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Primary Production of Benthic Microalgae in the Tropical Semi-enclosed Brackishwater Pond, Southwest Coast of India

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Abstract

The present study was carried out in a semi-enclosed brackishwater pond of Nethravathi estuary southwest coast of India. A semi-enclosed brackishwater pond is a mangrove fringed shallow water body with an average depth of 1m and an area of 5 ha directly connected to the estuary, that opens into the Arabian Sea. The study involved both field and laboratory work. Samples were collected in triplicate from four stations during the low tide period once a month for one year from February 1998 to January 1999. The study site is an important nursery ground for most of the finfish and shellfish and also serves as a center for fish seed collection. Benthic primary production, pigments, nitrogen and phosphorus of the sediment, benthic microalgae, sediment texture and physico-chemical characteristics of overlying waters such as water temperature, pH and dissolved oxygen were studied. Sediment temperature was found to be varied between 26.7 and 26.0°C while water temperature fluctuated from 26.3 to 37.6°C. The pH of the overlying water ranged between 6.65 and 7.7. The dissolved oxygen concentration of overlying waters were in the range of 3.52 and 4.87 ml·l. Data on sediment nitrogen ranged between traces and 1.296 mg·g⁻¹ sediment, while phosphorus ranged between 0.85 and 15.5 mg·g⁻¹ sediment. The sediment chlorophyll -a concentration fluctuated from 0.39 to 1.48 mg·g⁻¹ sediment with no clear pattern of seasonal variation, although higher values were found in May, December and September. The phaeopigments varied from 3.48 to 17.92 mg·g⁻¹sediment. The total annual benthic production was found to be 33.59 gC·m⁻², while primary production of water was only 10.51 gC·m⁻². Seasonal benthic primary production showed three peaks with a primary peak in May followed in December and September. The estimation of benthic primary production serves as a useful tool in future analyses of the productive component of estuarine benthic community.

Introduction

Primary production is defined as the rate of synthesis of organic matter by the phytoplankton through the uptake of carbon and utilization of solar radiation as a source of energy (Odum 1971, UNESCO 1973, Barnes and Mann 1980 and Parsons et al. 1984). To understand the ecology of an ecosystem, information on the phytoplankton biomass as food and rate of primary production is required.

The productivity of microbenthic algae accounts for a large percentage of the total primary production in shallow estuarine environments. An accurate estimate of benthic productivity is necessary for an understanding of the carbon flow in different aquatic environments. Microbenthic algae are known to regulate the cycle of nutrients from the sediment to the water column (Hargrave et al. 1983, Shaffer and Onuf 1983, Rizzo 1990, Sundback et al 1991, Wiltshire 1992, Barranguet 1997). As producers of new organic matter they can enter the benthic and pelagic food web, thus the microphytobenthos forms the key component of the carbon cycle in benthic environments. In shallow aquatic ecosystems, benthic microalgae provides a major food source for meio- and macro-invertebrate grazers such as annelids, nematodes, flat worms, crustaceans, mollusks and some demersal fishes and larvae.

A lot of information are available on phytoplankton productivity of waters (Verlencar 1984, Devassy and Goes 1989, Gupta et al. 1990 and Nayar et al. 1999), while information on benthic productivity in shallow waters and intertidal environments are scanty. More scientific investigations were carried out in the Ythan estuary, Scotland (Leach 1970), EMS – Dollard estuary (Colijn and Dejonge 1984), Australian estuarine system (Lukatelich and Mc Comb 1986) and Langebaan lagoon, South Africa (Fielding et al. 1985). The benthic microalgal community has been largely ignored in studies of the shallow intertidal ecosystem of India. Hence this paper attempts to study the in-situ photoautotrophic carbon fixation in the brackishwater environment. The study included the measurement of chlorophyll–*a* at the top layer of sediment, phytoplankton biomass, nutrients and texture of sediment and some important water quality parameters.

Study area

The semi-enclosed brackishwater ponds of Nethravathi estuary (12° 50' N, 74° 59' E) are mangrove fringed shallow water bodies with an average depth of 1 m and an area of 5 ha directly connected to the estuary, that opens into the Arabian Sea. It receives freshwater from the Nethravathi river during the southwest monsoon and part of the postmonsoon while during other seasons, it receives sea water from the estuary.

