BRAZILIAN JOURNAL OF OCEANOGRAPHY, 63(1):1-15, 2015

SPATIAL AND TEMPORALVARIATION OF DISSOLVED INORGANIC NUTRIENTS, AND CHLOROPHYLL-*a* IN A TROPICAL ESTUARY IN NORTHEASTERN BRAZIL: DYNAMICS OF NUTRIENT REMOVAL

Maria Aparecida Macedo Silva, Marcelo F. L. Souza and Paulo C. Abreu*

Universidade Estadual de Santa Cruz – UESC
Laboratório de Biogeoquímica Marinha
(Rodovia Ilhéus-Itabuna km 16, 45650-000 Ilhéus, BA, Brasil)

*Corresponding author: cidahmacedo@yahoo.com.br

http://dx.doi.org/10.1590/S1679-87592015064506301

ABSTRACT

Monthly sampling campaigns were carried out between February 2010 and January 2011 to evaluate the spatial and temporal distribution of nutrients (ammonium, nitrite, nitrate, dissolved organic nitrogen, phosphate, dissolved organic phosphorus and silicate) and chlorophyll-*a* along a salinity gradient in the tropical Cachoeira River estuary, subject to the untreated effluents of a sewage treatment plant (STP). During the study period the lowest and highest river discharge occurred in February and April 2010, respectively. High river outflow promoted increased concentrations of inorganic nitrogen and silicate but did not affect the concentration of phosphate. Based on the chlorophyll-*a* concentration the estuary may be classified as eutrophic / hypereutrophic in its inner portion and mesotrophic in the lower region. The inner portion is more affected by the nutrient load carried out by the river and STP, while dilution by seawater contributed to the reduction of the nutrient concentrations in the lower reaches of the estuary. The results indicate that nutrient uptake by the phytoplankton is the most effective dissolved inorganic nutrient removal processes, especially for phosphate. Mixing diagrams suggest that the coupling of nitrification and denitrification processes is also responsible for the elimination of nitrogen from this ecosystem.

RESUMO

Campanhas de amostragens mensais foram realizadas entre fevereiro de 2010 e janeiro de 2011 para avaliar a distribuição espacial e temporal de nutrientes (amônia, nitrito, nitrato, nitrogênio orgânico dissolvido, fosfato, fósforo orgânico dissolvido e silicato) e clorofila-*a*, ao longo do gradiente de salinidade no estuário tropical do Rio Cachoeira. Este estuário é sujeito aos efluentes de esgotos não tratados de uma estação de tratamento de esgoto (ETE). No período estudado a maior e menor vazão do rio ocorreram em fevereiro e abril de 2010, respectivamente. A alta vazão do rio promoveu aumento das concentrações de nitrogênio inorgânico e silicato, mas não afetou as concentrações de fosfato. Baseado nas concentrações de clorofila-*a*, o estuário pode ser classificado como eutrófico/hipereutrófico na porção interna e mesotrófico na região externa. A porção interna é mais afetada pela carga de nutrientes do rio e da ETE, enquanto a diluição pela água marinha contribuiu para diminuir as concentrações de nutrientes na porção externa. Os resultados indicam que a absorção de nutrientes pelo fitoplâncton é o processo mais eficiente na remoção desses nutrientes, especialmente do fosfato. No entanto, os diagramas de mistura sugerem que a nitrificação e denitrificação acopladas no rio também são responsáveis pela eliminação do nitrogênio do ecossistema.

Descriptors: Eutrophication, Sewage, Mixing diagrams, Ammoniacal nitrogen, Nitrate, Phosphate.
Descritores: Eutrofização, Esgoto, Diagrama de mistura, Nitrogênio amoniacal, Nitrato, Fosfato.

INTRODUCTION

Estuaries are coastal ecosystems with high biological productivity mainly due to the input of nutrients (N and P) that are fundamental to the development of primary producers. Besides their biological importance, estuaries provide numerous services such as fishery, recreational and harbor activities (FLEMER; CHAMP, 2006) and are widely used as the end point for domestic and industrial sewage that may, or not, be treated in sewage treatment plants (STP). The ability of estuaries to receive, assimilate and disperse contaminants from natural and anthropogenic sources is well known. However, they have a limited capacity of purification that, when exceeded, can compromise the quality of water and sediment of this ecosystem (LEVENSON, 1987; RABALAIS et al., 2002; TAPPIN, 2002.).

In many sewage treatment plants the removal of nitrogen and phosphorus is incomplete (due, e.g., to the primary and secondary treatment of sewage), generating effluents with high concentrations of these nutrients in the dissolved form (BIE et al., 2001; PEREIRA-FILHO et al., 2003; MALLIN et al., 2007). The difficulty in removing nitrogen and phosphorus from wastewater discharge causes severe eutrophication in the body of water that receieves it (RABALAIS, 2002; XIANGLI et al., 2008). Primary producers are the first component in the ecosystem to respond to increased nutrient loads by the growth of biomass. The decomposition of the biomass produced generates an anoxic or hypoxic condition and also leads to a decrease of biodiversity and the occurrence of harmful algal blooms. These conditions may generate a disruption of ecosystem functioning, loss of habitats, shifts in food webs and decrease of fisheries (SMITH et al., 1999; RABALAIS, 2002). In addition, excess nutrients released from sewage treatment plants and by other human activities in estuaries increase the flow of N and P to the adjacent coastal areas, extending similar environmental impacts to this region (VALIELA; BOWEN, 2002; FLEMER; CHAMP, 2006).

Tropical estuaries are under increasing pressure due to environmental impacts caused by population growth. However, these environments are much less studied than are temperate estuaries (WATTAYAKORN et al., 2001). For instance, BURFORD et al. (2008) highlighted the lack of data to compare productivity and respiration in tropical and temperate estuarine environments and CLOERN; JASSBY (2010) studied the seasonal components that control the phytoplankton variability in many estuaries and havesuggested that patterns of variability change along a latitudinal gradient. However, the corroboration of this hypothesis requires more observations of estuaries in tropical and subtropical regions. Thus, studies in tropical regions are extremely important to better understand the diversity of processes that occur on different spatial and temporal scales in these ecosystems located at low latitudes. Moreover, the lack of information on tropical estuaries prevents the comparison of processes at the global level and the identification of patterns common to all estuaries around the world.

Differently from the case of many other tropical estuaries, some studies on the dynamics of dissolved inorganic nutrients and chlorophyll-*a* have already been undertaken in the Cachoeira River estuary, a typical tropical estuary dominated by mangroves, located on the Eastern Brazilian Coast. SOUZA (2005) and SOUZA et al. (2009) report data from four sampling surveys performed between February 2000 and August 2001, and which showed considerable changes in the nutrient dynamics of this ecosystem especially after September 2000, when a Sewage Treatment Plant (STP) (Fig. 1) became operational. A mass balance approach (SOUZA, 2005) reveals a decrease in net autotrophy and net N fixation rates during this period. The observed increase in phosphate, though not of ammonium, was unexpected since this STP has only primary treatment. These changes resulted in a low DIN:DIP molar ratio. The authors considered that the low ammonium concentration was the result of a rapid ammonium uptake by the phytoplankton, or the transformation of this element into more oxidized forms such as nitrite and nitrate. Unfortunately, the only survey made before the STP came into operation was conducted in February 2000.

SILVA et al. (2013) showed that these trends continued in surveys carried out during a transition from the dry to the rainy period in 2003-2004. Low DIN:DIP and ammoniacal nitrogen in the lower estuary indicated phytoplankton limitation by N, while eutrophication in the inner estuary increased (up to 368 Chl-*a* µg/L).

All these studies were carried out during spring high tide. The aim of this study was to evaluate the spatial and temporal distribution of inorganic nutrients (N, P and Si) and chlorophyll-*a* during low tide, in order to characterize the most important mechanisms of nutrient removal in this tropical estuary.

MATERIAL AND METHODS

Study Area

The Cachoeira River estuary, located in northeastern Brazil (14°45'S-39°01'W and 14°50'S-39°05'W) (Fig. 1), has an area of approximately 16 km^2 and a drainage basin of 4,600 km^2. The estuary has a semidiurnal tidal cycle of nearly 2 m amplitude. Salt intrusion can reach the entire estuary during the dry season (SOUZA et al., 2009). The predominant tropical humid climate of the area is characterized by an average temperature of 24.6ºC and annual precipitation of between 1,500 and 2,000 mm (KLUMPP et al., 2002). The rain and fluvial discharges are extremely irregular, despite the well-defined dry and rainy seasons. The average annual river discharge in the basin is 24.1 m^3. s^{-1}, varying from 0.2 to 1,460 m^3. s^{-1}, responding quickly to precipitation. Historically the highest pluvial rates occur between November and January (BAHIA, 2001), but with large interannual variability. Some rainy events out of season may have a rapid impact on river discharges, due to the significant drainage area.

The estuary of the Cachoeira River receives domestic and industrial effluents from two urban centers, the cities of Ilhéus and Itabuna, each with a population of ca. 200,000 inhabitants. In the year 2000 a sewage treatment plant (STP) was installed on the inner portion of the estuary. It is there that the sewage from the city of Ilhéus receives primary level treatment, while the untreated sewage from the city of Itabuna is released directly into the Cachoeira River, 10 km above the STP. The overgrowth of aquatic weeds that often covers the water surface of this estuary (FIDELMAN, 2005), and the decomposition of macrophytes generates an oxygen deficit (0.4 -1.2 mg/ L^{-1}) or even anoxia. In the rainy season the macrophytes are transported to the estuary, also acting as a source of nutrients and detritus for the ecosystem (KLUMPP et al., 2002).

