

Chemical composition of phytoplankton from the estuaries of Eastern Amazonia

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ABSTRACT

Phytoplankton is important bioindicator of chemical and biological modifications of natural ecosystems. The objective of this study was to determine the total chemical composition of the phytoplankton of the Pará and Mocajuba estuaries on the eastern coast of the Amazon region in the Brazilian state of Pará. The chemical composition of the surface water, bottom sediments (total sample and bioavailable fraction), and the phytoplankton were determined by inductively coupled plasma optical emission spectrometry. Phytoplankton contained high concentrations of Ca, P, Mn, Fe, Zn, Al, Ba, and Pb. The phytoplankton of the Mocajuba estuary is rich in Fe (2,967-84,750 $\mu\text{g g}^{-1}$), while those from the Pará is rich in Al (1,216-15,389 $\mu\text{g g}^{-1}$), probably reflecting divergent anthropogenic inputs. Both samples indicated a high bioconcentration factor derived from both the water and the bioavailable fraction, reflecting the efficiency of these organisms in the concentration of metals.

KEYWORDS: Sediments, trace metals, plankton, surface water

Composição química do fitoplâncton de estuários da Amazônia oriental

RESUMO

O fitoplâncton é de grande importância para os estudos das interações solo-água, como indicadores de modificações químicas e biológicas nos ecossistemas naturais. O objetivo desse estudo foi determinar a composição química total do fitoplâncton nos estuários do rio Pará e Mocajuba (Pará, Brasil). As análises químicas foram realizadas na água superficial, sedimento de fundo (amostra total e fração biodisponível) e no fitoplâncton, por espectrometria óptica e de massa com plasma indutivamente acoplado. A composição química elementar do fitoplâncton é composta por elevadas concentrações de Ca, P, Mn, Fe, Zn, Al, Ba e Pb. O fitoplâncton do estuário do rio Mocajuba é rico em Fe (2.967 a 84.750 $\mu\text{g g}^{-1}$) e do rio Pará rico em Al (1.216 a 15.389 $\mu\text{g g}^{-1}$), provavelmente com contribuição antropogênica. O material fitoplanctônico apresentou elevado fator de bioconcentração proveniente tanto da água quanto da fração biodisponível, e reflete a eficiência desses organismos em concentrar metais.

PALAVRAS-CHAVE: Sedimentos, metais-traço, fitoplâncton, água superficial

INTRODUCTION

Rivers are the principal routes of transport of dissolved and particulate matter from inland areas to the oceans. This matter may be assimilated by planktonic organisms, which are one of the key components of the trophic dynamics of estuarine ecosystems (Furuya 1990; Kjerfve and Lacerda 1993). Bioactive metals in river water strongly influence the production and the chemical composition of the phytoplankton (Bruland *et al.* 1991) and may also affect cell structure and internal enzymatic activities (Viarengo 1985; Magdaleno *et al.* 1997). Phytoplankton may thus concentrate large quantities of chemicals in their structure (Martin and Knauer 1973), and each taxon or population may be metabolically distinct, depending on the specific chemical elements they have accumulated (Sigee *et al.* 1998). Given their short life cycle and sensitivity to environmental impact, phytoplankton provide an ideal model for the study of soil-water interactions, and can be used to identify and describe modifications in the physiological, biochemical, and ecological processes that occur in natural ecosystems (Gonzalez-Davila 1995; Petersen and Gustavson 1996; Luoma *et al.* 1998).

Data on the chemical composition of phytoplankton found in the shallow waters of the Amazon coast are rare, possibly due to their small size and the technical difficulties of preparing specimens. Amazonian phytoplankton studies have tended to focus on taxonomy or on explaining the high diversity and densities of phytoplankton found in the region (Figueiras and Pazos 1991; Leynaert *et al.* 1991; Paiva *et al.* 2006). The objective of this study was to determine the total chemical composition of the phytoplankton of the Pará and Mocajuba estuaries on the eastern coast of the Amazon region in the Brazilian state of Pará.

