Screening volatile organic compounds (VOCs) emissions from five marine phytoplankton species by head space gas chromatography/mass spectrometry (HS-GC/MS)

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Five marine cosmopolitan phytoplankton species namely; Calcidiscus leptoporus, Emiliania huxleyi, Phaeodactylum tricornutum, Chaetoceros neogracilis and Dunaliella tertiolecta were screened for emissions of selected VOCs using head space gas chromatography/mass spectrometry (HS-GC/MS) in single ion mode. The VOCs investigated included isoprene and various halogenated compounds. Among the different algae groups, the two diatoms Ch. neogracilis and P. tricornutum were the strongest emitters of methyl bromide (CH$_3$Br), and Ch. neogracilis was the strongest emitter of isoprene. Furthermore, we present evidence that several chlorinated organic compounds, normally considered as anthropogenic, can be produced from marine phytoplankton (namely chloroform, dichloromethane, trichloroethylene, tetrachloroethylene, chlorobenzene and dichlorobenzene).

Introduction

Volatile organic compounds (VOCs) are known to play a key role in the chemistry of both the troposphere and the stratosphere. In the presence of NO$_x$ (NO and NO$_2$) and sunlight, the photodecomposition of reactive VOCs (e.g., isoprene) produces tropospheric ozone, a radiatively-active gas that is toxic to both humans and plants. In contrast, halogen-containing organic species (e.g., CH$_3$Cl and CH$_3$Br) are known to contribute significantly to ozone destruction in the stratosphere. Furthermore, some VOC emissions, such as dimethyl sulfide (DMS), can be oxidized to form aerosol and may therefore have a direct effect on the Earth’s radiation budget. All the aforementioned VOCs (namely, isoprene, organohalogens and DMS) are known to have at least in part a marine source, yet in comparison to the terrestrial environment, species specific emissions are surprisingly poorly-characterised.

Recently, satellite technology has proven capable of providing ocean maps of where certain phytoplankton species dominate. Therefore, future global models can potentially improve ocean emissions significantly provided it is known which organic gases are emitted from which phytoplankton species. The first step in this characterisation is provided in this study.

Marine biota are known to produce a huge variety of halogen-containing organic compounds and many species between 1 and 30 carbon atoms have been documented. The potential to form halogenated compounds has been found in various species of bacteria, algae, mollusca, coelenterates and in several marine worms. Although the organochlorine compounds (CHCl$_3$, CH$_2$Cl$_2$, C$_2$Cl$_4$, C$_2$HCl$_3$, C$_2$H$_2$Cl$_2$, etc.), have been shown to have an oceanic source, their presence in the environment is usually related to human activities such as the use of pesticide, antifreezing agents, etc. Naturally-produced chlorinated compounds have also been found in terrestrial fungi, lichens and bacterial and macroalgae have been shown to produce trichloroethene and tetrachloroethene. Regarding isoprene, Bonsang et al. first presented evidence for an oceanic source, and several subsequent laboratory experiments based on phytoplankton cultures have confirmed that marine phytoplankton can emit isoprene.

Regarding isoprene, Bonsang et al. recently the global oceanic emission of isoprene has been estimated as 0.1 Tg C yr$^{-1}$ using satellite maps of the chlorophyll distribution. More recently, Mekhidze and Nenes proposed that phytoplankton-produced isoprene, can affect cloud properties over the Southern Atlantic ocean.

This paper presents the results of laboratory screening experiments on the phytoplankton production of isoprene and halogenated compounds. These experiments were conducted with cultures of five cosmopolitan marine phytoplankton species namely; Calcidiscus leptoporus, Emiliania huxleyi (both coccolithophorids), Phaeodactylum tricornutum, Chaetoceros neogracilis (both diatoms) and Dunaliella tertiolecta (chlorophyte), belonging to three different important algal classes of the coastal and open ocean. The analyses were achieved using head space gas chromatography/mass spectrometry (HS-GC/MS). In addition to the production of small halogenated species, we also investigate the possibility that phytoplankton can emit larger volatile species.
organochlorine compounds such as chlorobenzene and dichlorobenzene. While the potential climate impacts of various trace organic gases have been established, large uncertainties still exist over which species can be emitted from phytoplankton.