Water Sampling and Analysis

Sampling surveys were carried out monthly along the estuary from February 2010 to January 2011. Stations 1 and 2 were located in the inner portion of the estuary. This estuarine section has an average depth of about 2 meters. Station 1 is located upstream from the sewage treatment plant (STP). Stations 3 and 4 are located in the lower portion of the estuary, which has an average depth of 4 meters (Fig. 1). The sampling was made approximately at low tide, based on the visual observation of the flow. The tide gauge is located in Ilhéus Harbour, outside of and about 1 km from the estuary mouth. Personal observation of low tide slack water showed that it can occur more than an hour later than that of the tide table. The water sampling was undertaken in triplicate with a 5 L van Dorn bottle near the surface (0.5 m depth) and the bottom (1.0 m above the sediment). After collection the samples were stored in clean polyethylene bottles, pre-washed with HCl 50% and rinsed with distilled water.

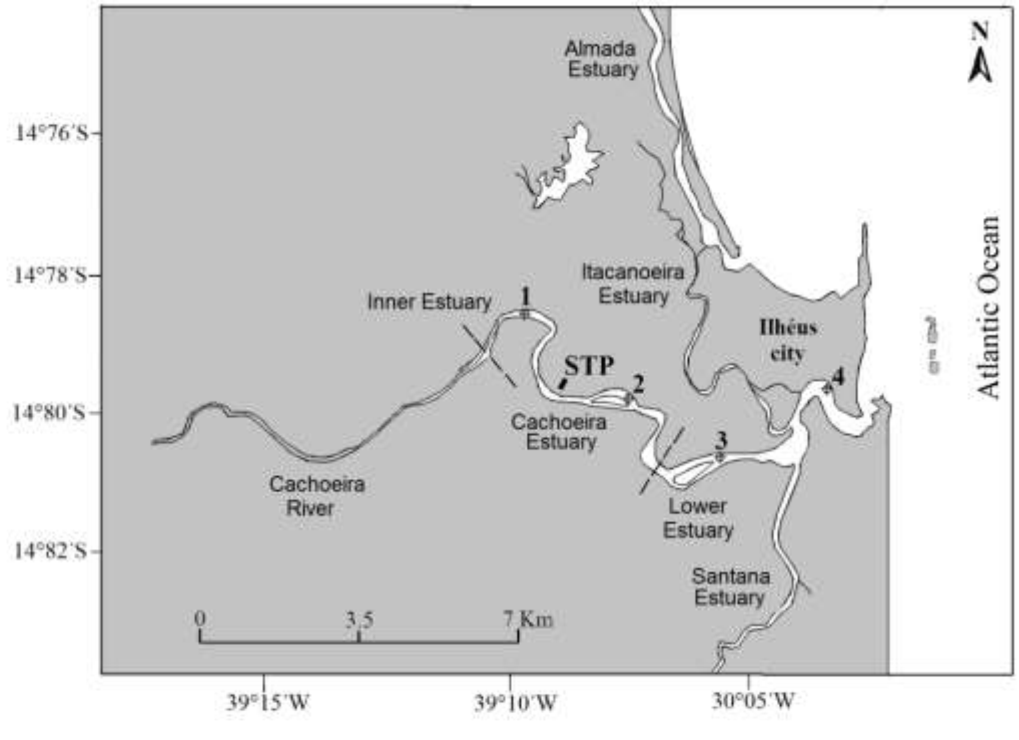

Fig. 1. Map of the study area showing the location of sampling stations in the Cachoeira River estuary and the sewage treatment plant (STP).

The water temperature, salinity and pH were measured *in situ* using a calibrated WTW Multiline P4. The dissolved oxygen was measured at the beginning with a calibrated Oxymeter (Hanna HI 9143). From October to December 2010 and in January 2011 the concentration of this gas was determined using the Winkler method (GRASSHOFF et al., 1983). Transparency of the water was estimated with a Secchi disk.

After collection the water was filtered through fiberglass filters (GF50/A) (Schleicher and Schuel), previously heated at 450°C, and the nutrients analyzed in filtered water. For chlorophyll-*a* analysis, water was filtered through GF934/AH (Schleicher and Schuel) glass fiber filters. Aliquots of filtered water samples were frozen for further analysis of phosphate, ammoniacal nitrogen, nitrite, and nitrate according to GRASSHOFF et al. (1983), while the silicate (DSi) concentration was determined using CARMOUZE's (1994) method. For analysis of dissolved organic phosphorus and nitrogen, the sample of filtered water was digested with potassium persulfate at 130°C for 50 minutes (GRASSHOFF et al., 1983). N and P were analyzed as nitrate and phosphate in the digested samples. The dissolved organic forms were determined by the difference between the concentrations obtained in samples after digestion with persulfate and of dissolved inorganic nitrogen and phosphorus analyzed previously.

Total suspended solids (TSS) were determined using a gravimetric method (STRICKLAND; PARSONS, 1972). Chlorophyll-*a* was analyzed using a spectrophotometric method (PARSONS et al., 1984). The calculation of the concentration of chlorophyll-*a* was made according to JEFFREY AND HUMPHREY (1975).

Estuarine Mixing Diagrams

Estuarine mixing diagrams were prepared using salinity as a conservative parameter (MIRANDA et al., 2002) in order to derive sources and sinks of nutrients deviating from conservative mixing under non-steady state conditions (MIDDELBURG; NIEUWENHUIZE, 2001; LIU et al., 2009).

Conservation of salt implies:

$$S = f\,.Sm+(1-f).Sr \qquad (1)$$

where S, Sm and Sr are the salinity of the sample, marine and freshwater end-member, respectively, and f is the seawater fraction:

$$f = S - Sr/Sm-Sr \qquad (2)$$

Conservation of total mass is given by:

$$\mathrm{Nx} = f\,.\,\mathrm{Nm} + (1-f).\mathrm{N}r \qquad (3)$$

Where Nx, Nm and Nr are the concentration (of nitrate) in the sample, marine and freshwater end-member, respectively.

Since true fresh and seawater end-members were not sampled, the concentration of nutrients in the samples were also salinity-normalized, in order to infer the processes influencing nutrient dynamics. The concentrations were normalized according to BOUILLON et al. (2007).

$$\mathrm{Nn} = \mathrm{Nx} - \alpha\,(\mathrm{Sx} - \mathrm{Sn}) \qquad (4)$$

Where Nn and Nx are the normalized and sample concentrations, α is the slope of the conservative mixing curve and Sx and Sn are the salinity of the sample and to which the data are normalized (in our study, the mean salinity of each survey). The mixing diagrams made with these salinity-normalized data allow an easier interpretation of the estuarine biogeochemical processes than non-normalized ones considering the spatial limitation of the sampling. Even a slight deviation from the expected straight horizontal line reflects a strong non-conservative signal, since normalization was made to a mean estuarine salinity instead of to low nutrient seawater.

Statistical Analysis

The Kruskall-Wallis nonparametric statistical test was used to examine significant differences (α=0.05) between stations, depth (surface and bottom) and seasons (dry and rainy) (ZAR, 1999).

RESULTS

The rainfall measured three days before sampling was high in March and April, as well as in September 2010 and January 2011 (Fig. 2a). Data of the monthly runoff of the Cachoeira River during the study period showed the highest discharges in April 2010, peaking at 8.79 x107 m^3. $month^{-1}$ (Fig. 2b). Estimates of water residence time, made in accordance with Gordon et al. (1996) in the dry season ranged from 7 to 41 days in the inner portion of the estuary, and <1 to 16 days in the lower portion (unpublished results). In the rainy season the residence time decreased to 3 days in the inner and <1 day in the lower portion.

The salinity varied from 0.30 to 22.8 in the inner portion. At the stations closer to the sea the salinity presented a minimum of 12.7 and maximum of 35.5. Stations 1 and 2 showed water column stratification most of the time, except between February and April 2010 (Fig. 3). In the lower reaches of the estuary the water column was always well mixed (the salinity at the bottom was almost same as at the surface) (Fig. 3).

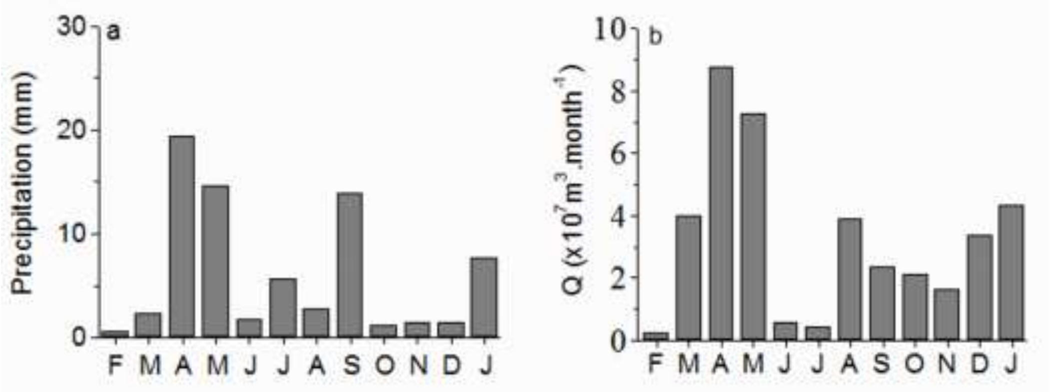

Fig. 2. Sum of precipitation 3 days before sampling (a) and monthly river discharge (b); National Water Agency: station No.53180000. Source: PROCLIMA INPE/CPET.

