Enhancement of Phytoplankton Community of Brackishwater System by Iron Fertilization

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Cell carbon content was studied in six major species of Coscinodiscus collected from three brackishwater ponds of Indian Sundarbans treated differently by the researchers of Department of Marine Science, University of Calcutta with the financial assistance of Department of Science and Technology (DST), Govt. of India during 2012. The carbon content of the species varied significantly with treatment ($p < 0.01$), which may be attributed to exposure of the phytoplankton community to different environmental conditions. The maximum values of phytoplankton volume and carbon in iron fertilized pond speaks in favour of phytoplankton bloom due to iron enrichment. Interestingly low nutrient concentrations coincided with maximum phytovolume, phytocarbon and phytopigment (Chl a) in the iron fertilized pond, which is in accordance with the HNLC (High Nitrate low Chlorophyll) concept – a common phenomenon in several large regions of the surface waters of world ocean.

Introduction

Phytoplankton form the base of the food chain in all types of aquatic ecosystem. The knowledge of their species composition, productivity and biomass are essential to understand the salient features of the aquatic systems and the effect of the hydrological parameters on the community. Cell volume of phytoplankton is a unique indicator of nutrient load and salinity of the ambient aquatic phase (Mitra et.al, 2012). The related parameters, such as cell size and conversion of carbon content from biovolume, and physiology are also important for marine ecosystem studies (Malone, 1980; Sournia, 1981; Chisholm, 1992).

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Phytoplankton cell size varies greatly among different genera or even between different individuals. Sizes range from a few micrometres (or even less than 1 mm) to a few millimetres. Hence, there is a wide range of nine orders in magnitude for cell biovolume of phytoplankton. Several automated and semi-automatic methods for biovolume estimation have been described in the literature, such as the Coulter Counter (Hastings et. al, 1962; Maloney et. al, 1962; Boyd et. al, 1995), the micrographic image analysis system (Gordon, 1974; Krambeck et. al, 1981; Estep et. al, 1986), flow cytometry (Olson et. al, 1985; Wood et. al, 1985; Steen, 1990) and holographic scanning technology (Brown et. al, 1989). In this programme we have estimated the biovolumes and carbon content of six major *Coscinodiscus* species from three brackishwater ponds in the Kakdwip region of Indian Sundarbans. These ponds were treated with iron salt ($FeSO_4$) and mangrove litter and one of the ponds was kept as control with only brackish water stored in it. Simultaneously the Chlorophyll *a* pigment was also estimated in the three ponds to monitor the growth of phytoplankton due to different types of treatment.

Methods

Study sites

Sundarbans delta is one of the dynamic mangrove dominated estuarine deltas of the world (Banerjee et. al, 2012), which is situated at the apex of Bay of Bengal. A major portion of this delta (62%) lies in Bangladesh and the remaining 38% is within the Indian sub-continent. In the Indian Sundarbans, approximately 2069 sq. km of area is occupied by the tidal river system or estuaries, which finally end up in the Bay of Bengal. These estuaries feed several brackishwater ponds in the area. We selected three ponds in the Kakdwip area of Indian Sundarbans (21^{0}52'35.7"N & 88^{0}11'55.0?E) and treated them differently to observe the effect of iron addition on cell volume and cell carbon of six major *Coscinodiscus* species available in the present study area (Mitra et. al, 2004). The variation of phytoplankton standing stock is reflected through phytopigment level and therefore chlorophyll *a* along with nutrients were also analysed simultaneously in these three ponds.

Salinity

The surface water salinity in the selected ponds was recorded by means of an optical refractometer (Atago, Japan) and cross-checked in laboratory using Mohr-Knudsen method. The correction factor was found out by titrating silver nitrate solution against standard seawater (IAPO standard seawater service Charlottenlund, Slot Denmark, chlorinity = 19.376 psu).