Materials and Methods

This study was conducted from February 1998 to January 1999. Four stations were selected at random throughout the semi-enclosed brackishwater pond. From each station, samples were collected in triplicate right after the sediment was exposed to air during low tide. Intact sediment samples were collected by gently pushing a plastic corer (2.9 cm ID) into the sediment. After removal, the top one cm sediment was separated and transferred into a stoppered glass tube (60 ml capacity) with prefiltered estuarine water collected in the same area and incubated with one ml of $NaH^{14}CO_3$ (1.11^{10^7} dpm) using a syringe. The samples were mixed well and incubated in the field (in-situ) for 3 hrs. After incubation the samples were fixed in neutral pH formalin concentration (4%) (Varela and Penas 1985). The samples were filtered through 0.45 mm membrane filter using Manifold filtering unit. The filters with filtrate were kept in a cold (4°C) dark condition. The samples were then prepared for the counting of radio isotopes (Marshall et al. 1973). The filters were labeled, stored in planchets and later used for the radio assay. The samples cannot be counted directly in the Liquid Scintillation Counter because the sediment causes severe quenching (Marshall et al. 1973). Instead, 4% W/V cab-o-sil is used. This produces thixotopic gel when mixed with toluene scintillant and results in a uniform dispersion of sediment particles with good counting properties. Counting was done using a Liquid Scintillation Counter. Annual production was calculated by taking the average of 6 hrs daylight and then extrapolated for a month and then to a vear.

Primary production of the water column was measured using two light and one dark bottles, following the in-situ method. The bottles were incubated with 1 ml of $NaH^{14}CO_3$ for 3 hrs. After incubation, samples were filtered through 0.45 mm membrane filter. A scintillation cocktail specific for aqueous samples was used. Uptake of ¹⁴C was measured using a liquid scintillation counter (Wallen and Geen 1968).

Separate core samples were taken for the identification of benthic microflora and other associated flora in the sediment. The algae present in the sediment were separated and concentrated using floatation technique or density gradient method (Jonge 1979). Differences in specific weight between intact benthic phytoplankton and other sediment components were used to separate these fractions with Ludox–TM (series of colloidal silica polymers). The measuring cylinder having 70% Ludox–TM is mixed with the sample. The cylinder was shaken for 60 seconds at high speed. After shaking, sand grains were allowed to settle for about 15 seconds. The supernatant having benthic phytoplankton was taken for the analysis. Plankton were counted under the microscope and expressed as cells·m⁻².

Two replicate sediment cores (2.9 cm ID) per sample were taken for pigment analysis. The top one centimeter of each core was collected and chlorophyll–a concentration in the sediment was measured (Cadee and Hegeman 1974b, Riznyk et al. 1978). The pigments were extracted with 90% acetone in a cold (4°C) and dark condition for 24 hrs, and subsequently centrifuged at 3000 rpm for 10 min (Whitney and Darely 1979). The use of MgCO₃ was avoided, since it may prevent the complete phaeophytinization of the acidified extracts and it tends to absorb significant quantities of chlorophyll–a and phaeophytine (Moed and Hallegraeff 1978). Pigment analysis

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of the water column was estimated by filtering 1.0 to 1.5 l of water sample through Whatman GF/C filters. These filters were used to extract chloro-phyll–a and phaeopigments (Parsons et al. 1989).

Sediment sample was collected and temperature was immediately measured using standard thermometer. Organic content of the sediment was determined following the wet oxidation method (El–Wakeel and Riley 1957) and results were expressed in terms of percentage of organic matter. Sediment nitrogen was measured following the modified Kjeldahl method using selenium method (De 1962). For analysis of total phosphorus in the sediment, the method given by Solar Zoano et al. (1980) was used. The method recommended by the International Biological Program (Buchanan and Kain 1971) was followed for the particle size analysis of the sediment.

Results

Primary production

The average monthly production rates from 1998 to 1999 were 0.71 and 39.23 mgC·m⁻²·hr⁻¹. The data exhibits a trimodal pattern of distribution with the peak production in May followed in December and September during an annual cycle (Fig. 1). Lower values were recorded in the month of August. However, benthic primary productivity was observed to be generally higher during the premonsoon season (18.84 to 39.23 mgC·m⁻²·hr⁻¹). The post monsoon period (October to January) recorded moderate values of phytobenthic production ranging from 0.71 to 23.95 mgC·m⁻²·hr⁻¹. Carbon uptake was low during monsoon (June-September) and ranges between 1.03 and 18.23 mgC·m⁻²·hr⁻¹.