The water temperature ranged from 24.9ºC to 34.3ºC over the study period, showing only a small seasonal variation between the respective minimum and maximum values in the Austral winter and summer (Fig. 3). High temperatures were already recorded in the inner portion of this estuary, even during high spring tide (Silva et al., 2013). The pH values showed little variation throughout the year (Fig. 3).

Higher concentrations of dissolved oxygen in surface samples were obtained in the inner portion of the estuary (Fig. 3). However, this area also showed the lowest oxygen concentrations at the bottom. Dissolved oxygen in the inner portion varied from a minimum of 1.9 mg L^{-1} at the bottom at station 2 in July to a maximum of 10.8 mg L^{-1} in January at the surface at that same station. In the lower portion dissolved oxygen varied from a minimum of 3.1 mg L^{-1} in May to a maximum of 9.7 mg L^{-1} in April at station 3.

Dissolved oxygen saturation varied from 25.3 to 153 %, but the estuarine waters were below 100 % saturation at all the sampling stations most of the time (Fig. 3). The surface samples from station 2 presented most of the values above or near 100 % saturation, denoting that this is the region with higher primary production. The difference between surface and bottom was higher at stations 2 and 1, with more saturated surface water. Stations 3 and 4 showed almost the same dissolved oxygen saturation at the surface and the bottom.

Greater transparencies were observed in October and December 2010 and January 2011. The highest values of water transparency (Secchi disk depth) were observed throughout the study at station 4, with a minimum 0.70 m and a maximum of 1.85 m. Stations 1 and 2 had the lowest transparency, with a minimum of 0.25m in August. Higher concentrations of total suspended solids (TSS) were measured at station 2 ($p<0.05$), except in April, when station 3 presented a maximum of 162 mg L-^{1} at the bottom.

The concentrations of ammoniacal nitrogen ranged from 0.04 to 56.9 µM. Higher concentrations were measured in the rainy season ($p<0.05$). High concentrations of ammoniacal nitrogen were also recorded in July, considered a dry month. The higher levels were recorded at stations 1, 2 and 3 ($p<0.05$). Station 4 had the lowest concentrations of this nutrient (Fig. 4).

Nitrite concentrations showed little spatial variation over time, except for the month of July, when very high values were observed at stations 1, 2 and 3 with a maximum of 41.8 µM (Fig. 4). On the other hand, nitrate showed higher concentrations in the inner portion of the estuary, especially at station 1 ($p<0.05$). The surface samples showed the higher values in most months ($p<0.05$). Average concentrations ranged from close to 0 in April at the surface at station 4, to 97.7 µM in January at station 1 (Fig. 4). A significant increase in nitrate concentrations was observed in the rainy season ($p<0.05$).

The dissolved organic nitrogen showed a similar pattern at all the stations with low concentrations in the months of higher river discharge (April to May; $p<0.05$), an increase in June, and smaller values in October. In general concentrations ranged from lower than the detection limit.

The mixing diagrams for ammonium, nitrite and nitrate with the standardized values for salinity show that these elements presented non-conservative behavior throughout the study period (Fig. 5). An inverse non-conservative signal of nitrate as compared to nitrite and ammoniacal nitrogen was observed in March, June, July and from October to January. Removal of both ammoniacal nitrogen and nitrate was observed especially in August and September.

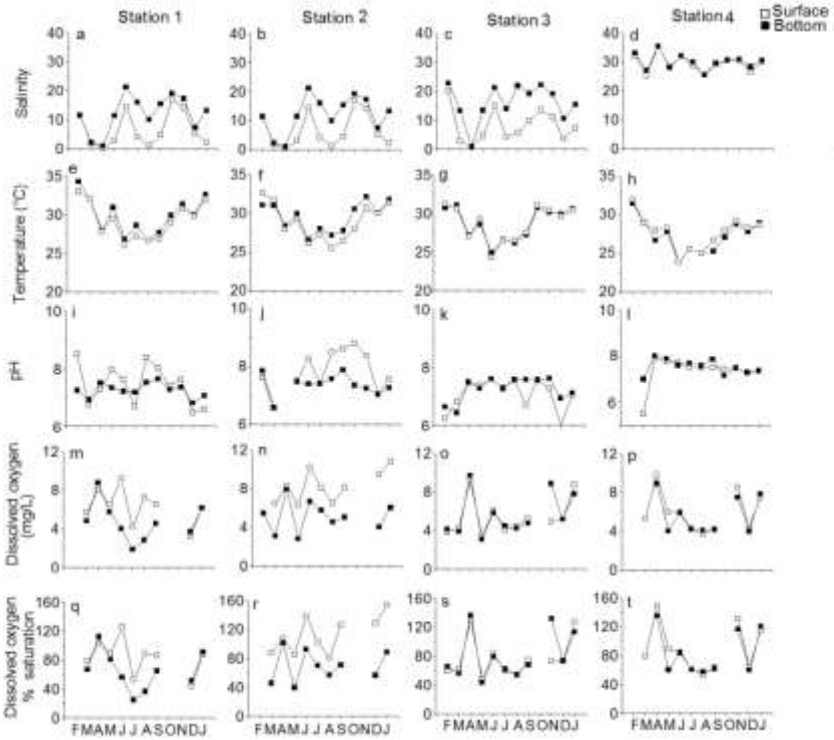

Fig. 3. Values of salinity (3 a-d), temperature (3 e-h), pH (3 i-m), dissolved oxygen (3 m-p) and dissolved oxygen % saturation (3q-t), at sampling stations (1-4) in the Cachoeira River estuary between February 2010 and January 2011. Error bars indicate the Standard Deviation. Surface samples (open square) and bottom samples (closed square).

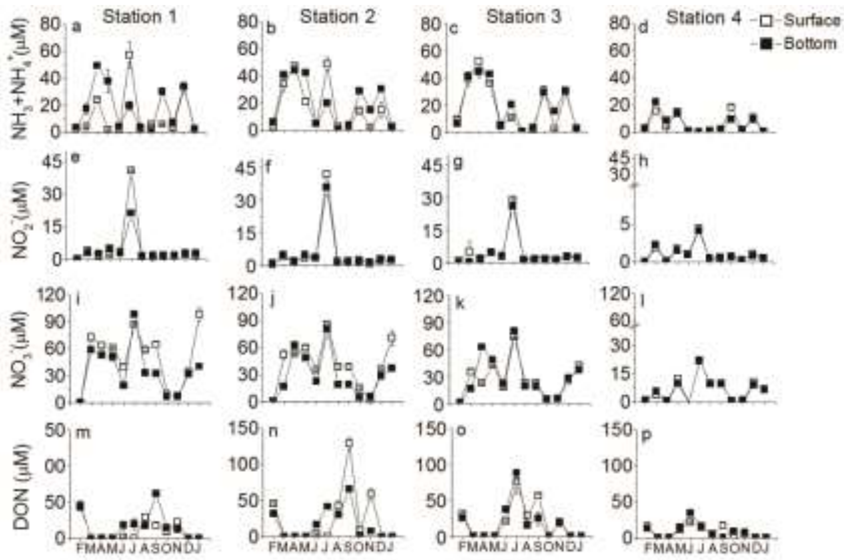

Fig. 4. Mean values (µM) of dissolved organic and inorganic nitrogen: NH_3+NH_4^+ (5 a-d), NO_2^-(5 e-h) NO_3^- (5 i-l) and DON (5 m-p), at sampling stations (1-4) in the Cachoeira River estuary between February 2010 and January 2011. Error bars indicate the Standard Deviation. Surface samples (open square) and bottom samples (closed square).

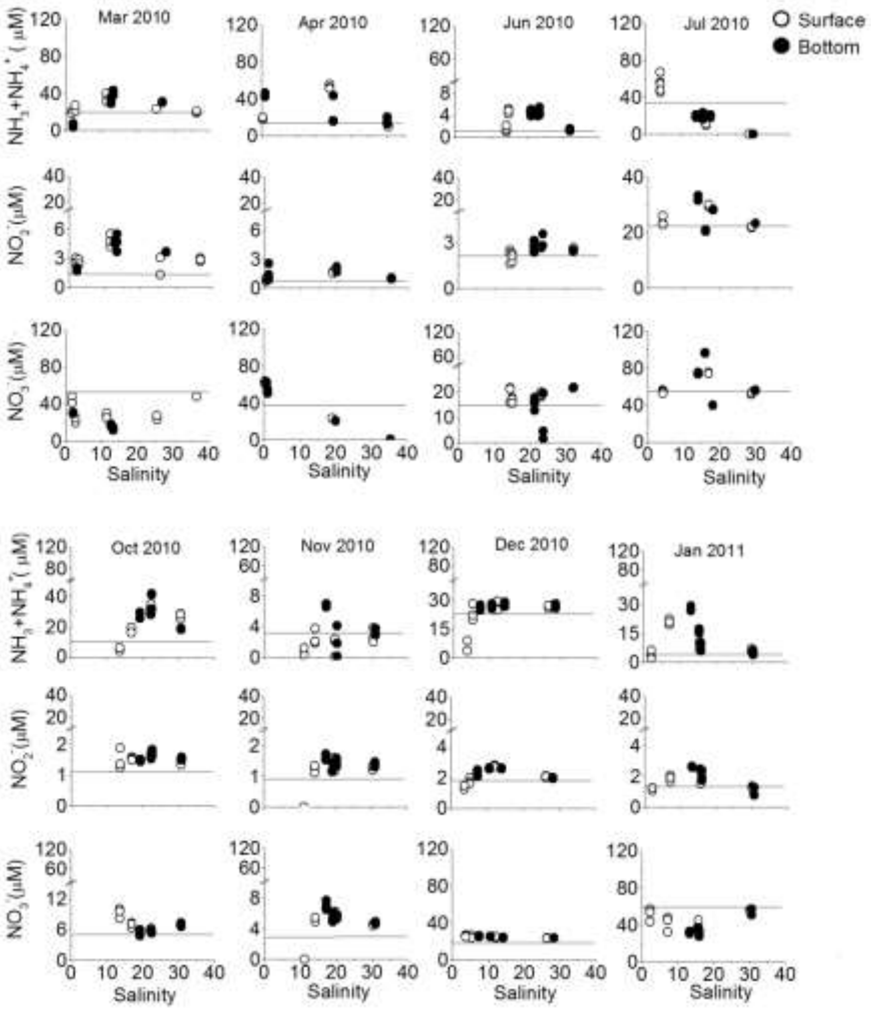

Fig. 5. Mixing diagram of salinity normalized ammonium, nitrite and nitrate concentrations in the Cachoeira River estuary between February 2010 and January 2011. Note the difference in scale and break on the y axis. The horizontal line represents the nutrient concentration in mean salinity. Surface samples (open circle) and (closed circle) bottom samples.