MATERIALS AND METHODS

Study areas

Sampling took place in two estuaries east of the mouth of the Amazon River, in the northeast of the state of Pará: the Mocajuba River is strongly influenced by the marine environment, while the Pará River is primarily influenced by fluvial dynamics. Furthermore, while the Mocajuba estuary is relatively well preserved, the Pará estuary is subject to a variety of anthropogenic impacts derived from the transportation of bulk cargoes such as mineral ores, fuel oils, and caustic soda related to the activities of local industries (Albras, Alunorte, Imerys rio Capim and Pará Pigmentos) and from the busy Vila do Conde shipping terminal (Figure 1, A and B).

The two estuaries have a similar tropical climate, characterized by intense rainfall (mean annual precipitation of 3000 mm), heat (mean temperature 27.7 °C), and humidity (typically between 80 and 85%). Rainfall is most intense

between January and June, and contributes to the run-off of organic and inorganic detritus, and the discharge of freshwater into the estuaries. During the dry season (August–November) freshwater discharge decreases considerably, resulting in an influx of oceanic water, although this process is relatively weak in the Pará estuary.

The geology of the study region is characterized by lateritized clastic sedimentary rocks (mudrocks and sandstones) of the Miocene–Pliocene Barreiras Formation, as well as Quaternary sediments represented by beaches, dunes and paleodunes, swamps, saltmarshes, coastal and muddy tidal plains, sandbanks and mud flats, as well as alluvial deposits (Costa *et al.* 2004; Siva *et al.* 2009).

The vegetation of the Mocajuba estuary is dominated by secondary forests on latosols, and mixed mangrove forests composed of *Rhizophora mangle* and *Avicennia germinans* on the tidal plains (Silva *et al.* 2009). By contrast, the Pará estuary is more typical of a lower Amazon floodplain, with freshwater swamps dominated by *Euterpe oleracea* and *Mauritia flexuosa* palms, and in particular *Montrichardia arborescens* Schott (Macedo *et al.* 2005).

Samples of water, sediments, and phytoplankton were collected in each estuary in January, April, August, and November, 2011. The samples were obtained at three points (P1C, P2C, and P3C) in the Mocajuba estuary (Figure 1A), and four (P1B, P2B, P3B, and P4B) points in the Pará (Figure 1B).

Surface water samples were collected during both the flood and ebb tides, and stored in one-liter polyethylene bottles before being vacuum-filtered through an acetate filter with a porosity of 0.45 µm. The samples were stored in nitric acid at pH < 2 for the measurement of dissolved chemical elements. Physico-chemical and chemical parameters (pH, salinity, temperature, and nitrate concentrations) were measured *in situ* using a (Horiba W-23XD multi-probe Kyoto, Japan). The sediment samples were collected with a stainless steel Petersen-type bottom grab, maintained under refrigeration (4 °C) before being dried to a constant weight in a stove at 50 °C, and then macerated in an agate mortar.

Phytoplankton was collected in horizontal trawls at a depth of 50 cm during the flood tide, using a standard plankton net with a 64 µm mesh (this mesh size is needed due to the strong local hydrodynamics). The wet samples were then sieved and passed through a 20 µm mesh before being washed with distilled water. Each sample was examined under a binocular microscope (Zeiss SV6 Binocular Stereo-microscope, Goettingen, Germany) to remove residues (e.g., leaves, stalks, and shells), and then lyophilized (Labconco, Freeze Dry Systems, 7752000, Kansas City, Missouri).

Subsamples (250 mL) of the plankton were fixed in a 4% formaldehyde solution and identified to the species level using

