# MICROPHYTOBENTHIC PRIMARY PRODUCTION, BIOMASS, NUTRIENTS AND POLLUTANTS OF SANTOS ESTUARY (24°S, 46°20'W). SÃO PAULO, BRAZIL

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#### **ABSTRACT**

The present study aimed to evaluate the seasonal variation of microphytobenthic biomass and its primary production in the intertidal zone in Santos Estuary (São Paulo, Brazil). This estuary is on the tropical region and it is regarded as a polluted area. The samples were collected at 3 sites, and environmental parameters, nutrients and heavy metals were analyzed. The primary production was evaluated according to GR NTVED (1960) method whereas chlorophyll *a* and phaeopigment *a* were estimated by TETT *et al.* (1975) method. Total nitrogen K ranged from 20.0 to 115.0 μg·g<sup>-1</sup> while total phosphate ranged from 26.0 to 223.0 μg·g<sup>-1</sup>. No significant seasonal variation of benthic primary production or chlorophyll *a* and phaeopigment *a* were observed. Significant positive correlation was found for microbenthic primary production and chlorophyll *a*. Primary production showed lower correlations whit nitrate, total nitrogen K, total phosphate and organic matter in the sediment. The values of phaeopigment *a* were higher than chlorophyll *a*. The means for chlorophyll *a* were 10.4 μg·m<sup>-2</sup>, 14.9 μg·m<sup>-2</sup>, 28.6 μg·m<sup>-2</sup>, 39.7 μg·m<sup>-2</sup> and 26.7 μg·m<sup>-2</sup>. The mean values for primary production were 40.7 mgC·m<sup>-2</sup>·h<sup>-1</sup> (summer); 51.3 mgC·m<sup>-2</sup>·h<sup>-1</sup> (autumn); 84.3 mgC·m<sup>-2</sup>·h<sup>-1</sup> (winter); 140.0 mgC·m<sup>-2</sup>·h<sup>-1</sup> (spring) and 60.8 mgC·m<sup>-2</sup>·h<sup>-1</sup> (summer).

*Key words*: biomass, primary production, microphytobenthic, intertidal, pollutants; biomassa, produção primária, microfitobentos, entremarés, poluentes.

# **INTRODUCTION**

The importance of sediment associated microalgae (microphytobenthos) for primary production in shallow waters, specially intertidal and coastal ecosystems, has been demonstrated by many researchers (SUNDBÄCK & JÖNSON, 1988). Several authors (CADÉE & HEGEMAN, 1974; SOURNIA, 1977; JOINT, 1978; ASMUS, 1982; VARELA & PENAS, 1985; PLANTE-CUNY & BODOY, 1987) compared pelagic and microphytobenthic primary production and all them pointed out the importance of microphytobenthic primary producers.

The microphytobenthos is important in estuarine systems where its contribution to total primary production is often greater than that of phytoplancton (HARGRAVE, 1969; LEACH, 1970; PLANTE-CUNY & BODOY, 1987).

Santos estuarine system is regarded as a highly disturbed and polluted environment due to high density of human population, industrial development and intense port activities. Many studies with physical, chemical and biological aspects have been carried out there, but only SOUSA (1979, 1983 and 1985) made a relationship among microphytobenthos and these parameters. The aim of this study was to evaluate the seasonal variation of microphytobenthic primary production and biomass on the intertidal region of Santos Estuary and their correlation with some environmental parameters and contaminants.

### THE SAMPLING AREA

Santos estuarine system is located on the coast of São Paulo, Southeastern Brazil (Fig. 1). It is situated in a tropical region with annual means of 20###C for temperature, rainfall ranging from 2.000-2.500mm, and insolation of 155 hours monthly in summer and 164 hours monthly in winter (SANTOS, 1965).

The three studied sampling stations presented different environmental conditions. Station 1 was located in the entrance of Santos Channel, under the influence of brackish water, industrial pollutants, wastes from port activities and sewage. Many Polychaeta tubes from the genera *Onuphis* and *Diopatra* could be seen on the sediment surface. Station 2 is the most exposed of these beaches, showing a clean sediment. Station 3 is located in front of the port area, in the middle of the estuary. Here, the salinity is influenced by fresh water coming from land and by marine water (SOUSA, 1985). This is a sheltered beach were organic wastes of domestic origin can be seen and a permanent thin layer of oil covers the sediment surface.