Experimental
Algae culture and species
Phytoplankton culture preparation conditions are described in detail in our previous work (see Yassaa et al.39). Briefly, all emission analysis experiments were carried out with batch cultures of algae provided by IFM-GEOMAR Kiel. Prior to the experiments, all cultures were kept at room temperature between 20–25 °C and adapted to a 12–12 hour light–dark cycle. The two coccolithophorids, *Calcidiscus leptoporus* (strain AC365, from South Atlantic off South Africa, CODENET culture collection, ALGObank (http://www.unicaen.fr/algobank) and *Emiliania huxleyi* CCMP 371, the two diatoms *Chaetoceros neogracilis* CCMP1318 and *Phaeodactylum tricornutum* (Phaeo, originating from the Falkowski laboratory) and the chlorophyte *Dunaliella tertiolecta* (DUN, originating from the Falkowski laboratory) were grown in f/2-medium.39 These last four cultures were kept axenic (bacteria free) prior to the experiment. These cultures are constantly used in IFM-GEOMAR Kiel for various purposes and checked regularly with “Marine Broth” for contamination of bacteria. So, *Calcidiscus leptoporus* was not axenic, but all CCMP1318 and CCMP 371 were axenic, according to the CCMP culture collection list, as well as *Phaeodactylum tricornutum* and the chlorophyte *Dunaliella tertiolecta*, our home growth axenic cultures. However, it cannot be ruled out that in the course of the experiment they became contaminated with ambient bacteria, since the equipment used for the experiment could not be sterilized. All algae were in the transition from the exponential to the stationary phase of growth. This was measured using a PhytoPAM (WALZ).

The light intensity was approximately 250 μE s⁻¹ m⁻² and the samplings of VOC emissions were performed in the middle of the light cycle (between 10:00–14:00). Chlorophyll a was measured according to the following procedure: 10 ml of the cultures were filtered through GF/F filters and frozen at −20 °C. Chlorophyll was extracted with 10 ml 90%-acetone, centrifuge and the supernatant was measured fluorometrically with a Turner fluorometer according to Welschmeyer.31

Blank tests
Prior to the transfer of algae and control from Nalgene to the Duran bottles, blank tests were performed with empty bottles. Further controls were performed with bottles containing only the f/2 medium, which was used to cultivate the phytoplankton species. Most plastics are permeable to light hydrocarbons, and several have been shown to actively absorb these chemicals, and sometimes subsequently to re-emit them.32 For these reasons glass vessels were preferred in our experiments. The VOC mixing ratios in the headspace (HS) above f/2 medium were defined as “control”. The emissions from phytoplankton were defined as occurring only when the HS mixing ratio for a given organic compound was higher than the control HS mixing ratio.

Sampling and analytical procedure
First, 200 ml of algae suspension from each species was separately transferred from Nalgene incubation bottles into 250 ml Duran glass bottles fitted with a PTFE-septum. A further identical glass bottle was left empty as a gas blank and one was filled with the same liquid concentration of f/2 medium. A head space gas chromatograph/mass spectrometer (HS-GC/MS) instrument (GC 6890 and MS 5973, both Agilent Technology, Palo Alto, CA, USA) was used for the analysis of VOCs in the headspace of the algae species. A volume of 10 ml of headspace sample was cryogenically concentrated at −70 °C (Neslab cc-100 circulation cooler, Portsmouth, USA) in a stainless steel microtrap packed with porous silica beads (Unibeads 1S, 80/100 Mesh, Alltech) under a flow-rate of 40 mL min⁻¹. A RTX-VMS capillary column (40 m-long, 0.18 mm ID, 1 μm film thickness) supplied by J & W Scientific (California, USA) was used for the separation of sampled compounds. After sample injection, the column oven was maintained at 50 °C for 4 min. After the initial isothermal step, the temperature was first increased to 100 °C at 9 °C min⁻¹ and then from 100 to 230 °C (2 min) at a rate of 40 °C min⁻¹. The mass spectrometer detector was operated in electron impact mode with the following conditions: potential ionization 70 eV; source temperature 230 °C; and selected ion monitoring (SIM) mode. The detection limit was in the range of 0.05 to 5 pptv and the uncertainty was 15%. The sampling was performed during 4 days for *Chaetoceros neogracilis* and *Emiliania huxleyi* and during 3 days for the rest. For each phytoplankton species more than 5 replicates were collected and analysed.

Results and discussion
By comparing the samples from each alga to the control, one can define whether emission of an organic compound has occurred in each case. Among the studied compounds, there was evidence of significantly enhanced levels of several VOCs in the headspace above certain cultures. To be deemed significant the emission in the sample had to be 2 times the value of the control. For certain other VOC compounds studied here, no production occurred (e.g., methylchloroform, CCl₄, CFCs). The histograms displayed in Fig. 1 to 4, compare the VOC mixing ratios in the headspace (HS) of the five algae species (Fig. 1 for isoprene, Fig. 2 for volatile halogenated compounds, Fig. 3 for organochlorine compounds and Fig. 4 for chlorobenzene).