Dissolved iron

Surface water samples were collected from the three ponds using 10-l Teflon-lined Go-Flo bottles fitted with Teflon taps and deployed on a rosette or on Kevlar line, with additional surface sampling carried out by hand. Shortly after collection, samples were filtered through Nuclepore filters (0.4 μm pore diameter) and aliquots of the filters were acidified with sub-boiling distilled nitric acid to a pH of about 2 and stored in cleaned low-density polyethylene bottles. Dissolved Fe was separated and pre-concentrated from the brackishwater using dithiocarbamate complexation and subsequent extraction into Freon TF, followed by back extraction into HNO_3 (Danielsson et. al, 1978). Extract was analysed for dissolved Fe by Atomic Absorption Spectrophotometer (Perkin Elmer: Model 3030). The accuracy of the dissolved heavy metal determinations is indicated by good agreement between our values and reported for certified reference seawater materials (CASS 2) (Table 1).

Table 1

Element	Certified value($\mu g\ l^{-1}$)	Laboratory results ($\mu g\ l^{-1}$)
Fe	2.97 ± 0.12	2.61 ± 0.14

Analysis of reference material for near shore seawater (CASS 2)

Nutrient analyses

Surface waters for nutrient analyses were collected in clean TARSON bottles and transported to the laboratory in ice-freezed condition. Triplicate samples were collected from the same collection site to maintain the quality of the data. The standard spectrophotometric method of Strickland and Parsons (1972) was adopted to determine the nutrient concentration in surface water. Nitrate was analysed by reducing it to nitrite by passing the sample with ammonium chloride buffer through a glass column packed with amalgamated cadmium filings and finally treating the solution with sulphanilamide. The resultant diazonium ion was coupled with N - (1-napthyl)- ethylene diamine to give an intensely pink azo dye. Determination of the phosphate was carried out by treatment of an aliquot of the sample with an acidic molybdate reagent containing ascorbic acid and a small proportion of potassium antimony tartarate. Dissolved silicate was determined by treating the sample with acidic molybdate reagent. The resultant silico-molybdic acid was reduced to molybdenum blue complex by ascorbic acid and incorporation of oxalic acid prevented formation of similar blue complex by phosphate.

Cell volume

Net samples for phytoplankton were collected around 12.00 noon with a conical nylon net bag (30 cm diameter) made of a 30 No. bolting silk from the three selected ponds and preserved in 4% neutral formaldehyde. Phytoplankton samples were observed with a ZEISS research microscope coupled with an image analyzing system. Phytoplankton cell identifications were based on standard taxonomic keys (Verlencar 2004; Botes 2003). Linear dimensions of the phytoplankton species were measured on the basis of taxonomic information and shape code as provided by Sun & Liu (2003). For each species of Coscinodiscus the best fitting geometric shape (cylindrical) and corresponding equation was used to calculate the cell volume.

Cell carbon

The cell volume of diatoms was converted into cell carbon as per the expression cell carbon (pg) = 0.288 [live cell volume μm^3)]$^{0.811}$, which is the standard expression for transforming cell volume into cell carbon (Montagnes et. al, 1994).

Chlorophyll a

For cholorophyll *a* analysis, 1 liter of surface water, collected from each of the pond was filtered through a 0.45 μm Millipore membrane fitted with a vacuum pump. The residue along with the filter paper was dissolved in 90% acetone and kept in a refrigerator for about 24 hours in order to facilitate the complete extraction of the pigment. The solution was centrifuged for about 20 min under 5000 rpm and the supernatant solution was considered for the determination of the chlorophyll pigment by recording the optical density at 750, 664, 647 and 630 nm with the help of SHIMADZU UV 2100 spectrophotometer. All the extinction values were corrected for a small turbidity blank by subtracting the 750 nm signal from all the optical densities, and finally the phytoplankton pigments were estimated as per the following expression of Jeffrey and Humphrey (1975).

$$\text{Chl a} = 11.85\ OD_{664} - 1.54\ OD_{647} - 0.08\ OD_{630}$$

The values obtained from the equations were then multiplied by the volume of the extract (in ml) and divided by the volume of the water (in litter) filtered to express the chlorophyll content in $mg.m^{-3}$. All the analyses were done in triplicate on the basis of collection of three samples from the same site in order to ensure the quality of the data.