#### **METHODS**

In order to estimate microphytobenthic primary production in the intertidal zone, five samples comprising the uppermost centimeter of sediment from each station were collected seasonally during ebb-tide and analyzed according to the method of GR NTVED (1960). These five samples were mixed and two sub-samples of 1cm<sup>2</sup> were taken and put in light and dark botles

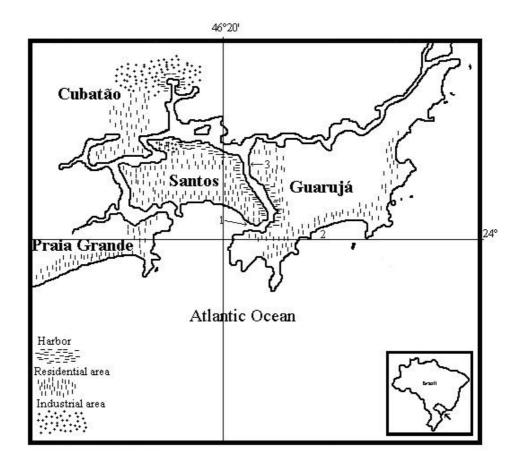


Figure 1. Santos region showing the sampling stations positions (1, 2, 3), the cyties and harbor, residential and industrial areas.

respectively. Each bottle was filled up with filtered sea water (Millipore, HA-0.45  $\mu$ m) from the sampling place. Incubation took place in the laboratory for two hours. The incubator was provided with fluorescent lamps of light intensity of 30.6·10<sup>15</sup> quanta·cm<sup>-2</sup>·s<sup>-1</sup> and constant temperature. Afterwards, these samples were strained through a Millipore HA-45 pore filter to obtain the material containing the assimilated <sup>14</sup>C.

The amount of assimilated 14C was measured using a Liquid Scintillation Spectrophotometer Model LKB ultra beta 1210. Therefore each sample was put in a vial with a scintillation solution. This solution was prepared with Toluol and Renex (2:1), 7.000 g PPO and 0.350 g POPOP per liter (BUNT et al, 1972).

Chlorophyll a and phaeopigment a were measured according to TETT et al. (1975) method. The pigments were extracted with hot methanol (75°C) and analyzed in spectrophotometer in 750 and 665 nm for chlorophyll a and after acidification with chloridric acid (8%) for phaeopigment a, respectively.

Conductivity measurements were carried out using a Kalshico Induction Salinometer Model 118 WA 200. Organic matter and grain size of the uppermost centimeter of sediment were determined according to the technique described by SUGUIO (1973). The frequency distribution of grain size was classified by FOLK & WARD (1957) method. Nitrate, total nitrogen K and total phosphate were determined according to PARSONS et al. (1984) method. Determinations of zinc, cooper and mercury were determined according to ENVIRONMENT CANADA (1979).

To the primary production and biomass results were applied the Kruskal-Wallis test (SOKAL & ROHLF, 1979), comparing the differences (p < 0.01) among the stations and among the sampling periods. Spearman correlation (p < 0.01) was applied between biological parameters and among them and the other measured parameters.

## RESULTS AND DISCUSSION

Values for primary production, biomass and organic matter content are presented in Tab. I, water and sediment temperatures, water salinity, total nitrogen K, nitrate and total phosphate and values for zinc, cooper and mercury are presented in Tab. II.

Benthic primary production values for this period ranged from 10.0 mgC·m<sup>-2</sup>·h<sup>-1</sup> to 297.0 mgC·m<sup>-2</sup>·h<sup>-1</sup> (Tab. I), with a mean value of 78.83 mgC·m<sup>-2</sup>·h<sup>-1</sup>.

Chlorophyll a values ranged from 2.0 to 95.6 mg·cm<sup>-2</sup> whereas phaeopigment a ranged from 9.4 to 135.1mg·cm<sup>-2</sup>. The respective mean values were 24.0 and 42.3 mg·cm<sup>-2</sup>.