**Fig. 1** Comparison of average isoprene emission by five phytoplankton species (expressed in pptv) using HS-GC/MS.
The initial concentration of each VOC in the water ($C_{0\text{aq}}$) was calculated as follows:

$$C_{0\text{aq}} = 10^{-12} \cdot C_{\text{pptv}} \cdot \frac{V_g}{V_{\text{mol}}} \left( \frac{K_HRT}{V_g} + \frac{1}{V_{0\text{aq}}} \right)$$

with

- $C_{\text{pptv}} = \text{pptv(HS-phytoplankton)} - \text{pptv(control)}$ (pptv(HS-phytoplankton) = mixing ratio in pptv in the head space above a specific phytoplankton culture; and pptv (control) = mixing ratio in the head space above the control).
- $V_g = \text{volume of the gas phase (L)}$
- $V_{\text{mol}} = 24 \text{ L mol}^{-1} \text{ at } 25 \degree \text{C}$
- $V_{0\text{aq}} = \text{volume of the liquid phase (L)}$
- $K_H = \text{Henry law constant (mol L}^{-1} \text{ atm}^{-1})$
- $R = 0.08206 \text{ L atm deg K}^{-1} \text{ mol}^{-1}$
- $T = \text{ambient temperature = 298.15 K}$

The biomass (chlorophyll a (Chl a), $\mu$g L$^{-1}$) and the biomass-normalised concentration (pmol L$^{-1}$/Chl a) in each culture are reported in Table 1. We chose Chl a to calculate biomass-normalised concentration because Chl a concentrations are recognised as a reasonably good indicator of phytoplankton biomass. This biomass-normalised concentration was calculated as follows: $C_{0\text{aq}}$/Chl a (pmol L$^{-1}$/Chl a).

**Isoprene**

Isoprene was identified in all cultures and a low but measurable level was determined in the control sample (Fig. 1). These results and those of Moore et al., Milne et al., and Shaw et al. from laboratory experiments support the proposal made by Bonsang et al. that marine algae are a source of isoprene. In the current study we show that the diatom *Ch. neogracilis* is a strong emitter of isoprene (28.48 pmol L$^{-1}$/Chl a). In addition, the two coccolithophorids (*E. huxleyi* and *C. leptoporus*) also emit isoprene strongly (see the biomass-normalised concentration of isoprene, 11.45 and 5.40 pmol L$^{-1}$/Chl a, respectively, in Table 1). This implies that the emission of isoprene from phytoplankton is not specific to diatoms, but can occur in other algae species like coccolithophorids too, which are known to build worldwide blooms.

Small quantities of marine photochemically produced isoprene (a few pptv) have been shown to impact the formaldehyde budget at a remote coastal site in the Southern Hemisphere. This is despite the estimated global marine source of isoprene (0.1 Tg yr$^{-1}$) being dwarfed by the terrestrial source (ca. 500 Tg yr$^{-1}$). However, in regions of high biological activity, e.g. the North Atlantic in summer, upwelling regions, or at oceanic fronts, the isoprene emissions could be of importance to local photochemistry. These regions of high biological productivity are generally dominated by diatoms, the family of plankton found here to be the most active emitters of isoprene. A further consideration is that the atmospheric oxidation products of these marine emissions, albeit minor in the absolute sense, may condense on existing aerosols and thereby change their physical properties, such as reflectivity and hydrosopicity.
Halogenated compounds

As stated in the introduction, several studies have been made to estimate the fluxes of halogenated compounds between the ocean and the atmosphere. All algae species studied in this work were emitters of CH$_3$Cl, CHBr$_3$, and CH$_3$Br (Fig. 2 and 3). For the other compounds, the measured mixing ratio clearly depends on the algae species. Previous studies confirmed the production of CH$_3$Cl and CH$_3$Br from the marine phytoplankton.\textsuperscript{11–12,35–37} C. leptoporus was the major CH$_3$Cl emitter followed by Ch. neogracilis. No significant CH$_3$Cl mixing ratio was observed from the control medium.