Fluorescence study

Fluorescence study was done with fluorescence microscope (Olympus IX71, Tokyo, Japan) after staining with Acridine Orange (AO), which is a metachromatic dye that differentially stains double-stranded (ds) and single-stranded (ss) nucleic acids of the phytoplankton. When AO intercalates into dsDNA it emits green fluorescence on excitation at 480-490 nm.

Statistical analysis

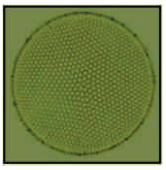
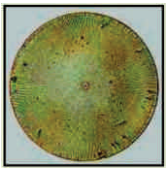
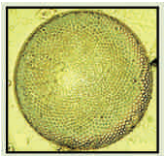
To explore the relationships between phytoplankton cell volume and cell carbon, scatterplots and allometric equations were computed. To assess whether cell volume, carbon content and environmental variables varied significantly between the ponds, two-way ANOVA was performed. All statistical calculations were performed with SPSS 9.0 for Windows.

Results

1. The average cell volume of phytoplankton ranged from (3839.30 μm^3 in Coscindiscus oculusiridis in control pond) to (70918.19 μm^3 in Coscindiscus radiatus in $FeSO_4$ treated pond) (Table 2).
2. Phytoplankton cell volume was maximum in $FeSO_4$ treated pond (average value 28042.42 μm^3) followed by mangrove litter treated pond (average value 13789.43 μm^3) and control pond (average value 13253.95 μm^3) (Table 2).
3. The average cell carbon of phytoplankton ranged from (232.39 picogram in Coscindiscus oculusiridis in control pond) to (2473.80 picogram in Coscindiscus radiatus in $FeSO_4$ treated pond) (Table 2).\
4. Phytoplankton cell carbon content was maximum in $FeSO_4$ treated pond (average value 1108.38 picogram) followed by mangrove litter treated pond (average value 612.51 picogram) and control pond (average value 591.87 picogram) (Table 2).
5. The phytopigment concentrations was maximum in $FeSO_4$ treated pond (75.2 mg/m^3), followed by mangrove litter treated pond (46.5 mg/m^3) and control pond (29.2 mg/m^3) (Fig. 1).
6. The nitrate concentrations ranged in the order control pond (28.22 μgat/l) > mangrove litter treated pond (14.65 μgat/l) > $FeSO_4$ treated pond (5.28 μgat/l). Phosphate and silicate exhibited similar trends with highest values in the control ponds (7.57 μgat/l and 68.67 μgat/l respectively), followed by mangrove litter pond (2.19 μgat/l and 39.17 μgat/l respectively) and $FeSO_4$ treated pond (0.33 μgat/l and 21.44 μgat/l respectively) (Fig 1).

7. Maximum fluorescence was observed in $FeSO_4$ treated pond (Fig 2c) followed by mangrove litter treated pond (Fig 2b) and control pond (Fig 2a).

Table 2

No.	Species	Treatment	Average Cell volume 'V' (in μm^3)	Average cell carbon (in picogram)
1.		*Control pond Mean salinity= 3.5 psu*	10637.39	531.38
		$FeSO_4$ treated pond Mean salinity = 4.1 psu	44968.71	1709.60
		Mangrove litter treated 1pond Mean salinity = 3.8 psu	12477.54	604.44
2.		*Control pond Mean salinity=3.5 psu*	12132.22	590.84
		$FeSO_4$ treated pondMean salinity=4.1 psu	24996.13	1061.90
		Mangrove litter treated pond Mean salinity = 3.8 psu	12336.21	598.88
3.		*Control pondMean salinity= 3.5 psu*	4196.70	249.79
		$FeSO_4$ treated pond Mean salinity = 4.1 psu	10778.1	536.80
		Mangrove litter treated pond Mean salinity = 3.8 psu	4317.48	255.60
4.		*Control pond Mean salinity= 3.5 psu*	44451.19	1693.66
		$FeSO_4$ treated pond Mean salinity = 4.1 psu	70918.19	2473.80
		Mangrove litter treated pond Mean salinity = 3.8 psu	45240.20	1717.99
5.		*Control pond Mean salinity= 3.5 psu*	4266.90	253.17
		$FeSO_4$ treated pond Mean salinity = 4.1 psu	8042.07	423.30
		Mangrove litter treated pond Mean salinity = 3.8 psu	4387.07	258.94
6.		*Control pondMean salinity= 3.5 psu*	3839.30	232.39
		$FeSO_4$ treated pond Mean salinity = 4.1 psu	8551.3	444.90
		Mangrove litter treated pond Mean salinity = 3.8 psu	3978.08	239.18