Nitrate values ranged from 20.0 g  $\cdot$ g<sup>-1</sup> to 115.0 g  $\cdot$ g<sup>-1</sup>; total nitrogen K ranged from 2.0 g  $\cdot$ g<sup>-1</sup> to 5.5 g  $\cdot$ g<sup>-1</sup>; whereas total phosphate values ranged from 26.0 g  $\cdot$ g<sup>-1</sup> to 223.0 g  $\cdot$ g<sup>-1</sup>

The values of mercury in sediment ranged from <0.090 g  $\cdot$ g<sup>-1</sup> to 1.72 g  $\cdot$ g<sup>-1</sup>, whereas zinc concentration ranged from 2.10 g  $\cdot$ g<sup>-1</sup> to 90.00 g  $\cdot$ g<sup>-1</sup>. Cooper values ranged from < 0.24 g  $\cdot$ g<sup>-1</sup> to 114.00 g  $\cdot$ g<sup>-1</sup>.

The sediments grain size from stations 1 ( $\phi$  = 2,83) and 2 ( $\phi$  = 2,96) were classified as fine sand and station 2 ( $\phi$  = 1,86) was classified as medium sand.

Table I. Biological data for all stations during the period. Primary production (PrPr), chlorophyll a (CHL) and phaeopigment a (PHEO).

	Stations	PrPr	CHL	PHEO		
		$(mgC/m^2/h)$	(mg/cm <sup>2</sup> )			
Summer	1	10.0	3.3	36.6		
1981	2	46.0	10.5	19.4		
	3	66.0	17.5	46.9		
Autumn	1	31.0	2.0	35.4		
1981	2	75.0	8.5	11.0		
	3	48.0	34.2	83.6		
Winter	1	85.0	18.3	20.3		
1981	2	57.0	2.2	13.7		
	3	111.0	65.3	135.1		
Spring	1	89.0	21.0	50.8		
1981	2	54.0	2.6	9.4		
	3	297.0	95.6	98.4		
Summer	1	22.5	15.9	14.2		
1982	2	69.0	3.4	15.0		
	3	91.0	60.9	45.1		

Table II. Environmental data for all stations during the period. Organic Matter (OM), water (TW) and sediment (TS) temperatures, salinity (Sal), total nitrogen (Nt), nitrate ( $NO_2$ ), total phosphate ( $PO_4$ ), and metals (Cu, Rg and Rg).

										Metals	
	Stations	OM	TW	TS	Sal	Nt	$NO_2$	$PO_4$	Cu	Hg	Zn
		(%)	(°	<u>C)</u>			(mg/g)		•		
Summer	1	0.60	27.0	28.0	29.5	62.0	2.6	158.0	25.20	0.42	24.90
1981	2	0.08	25.5	28.0	33.0	20.0	5.0	30.0	14.70	0.21	19.20
	3	1.30	28.0	27.0	21.0	102.0	3.0	200.0	24.00	0.84	23.40
Autumn	1	0.65	21.5	23.0	23.5	70.0	2.5	161.0	31.60	0.09	20.00
1981	2	0.05	22.0	24.0	26.0	25.0	4.5	26.0	0.24	0.26	21.60
	3	1.00	22.0	24.0	15.5	98.0	3.6	215.0	3.70	0.09	2.10
Winter	1	1.03	20.0	22.0	19.5	68.0	1.9	165.0	2.90	0.16	2.75
1981	2	0.09	20.0	23.0	30.0	35.0	4.1	28.0	6.90	0.20	2.98
	3	1.68	20.0	22.0	14.0	115.0	2.0	219.0	0.24	0.09	2.10
Spring	1	0.50	23.0	24.5	23.0	60.0	2.2	161.0	5.50	0.14	2.90
1981	2	0.09	23.0	24.0	30.0	37.0	4.3	29.0	86.00	1.72	90.00
	3	1.51	22.0	22.0	15.0	107.0	2.5	223.0	91.00	1.54	84.00
Summer	1	0.40	28.0	28.0	24.0	74.0	3.6	151.0	103.00	1.67	77.20
1982	2	0.07	27.0	27.0	34.0	29.0	5.5	31.0	94.00	1.68	83.10
	3	1.78	26.0	27.0	17.0	105.0	3.0	194.0	114.00	1.65	85.00

The Spearman correlation (p < 0.01) between benthic primary production and chlorophyll a with the measured parameters are presented on the Tab. III.