The main source of methyl chloride to the atmosphere is currently considered to be from tropical terrestrial biomass.\textsuperscript{38–40} However, a significant fraction of ca. 25% is thought to be oceanic and little is known about mechanism of CH$_3$Cl production in seawater. Two indirect pathways of CH$_3$Cl production in seawater have been suggested by Zafiriou.\textsuperscript{41} The latter demonstrated that chlorine substitution of CH$_3$I was a mechanism of CH$_3$Cl formation in seawater.\textsuperscript{41} This hypothesis can be ruled out by the experiments presented here, as only very low concentration of CH$_3$I were encountered in our cultures. A second possible pathway is the reaction of DMSP with chlorine to produce CH$_3$Cl.\textsuperscript{42} Tait et al.\textsuperscript{43} showed in laboratory experiments that this pathway (DMSP + chlorine) is unlikely to explain the CH$_3$Cl production from P. tricornutum cultures and suggested that direct production of CH$_3$Cl as a by-product of phytoplankton metabolism could occur.\textsuperscript{43} In the present study, the direct emission of CH$_3$Cl by phytoplankton has been demonstrated from five different cosmopolitan phytoplankton species comprising of diatoms, green algae and coccolithophorids species indicating that the release of CH$_3$Cl is not unique to a single planktonic group or species and might explain the relative high oceanic contribution of this compound.

Methyl bromide was also screened in the phytoplankton emissions examined here. In our experiments the two diatoms Ch. neogracilis and P. tricornutum were the strongest emitters of CH$_3$Br (0.007 and 0.002 pmol L$^{-1}$/Chl a). The coccolithophorid E. huxleyi has a biomass-normalised mixing ratio of 0.002 pmol L$^{-1}$/Chl a. The CH$_3$Br biomass-normalised production rates of 31 and 32 nmol g Chl a$^{-1}$ d$^{-1}$ recorded from E. huxleyi and P. tricornutum, respectively were established by Moore et al.\textsuperscript{37} Interestingly, the ratio CH$_3$Cl : CH$_3$Br of biomass-normalised production rate obtained was around 6 for both phytoplankton species. In the present work, the ratio CH$_3$Cl : CH$_3$Br of biomass-normalised concentrations was also close to 6 for P. tricornutum and approximately 15 for E. huxleyi. The difference in either the temperatures experienced in the cultures or the state of the growth could explain the discrepancy between the two studies, since a slow production of CH$_3$Cl from P. tricornutum has been noted during the exponential growth phase,\textsuperscript{44} and methyl chloride production increased with the onset of the stationary growth phase.\textsuperscript{44}

Bromoform concentrations were found in the range of 5 to 6 pptv in vessels containing algae and less than 1.5 pptv in the control sample. C. leptoporus was the strongest biomass-normalised emitter (0.0038 pmol L$^{-1}$/Chl a) of bromoform. E. huxleyi and Ch. neogracilis were in the same order of magnitude (around 0.002 pmol L$^{-1}$/Chl a) while D. tertiolecta and P. tricornutum were somewhat weaker (0.0001).

![Fig. 3](image_url) Comparison of average halogenated compounds emission by five phytoplankton species (expressed in pptv) using HS-GC/MS.

![Fig. 4](image_url) Comparison of average chlorobenzene and $p$-dichlorobenzene emissions by five phytoplankton species (expressed in pptv) using HS-GC/MS.
Table 1  Phytoplankton biomass (chlorophyll a in μg L⁻¹) and initial biomass-normalised concentration (pmol L⁻¹/Chl a) of VOCs in the water in each phytoplankton culture

<table>
<thead>
<tr>
<th>Chlorophyll a/μg L⁻¹</th>
<th>Emiliania huxleyi</th>
<th>Calcidiscus leptoporus</th>
<th>Phaeodactylum tricornutum</th>
<th>Chaetoceros neogracilis</th>
<th>Dunaliella tertiolecta</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOCs/μmol L⁻¹/Chl a</td>
<td>87</td>
<td>87</td>
<td>683</td>
<td>134</td>
<td>432</td>
</tr>
<tr>
<td>Isoprene</td>
<td>11.45</td>
<td>5.40</td>
<td>2.85</td>
<td>28.48</td>
<td>2.85</td>
</tr>
<tr>
<td>CH₃Cl</td>
<td>0.0255</td>
<td>0.1744</td>
<td>0.0022</td>
<td>0.0697</td>
<td>0.0055</td>
</tr>
<tr>
<td>CH₃Br</td>
<td>—</td>
<td>0.0000</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CH₃I</td>
<td>0.0020</td>
<td>0.0199</td>
<td>0.0004</td>
<td>0.0072</td>
<td>0.0002</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>0.3792</td>
<td>0.0287</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₂H₅Cl</td>
<td>0.0040</td>
<td>0.1131</td>
<td>0.0004</td>
<td>0.0811</td>
<td>0.0005</td>
</tr>
<tr>
<td>1.1-Dichloroethane</td>
<td>0.0880</td>
<td>0.0179</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.2-Dichloroethane</td>
<td>0.0302</td>
<td>0.0179</td>
<td>0.0002</td>
<td>0.0007</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*C. leptoporus* was the only emitter of CH₃I in this study (0.0005 pmol L⁻¹/Chl a). *E. huxleyi*, *D. tertiolecta* and *P. tricornutum* were also previously found not to emit CH₃I in at least one previous study. CH₃Cl, CH₃Br, CH₃I and CHBr₃ have been quantified in cultures of several other species indicating that the production of halogenated compounds is possible in many phytoplankton species, although the direct source is not always easy accessible.