Primary production in the water column (Fig. 2) showed a maximum value in May and smaller peaks in November and December. The average monthly production was 4.89 gmC·m⁻²·hr⁻¹. Table 1 shows values of production rates for water and sediment and were estimated using the day length factor (Leach 1970). Total year round production was estimated to be 10.51 gmC·m⁻²·yr⁻¹ for the water column and 33.59 gmC·m⁻²·yr⁻¹ for the sediments. This gives an estimated total production of 44.1 gmC·m⁻²·yr⁻¹ for the area studied.

Monthly values of benthic primary production showed significant correlation (Table 2) with sediment temperature (r = 0.72), chlorophyll–*a* (r = 0.68) and microalgae (r = 0.5230) present in the sediment. Production showed no significant correlation with phaeopigment (r = -0.36), sediment nitrogen (r = 0.27) and sediment phosphorus (r = -0.1611).

Pigments

Variation of chlorophyll–a and phaeophytin content of water is shown in figure 4. Higher chlorophyll–a values were observed in May and lower values in January. However the seasonal trends of phaeophytin are more irregular and varied from 0.54 mg·m⁻³ to 19.04 mg·m⁻³ exhibiting a trimodal distribution with peaks in March, November and June.

The sediment chlorophyll–*a* concentration fluctuated from 0.39 mg·g⁻¹ to 1.48 mg·g⁻¹ sediment with no clear pattern of seasonal variation, although higher values were found in May, December and September (Fig. 3). The phaeopigments varied from 3.48 to 17.92 mg·g⁻¹ sediment. Higher values were observed during the months of August and November.

Chlorophyll–*a* showed significant correlation (Table 2) with benthic primary production (r = 0.68) and microalgae (r = 0.91), while no significant correlation with temperature (r = 0.29), phaeopigment (r = -0.41), sediment nitrogen (r = 0.088) and sediment phosphorous (r = 0.24) was observed.



Fig. 1. Monthly variation of benthic primary productivity in the semi-enclosed brackishwater pond.

Fig. 2. Monthly variation of primary productivity of overlying waters of semienclosed bracki shwater pond.

Table	1. M	ean	mon	thly	prima	ary j	prod	lucti	vity	for	water	co	lumn	and	sedimer	nt and	l est	timated	an-
nual	produ	ictio	n at	brac	kishv	vate	r po	nds	of N	Vetl	hravat	hi	estua	ry.					

	Mean produ (mgC∙m	uction rate 1 ⁻² ·h ⁻¹)	Mean calculated primary productivity $(mgC \cdot m^{-2} \cdot month^{-1})$				
Month	Water column	Sediment	Water column	Sediment			
February, 1998	01.73	24.48	311.4	4406.4			
March	01.76	18.84	316.8	3391.2			
April	04.36	18.95	784.8	3411.0			
May	14.24	39.23	2563.2	7061.4			
June	04.71	18.23	847.8	3281.4			
July	02.78	09.30	500.4	1674.0			
August	03.78	01.03	680.4	1854.0			
September	11.45	02.02	2061.0	363.6			
October	04.13	00.71	743.4	127.8			
November	03.41	01.43	565.2	257.4			
December	02.49	23.94	448.2	4309.2			
January, 1999	03.85	19.16	693.0	3448.8			

Annual production (gC. m⁻²) Water column - 10.51 Sediment - 33.59 Total - 44.10

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BENTHIC DIATOM

A total of 40 diatom species were observed, among which Amphora lineolata, Diploneis smithii, Nitzschia longissima, N. sigma, Navicula monilifera, N. lyra, N. permagna, Pleurosigma angulatum, P. directum, Surirella fluminensis, Synedra ulna and Triceratium robersterinium were dominant. The biomass was higher in May and lower in August. A gradual increase was observed from November to December. Benthic diatom showed significant correlation with primary production and chlorophyll–a in the sediment.

NUTRIENTS

The data on sediment nitrogen gathered during the present study revealed that the values ranged between traces and 1.296 mg·g⁻¹ of sediment, while phosphorus ranged between 0.85 and 15.5 mg·g⁻¹ sediment. These values suggest that there should not be any nutrient limitations for microalgal growth in the sediment. Sediment at the study area was relatively rich in dead and decayed vegetation of the mangrove. The most predominant fraction was found to be sand, followed by silt and clay in space and time.



