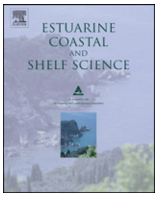
Estuarine, Coastal and Shelf Science 95 (2011) 110–118

Contents lists available at SciVerse ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



The relationship between volatile halocarbons and phytoplankton pigments during a *Trichodesmium* bloom in the coastal eastern Arabian Sea

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ARTICLE INFO

Article history:
Received 15 June 2010
Accepted 20 August 2011
Available online 27 August 2011

Keywords:
phytoplankton composition
photosynthetic pigments
Trichodesmium
halocarbons
Arabian Sea
Indian coast

ABSTRACT

Eukaryotic phytoplankton such as diatoms and prymnesiophytes produce biogenic halocarbons in the ocean that serve as important sources of chlorine and bromine to the atmosphere, but the role of cyanobacteria in halocarbon production is not well established. We studied distributions of chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), methylene bromide (CH_2Br_2) and bromoform ($CHBr_3$) in relation to phytoplankton composition, determined from pigment analysis complemented by microscopic examination, for one month in coastal waters of the eastern Arabian that experienced a *Trichodesmium* bloom that typically occurs during the Spring Intermonsoon season. High concentrations of zeaxanthin (23 $\mu g\ l^{-1}$), alpha beta betacarotene (6 $\mu g\ l^{-1}$) and chlorophyll *a* (67 $\mu g\ l^{-1}$) were found within the bloom whereas the marker pigment concentrations were low outside the bloom. $CHCl_3$ and CCl_4 occurred in relatively high concentrations in surface waters whereas CH_2Br_2 and $CHBr_3$ were restricted to the subsurface layer. Chlorinated halocarbons were positively inter-correlated and with $CHBr_3$. The observed spatial and temporal trends in brominated compounds appear to be related to the abundance of *Trichodesmium* although correlations between concentrations of brominated compounds with various marker pigments were poor and statistically non-significant. The results support the existence of multiple sources and sinks of halogenated compounds, which might obscure the relationship between halocarbons and phytoplankton composition.

1. Introduction

Phytoplankton are known to produce various halocarbon compounds that are environmentally important because of their greenhouse warming potential and ability to destroy ozone in the stratosphere (Lovelock, 1975; Salawitch, 2006; Quack and Wallace, 2003). Oceanic fluxes of biogenic halocarbons contribute significantly to the atmospheric halocarbon budgets (Abrahamsson et al., 2004). The long-lived bromine- and chlorine-containing species are particularly important as they are involved in stratospheric ozone depletion (Chameides and Davis, 1980). Production of these compounds by phytoplankton is species-dependent (Tokarczyk and Moore, 1994). However, in addition to production by phytoplankton (Singh et al., 1983; Tokarczyk and Moore, 1994; Tait and Moore, 1995; Moore et al., 1996; Scarratt and Moore, 1998) and macroalgae (Manley et al., 1992; Ekdahl et al., 1998), anthropogenic activities could also be the source of these compounds in the coastal areas (Mills et al., 1998; Christof et al., 2002) making it difficult to identify their origin. Despite this, co-variation of halogenated compounds and photosynthetic pigments has been taken to support their production by autotrophs (Schall et al., 1997; Yamamoto et al., 2001; Quack et al., 2007; Bravo-Linares and Mudge, 2008). Production of brominated and chlorinated methanes in the open ocean has often been linked to phytoplankton, particularly diatoms (Class and Ballschmiter, 1988; Klick and Abrahamsson, 1992; Baker et al., 2000; Plummer and Edzwald, 2002).