**Further organochlorine compounds**

Several chlorine containing organic compounds, commonly considered as anthropogenic compounds, have been shown to have a biogenic source in this work. The presence of chloroform, C₂H₃Cl, 1,1-dichloroethane, 1,2-dichloroethane and trichloroethene was noted in the headspace over each culture with a mixing ratio higher than in the control vessel (Table 1). Tetrachloroethene was also present in all species measured, the formation of trichloroethene was more important than that of tetrachloroethene. The mechanism behind the formation of these compounds is not known although chlorination of ethane through addition and elimination reactions driven by peroxidase activity is one possibility.

**Chlorobenzenes**

Chlorobenzenes are normally considered as anthropogenic emissions. Due to their slow chemical reactivity (lifetime of several days) and high-toxicity, polychlorinated benzenes are included in the list of persistent organic pollutants (POPs). They have many applications, e.g. as reagents in the chemical industry, and as biocides and additives. They have been characterised in the marine environment both in seawater, and bioaccumulated in phytoplankton and fish lipid tissues. In all cases, their occurrence was related to land-based sources which introduce them through rivers, estuarine and coastal waters, and through sea-based activities, such as shipping and exploitation of offshore resources. They can also be subject to long-range atmospheric transport by advection. However, to our knowledge phytoplankton production of chlorobenzenes has not yet been reported.

Chlorobenzene and p-dichlorobenzene were identified in the headspace above the five cultures, although the emission was significant (i.e. twice the blank and f/2 medium in the species *D. tertiolecta*, *C. leptoporus* and *P. tricornutum*, Fig. 4). *D. tertiolecta* was the strongest emitter of chlorobenzene and p-dichlorobenzene followed by *C. leptoporus* and *P. tricornutum*. An estimate of the mixing ratio for chlorobenzene and p-dichlorobenzene in the headspace was obtained, relative to a secondary standard filled with ambient air from Mainz (Germany). The resultant mixing ratios were up to 1.4 ppbv of chlorobenzene in the *Dunaliella* sample and 140 pppt of para-dichlorobenzene. *meta-* and ortho-Dichlorobenzenes were also observed as peaks in the chromatogram from each of the cultures but could not be quantified because their mixing ratios were below the detection limit.

As far as we are aware, these results constitute the first evidence of chlorobenzene and dichlorobenzene production and emission by phytoplankton. The biological and chemical mechanisms behind their formation, as well as the significance of the production of these substances, warrants further research.

**Conclusion**

The results of laboratory experiments conducted on seawater containing five cosmopolitan phytoplankton monocultures show that several atmospherically-important VOCs are emitted by specific phytoplankton species. It is shown that the investigated microalgae of three different algae classes have the potential to produce isoprene, halogenated compounds, chlorobenzene and dichlorobenzene to varying extents. However, no simple algae cell-associated typical VOC emission scheme could be observed. For some VOCs (e.g. isoprene, CH₃Cl, CHBr₃ and CH₃Br) emission was determined in all phytoplankton samples tested whereas for others (e.g. methyl iodide) it was identified in one or two of the species (e.g. *C. leptoporus*). Thus, for future studies it is important to isolate the key species of a given oceanic or coastal region.

This study represents the first step in assessing phytoplankton VOC emissions on a global scale. These screening experiments have yielded a list of target compounds (some known, some new) to be monitored in the next stage of experiments.
Before extrapolation to the global scale can occur, the emission rates as a function of temperature, pH, growth stage amongst other factors needs to be assessed and actual production rates are essential. Experiments with mixed species assemblages or mesocosms on the natural ocean will be necessary, since emissions from one species may be consumed by another. The results presented here indicate that the natural production in the marine environment should not be neglected, particularly in regions of high biological activity such as in upwelling regions and seasonal phytoplankton blooms. Such experiments including both laboratory and field components are planned within on-going field projects.

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