Fig. 3. Monthly variations of chlorophyll- α and phaeopigments in the sediments of brackishwater pond.

Fig. 4. Monthly variations of chlorophyll- α and phaeopigments in the water column of bracki shwater pond.

	Temperature	BPP	Chlorophyll-a	Phaeopigment	Periphyton	Sediment nitrogen	Sediment phosphorus	Organic carbon
Temperature	-	0.72*	0.29	-0.24	0.14	-0.18	-0.64*	0.52
BPP**	-	-	0.68*	-0.36	0.52	0.27	-0.16	0.39
Chlorophyll-a	-	-	-	-0.41	0.91*	0.08	0.24	-0.10
Phaeopigment	-	-	-	-	-0.13	-0.14	0.01	0.21
Periphyton	-	-	-	-	-	0.008	0.28	-0.04
Sediment nitrogen	-	-	-	-	-	-	0.61*	-0.03
Sediment phosphorus	-	-	-	-	-	-	-	-0.18
Organic carbor	1 -	-	-	-	-	-	-	-

Table 2. Correlation coefficient for the parameters studied.

*r significant at P \pounds 0.05

**BPP - Benthic Primary Productivity

Temperature of the overlying water was in the range of 26.3 to 37.6°C while for the sediment it fluctuated between 26.7 and 36.0°C. The pH values were found to vary between 6.65 and 7.7. Dissolved oxygen concentration was in the range of 3.52 and 4.87 ml·l.

Discussion

The estimated annual production of the benthic microalgae, 33.59 gC·m⁻², is a moderate value compared to other areas (Riznyk et al. 1978, Varela and Penas 1985 and Barranguet 1997). The phytobenthic production showed a marked seasonal variation. Comparison with other data is restricted by the wide variability of procedures used: different methods (O_2 , ¹⁴C), treatment of samples (intact, altered), preservatives (buffered formalin, Lugol's solution, H_3Po_4), sample processing for ¹⁴C uptake determinations (wet digestion, combustion) and measurement of radioactivity (Geigermuller counter, Liquid Scintillation counter). In some cases both the low tide and high tide productions were used while in some, only the low tide or high tide values were used which makes a difference.

In the present study, epibenthic production m^{-2} was about 3 folds greater than that of the productivity of water column. Cadee and Hegeman (1974a) and Verala and Penas (1985) found epibenthic productivity to be 10 times greater than that of the water column, while Matheke and Horner (1974) found epibenthic production to be only twice as high as that of plankton.

Chlorophyll–a values supported three peaks of benthic primary production from April to May, December to January and September to October. However, the degraded product of chlorophylls (phaeopigment) was recorded to be less during peak periods of microbenthic productivity and higher during lower production. Statistically, chlorophyll–a values showed a positive significant correlation with benthic production, thus indicating chlorophyll–a to have direct bearing on the seasonal variation of microphytobenthic productivity. In comparison, various authors (Cadee and Hegeman 1974b and 1977, Colijn 1978, Davis and Mc Intyre 1983, Colijn and de Jonge 1984) have found a positive significant correlation between sediment chlorophyll–a and benthic primary productivity.

The seasonal distribution of benthic diatom supported the peak periods of phytobenthic productivity. In comparison, Colijn and Venekamp (1977) observed significant positive correlation between algal biomass and microphytobenthic productivity in EMS–Dollard estuary. Species composition of plankton in water and sediment showed predominance of pennate diatoms during the period of study whereas less contribution of centric diatoms was noticed.

Total nitrogen and total phosphorus in the sediment did not show any marked variation between seasons. The higher phytoplankton production coincided with lower nitrogen and phosphorus content. This indicates that the available nutrients has been used by the phytoplankton for their growth to reach peak production and thus indicated lower values of nutrients during this period. The same could be related to higher benthic primary production during the period of lower nutrient concentrations. In support of this, Lukatelich and Mc Comb (1986) found an inverse relationship between sediment nutrients and benthic primary production in shallow estuarine system in Australia. In contrast, Varela and Penas (1985) hardly observed any correlation between nutrient concentration and algal growth.