Unlike eukaryotic phytoplankton and macroalgae, information on halocarbon production by cyanobacteria is limited. Karlsson et al. (2008) recently demonstrated the potential of cyanobacteria in the Baltic Sea in the production of halogenated (especially brominated) compounds. *Trichodesmium* is inarguably the best known of all cyanobacterial genera in the ocean. However, little is known about the halocarbon production by this genus, which occurs widely in tropical waters often forming large blooms. These blooms are, however, quite patchy, both temporally and spatially. The patchy distribution is usually connected to the physical characteristics of surface waters (Kononen and Leppänen, 1997). In the Arabian Sea, for example, *Trichodesmium* blooms are mostly confined to the Spring Intermonsoon (SI) period (Qasim, 1970; Devassy et al., 1979; Capone et al., 1998; Poulton et al., 2009; Krishnan et al., 2007). The high surface temperature, calm weather and oligotrophic conditions in surface waters seem to favor growth of *Trichodesmium* during the

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doi:10.1016/j.ecss.2011.08.025

SI season (SenGupta and Naqvi, 1984). In order to study the hitherto unknown potential of *Trichodesmium* in halocarbon production, phytoplankton pigments and halocarbons ($CHCl_3$, CCl_4, CH_2Br_2 and $CHBr_3$) were estimated in the eastern Arabian Sea for one month during this season.

2. Materials and methods

A patchy *Trichodesmium* bloom was observed between 3rd and 5th April 2007 during regular visits to the Candolim Time Series (CaTS) station G3 located in the eastern Arabian Sea off Goa, India (Fig. 1a). In order to investigate the phenomenon in detail and to extend the observations further offshore, three cruises of the coastal research vessel *Sagar Sukti* (SaSu) were undertaken: SaSu 135 from 17th to 18th April., SaSu 139 from 4th to 5th May and SaSu 141 from 10th to 11th May 2007. The bloom extended up to Sta. G8. Monthly composite images of chlorophyll (Chl *a*) and sea surface temperature (SST) prepared with the MODIS data (http://daac.gsfc.nasa.gov/giovanni) are shown in Fig. 2. Niskin bottles (5 L) fixed on polyvinyl chloride (PVC)-coated hydrowire were used for sampling of water for routine measurements as well as for halocarbons and phytoplankton pigments.

2.1. Halocarbons extraction

Subsamples for halocarbons were collected immediately after subsampling for oxygen in 100 ml volumetric flasks by following the standard protocol for dissolved gases, i.e. bubbling was avoided and 200–300 ml of water was allowed to overflow. Triplicate samples were taken during 3rd-5th April but only one sample was collected subsequently from each depth. Samples were preserved immediately at 4 °C in dark until extraction. Halocarbon extraction followed the procedure of Abrahamsson and Klick (1990). For checking the detection limit, water was purged with ultra pure grade nitrogen (purity 99.9995%) and analyzed for any background contamination. Detection limits, which is defined here as signal to noise ratio (S/N=5), were determined by spiking the N_2-purged water with the halogenated compounds being analyzed. The precision was determined as the relative standard deviation of quadruplicate analyses of the spiked samples. The detection limit ranged from 0.01 to 1.2 ng l^{-1} while the precision varied between 7.2 and 9.7%. The lowest detection limit was for the chlorinated compounds ($CHCl_3$ and CCl_4) whereas it was the highest for dibromomethane. Bromoform could be detected at 0.3 ng l^{-1}. A 4–5 point external standard calibration (ESTD) was performed by using pure liquid standards obtained from Sigma Aldrich (Germany). The peak area verses amount of standard (dissolved in methanol (Chromasolv) procured from E. Merck) injected was used to prepare calibration curves. Linearity through the origin was assumed and average calibration factor was taken for quantification. See Roy (2010) for further details.