The phosphate concentration varied between 3.20 and 36.4 µM. The phosphate concentrations were higher at stations 1 and 2, in the inner portion of the estuary ($p<0.05$). The surface samples in this estuarine areashowed higher levels of phosphate (Fig. 6). The highest concentration (36.4 µM) was measured at station 1 during February. In the lower portion of the estuary, phosphate was low attaining a maximum concentration of 12.0 µM at station 3 and 3.76 µM at station 4 (Fig. 6c-d). The lowest phosphate concentration was 0.35 µM, observed at station 4 in April. Regarding the behavior of phosphate along the salinity gradient, this element also showed a non-conservative pattern in all the months, removal prevailing (Fig. 7).

The silicate concentrations ranged from 12.0 to 314 µM, with higher concentrations at stations 1 and 2 ($p< 0.05$). The concentration of silicate was higher in the rainy than in the dry season ($p<0.05$). Values were similar at the surface and bottom, although station 2 showed higher concentrations at the surface (Fig. 6). Station 4 presented the lowest concentration. The highest value (84.5 µM) of silicate at this station was recorded in May (Fig. 6). Like other nutrients, silicate showed a non-conservative behavior during most of the study period, with a probable sink of this nutrient in oligohaline waters (Fig. 8).

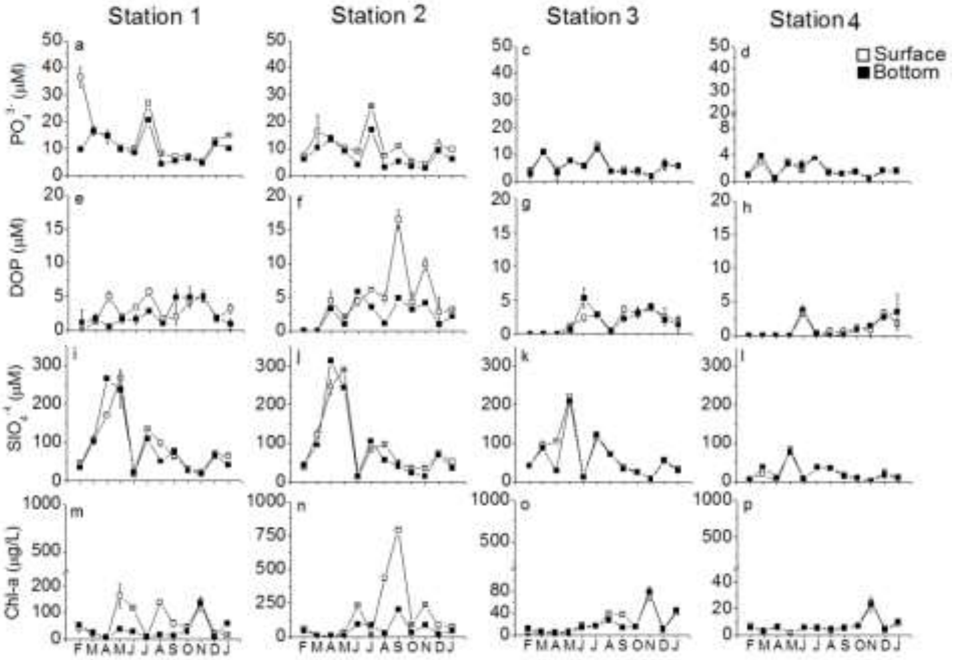

Fig. 6. Mean values (µM) of phosphate (6 a-d), dissolved organic phosphorus (DOP) (6 e-h), silicate (6 i-l) and chlorophyll-*a* (µg/L) (6 m-p), at sampling stations (1-4) in the Cachoeira River estuary between February 2010 and January 2011. Bars indicate the Standard Deviation. Surface samples (open square) and bottom samples (closed square).

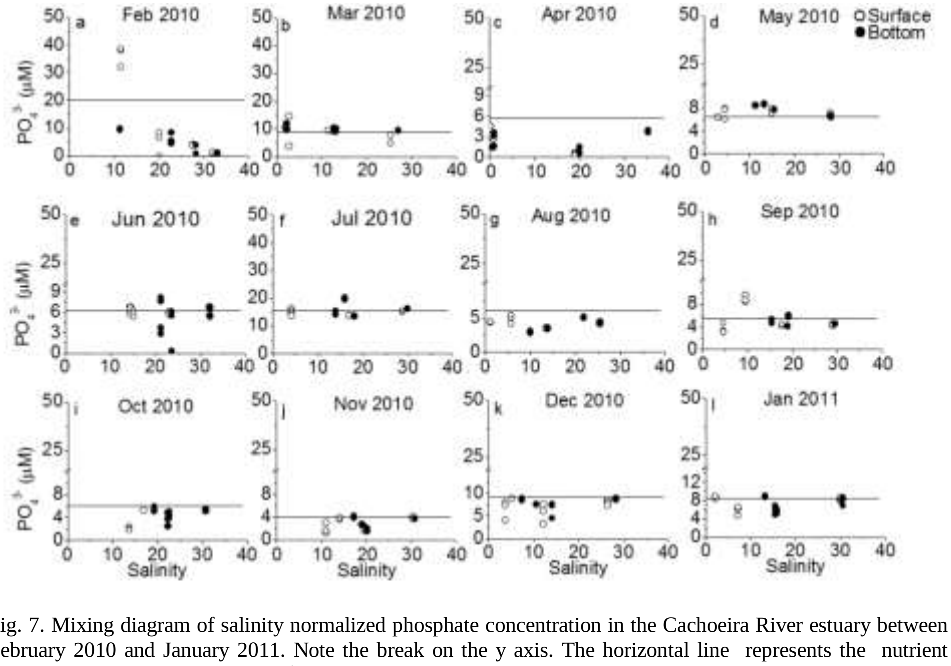

Fig. 7. Mixing diagram of salinity normalized phosphate concentration in the Cachoeira River estuary between February 2010 and January 2011. Note the break on the y axis. The horizontal line represents the nutrient concentration in mean salinity. Surface samples (open circle) and (closed circle) bottom samples.

The chlorophyll-*a* concentration was higher in the inner portion of the estuary (Stations 1 and 2), and significantly higher in the dry than the rainy season ($p<0.05$). Bottom samples collected at station 1 presented higher values than those at the surface in the months of February and March 2010 and January 2011. The highest concentration of chlorophyll-*a* was observed at station 2 in August (793 µg/L; Fig. 6). Stations 3 and 4 hadsimilar values of chlorophyll in the surface and bottom samples, with a low concentration (minimum 20 µg/L) from February to July at station 3 (Fig. 6).

DISCUSSION

Unlike temperate regions, where seasonality is marked by changes in the water temperature, the observed variability of nutrients and chlorophyll-*a* in the Cachoeira River estuary was mainly conditioned by meteorological and hydrological events, such as rain and river discharge, with higher fluvial inputs of nutrients during the rainy periods. In the Cachoeira River estuary, freshwater outflow significantly increased the amount of ammonium, nitrate and silicate, resulting in higher concentrations of these nutrients during the rainy season. Preliminary estimates of fluvial fluxes of DIN to the estuary (unpublished results) ranged from 0.3 x 106 mmol d^{-1} in February to 305 x 106 mmol d^{-1} in April, with higher inputs during the rainy months. Except in February, March, October and November, nitrate is the main form in fluvial inputs, which may be responsible for the high concentrations found in the estuary. Fluvial inputs of DIP were estimated as 1.2 x 106 mmol d^{-1} in February and 44 x 106 mmol d^{-1} in April.

The pattern of increasing nutrients during rainy periods observed in the Cachoeira River estuary is also common in other tropical estuarine ecosystems (EYRE; BALLS, 1999; SARMA et al., 2010), including those located in northeastern Brazil (DITTMAR et al., 2001; BASTOS et al., 2011). However, other estuaries in the same region presented higher nutrient concentrations during the dry period (BRANCO et al., 2002; FLORES MONTES et al., 1998; SANTOS et al., 2009). This fact is probably related to the massive input of organic matter and nutrients from anthropogenic sources, and the increased water residence time that retains most of the nutrients originating from the decomposition of this material.