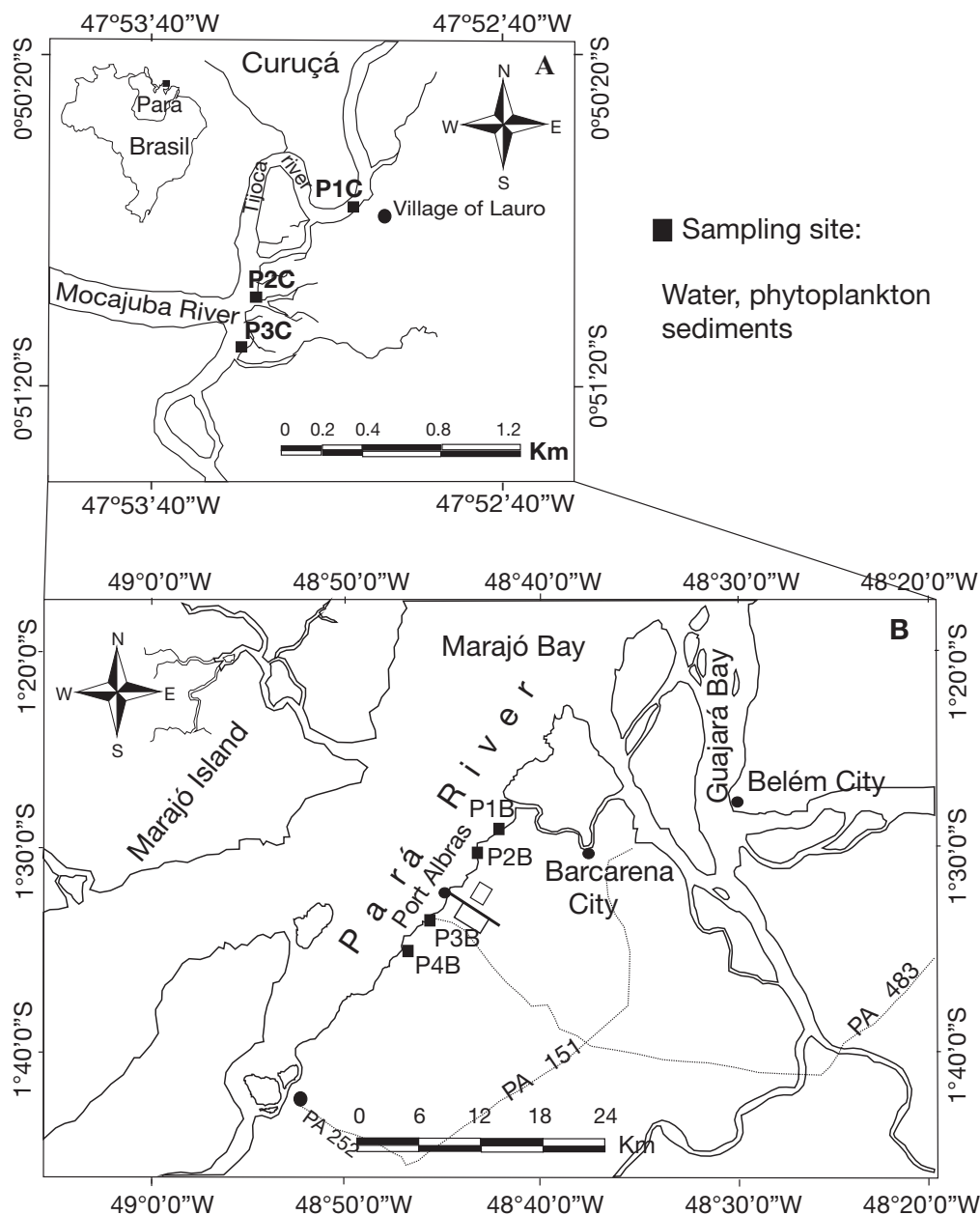


Figure 1. Location of the sampling points in the two study areas

the specialized literature for each taxonomic group. Density was measured as the number of organisms per liter of water (Utermöhl 1958). Samples were analyzed under an (Olympus IX70 inverted microscope, New York USA).

The mineralogy of the sediment was determined by X-ray diffraction at the Institute of Geosciences, Federal University of Pará, using a diffractometer (PW 3040, PANalytical, Almelo, The Netherlands), with a copper anode ($k\alpha_1 = 1.54060 \text{ \AA}$), tension generator and current adjusted to 40 kV

and 30 mA, respectively. Diffractions of total samples ranged from 5 to 75° 2θ , with step size of 0.02° - 10 s. The results were interpreted using the X'Pert HighScore 2.1 software and the International Center for Diffraction Data database.