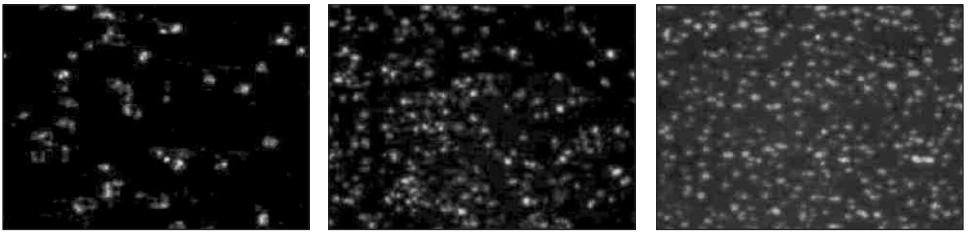
Cell volume and cell carbon of six major species of Coscinodiscus sp.

Discussion

The persistence of High Nitrate Low Chorophyll (HNLC) conditions in the surface waters of several large regions of the world's oceans comprises a familiar enigma in oceanography (Chisholm et.al, 1991). The factors that prevent the utilization of nitrate also regulate the rate at which carbon dioxide is taken up by phytoplankton and, ultimately, the amount of carbon exported from the surface waters. The oceans are both a major source and sink for atmospheric carbon dioxide, and processes that control the balance of these fluxes are thought to have a major effect on global climate (Siegenthaler, 1986). Understanding the factors that limit the uptake of excess plant nutrients is, therefore, a key to understanding climate change. Grazing pressure exerted on phytoplankton by rapidly reproducing microzooplankton and micronutrient (iron) deficiency may function jointly in these HNLC waters (Price et. al, 1991); yet the relative importance of each of these factors in controlling the biomass and rates of phytoplankton production has remained contenious (Landry et.al, 1997). The experimental tools available to the oceanographer have, until recently, been inadequate to resolve the relative importance of these processes. In vitro enrichment experiments (Martin et.al, 1990, Coale 1991, De Baar et.al, 1990, Price et.al, 1994) where iron is added at nanomolar levels to samples of seawater, invariably do not represent the in situ phytoplankton grazer community. The present study exhibits considerable growth of phytoplankton volume, phytoplankton carbon and chlorophyll a level in iron sulphate treated pond along with significant lowering of nutrients (NO_3, PO_4 and SiO_3). On contrary in the control pond, all species of Coscinodiscus showed lowest cell volume, carbon and chlorophyll a that speak in favour of the role of iron fertilization in enhancing the bloom condition of phytoplankton and utilization of nutrients from ambient water.

Fluorescence measurements also confirmed the increase of phytoplankton standing stock in iron fertilized pond (Figs 2a-2c).