Table III. Spearman correlation for primary production and biomass (Chlorophyll a), with the other measured parameters.

Spearman correlations ( $p < 0.01$ )						
	Primary Production	Chlorophyll a				
Chlorophyll a	0.687					
Phaeopigment a	0.432	0.753				
Organic matter	0.470	0.670				
Temperature of water	-0.353	-0.051				
<b>Temperature of sediment</b>	-0.551	-0.054				
Salinity	-0.218	-0.215				
Total nitrogen	0.367	0.675				
Nitrate	-0.372	-0.426				
Total phosphate	0.445	0.779				
Cu	-0.118	-0.011				
Hg	-0.039	-0.132				
Zn	-0.041	-0.175				

Boldfaced numbers are significant correlations (positive or negative)

Santos estuarine ecosystem comprises six cities. One of them, Cubatão, has a great industrial center with oil refinery, petrochemical chemical and fertilizers industries and a big steel plant and Santos has the greatest Brazilian harbor. Sewage from those cities and industrial wastes have caused environmental and ecological disturbances. Thus, this ecosystem is considered polluted (GIANESELLA-GALVÃO, 1978; TOMMASI, 1979; BOLDRINI & PEREIRA, 1987; TANIGUSHI, 1995).

The three sampling stations presented different conditions of pollutants aport. The station 3 receives the effects of the pollutants from the industrial park and from the port. The amount of pollutants in the water at station 1 is lower, due to its distance from the pollution sources. There is a pollution gradient in Santos Channel waters, with greater values on the high estuary (TOMMASI, 1979). The values obtained for mercury, copper and zinc and nutrients confirm TOMMASI (*op.cit.*) and BOLDRINI & PEREIRA (1987) studies.

The lower hydrodinamism and grain size with higher values of organic matter and nutrients on station 3, probably were responsible for the higher values of microphytobenthic

biomass and primary production in this site. Several studies showed the association between increase of chlorophyll a and increase of silt fractions on sediment (CADEÉ & HEGEMAN, 1977; COLIJN & DIJKEMAN, 1981; KNOX, 1986). Microphytobenthic primary production and chlorophyll-a higher values were also obtained at station 3 by SOUSA (1983, 1985). SOUSA & DAVID (1996) considered hydrodinamism the main agent responsible for the changes of intertidal microphitobenthic biomass. Moreover, Plante-CUNY & BODOY (1987) observed values of microphytobenthic primary production on a sheltered place, four times higher than those from the exposed place.

The higher hidrodinamism, grain size, values of salinity and lower values of Cu, Hg, organic matter, PO<sub>4</sub>, chlorophyll-*a* and phaeopigment-*a* observed on station 2 probably were due to its geographical position. This beach is oceanic, as is shown in Fig. 1. Thus, it may be compared with the other stations in order to observe the differences between oceanic and estuarine influences.

During the studied period, the microphytobenthos primary production were higher in August and October, coinciding with the beginning of spring. High seasonal variations were observed in cold and temperate regions (CADEÉ & HEGMAN, 1977; COLIJN & DIJKEMA, 1981). Despite that, the Kruskal-Wallis test (SOKAL & ROHLF, 1979) did not show significant differences, suggesting an absence of seasonallity in the microphytobenthos primary production in Santos Estuary.

For each station, fluctuations on the benthic primary production were observed within the same sampling and period. These fluctuations were probably related to local conditions or heterogenic distribution of pigments on the sediment (PLANTE *et al.*, 1986). SOUSA (1985) observed variations of ten times for samples taken with 1 meter of distance in a same site. HICKLING (1970, *apud* PLANTE-CUNY, 1984) found the mean value of 1.22 gC·m<sup>-2</sup>·day<sup>-1</sup>, and SOURNIA (1976, *apud* PLANTE-CUNY, 1984) found 1.13 gC·m<sup>-2</sup>·day<sup>-1</sup> for benthic primary production of intertidal zone at tropical regions. In the present work the found mean value was 0.67 mgC·m<sup>-2</sup>·day<sup>-1</sup>.