Abundance and distribution of benthic organisms are mainly related to sediment texture, water movement and prevailing physical forces such as tide. The size fraction of sediments such as sand, silt and clay therefore plays an important role in the benthic production. Our results show that phytobenthic production has a direct relationship with silt fraction (r = 0.30) of sediment, while sand and clay content failed to show any marked variations with benthic primary productivity. Davis and Mc Intyre (1983) on the other hand, estimated the annual mean rates of benthic primary production to vary in the range of 38 gC·m⁻², 53 gC·m⁻² and 21 gC·m⁻² in sand, fine sand and silty sediments, respectively.

Conclusion

Benthic diatoms may be the main food source for many of the meio- and macrofaunal grazers in shallow estuarine systems. The works of Fenchel and Kofed (1976), Levinton and Bianchi (1981) and Barranguet (1997) indicated that for hydrobia snails and mussels, benthic diatoms are likely to be the major sources of nutrition. The benthic diatoms, however, are capable of growing into dense mats with high carbon biomass and ultimately of producing microalgal populations. The estimation of benthic primary production and biomass of benthic algae should serve as a useful tool in future analyses of this productive component of the estuarine benthic community.

References

- Barnes, R.K. and K.H. Mann 1980. Prologue. In: R.S.K. Fundamentals of aquatic ecosystems, Blackwell Scientific Publicatins, Oxford. pp. 295.
- Barranguet, C. 1997. The role of microphytobenthic primary production in a Mediterranean Mussel culture area. *Estuarine Coastal and Shelf Science*, 44: 753-765.
- Buchanan, J.B. and J.M. Kaim 1971. Measurement of the physical and chemical environment. In: Holme, N.A., Mc Imtyre, A.D. (ed.) Methods for the study of marine benthos. Blackwell Scientific Publications, Oxford and Edinburgh, p. 30-58.
- Cadee, G.C. and J. Hegeman. 1974a. Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea, Netherlands. *Journal of Sea Research.* 8: 260-291.
- Cadee, G.C. and J. Hegeman. 1974b. Primary production of the phytoplankton in the Dutch Wadden Sea, Netherlands. *Journal of Sea Research.* 8: 240-259.
- Cadee, G.C. and J. Hegeman. 1977. Distribution of primary production of the benthic microflora and accumulation of organic matter on a tidal flat area, Balgzand, Dutch Wadden Sea, Netherlands. *Journal of Sea Research*. 11: 24-41.
- Colijn, F. 1978. Primary productivity measurements in the EMS-Dollard estuary during 1975 and 1976. Biologisch Onderzoek Ems-Dollard Estuarium, Publikaties en Verslagen, 1978-1, p.1-14.