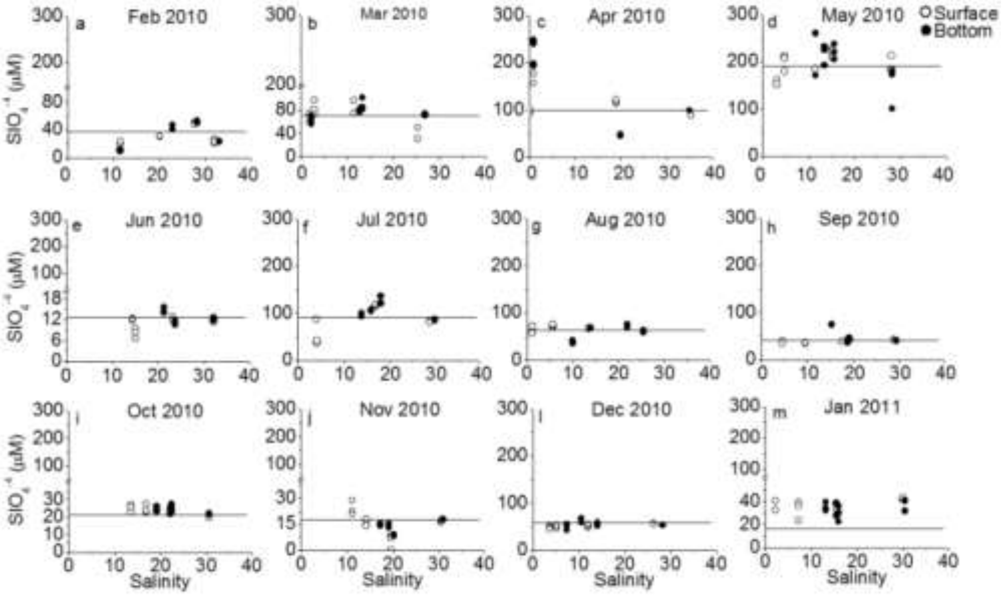

Fig. 8. Mixing diagram of salinity normalized silicate concentrations in the Cachoeira River estuary between February 2010 and January 2011. Note the break on the y axis. The horizontal line represents the nutrient concentration in mean salinity. Surface samples (open circle) and (closed circle) bottom samples.

The higher water residence time in the inner portion makes the non-conservative characteristics of nutrients more evident. In addition to internal biogeochemical processes, the sewage treatment plant and the effluents discharged directly into the river provided the system with nutrients most of the time. The STP's input of dissolved inorganic nutrients into the estuary was estimated to be about 0.7 x 106 mmol d^{-1} (DIP) and 1.1 x 106 mmol month-1 (DIN). Most of this DIN input was in the form of ammoniacal nitrogen, which also contributed to some of the high concentrations observed in the estuary (SILVA et al., 2013). During the dry season the nutrient inputs from the STP were of the same order of magnitude as the fluvial ones, but slightly greater than the DIN. Greater fluvial discharges diminish the importance of the STP inputs, but still present a DIN:DIP ratio lower than 16. Despite the low DIN:DIP ratio of the inputs, estuarine nitrogen fixation in a high water residence time environment should compensate for this nitrogen “deficit” and contribute to a higher DIN:DIP ratio. Only at station 2 was a DIN:DIP ratio higher than 16:1 observed, in April and October (Fig. 9)

Differently from the inner portion, the lower area of the estuary is a well-mixed region, strongly influenced by sea water. The greater water exchange favors the dilution of nutrients by the seawater that, together with the greater distance of the main sources of nutrients, leads to lower concentrations of nutrients in this region. Thus, the estuary has two compartments with different characteristics. The inner portion being more stratified with high concentrations of nutrients and biomass, and the lower portion well mixed, with lower concentrations of nutrients and biomass. The concentration of chlorophyll-*a* observed in this study indicates that the estuary is eutrophic/hypertrophic in the inner portion and mesotrophic in the lower portion, according to the criteria proposed by various authors (SMITH et al., 1999; WHITALL et al., 2007; BOYER et al., 2009).

The difference between surface and bottom dissolved oxygen saturation at stations 2 and 1, with more saturated surface water at this latter, is the result of stratification in the inner estuarine portion and of aerobic remineralization of organic matter at the bottom. Stations 3 and 4 showed almost the same dissolved oxygen saturation at the surface and the bottom due to the more efficient vertical mixing. The maximum dissolved oxygen value (9.7 mg L^{-1} in April at station 3) was not associated with high chlorophyll-*a* concentrations, suggesting some other origin than phytoplanktonic primary production. Extensive mudflat areas are covered with a brownish/golden microphytobenthic layer, probably with high diatom biomass. The water was almost depleted of dissolved silicate. This benthic production during the ebb tide could be responsible for the high dissolved oxygen.

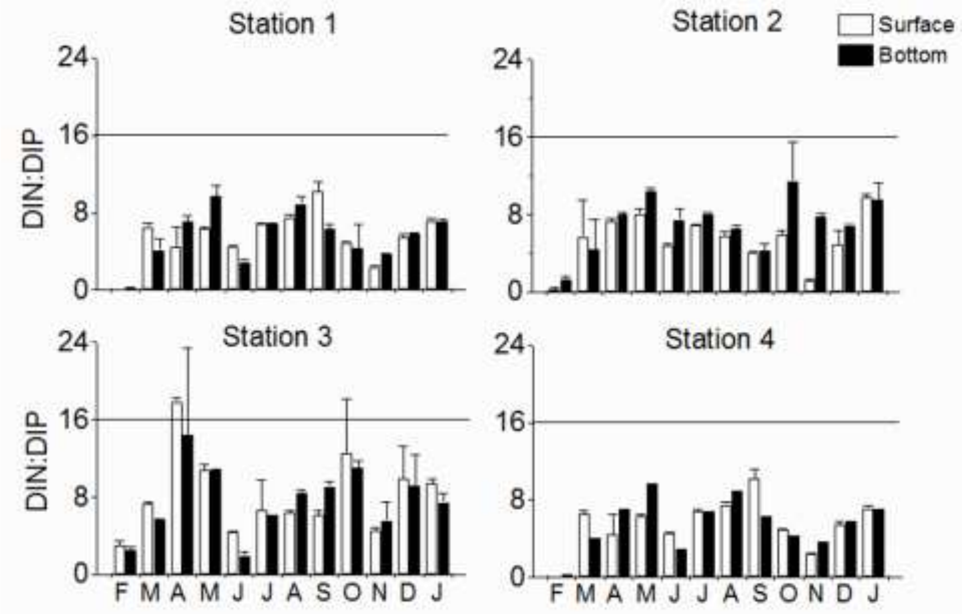

Fig. 9. Mean values of the Redfield molar ratio (16N:1P) in the Cachoeira River estuary between February 2010 and January 2011. Bars indicate the Standard Deviation. Surface samples (open square) and bottom samples (closed square).

Differently from NID and silicate, the phosphate in the Cachoeira River estuary did not show any clear relationship with the rainfall. Moreover, the behavior of phosphate along the salinity gradient corresponds to that observed in previous studies, where the highest concentrations were measured in oligohaline waters (SILVA et al., 2013; SOUZA et al., 2009). Except in May, a widespread removal of phosphate was observed along the estuary. The high levels of phosphate found in the Cachoeira River estuary are, for sure, related to a constant input of this element from the Sewage Treatment Plant. However, the lack of seasonal variability and the absence of a conservative pattern of this element regarding the salinity distribution indicate that in the Cachoeira River estuary the phosphate dynamics is related to other processes such as the uptake by the phytoplankton and adsorption/desorption of this element to suspended particles. The high chlorophyll-*a* concentrations suggest that uptake by the phytoplankton is by far more important. The non-conservative removal of both nitrate and ammoniacal nitrogen in August and September corroborate the importance of this process.

The coastal zone around the estuary of Rio Cachoeira is oligotrophic (Eça et al., 2014), with low concentrations of chlorophyll-a (< 1 µg/ L), phosphate (< 0.2 µM), dissolved silica (< 6 µM), nitrate and ammoniacal nitrogen (< 1 µM). Those authors reported a DIN:DIP ratio below 16:1 in two surveys, but up to 60:1 in March 2006. This marine endmember surely influences the estuarine waters, especially in the lower portion.

The chlorophyll-*a* values currently observed in the estuary of the Rio Cachoeira (Table 1) are similar to those reported in other estuaries of Northeastern Brazil (FEITOSA et al., 1999; SANTOS et al., 2009). However, a comparison of our dataset with the chlorophyll-a values measured in previous studies in this same estuary (SILVA et al., 2013; SOUZA et al., 2009) reveals an increase in this variable since the establishment of the Sewage Treatment Plant. SOUZA et al. (2009) reported maximum valuesof chlorophyll-*a* varying between 42 and 60 µg/L, while SILVA et al. (2013) observed 5.7 and 369 µg/L in the lower and inner estuary, respectively. In the present study, the maximum chlorophyll-*a* concentration in these areas varied between 20 and 793 µg/L.

The uptake of nutrients (N, P) by the phytoplankton contributed to a decrease in the concentrations of these elements in the Cachoeira River estuary, generating the high chlorophyll-*a* values measured in this system. The normalized mixing diagrams show that this is the main DIN removal mechanism in August and September. However, during periods of high river discharge (April, May and December 2010 and January 2011) a decrease in the phytoplankton biomass resulting in significant differences in concentrations of chlorophyll-*a* as between the dry and rainy seasons was observed**.** Very high levels of rainfall can depress the accumulation of phytoplankton biomass since they reduce the water residence time, washing out most of the organic matter produced and impeding the accumulation of phytoplankton, as observed in other estuaries (ABREU et al., 2010; ZINGONE et al., 2010). The tidal renewal of water and strong fresh water inflow due to precipitation can also promote a dilution of nutrient concentrations in the estuary, but the effects of these processes are prevented in the analysis by the use of the normalized mixing diagram.

