Available online at: www.mbai.org.in doi:10.6024/jmbai.2023.65.1.2225-13

Transparent exopolymer particle production by diatoms and its relation with CO_2 flux in mangrove waters of Sundarban, West Bengal

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Received: 24 Aug 2020 Revised: 18 Aug 2022
Accepted: 24 Aug 2022 Published: 30 Apr 2023

Short communication

Abstract

Diatoms play an important role in the process of the photosynthetic conversion of carbon dioxide to transparent exopolymer (TEP). This study revealed that the formation of TEP is encouraged during the maximum population of diatom with a greater relative abundance of *Skeletonema* cf *costatum, Dytilum brightwellii, Chaetoceros* sp. in the post-monsoon. The concentration of TEP ranged from 120.4 to 203.5 μg gum xanthan equivalent/l with the maximum level in the post-monsoon, while fugacity of CO_2 (fCO_2, μ atm) was minimum (304.5 μ atm) and the study area acted as a sink for atmospheric CO_2 (-5.25 μ M/m^2/h) in contrast to the pre-monsoon (364.0 μ atm; 13.3 μ M/m^2/h) and monsoon (432.5 μ atm; 10.6 μ M/m^2/h). Thus TEP production by diatom is related to the uptake of CO_2 during available phosphorus-limited environments and may participate in other ecological processes.

***Keywords**: Diatom, transparent exopolymer particle, carbon dioxide, estuary, sundarban mangroves*

Introduction

The TEP is microscopic, transparent organic particle that consists mainly of acid polysaccharides and is an exopolymer of diatom and bacteria (Passow, 2000; Passow, 2002a). Grossart and Simon (1997) and Berman-Frank *et al.* (2007) observed TEP formation in senescent or nutrient-stressed algae and cyanobacteria. TEP production by diatom could be related to the uptake of CO_2 under nutrient limitation (Raven and Johntson, 1991). The TEP may be an important component of carbon cycles, as its concentration tends to increase along productivity gradients from oceanic to coastal regions. Bhaskar and Bhosle (2006) reported that the mean % of TEP-C contribution to the annual average organic carbon production for 1998-2000 was 6.9% ±5.8%, next only to phytoplankton-C (33.1±22.1%) and greater than bacterial-C (4.6%±4.6%) or Carbon coming from other sources (<3.8%) in the west coast of India. The TEP carbon contributes to 16% of the total organic carbon pool (Wetz *et al.*, 2009) and their aggregation and sinking could contribute significantly to the flux of carbon to depths and may have far-reaching consequences for the sequestration of carbon.

In the northeastern region of the Bay of Bengal, the estuarine systems of the river Ganges are dominated by the highly productive Sundarban mangrove ecosystems. Here, seasonal variability of diatom showed the highest levels during post-monsoon periods with a greater number of definable Bacillariophyceae species over Dinophyceae taxa (De *et al.*, 1994; Biswas *et al.*, 2010). The Biological pump for CO_2 has a very active presence here (Biswas *et al.*, 2004) and the organic carbon production of small-sized diatom was found by De *et al.* (1991) to be more than 70% of the total primary production. Copepods were the most abundant taxa (54.6% of total zooplankton abundance) and Chowdhury *et al.* (2012) explained the inter-annual variations in diatom abundance by a simple predator-prey relationship with zooplankton. Passow and Alldredge (1999) reported that the zooplankton genera, *Euphausia pacifica* preferred TEP rather than grazing small-sized diatom *Thalassiosira weissflogii*. The previous study by Chowdhury *et al.* (2016) in the Sunderban Mangrove waters was mainly based on 1) finding possible sources and 2) temporal and seasonal variation of TEP in the water. This study reports the TEP formation relating diatom production with increased CO_2 uptake under a limited supply of available nutrients in the estuarine environment of the Sundarban mangrove ecosystem and addressed the influence of TEP production on the seasonal changes of CO_2 flux.