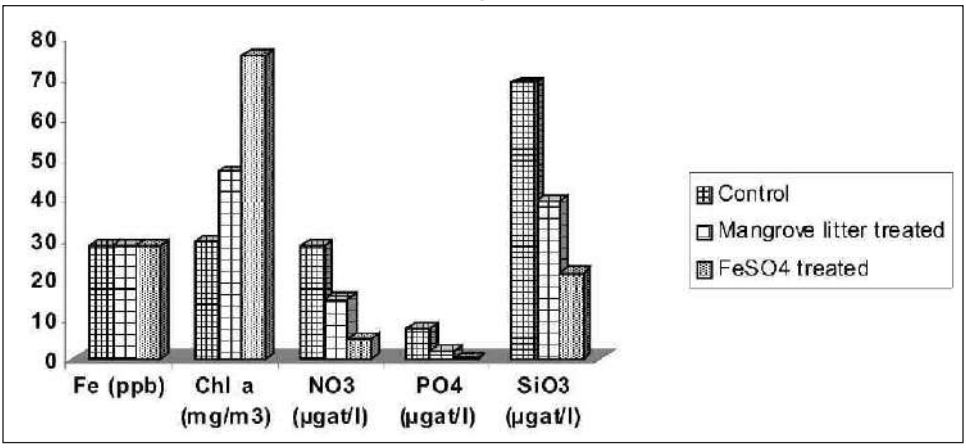
Fig 2 (a) - (c)



Fluorescence microscopy images of phytoplankton standing stock

All the hydrological variables showed a distinctive response to iron after the seven days experimental period. Compared to control pond, the mangrove litter treated pond and iron sulphate treated pond showed 59.25 % and 157.53 % increase respectively in chlorophyll a concentration, the nitrate value decreased by 48.09 % in mangrove litter treated pond and 81.29 % in $FeSO_4$ treated pond compared to control pond. Similar trend was also observed for phosphate and silicate. The phosphate decreased by 71.07 % in mangrove litter treated pond and by 99.56 % in $FeSO_4$ treated pond. The silicate decreased by 42.96 % in mangrove litter treated pond and by 68.78 % in $FeSO_4$ treated pond (Fig. 3).

Fig 3



Variations of hydrological parameters due to different treatment

The increase in phytoplankton carbon content in $FeSO_4$ treated pond also confirms the role of iron in accelerating CO_2 uptake from the ambient waters. ANOVA results also confirm significant variation of cell volume, cell carbon, chlorophyll a, nitrate, phosphate and silicate between the ponds (Table 3).

Table 3

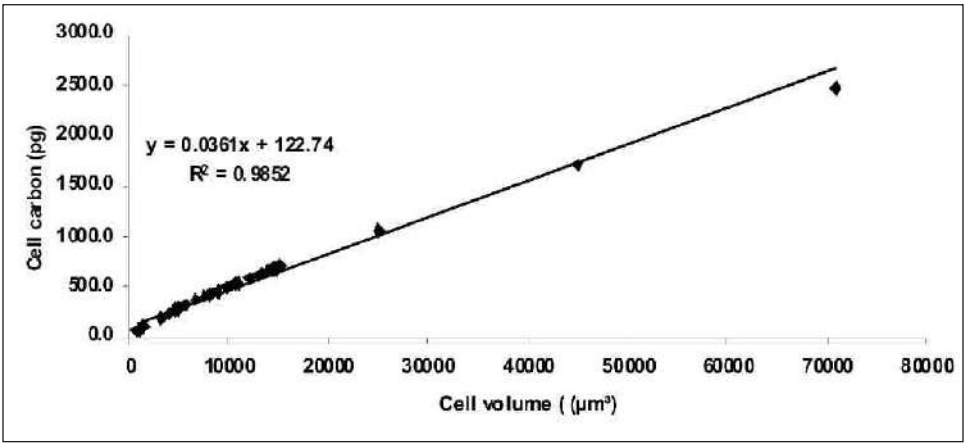
Variable	F_{cal}	$F_{crit}(p<0.05)$
A) Cell volume Between pond	4.15	4.10
B) Cell carbon Between pond	10.56	4.10
C) Chl (mg/m^3) Between pond	65535	4.10
D) NO_3 (μgat/l) Between pond	1752.6	4.10
E) PO_4 (μgat/l) Between pond	36298	4.10
F) SiO_3(μgat/l) Between pond	71805.9	4.10

ANOVA exhibiting significant variation of phytoplankton cell volume, cell carbon and hydrological parameters between ponds

Our observation synchronizes with the works of Kumar et. al (1995) who observed increased export of carbon to sub-Antarctic sediments during the Last Glacial Maximum at times of higher iron flux.