Table 1. Mean values (N=3), standard deviation and minimum and maximum of chlorophyll-*a* (µg/L), at sampling stations (1-4) in the Cacheira River Estuary between February 2010 and January 2011.

	Station 1		Station 2		Station 3		Station 4	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Feb	43.3 ± 15.5 (31.4 - 60.8)	52.2 ± 3.8 (47.0-54.9)	61.7 ---	45.6 ± 0.6 (45.1-45.9)	5.40 ± 2.2 (6.90-3.86)	11.9 ± 2.8 (8.71 - 14.0)	7.0 ---	5.70 ± 0.6 (5.10 - 6.27)
Mar	17.2 ± 4.0 (14.2 - 21.7)	25.4 ± 10.2 (18.1 - 32.6)	7.8 ± 1.2 (6.70 - 8.71)	9.8 ± 1.4 (8.83 - 10.7)	4.3 ±0.3 (3.98 - 4.64)	7.0 ± 4.80 (3.50 - 10.5)	2.90 ± 0.50 (2.60 - 3.30)	3.1 ± 1.2 (1.95 - 4.35)
Apr	6.3 ± 1.7 (2.04 - 5.96)	3.6 ± 0.80 (6.50 - 7.63)	4.70 ± 1.20 (7.04 - 7.51)	6.60 ± 2.20 (7.85 - 8.13)	3.70 ± 0.20 (3.53 - 3.85)	4.70 ± 1.30 (3.18 - 5.33)	6.0 ± 1.0 (5.30 - 6.72)	6.60 ± 0.30 (3.36 - 6.76)
May	163 ± 43.4 (132 - 193)	36.3 ± 1.90 (34.2 - 37.7)	32.8 ± 16.0 (16.5 - 48.5)	9.70 ± 0.30 (9.41 - 9.97)	5.20 ± 2.60 (2.97 - 8.0)	6.70 ± 3.1 (3.41 - 9.60)	1.80 ± 1.0 (1.0 - 2.47)	2.18 ± 1.50 (1.12 - 3.24)
Jun	118 ± 3.6 (114 - 121)	26.7 ± 3.2 (24.4 - 30.4)	233 ± 4.10 (229 - 235)	90.9 ± 28.6 (65.2 - 121)	18.2 ± 3.0 (15.3 - 21.3)	14.4 ± 0.4 (14.0 - 14.8)	5.6 ± 0.5 (5.07 - 5.98)	5.80 ± 0.2 (5.60 - 5.90)
Jul	9.30 ± 1.20 (7.90 - 10.3)	8.0 ± 0.1 (7.90 - 8.06)	13.4 ± 3.70 (9.70 - 17.3)	84.7 ± 14.6 (86.1 - 92.5)	16.3 ± 1.50 (14.7 - 17.5)	16.2 ± 1.0 (15.3 - 17.3)	5.20 ± 1.60 (3.54 - 6.80)	5.70 ± 0.2 (5.44 - 5.77)
Aug	138 ± 9.65 (131 - 145)	15.6 ± 1.90 (13.4 - 16.9)	436 ± 19.9 (422 - 450)	28.5 ± 2.1 (27.0 - 30.8)	38.3 ± 2.4 (35.5 - 39.8)	27.0 ± 3.1 (24.1 - 30.3)	3.5 ± 0.7 (2.91 - 4.24)	4.6 ± 1.0 (3.90 - 5.80)
Sep	58.7 ± 14.9 (45.2 - 74.7)	13.8 ± 5.0 (8.85 - 18.8)	793 ± 14.2 (782 - 802)	198 ± 1.0 (197 - 199)	37.4 ± 3.0 (34.0 - 39.3)	14.5 ± 1.6 (12.6 - 15.7)	5.5 ± 0.8 (4.97 - 6.39)	5.2 ± 0.5 (4.92 - 5.82)
Oct	47.6 ± 5.1 (43.3 - 53.2)	29.4 ± 0.3 (29.1 - 29.6)	93.4 ± 1.4 (92.4 - 94.4)	30.9 ± 8.5 (25.0 - 37.0)	15.7 ± 3.2 (12.2 - 18.4)	15.8 ± 4.0 (11.2 - 18.2)	7.2 ± 0.4 (6.30 - 7.70)	7.2 ± 1.6 (5.40 - 8.24)
Nov	138 ± 14.0 (130 - 154)	132 ± 25.0 (103 - 149)	238 ± 10.9 (230 - 245)	86.7 ± 22.9 (70.5 - 102)	74.9 ± 13 (60.3 - 85.4)	78.8 ± 1.3 (77.6 - 80.1)	24.3 ± 2.7 (22.5 - 27.3)	23.2 ± 5.20 (19.5 - 27.0)
Dec	22.3 ± 7.2 (16.0 - 30.2)	10.5 ± 0.80 (9.70 - 11.2)	85.9 ± 22.0 (62.6 - 106)	19.1 ± 6.1 (12.0 - 23.0)	11.5 ± 5.3 (6.96 - 17.3)	8.9 ± 1.30 (7.81 - 10.3)	4.90 ± 0.5 (4.60 - 5.22)	3.90 ± 0.7 (3.14 - 4.42)
Jan	16.9 ± 0.6 (16.2 - 17.4)	58.5 ± 3.7 (54.4 - 59.4)	74.9 ± 8.20 (67.1 - 83.5)	45.9 ± 9.7 (37.2 - 56.4)	40.6 ± 4.3 (37.0 - 45.3)	45.2 ± 2.0 (43.5 - 47.3)	9.10 ± 0.50 (8.60 - 9.50)	9.90 ± 0.6 9.60 - 10.6

It is also noteworthy that DON and DOP did not present the same temporal variation as that observed for the inorganic dissolved forms. Actually, the highest DON and DOP were measured after an increase in chlorophyll-*a*. According to WARD; BRONK (2001), an excess DON release by the phytoplankton was observed when nitrate was the main nitrogen source. It is thus possible that the high DON concentration measured is related to the phytoplankton activity in the inner estuarine region. Similarly, we consider that the high DOP values measured in the estuary were related to the phytoplankton production, as observed elsewhere (NAUSCH; NAUSCH, 2011). The remineralization of these dissolved organic pools is another internal source of DIN and DIP. It might be supposed that the remineralization rate of DON is slower than that of DOP due to the more refractory nature of these compounds. This could also be responsible for the low DIN:DIP ratio and high nutrient concentrations.

The high nitrate concentrations measured during this study suggest that the nitrification process can reduce the impact of ammonium in the estuary, preventing the acceleration of eutrophication due to the coupling of nitrification and denitrification**.** In the nitrogen cycle, nitrification is the transformation of ammonium by bacteria into more oxidized elements such as nitrite and nitrate (XIANGLI et al., 2008; BRION et al., 2000). On the other hand, denitrification may occur under low oxygen conditions, and also result in the loss of the nitrogen that enters the system in the form of ammonium (SEITZINGER, 1988; WARD, 1996). Actually these two processes are widely used in the removal of N in sewage treatment plants (TALLEC et al., 2006; DINÇER et al., 2000), but still result in high ammoniacal nitrogen inputs with low DIN:DIP. Denitrification is also removing DIN in the impounded waters of the River Cachoeira (which means “waterfall” as the river runs over a rocky bottom and presents several small rapids and impoundments), especially during the dry season.

The high stock of ammoniacal nitrogen in the estuarine waters, from fluvial and STP inputs and the remineralization of organic matter, is available for nitrification. The non-conservative release of nitrite and ammoniacal nitrogen, simultaneously with the nitrate removal observed in the mixing diagram, reveals that this process prevails in July, November and December. An inverse distribution was observed in March, April, June, October and January, suggesting that during these months denitrification was an efficient mechanism of DIN removal. High concentrations of nitrite were observed in July (> 40 µM). This uncommon nitrite accumulation may occur due to phytoplankton excretion under light limitation, and rate-limitation of the second step of either nitrification or denitrification (LOMAS; LIPSCHULTZ, 2006; MORDY et al., 2010). Nitrate concentrations were also high during this survey. MORDY et al. (2010) also described how an increase of nitrate concentrations in the water can result in a decrease in nitrite reductase activity, intracellular accumulation and in a further release of nitrite.

One could argue that denitrification is a process characteristic of anoxic environments, and dissolved oxygen was always high in the estuary with a minimum of 2.8 mg L-1. This process should prevail in tidal and marginal sediments (TAPPIN, 2002), as in mangrove sediment and estuarine mudflats, and is reflected in the shallow water column due to an efficient sediment-water coupling and lateral transport from the mangrove swamps. Anoxic microzones can also occur in suspended particles in well oxygenated waters, where anaerobic denitrifying bacteria can act (PAERL; PINCKNEY, 1996). HERBERT (1999) describes that the presence of macrophytes can stimulate denitrification due to the release of organic debris during decomposition. Freshwater macrophytes (mainly *Eicchornia crassipes*) are commonly found in the River Cachoeira estuary during the rainy season. Some authors have also reported the ability of bacteria (*Thiosphaera pantotropha, Pseudomonas aeruginosa, Pseudomonas stutzeri, Paracoccus denitrificans, Alcaligenes faecalis*) to denitrify even in the presence of low levels of dissolved oxygen (BERNAT et al., 2003; CHEN et al., 2003; TAKAYA et al., 2003; JOO et al., 2005).