Material and methods

Between January 2011 and December 2013, surface water samples were collected monthly from the confluence of the Saptamukhi River and the Bay of Bengal, Lothian island (21° 45.22′ N and 88° 20.45′E). Samples were analyzed for the numerical abundance of diatom and their composition, along with the related chemical parameters (NH_4^+, NO_3^-, NO_2^-, $Si(OH)_4$, PO_4^{-3}, salinity) and physical parameters (temperature, Secchi depth). Water samples for fugacity of CO_2 (fCO_2) were collected in separate glass bottles and were preserved with $HgCl_2$ and sealed airtight. They were kept cool and in dark in an ice box for analysis in the laboratory. The fCO_2 was determined by equilibrating the sample with nitrogen (Millero, 2000). The CO_2 in the equilibrated nitrogen was measured using gas chromatography with a relative uncertainty of ± 0.073. The fCO_2 in Estuarine air was measured using gas chromatography (Varian CP 3800GC) connected to a methanizer (Nickel catalyst system) for catalytic reduction of CO_2 followed by its subsequent determination with FID. The air-water CO_2 flux ($\mu M/m^2/h$) was computed as $FCO_2 = \alpha k \Delta fCO_2$, where FCO_2 is the air-water CO_2 flux, α is the CO_2 solubility coefficient ($mol/m^3/atm$), k is the gas transfer velocity (m/d) and $\Delta f CO_2$ is the air-water CO_2 gradient, (fCO_{2water}-fCO_{2air}), k was computed according to a parameterization as a function of wind speed, water current and depth (Borges *et al*., 2004), $k_{600} = 0.24 + 0.4126\ w^{0.5}\ h^{-0.5} + 0.619\ u_{10}$ where k_{600} is the gas transfer velocity of CO_2 normalized to a Schmidt number (S_c) of 600 in $m.d^{-1}$,w is the water current (cm/s), h is the water depth (25 m) and u_{10} is the wind speed (m/s) referenced at a height of 10 m. Water current data were collected using a current meter covering tidal cycles.

Nutrients were analyzed using a spectrophotometer (Systronics, UV-VIS Spectrophotometer, 117), (Grasshoff *et al*., 1983). The dissolved Nitrate estimation method was based on the formation of azo dye by using sulphanilamide. the resultant diazonium ion was coupled with 1ml of N - (1-naphthyl) ethylene diamine dihydrochloride to give an intensely pink dye. The absorbance of the resulting pink solution was measured spectrophotometrically at 543 nm against a reagent blank. The concentration of nitrate was computed from the calibration curve. Ammonia nitrogen in water was determined following the Phenol-hypochlorite method (Solorzano, 1969). The method essentially consists of the formation of mono chloramine by the reaction of ammonium with hypochlorite, which on reaction with phenol in an alkaline medium ($pH >= 10$) in presence of trisodium citrate and nitroprusside (catalyst) gave an intensely coloured indophenol blue. The absorbance of the resulting blue colour was measured at 630 nm against a reagent blank and the concentration of NH_4^+-N was computed from the calibration curve. The dissolved Phosphate of sample water was determined using acidified molybdate solution and ascorbic acid. The absorbance of the resultant molybdenum blue was measured spectrophotometrically at 882 nm against a reagent blank. The dissolved Silicate of the sample was determined by using acidified molybdate solution and an ascorbic acid solution. The blue silicomolybdic complex was formed within 30 mins and stable for hours. The absorbance of the blue complex was measured photometrically at 810 nm against a reagent blank and the concentration of SiO4 - Si was computed from the calibration curve.

Results

From a seasonal perspective, the monsoon period was different from other seasons. Nutrient concentrations were at maximum (Mean DIN = 24.2 ± 0.07 μM, DIP = 1.00 ± 0.06 μM and Silicate = 47.3 ± 6.29 μM) during monsoon but were at a minimum during pre-monsoon (Mean DIP = 0.6 ± 0.09 μM; DIN =18.8 ± 0.42 μM) and intermittent during post-monsoon. (Mean DIN= 21.8 ± 0.55 μM; DIP=0.8 ± 0.01 μM; Silicate=33.8 ± 1.63 μM). a similar relationship was obtained as observed earlier (Chowdhury *et al*., 2016).

The mean range of fCO_2 in mangrove water was decreased from 364.0 and 482.5 μatm during the pre-monsoon and monsoon to 304.5 μatm in the post-monsoon in consistence with the reversal exchange flux of CO_2 from the source in the pre-monsoon (13.3 $\mu M/m^2/h$) and monsoon (10.5 $\mu M/m^2/h$) to sink in the post-monsoon (-5.25 $\mu M/m^2/h$) when diatoms reached maximum abundance and water showed increased values of pH and transparency along with decreased values of available nutrients. The total population of diatoms ranged from 2,219 to 2,25,525 cells/l, with an average maximum of 55,108 ± 26,004 cells/l in the post-monsoon and an average minimum of 21,547 ± 10,542 cells/l in the monsoon. The abundance of small-sized diatoms was found greater (RA 51.05-64.41%) than the bigger one and 14 defined species < 20 μm were observed. 8 *Chaetoceros* species present in the water (Table 1) may be the main contributors to TEP production in the water of the study area which is in agreement with a study by Passow (2002) where he observed the considerable amount of TEP production by different *Chaetoceros* species in the laboratory cultural set up.