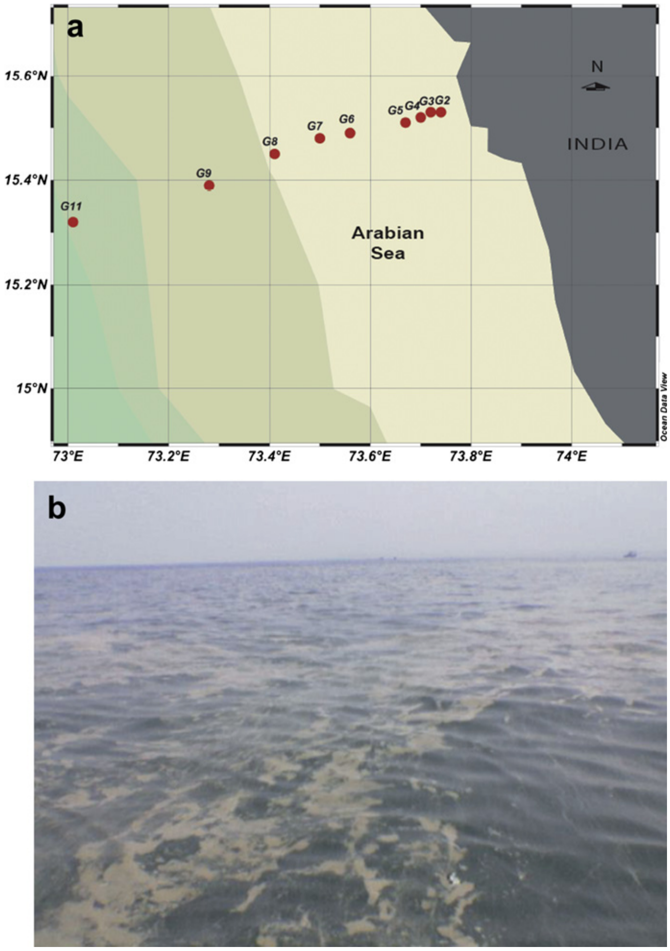

Fig. 1. a) Sampling locations in the eastern Arabian Sea off Goa; b) picture showing patchy *Trichodesmium* bloom observed at CaTS station G3. April.

2.2. Pigments extraction and speciation

For the analysis of phytoplankton pigments, water samples (10–800 ml) were filtered through Whatman GF/F (25 mm, 0.7 μm) filters that were stored at −85 °C until analysis. Pigments were extracted from 3 ml of 100% acetone for 5 min in an ultrasonic bath filled with ice-water. The extracts were stored overnight at −20 °C until analysis. The HPLC analysis was carried out following the method of Roy et al. (2006) however the buffer used was 28 mM tetra butyl ammonium acetate. The method did not separate alpha and betacarotene and hence these compounds were grouped. We also used the weighted sum of different marker pigments as proposed by Utiz et al. (2006) to reconstruct the proportion of various phytoplankton groups for this study. The sum of 19′-hexanoyloxyfucoxanthin (19′HF), 19′-butanoyloxyfucoxanthin (19′BF) and alloxanthin (Allo) was used to indicate nanoflagellates abundance whereas the sum zeaxanthin (Zea) and chlorophyll *b* (Chl *b*) have been used to represent picoplankton group. Microplankton population was represented by the sum of fucoxanthin (Fuco) and peridinin (Perid). It should be noted that the pigment grouping reported here does not strictly represent the true size class and has already been acknowledged by several authors (Vidussi et al., 2001; Roy et al., 2006; Uitz et al., 2006). For calculating the sum of all weighted diagnostic pigments, $\sum$DPw, is expressed as:

$$\begin{aligned}\sum \mathrm{DPw} = {} & 1.41[\mathrm{Fuco}] + 1.41[\mathrm{Perid}] + 1.27[19'\mathrm{HF}] \\ & + 0.35[19'\mathrm{BF}] + 0.60[\mathrm{Allo}] + 1.01[\mathrm{chl}\ b] + 0.86[\mathrm{Zea}]\end{aligned}$$

In comparison to DP, $\sum$DPw represents the chlorophyll *a* concentration, which can be reconstructed from the knowledge of the concentration of the seven other pigments (Uitz et al., 2006). The fractions of the chlorophyll *a* concentration associated with each of the three phytoplankton classes (f_{micro}, f_{nano} and f_{pico}) were then derived according to the following ratios.

$$\begin{aligned}f_{\mathrm{micro}} &= (1.41\ [\mathrm{Fuco}] + 1.41[\mathrm{Perid}])/\sum \mathrm{DPw} \\ f_{\mathrm{nano}} &= (1.27[19'\mathrm{HF}] + 0.35[19'\mathrm{BF}] + 0.60[\mathrm{Allo}])/\sum \mathrm{DPw} \\ f_{\mathrm{pico}} &= (1.01[\mathrm{Chl}\ b] + 0.86[\mathrm{Zea}])/\sum \mathrm{DPw}\end{aligned}$$

We used these ratios to derive the proportions of the three groups for our sampling period. For the qualitative and quantitative