A previous study using a mass balance approach has described a decrease in net N fixation rates in the Cachoeira River estuary, probably caused by an increase in denitrification (SOUZA, 2005). The normalized mixing diagrams in fact indicate that denitrification, nitrification and phytoplankton assimilation alternate as the main non-conservative processes involving dissolved inorganic nitrogen. The coupling of these processes can control the DIN concentrations and maintain the low DIN:DIP ratio observed in the estuary (Fig. 10a and b). If N fixing and nitrification prevailed, an increase in DIN concentrations and the DIN:DIP ratio would be expected (Fig. 10a), especially in periods with a high water residence time and limited exchange with the coastal waters. When denitrification and N2 loss to the atmosphere predominate, there is a negative feedback, tending to decrease the nitrate concentration and the DIN:DIP ratio (Fig. 10b). This seems also to be the case in the Mandovi estuary (India), subject to inputs of ammoniumnitrate (mining explosives) and treated sewage. This estuary maintains a low DIN:DIP ratio, and the importance of denitrification (PRATIHARY et al., 2009) and phytoplankton assimilation (SOUSA, 1999, 2006) for the decrease of DIN concentrations have been assessed by those independent studies. Estuarine denitrification can act by controlling eutrophication, reducing the dissolved inorganic nitrogen available to phytoplankton.

CONCLUSION

The Cachoeira River estuary presents lower nutrient concentrations than had been expected, in view of the high nutrient loading and high water residence time, especially in the inner portion. An effective mixing with coastal waters is responsible to the lower nutrient concentrations in the lower portion. Episodic flooding also flushed the entire estuary, preventing continuous eutrophication. The estuary presents lower nutrient concentrations during the dry periods, which is consistent with high rates of biogeochemical removal. The coupling of nitrification, phytoplankton assimilation and denitrification reduces DIN concentration, sustaining a low DIN:DIP ratio. There is evidence in the literature that this coupling mechanism occurs in other tropical estuaries. Denitrification, as well as the physical driving forces, acts by controlling estuarine eutrophication. The results of this study show many similarities in the functioning of this tropical estuary to those of temperate regions. The main point is that, as occurs in other, temperate systems, phytoplankton in the Cachoeira River estuary plays an important role in the control of nutrients in this ecosystem. However, differently from other temperate systems where water temperature and light are the main driving forces controlling the phytoplankton, in this tropical estuary hydrology seems to be of greater importance.

ACKNOWLEDGMENTS

We thank Mr. Ruy Cavalcanti and his laboratory colleagues for their assistance in the field work and Fernanda S. Silva for her help in the chemical analysis. M.A.M. Silva would also like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Edital CAPES Pro-Amazônia Azul 04/2006 for granting the support for her doctorate (Proc. No. 004 ED04/2006). The Universidade Estadual de Santa Cruz for support while the study was underway. We also acknowledge the anonymous reviewer whose valuable comments and suggestions greatly improved the manuscript.

REFERENCES

ABREU, P. C. M.; BERGESCH, L. A.; PROENÇA, C. A.; GARCIA, E.; ODEBRECHT,C. Short- and long-term chlorophyll-*a* variability in the shallow microtidal Patos Lagoon Estuary, Southern Brazil. **Estuar. Coast.,** V. 33, p. 554-569, 2010.

BAHIA (Estado). Superintendência de Recursos Hídricos. **Programa de recuperação das bacias dos rios Cachoeira e Almada: diagnóstico regional, caracterização Hidrológica.** Ilhéus, SRH/UESC, v. 1, Tomo IV, 2001.

BASTOS, R. B.; FEITOSA, F. A. N.; KOENING, M. L.; MACHADO, R. C. A.; MUNIZ, K. Caracterização de uma zona costeira tropical (Ipojuca, Pernambuco-Brasil): Produtividade fitoplanctônica e outras variáveis ambientais. **Braz. J. Aquat. Sci. Technol.,** V. 16, p.1-11, 2011.

BERNAT, K.; WOJNOWSKA-BARYŁA, I.; DOBRZYŃSKA, A. Nitrogen oxidation and reduction in aerated single-stage activated sludge process. **Pol. J. Environ. Stud.,** V. 12, p. 387-394. 2003.

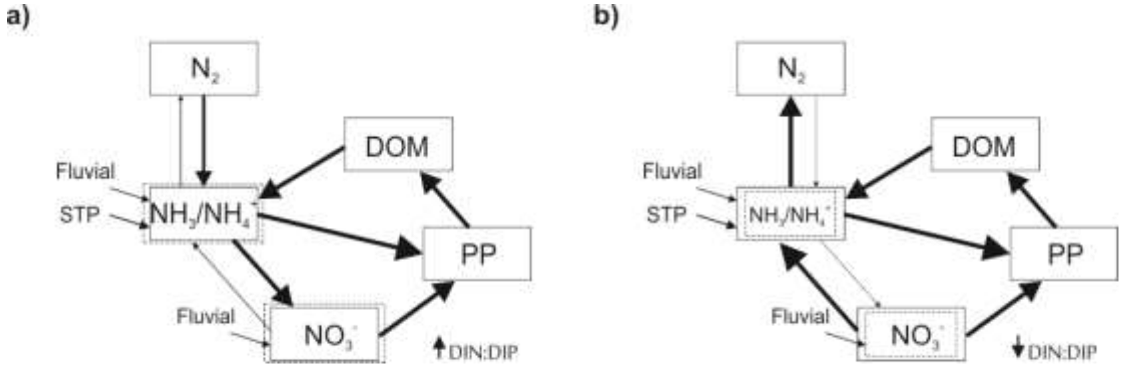

Fig. 10. Simplified conceptual model of nitrogen cycling in the estuary at different scenarios: a) prevailing nitrogen fixation and nitrification; b) Prevailing denitrification. Arrows thickness indicate the relative magnitude of the rates; dashed boxes indicate the trend to increase or decrease nutrient stock. Fluvial and STP inputs assumed as constant and exchanges with sea neglected.

BIE, M. J. M.; SPEKSNIJDER, A. G. C. L.; KOWALCHUK, G.A.; SCHUURMAN, T.; ZWART, G.; STEPHEN, J. R.; DIEKMANN, O. E.; LAANBROEK, H.J. Shifts in the dominant populations of ammonia oxidizing β-subclass Proteobacteria along the eutrophic Schelde estuary. **Aquat. Microb. Ecol.,** v. 23, p.225–236, 2001.

BOYER, J. N.; KELBLE, C. R.; ORTNER, P. B.; RUDNICK, D. T. Phytoplankton bloom status: Chlorophyll a biomass as an indicator of water quality condition in the southern estuaries of Florida, USA. **Ecol. Ind.,** v. 95, p. 556–5 67, 2009.

BRANCO, E. S.; FEITOSA, F. A. N.; FLORES MONTES, M. J. Variação sazonal e espacial da biomassa fitoplanctônica relacionada com parâmetros hidrológicos no Estuário de Barra das Jangadas (Jaboatão dos Guararapes, Pernambuco – Brasil). **Trop. Oceanogr.,** v. 30, n. 2, p.79–96, 2002.

BRION, N.; BILLEN, G.; LOIC, G.; FICHT, A. Distribution of nitrifying activity in the Seine River (France) from Paris to the Estuary. **Estuaries,** v. 23, p.669–682, 2000.

BOUILLON, S. F.; DEHAIRS, VELIMIROV, B.; ABRIL, G.; BORGES, A. V. Dynamics of organic and inorganic carbon across contiguous mangrove and seagrass systems (Gazi Bay, Kenya). **J. Geophys. Res.,** 112, G02018, doi:10.1029/2006JG000325, 2007.

BURFORD, M. A.; ALONGI, D. M.; MCKINNON, A. D.; TROTT, L.A. Primary production and nutrients in a tropical macrotidal estuary, Darwin Harbour, Australia. **Estuar. Coast Shelf. Sci**., v. 79, p.440–448, 2008.

CARMOUZE, J. P. **O metabolismo dos ecossistemas aquáticos. Fundamentos teóricos, métodos de estudo e análises químicas.** São Paulo: Ed. Edgar Blücher/SBL/FAPESP,1994.

CHEN, F.; XIA, Q.; JU, L. Aerobic denitrification of *Pseudomonas aeruginosa* monitored by OnlineNAD(P)H fluorescence. **Appl. Environ. Microbiol.,** v. 69, p. 6715–6722, 2003.

CLOERN, J. E.; JASSBY, A. D. Patterns and scales of phytoplankton variability in estuarine–coastal ecosystems. **Shelf Sci., Estuar. Coast**., v. 33, p. 230–241, 2010.

DINÇER, A.R.; KARG, F. Kinetics of sequential nitrification and denitrification processes. Enzyme **Microbiol. Tech**., v. 27, p. 37–42, 2000.

DITTMAR, T.; LARA, R. J.; KATTNER, G. River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. **Mar. Chem**., v. 73, p. 253–271, 2001.

EÇA, G. F.; LOPES, J. B. B. S.; SOUZA, M. F. L;, BELÉM, A. L. Dissolved inorganic nutrients and chlorophyll on the narrow continental shelf of eastern Brazil. **Braz. J. Oceanogr.,** v. 62, p. 11-21, 2014.

EYRE, B.; BALLS, P. A comparative study of nutrient behavior along the gradient of tropical and temperate estuaries salinity. **Estuaries,** v.22, p.313–326, 1999.

FIDELMAN, P. I. J. Contribuição para a mitigação dos impactos da macrófita aquática *Eichhorniacras sipes* sobre a zona costeira da região Sul da Bahia. **Gerenc. Cost. Int.,** v.4, p. 1–5, 2005.

FEITOSA, F. A. N.; NASCIMENTO, F. C.; COSTA, K. M. P. Distribuição espacial e temporal da biomassa fitoplanctônica relacionada com parâmetros hidrológicos na Bacia do Pina (Recife – PE). Trab. **Oceanogr. Univ. Fed. PE**, **Recife.,** v. 27, p.1–13, 1999.