The TEP present in the surface water followed a seasonal trend *i.e.* highest concentration during post-monsoon period (203.5 ± 53.9 μg Gum Xanthan equivalent/l) followed by a decrease to 193.4 ± 34.1 μg Gum Xanthan equivalent/l in the pre-monsoon

Table 1. Name of some important Diatom genera with the average cell radius

Sl No	Name of Diatom	Ave. Cell radius (μm)
1.	*Skeletonema* cf *costatum*	2.7
2.	*Thalassiosira decipiens*	2.5
3.	*Chaetoceros decipiens*	2.5
4.	*Chaetoceros curvisatus*	2
6.	*Chaetoceros compressus*	1.5
7.	*Chaetoceros atlanticus*	2.5
8.	*Navicula rhombica*	3.0
7.	*Cocconies*	2.0
8.	*Chaetoceros loranzianus*	2.5
9.	*Thalassionema*	2.5
10.	*Chaetoceros* sp.	2.5
11.	*Chaetoceros didymus*	2.5
12.	*Chaetoceros affinis*	2.5
13.	*Bacillaria* sp.	1.5

and further decreased again to an average minimum of 120.4 ± 36.4 μg Gum Xanthan equivalent/l in the monsoon.

Discussion

In the post-monsoon season, there was about 2.6 fold increase in the diatom population compared to the monsoon. The relative abundance of *Skeletonema* sp. and *Chaetoceros* sp. reached a maximum level of 59.9% during the post-monsoon when fCO_2 of water was decreased by 128 μatm. An inverse flux of CO_2 from the atmosphere to mangrove water was observed along with reduced water fCO_2. Integrating over the year, the surface waters of the Sundarban mangrove (1781 km^2) exported 1.5 x 10^6 kg C, 22.4% of which was pumped out by the biological activity in the water annually. This corresponds to a CO_2 flux of 147.1 μM/m^2/d over the water area of the Sundarbans and it acted as a perennial source of CO_2. A previous study by Biswas *et al.* (2004) reported that air-water exchange flux of CO_2 was reversed in post-monsoon in estuarine water when diatom reached the highest population. The flux of CO_2 observed in this study agrees with the coastal Bay of Bengal, which acts as a net source of CO_2 to the atmosphere (200 μ M/m^2/d) (Sarma *et al.*, 2012). Engel (2002) observed the production of TEP was significant while there was greater CO_2 uptake by the natural diatom community.

During post-monsoon, the relative abundance of *Skeletonema* cf *costatum* was found at maximum (53.71-64.41%) concerning other times of the year and *Skeletonema* cf *costatum*, *Thalassiosira decipiens* and *Chaetoceros* sp. were major species amongst small-sized diatom. This suggested that the high production of extra polymeric substances by these diatom species could be an important source of TEP. Engel (2000), Thornton (2002) and Bar-Zeev *et al.* (2009) suggested that the higher abundance of small-sized diatom, *Chaetoceros* sp. and *Skeletonema* sp. increased the TEP concentration in water. According to Passow (2002), TEP is generated during growth, stationary phase and senescence of some phytoplankton species, including the diatom *Chaetoceros affinis* and the prymnesiophyte *Phaeocystis antarctica* (Hong *et al.,* 1997) while *Skeletonema* cf *costatum* produced TEP during the advanced stage of senescence (Engel, 1998). The highest TEP concentration in the month of January (267.5 μg Gum Xanthan equivalent/l) and lowest concentration (83.0 μg Gum Xanthan equivalent/l) in the month of July suggested the origin of TEP as a diatom exudates product of photosynthesis. The monthly variation of TEP exhibited a close resemblance with that of diatom in agreement with a similar relationship as observed earlier (Chowdhury *et al.*, 2016), but a reverse trend was observed for phosphate and CO_2 flux. Passow and Alldredge (1994) and Engel and Passow (2001) observed a greater concentration of TEP in the euphotic zone of the coastal sea compared to the open ocean. The former work by Chowdhury *et al.* (2016) in the Sundarban Mangrove waters (nearby stations of the present station) revealed that a greater abundance of phytoplankton, mainly diatom species like *Skeletonema* cf *costatum* and *Chaetoceros* sp. may increase TEP production and similar observations were found by Hong *et al.* (1997) and Engel (1998) in their respective studies. We observed variability in TEP and diatom along with physicochemical parameters used in VARIMAX-rotated factor

Table 2. VARIMAX-Rotated factor-loading matrix for TEP

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Communality
TEP	-0.815	-0.042	-0.020	0.471	-.269	0.962
Phosphate	0.177	-0.031	-0.931	-0.187	0.202	0.975
DIN	0.221	-0.467	-0.355	-0.075	0.762	0.980
Silicate	0.322	-0.388	-0.351	-0.767	0.048	0.969
Phytoplankton	-0.937	0.138	0.290	0.063	-0.043	0.987
Flux	0.041	-0.941	-0.016	-0.190	0.223	0.974
Variance	1.7284	1.2768	1.2016	0.8921	0.7480	5.8471
% of Variance	28.8	21.3	20.0	14.9	12.5	97.5

