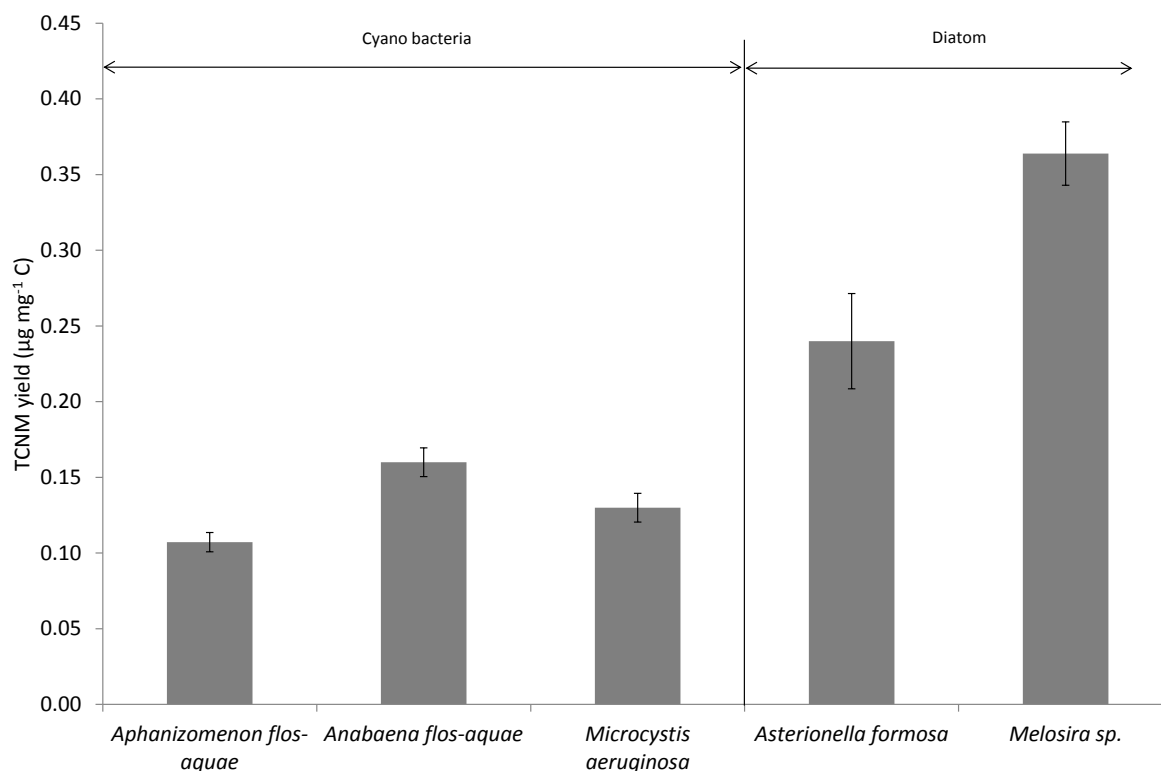


Figure 4: TCNM concentrations produced by the AOM from each algal species compared to literature values

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

4 Discussion

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

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

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

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

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

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

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	α -carbon	
	Histidine	2-(1-chloro-1H-imidazol-4-yl)-acetonitrile	α -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	β -keto acid such as 3-oxopentanedioic acid or cyanoacetic acid	Variable	Bond et al., 2009

nr – not reported

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

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

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

5 Conclusions

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

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

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

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

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