FLEMER, D. A.; CHAMP, M. A. What is the future fate of estuaries given nutrient overenrichment, freshwater diversion and low flows? **Mar. Pollut. Bull.,** v. 52, p. 247–258, 2006.

FLORES MONTES, M. J.; MACEDO, S. J.; KOENING, M. L.; CORREIA, I. L. Variação nictemeral do fitoplâncton e elementos nutrientes no canal de Santa Cruz, Itamaracá, PE **Trab. Oceanogr. Univ. Fed. PE Recife**, v. 26, n. 1, p. 13-26, 1998.

GRASSHOFF, K.; EHRARDT, M.; KREMLING, K. **Methods of Seawater Analysis**. Weinheim: Verlag Chemie, 419 pp, 1983.

HERBERT, R. A. 1999. Nitrogen cycling in coastal marine ecosystems. **FEMS Microbiol. Rev.,** v. 23, P. 563 - 590.

JEFFREY, S. W.; HUMPHREY, G. F. New spectrophotometric equations for determining chlorophylls a, b, c and c2 in higher plants, algae and natural phytoplankton. **Biochem. Physiol. Pflanzen**., v. 167, p.191–194, 1975.

JOO, H-S.; HIRAI, M.; SHODA, M. Characteristics of ammonium removal by heterotropic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4. **J. Biosci. Bioeng.,** v. 100, p. 184-191, 2005.

KLUMPP, A.; BAUER, B. K.; GERSTEIN, C. F.; MENEZES, M. Variation of nutrient and metal concentration in aquatic macrophytes along the Rio Cachoeira in Bahia (Brazil). **Environ. Int.,** v. 28, p.165–171, 2002.

LEVENSON, H. Estuaries and coastal waters need help. **Environ. Sci. Technol**., v. 21, p.1052–1054,1987.

LIU, X.; YU, Z.; SONG, X.; CAO, X.The nitrogen isotopic composition of dissolved nitrate in the Yangtze River (Changjiang) estuary, China. **Estuar. Coast. Shelf Sci.,** v.85, p. 641-650, 2009**.**

LOMAS, M. W.; LIPSCHULTZ, F. Forming the primary nitrite maximum: Nitrifiers or phytoplankton? **Limnol. Oceanogr.,** v. 51, p. 2453–2467, 2006.

MALLIN, M. A.; CAHOON, L. B.; TOOTHMAN, B. R.; PARSONS, D. C.; MCIVER, M. R.; ORTWINE, M. L.; HARRINGTON, R. N. Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary. **Mar. Pollut. Bull.,** v. 54, p. 81–88, 2007.

MIDDELBURG, J. J.; NIEUWENHUIZE, J. Nitrogen isotope tracing of dissolved inorganic nitrogen behaviour in tidal estuaries. **Estuar. Coast Shelf S**., v. 53, p. 385–391, 2001.

MIRANDA, L. B.; CASTRO FILHO, B. B. M. de; KJERVE, B. **Princípios de Oceanografia Física de estuários**. São Paulo: EDUSP, 414 p, 2002.

MORDY, C. W.; EISNER, L. B.; PROCTOR, P.; STABENO, P.; DEVOL, A. H.; SHULL, D. H.; NAPP, J. M.; WHITLEDGE, T. Temporary uncoupling of the marine nitrogen cycle: Accumulation of nitrite on the Bering **Sea Shelf. Mar. Chem**., v. 121, p. 157–166, 2010.

NAUSCH, M.; NAUSCH, G. Dissolved phosphorus in the Baltic Sea — Occurrence and relevance. **J. Marine Syst**., v. 87, p. 37- 46, 2011.

PAERL, H. W.; PINCKNEY, J. L. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. **Microbiol. Ecol.,** v. 31, p. 225–247, 1996.

PARSONS, T. R.; MAITA, Y.; LALLI, C. M. **A manual of chemical and biological methods for seawater analysis.** 2. ed. Oxford: New York: Pergamon Press, 1984. chap. 4. (Pergamon international library of science, technology, engineering and social studies).

PEREIRA-FILHO, J.; SPILLERE, L. C.; SCHETTINI, C. A. F. Dinâmica de nutrientes na região portuária do estuário do Rio Itajai-Acu, SC. **Atlântica,** v. 25, p.11–20, 2003.

PRATIHARY, A. K.; NAQVI, S. W. A.; NAIK, H.; THORAT, B. R.; NARVENKAR, G.; MANJUNATHA, B. R. AND RAO, V. P. Benthic fluxes in a tropical estuary and their role in the ecosystem. **Estuar. Coast Shelf Sci**., 85, p.387-398, 2009.

RABALAIS, N. N. Nitrogen in aquatic ecosystems. **Ambio,** v. 31, p.102–112, 2002.

SANTOS, T. G.; BEZERRA-JUNIOR, J. L.; COSTA, K. M. P.; FEITOSA, F. A. N. Dinâmica da biomassa fitoplanctônica e variáveis ambientais em um estuário tropical (Bacia do Pina, Recife, PE). **Rev. Bras. Enga. Pesc.,** v. 4, n. 1, p. 95–109, 2009.

SARMA, V. V. S. S.; PRASAD, V. R.; KUMAR, B. S. K.; RAJEEV, K.; DEVI, B. M. M.; REDDY, N. P. C.; SARMA, V. V.; KUMA, M. D. Intraannual variability in nutrients in the Godavari estuary, India. **Cont. Shelf Res.**, v. 30, 2005-2014, 2010.

SEITZINGER, S. P. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. **Limnol. Oceanogr**., v. 33, p. 702-724, 1988

SILVA, M. A. M.; EÇA, G. F.; SANTOS, D. F.; GUIMARÃES, A. G.; LIMA, M. C.; SOUZA, M. F. L. Dissolved inorganic nutrients and chlorophyll-*a* in an estuary receiving sewage treatment plant effluents: Cachoeira River estuary (NE Brazil). **Environ. Monit. Assess.**, v. 185, p. 5387-5399, DOI 10.1007/s10661-012-2953-x, 2013.

SMITH, V. H.; TILMAN, G. D.; NEKOLA, J. C. Eutrophication: impacts of excess nutrient inputs on freshwater, marine and terrestrial ecosystems. **Environ. Pollut.**, v. 100, p. 179–196, 1999.

SOUSA, S. N. Effect of mining rejects on the nutrient chemistry of the Mandovi estuary, Goa. **Indian J. Mar. Sci.**, v. 28, p. 355-359, 1999.

SOUZA, M. F. L. Nutrient biogeochemistry and mass balance of a tropical estuary: estuary of Cachoeira River, Northern Brazil. **Int. J. of Ecol. Environ. Sci**., v. 31, p.177–188, 2005.

SOUZA, M. F. L.; EÇA, G. F.; SILVA, M. A. M.; AMORIM, F. A. C.; LÔBO, I. P. Distribuição de nutrientes dissolvidos e clorofila-*a* no Estuário do Rio Cachoeira, Nordeste do Brasil. **Atlântica.**, v. 31, p.107–121, 2009.

STRICKLAND, J. D. H.; PARSONS, T. R. A pratical handbook of seawater analysis. 2.ed. **Fish. Res. Board Can.,** 167: 311p. 1972.

TALLEC, G.; GARNIER, J.; GOUSAILES, M. Nitrogen removal in a wastewater treatment plant through biofilters: nitrous oxide emissions during nitrification and denitrification. **Bioproc. Biosyst**., v.29, p. 323–333, 2006.

TAPPIN, A. D. An examination of the fluxes of nitrogen and phosphorus in temperate and tropical estuaries: current estimates and uncertainties. **Estuar. Coast. Shelf. Sci**., v. 55, p. 885–901, 2002.

VALIELA, I.; BOWEN, L. Nitrogen sources to watersheds and estuaries: role of land cover mosaic and losses within watersheds. **Environ. Pollut**., v. 118, p. 239–248, 2002.

WHITALL, D.; BRICKER, S.; FERREIRA, J.; NOBRE, A.M.; SIMAS, T.; SILVA, M. Assessment of Eutrophication in Estuaries: Pressure–State–Response and Nitrogen Source Apportionment. **Environ. Management**., v. 40 p.678–690, 2007.

WARD, B. B. Nitrification and denitrification: probing the nitrogen cycle in aquatic environments. **Microb. Ecol**., v. 32, p. 247–261, 1996.

WARD, B. B.; BRONK, D. A. Net nitrogen uptake and DON release in surface waters: importance of trophic interactions implied from size fractionation experiments. **Mar. Ecol. Prog. Ser**., v. 219, p.11–24, 2001.

WATTAYAKORN, G.; PRAPONG, P.; NOICHAREON, D. Biogeochemical budgetsand processes in Bandon Bay, Suratthani, Thailand. **J. Sea Res.,** v. 46, p.133–142, 2001.

XIANGLI, Q.; ZHENJIA, Z.; QINGXUAN, C.; YAJIE, C. Nitrification characteristics of PEG immobilized activated sludge at high ammonia and COD loading rates. **Desalination,** v. 222 p. 340–347, 2008.

ZAR, J. H. **Biostatistical analysis**. New Jersey: Prentice-Hall Inc**.** 2 ed. 1999.

ZINGONE, A.; PHLIPS, E. J.; HARRISON, P. J. Multiscale variability of twenty-two coastal phytoplankton time series: a global scale comparison. **Estuar. Coast. Shelf. Sci.**, v. 33 p. 224–229, 2010.

(Manuscript received 10 May 2013; revised 20 September 2014; accepted 26 September 2014)