Table 3. Multiple regression analysis with a stepwise variable selection. Dependent variable: TEP, the concentration of transparent exopolymer particle (µg gum xanthan equivalent l^{-1}) Independent variables: DIP, Dissolved inorganic Phosphate (µM); DIN, Dissolved inorganic nitrogen (µM); Silicate (µM), Phytoplankton cell number (cells. l^{-1}) and FCO_2, air-water CO_2 flux (µMm^{-2} h^{-1})

Predictor	R^2	P	F	n
DIP	0.166	0.014	6.78	36
DIN	0.407	0.0001	16.08	36
Silicate	0.562	0.0001	28.34	36
Phytoplankton cell number	0.762	0.0001	41.11	36
FCO_2	0.764	0.025	5.52	36

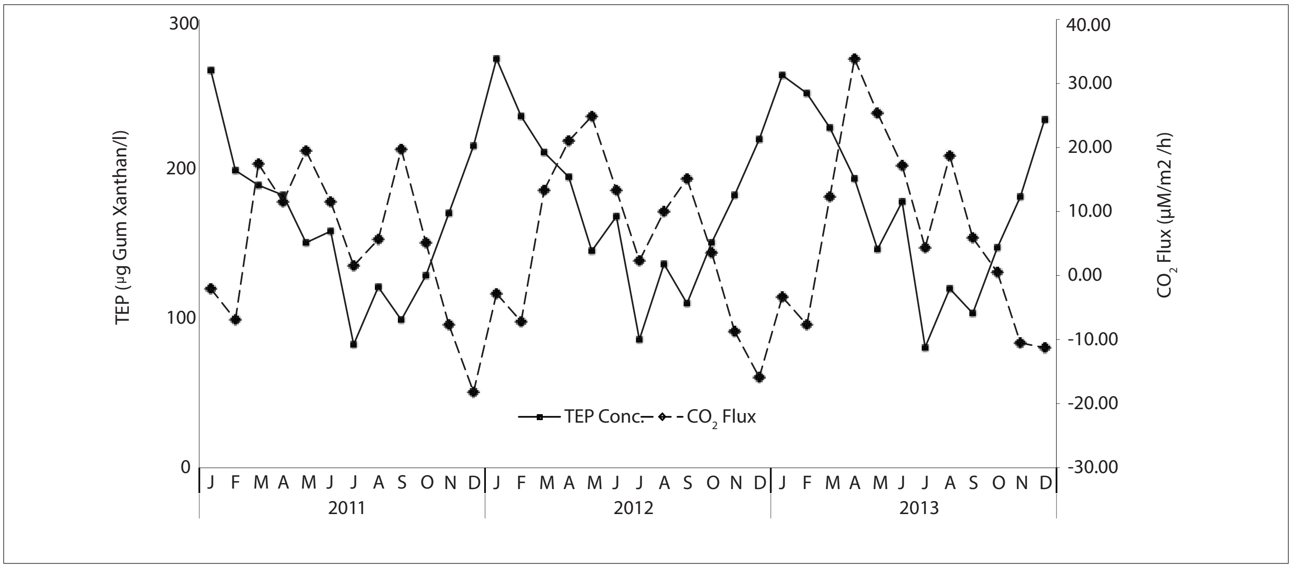

Fig. 1. Monthly variation of TEP in the river water and air-water exchange flux of CO_2 in the years 2011, 2012 and 2013

analysis and the results are given in Table 2. The Association of TEP with diatom and silicate in factor 1 indicated that TEP had a diatom source and was similar to the previous study in the nearby stations (Chowdhury *et al.*, 2016). The significance of the response of TEP concentration was tested by multiple regression analysis (Table 3). The dependent variables were TEP and the independent variables were phosphate, DIN, silicate, phytoplankton population and CO_2 flux. Statistical analysis revealed a significant correlation between TEP with an independent variable like diatom number and CO_2 exchange flux (*R2*= 0.764, p = 0.025, n = 36). Linear terms phosphate and diatom were positive with 0.366 explained variability of TEP. This suggested that the enhanced sequestration of CO_2 in terms of TEP by diatom occurs during the minimum availability of phosphorus in the post-monsoon when mangrove water acts as a sink to the atmospheric CO_2.

Conclusion

The amount of TEP production increased during post-monsoon when water exhibited sink for atmospheric CO_2 and the occurrence of diatom number was maximum with a higher relative abundance of small-sized diatom over bigger ones at relative phosphorus depleted conditions. *Chaetoceros* and *Skeletonema* cf *costatum* were more abundant during the production of TEP. TEP could play a significant role in the cycling of organic matter in shallow water mangrove ecosystems.

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