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- Colijn, F. and V.N. de Jonge. 1984. Primary production of microphytobenthos in the EMS-Dollard Estuary. *Marine Ecological Progress Series*, 14: 185-196.
- Colijn, F. and L. Venekamp. 1977. Benthic primary production in the EMS-Dollard estuary during 1975. *Hydrobiological Bulletin*, 11: 16-17.
- Davis, M.W. and C.D. Mc Intyre. 1983. Effects of physical gradients on the production dynamics of sediment associated algae. *Marine Ecological Progress. Series*, 13: 103- 114.
- De, S.K. 1962. Methods of soil analysis Narayan Publishing House, Allahabad 204 pp.
- Devassy, V.P. and J.I. Goes. 1989. Seasonal patterns of phytoplankton biomass and productivity in a tropical estuarine complex. Proceedings of Indian Academic Science, 99(5): 485-501.
- El-Wakeel. S.K. and J.P. Riley. 1957. The determination of organic carbon in marine muds. J. Cons. Perm. Inst. Explor. Mer., 22: 180-183.
- Fenchel, T. and H. Kofoed. 1976. Evidence for exploitative interspecific competition in mud snails. *Oikos.*, 27: 367-376.
- Fielding, P.J., J. Damstra and G.M. Branch. 1985. Benthic diatom biomass, production and sediment chlorophyll in Langebaan lagoon, South Africa. *Estuarine and Coastal Shelf Science*, 14: 414-425.
- Gupta, D.P., S.N. Pillai, L. Krishnan and K. Alagaraswamy. 1990. Studies on low productive rain fed brackishwater culture pond along the periphery of Chilka lake, Orissa. In: The Second Indian Fisheries Forum, 27-31 May, Mangalore, Karnataka.
- Hargrave, B.T., N.J. Prouse, G.A. Phillips and P.A. Neame. 1983. Primary production and respiration in pelagic and benthic communties at 2 intertidal sites in the upper bay of Fundy. *Canadian Journal of Fisheries and Aquatic Science*, 40 (Suppl. 1), 229-243.
- Jonge De, V.N. 1979. Quantitative separation of benthic diatoms from sediments using density gradient centrifugation in the colloidal silica Ludax – TM. *Marine Biology*, 51: 267-278.
- Leach, J.H. 1970. Epibenthic algal production in an intertidal mud flat. *Limnology and Ocean*ography, 15: 514-521.
- Lukatelich, R.J. and Mc Comb, A.J. 1986. Distribution and abundance of Benthic microalgae in a shallow south western Australian estuarine system. *Marine Ecological Progress. Series*, 27: 287-297.
- Marshall, N., D.M. Skauen, H.C. Lampe and C.A. Oviatt. 1973. Primary production of benthic microflora. In: A guide to the measurement of marine primary production under some special conditions. UNESCO Monographs on Oceanographic Methodology, 3: 37-44.
- Matheke, G.E.M. and R. Horner. 1974. Primary productivity of the benthic microalgae in the Chukchi sea near Barrow, Alaska. *Journal of Fisheries Research Board Canada*, 31: 6.
- Moed, G.R. and G.M. Hallegraeff. 1978. Problems in the estimation of chlorophyll –a and phaeopigments from pre- and post acidification spectrophotometric measurements. *Hydrobiology*, 63: 787-800.
- Nayar, S., G. Gowda, and T.R.C. Gupta. 1999. Size fraction primary productivity of a tropical coastal lagoon on the southwest coast of India. Asian Fisheries Science, 12(3): 217-222.
- Odum, E.P. 1971. Fundamentals of ecology. Saunders, New York, pp. 573.
- Parsons, T.R., Moitay and C.M. Lalli. 1989. A manual of chemical and biological methods of seawater analysis. Pergamon Press, New York pp. 173.
- Parsons, T.R., M. Takahashi. and Hargrave. 1984. Biological oceanographic processes. 3 ed. Pergamon Press, London, pp. 330.
- Riznyk, R.Z., J.I. Edens and R.C. Libby. 1978. Production of epibenthic diatoms in a southern California impounded estuary. *Journal of Phycology*, 14: 273-279.
- Rizzo, W.M. 1990. Nutrient exchanges between the water column and a subtidal benthic microalgal community. *Estuaries.*, 13: 219-226.
- Shaffer, G.P. and C.P. Onuf. 1983. An analysis of factors influencing the primary production of the benthic microflora in a southern California lagoon. *Netherlands Journal of Sea Research*, 17: 126-144.
- Solorzano, L. and J.H. Sharp. 1980. Total dissolved and particulate phosphorus. *Limnology and Oceanography*, 25: 754-758.
- Sundback, K., V. Enoksson, W. Graneli and K. Petterson. 1991. Influence of sub littoral microphytobenthos on the oxygen and nutrient flux between sediment and water: a laboratory continuous flow study. *Marine Ecological Progress Series*, 74: 263-279.
- UNESCO. 1973. A guide to the measurement of estuarine primary production under some special conditions. UNESCO, Paris.
- Varela, M. and E. Penas. 1985. Primary production of benthic microalgae in an intertidal sand flat of the Ria de Arosa, NW Spain. *Marine Ecological Progress Series*, 25: 111-119.

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- Verlencar, X.N. 1984. Dissolved organic nutrient and phytoplankton production in the Mandovi estuary and coastal waters of Goa. *Mahasagar*, 17(3): 141-149.
- Wallen, D.G. and G.H. Geen. 1968. Loss of radioactivity during storage of ¹⁴C labelled phytoplankton on membrane filters. *Journal of Fish Research Board Canada*, 25: 2219- 2224.
- Whitney, D.E. and W.M. Darley. 1979. A method for the determination of chlorophyll- *a* in samples containing degradation products. *Limonology and Oceanography*, 24: 183-186.
- Wiltshire, K.H. 1992. The influence of microphytobenthos on oxygen and nutrient fluxes between eulittoral sediments and associated water phases in the Elbe Estuary. Proceedings of the 25th EMBS, Colombo, G et al., eds. Fredensborg, Denmark, pp. 63-70