PLANTE-CUNY (*op.cit.*) suggested that on intertidal sediments of temperate areas the microphytobenthic primary production range between 30 and 180gC·m<sup>-2</sup>·year<sup>-1</sup>, whereas RIZZO & WETZEL (1985) suggested a mean of 128 ± 50 gC·m<sup>-2</sup>·year<sup>-1</sup>, regardless of the latitude and substrate. In tropical areas, primary production values measured on intertidal sediments were

about 300 gC·m<sup>-2</sup>·year<sup>-1</sup> (PLANTE-CUNY, 1984). For the present work the mean value was 225 gC·m<sup>-2</sup>·year<sup>-1</sup> (or 77 mgC·m<sup>-2</sup>·h<sup>-1</sup>).

The Spearman correlation between primary production and chlorophyll a was  $r^2 = 0.687$ . The best correlations for chlorophyll a were observed with phosphate, phaeopigment a (strong positive correlations), total nitrogen K and organic matter (positive correlations). The negative correlation between temperature and primary production may be explained by the lower efficiency of photosynthesis under high temperatures.

The microphytobenthic primary production and biomass contribution to the coastal zone is very important and can not be neglected due to the extension of the sediment surface around the word, specially in the tropical regions, where sediments are particularly productive in the estuaries. Therefore, we think that in Santos estuarine system, microphytobenthos has a significant contribution to the total primary production of the area.

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## **BIBLIOGRAPHY**

- ASMUS R. (1982). Field measurements on seasonal variation of the activity of primary producers on a sandy tidal flat in the northern Wadden Sea. **Netherlands Journal of Sea Research**, **16**:389-402.
- BOLDRINI, C.V. & PEREIRA, D.N. (1987). Metais pesados na Baía de Santos e São Vicente Bioacumulação. **Ambiente 1(3)**: 118-127.
- BUNT, J.S.; LEE, C.C. & LEE, E. (1972). Primary productivity and related data from tropical marine sediments. **Marine Biology**, **16**:28-36.
- CADÉE, G.C. & HEGEMAN, J. (1974). Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. **Netherlands Journal of Sea Research**, 8:260-291.
- CADÉE, G.C. & HEGEMAN, J. (1977). Distribution of primary production of the benthic microflora and accumulation of organic matter on a tidal flat area, Belgzand, Dutch Wadden Sea. **Netherlands Journal of Sea Research**, **11(1)**:24-41.
- COLIJN, F. & DIJKEMA, K. (1981). Species composition of benthic diatoms and distribution of chlorophyll <u>a</u> on an intertidal flat in the Dutch Wadden Sea. **Marine Ecology Progress Series, 4**:9-21.