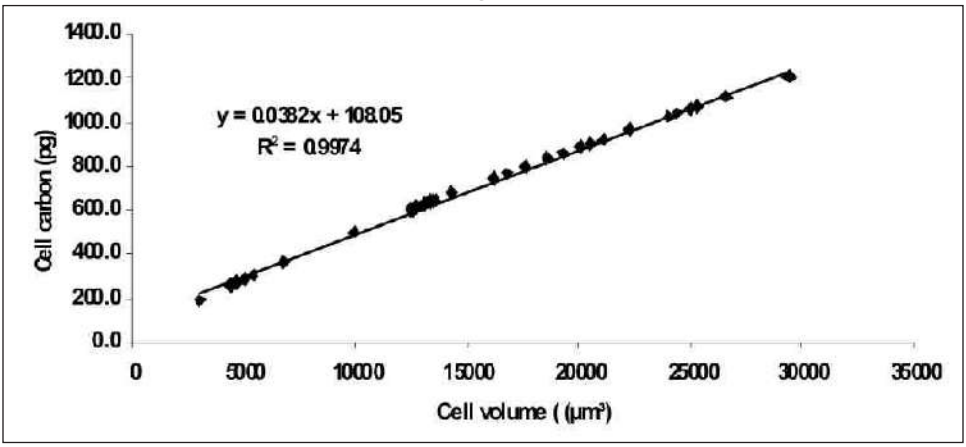
It is noteworthy that phytoplankton carbon content is accounted solely due to phytoplankton volume (Figs. 4-6).

Fig 4



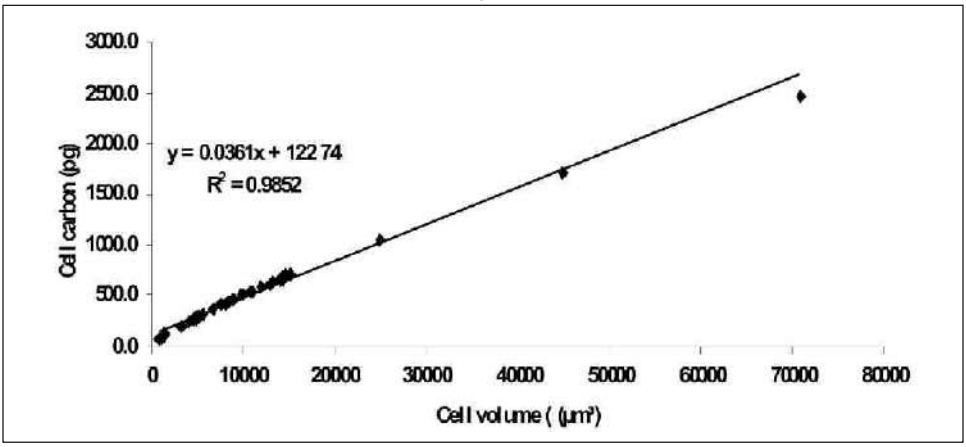
Allometric equation for phytoplankton in control pond

Fig 5



. Allometric equation for phytoplankton in mangrove litter treated pond

Fig 6



Allometric equation for phytoplankton in iron sulphate treated pond

The degree of dependency is, however, a function of ambient environmental condition. Thus, a uniform allometric equation representing the inter-relationship between phytoplankton carbon and volume cannot be established. The carbon sequestration in this unique free floating micro-producer community is a function of biomass production capacity that depends on the interaction between climate and environmental variables. Hence, results obtained at one aquatic system may not be equally applicable to another aquatic system.

The present study has great relevance in the field of pisciculture as the overgrowth of phytoplankton can act as direct natural feed for surface feeders (like Rohu, Catla) and bottom feeders (like giant fresh water Prawn) after the algal crash.

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