- ENVIRONMENT CANADA (1979). **Analytical Methods Manual**. Environment Canada, Inlands Waters Directore, Water Quality. Ottawa.
- FOLK, R. L. & WARD, W. C. 1957. Brazos river bar: a study in the significance of grain size parameters. **J. sedim. Petrology, 27**:3-26.
- GIANESELLA-GALVÃO, S.M.F. (1978). Produção primária da Baía de Santos, Estado de São Paulo: aspectos sobre a eficiência fotossintética em um ambiente marinho poluído. Dissertação de Mestrado. Universidade de São Paulo. Instituto Oceanográfico. 53p.
- GRØNTVED, J. (1960). On the productivity of microbenthos and phytoplankton in some Danish fjords. **Medelelser fra Danmarks Fiskeri Havunders, MS 3(3)**:55-92.
- HARGRAVE, B.T. (1969). Epibenthic algal production and community respiration in the sediments of Marion Lake. **Journal of Fisheries Research Board of Canada**, **26(8)**:2003-2026.
- JOINT, I.R. (1978). Microbial production of an estuarine mudflat. Estuarine Coastal and Marine Science, 7:185-195.
- KNOX, G.A. (1986). Estuarine Ecosystems: A Systems Approach VI. CRC Press Inc. Boca Raton, Florida, 289p.
- LEACH, J.H. (1970). Epibenthic algal production in and intertidal mudflat. **Limnology and Oceanography**, **15(4)**:514-521.
- PARSONS, T.M., MAITA, Y. & LALLI, C.M. (1984). A Manual of Chemical and Biological Methods for Seawater Analysis. Oxford, Pergamon Press. 173p.
- PLANTE-CUNY, M.R. (1984). Le microphytobenthos et son rôle à l'échelon primaire dans le milieu marin. **Oceanis**, **10**:412-427.
- PLANTE, R., PLANTE-CUNY, M.R. & REYS, J.P. (1986). Photosinthetic pigments of sandy sediments on the north Mediterranean coast: their spatial distribution and its effect on sampling strategies. **Marine Ecology: Progress Series, 34**:133-141.
- PLANTE-CUNY, M.R. & BODOY, A. (1987). Biomasse et production primaire du phytoplancton et du microphytobenthos de deux biotipes sableux (Golfe de Fos, France). **Oceanology Acta, 10(2)**:223-237.
- RIZZO, W.M. & WETZEL, R.L. (1985). Intertidal and shoal benthic community metabolism in a temperate estuary: studies of spatial and temporal scales of variability. Estuaries, 8:342-351.
- SANTOS, E.O. (1965). Características climáticas. In: H. Azevedo, **A Baixada Santista: Aspectos Geográficos.** São Paulo, EDUSP. Vol 1, p.95-150.
- SOKAL, R.R. & ROHLF, F.J. (1979). Biometria. Madrid. Blume Ediciones. 822p.
- SOURNIA, A. (1977). Analyse et bilan de la production primaire dans les récifs coralliens. **Annales de L'Institut Oceanographique de Paris**, **53(1)**:47-74.
- SOUSA, E.C.P.M. (1979). Produção primária bentônica da zona entre marés em praias da Baixada Santista. Dissertação de Mestrado. Universidade de São Paulo. Instituto Oceanográfico 168p.
- SOUSA, E.C.P.M. (1983). Primary production of the benthic microflora living on intertidal flats in the Santos estuarine system (24°S, 46°W) São Paulo, Brazil. **Boletim do Instituto Oceanográfico. São Paulo, 32(2)**:177-186.
- SOUSA, E.C.P.M. (1985). Estudos sobre a variação da produção primária bentônica da região entre marés de praias da Baixada Santista (23°30'-24°S e 46°05'-46°30'W) São Paulo Brasil. Tese de Doutorado. Instituto Oceanográfico. Universidade de São Paulo. 179p.
- SOUSA, E.C.P.M & DAVID, C.J. (1996). Variação diária dos pigmentos fotossintetizantes do microfitobentos da Praia de Aparecida, Santos, S.P. Brasil. **Revista Brasileira de Biologia**, **56(1)**:147-154.
- SUGUIO, K. (1973). Introdução à Sedimentologia. São Paulo, Ed. Edgard Blücher/EDUSP. 317p.
- SUNDBACH, K. & JONSSON, B. (1988). Microphytobenthic and biomass in sublittoral sediments of a stratified bay, southeastern kattegot. **Journal Experimental Marine Biology Ecology, 122**:63-81.
- TANIGUSHI, S. (1995). Pesticidas organoclorados e bifeniles policlorados em bivalves ao longo da costa brasileira Internacional Mussel Watch. Dissertação de Mestrado. Universidade de São Paulo. Intituto Oceanográfico. 65p.

- TETT, P.; KELLY, M.G. & HORNBERG, G.M. (1975). A method for the spectrofotometric measurements of chlorophyll <u>a</u> and pheophytin <u>a</u> in benthic microalgae. **Limnology and Oceanography, 20(5)**:887-896.
- TOMMASI, L.R. (1979). Considerações ecológicas sobre o sistema estuarino de Santos (SP). Tese de Livre-Docência. Universidade de São Paulo. Instituto Oceanográfico. 2 volumes.
- VARELA, M. & PENAS, E. (1985). Primary production of benthic microalgae in an intertidal sand flat of the Ria de Arosa, NW Spain. **Marine Ecology: Progress Series, 25**:111-119.

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