The images for micromorphological observations, textural, and semi-quantitative chemical analyses were obtained using a Scanning Electron Microscope, SEM (LEO 1450, London, England) coupled to an Energy Dispersive X-ray Spectroscopy (EDS) 500 DP, Gresham Scientific Instruments (Sirius 10/7.5,

London, England). The phytoplankton samples were placed in an aluminum holder, at a controlled temperature and metalized with gold. These analyzes were conducted in the Goeldi Museum laboratory in Belém, Brazil.

The total chemical analysis of the bottom and suspended sediments was based on the alkaline fusion of the samples with lithium metaborate/tetraborate, followed by solubilization with nitric acid. Finally, measurements were made by mass inductively coupled plasma mass spectrometry (ICP-MS) in the Acme Analytical Laboratory, Vancouver, Canada, following the Litho-Geochemical Standard Package codes and STD OREAS45CA reference materials. The bioavailable fraction of the major and trace chemical elements in the sediments was extracted by leaching with 0.1 N HCl under continuous agitation (16 hours) and measured by ICP-MS in the Acme Analytical Laboratory.

The concentrations of major and trace chemical elements in the water were also determined by ICP-MS in the Acme Laboratory, using the equipment specified above and STD TMDA-70 reference materials. The phosphate concentrations in the water were determined by the ammonium molybdate and silica method, with ammonium heptamolybdate, using a (Spectro Vision UV-VIS, DB-1880S, Shanghai, China) at 835 nm in the Goeldi Museum Laboratory in Belém.

The phytoplankton was analyzed by digesting 0.1 g (dry weight) subsamples in HNO₃ and HCl (3:1 v/v). This solution was then heated to 100 °C for 45 minutes until completely digested. Once cooled, samples were measured with Milli-Q water. The concentrations of Na, K, Ca, Mg, P, Mn, Fe, Cu, Zn, Se, Al, As, Ni, Cd, Ba and Pb ($\mu\text{g g}^{-1}$) were obtained by inductively coupled plasma optical emission spectrometry (ICP-OES, HORIBA, Jobin Yvan Ultima 2, Kyoto, Japan), in the Laboratory for Environmental Analysis and Geoprocessing Laboratory at the Brazilian Center for Nuclear Energy in Agriculture (CENA/USP) in Piracicaba. The accuracy was evaluated using Dogfish Muscle Certified Reference Material (DORM-2). The chemical concentration of each element in each sample was obtained from the arithmetic mean of the samples in triplicate. The blanks were prepared with the same quantities of reagents used in the chemical analyses and the same reference material, in order to assure the integrity of the digestive process. The recuperation of the certificated samples ranged from 92% to 102% for Na, K, Ca, Mg, P, Mn, Fe, Cu, Zn, Se, Al, Ni, As, and Ba, and 88% for Cd and Pb. The detection limits were 0.01 $\mu\text{g g}^{-1}$ for Na, K, Ca, Mg, P, Mn, Fe, Cu, Zn, Cd, and Ba, 0.02 $\mu\text{g g}^{-1}$ for Ni, Se, and As, and 0.04 $\mu\text{g g}^{-1}$ for Pb and Al.

The bioconcentration factor (BCF) for the phytoplankton represents the ratio between the concentration of chemical elements in the phytoplankton biomass (dry weight in $\mu\text{g g}^{-1}$) and the concentration of these elements in the water (μg

mL^{-1}). The sediment bioconcentration factor (BSCF) is the ratio between the total chemical concentration in the sediment and the bioavailable fraction (dry weight in $\mu\text{g g}^{-1}$) following Kenaga (1980). The concentrations of trace elements in the phytoplankton were normalized by the concentrations of the phosphate ion ($\mu\text{g mL}^{-1}$ trace element: $\mu\text{g mL}^{-1}$ phosphate), and the chemical concentrations of the sediments were normalized by the phosphorus ($\mu\text{g g}^{-1}$ trace element: $\mu\text{g g}^{-1}$ P). The BCF and BSCF values are expressed as logarithms. A linear regression analysis was used to quantify the relationships between the concentrations of trace elements in the water and the BCF (significance level $p = 0.05$). A principal coordinates analysis (PCoA) was run in PC-ORD, version 6.0 (McCune and Mefford 2011), using the logarithmic [$\log(x+1)$] data.

RESULTS

Bottom sediments

The silt/clay sediments of the Mocajuba estuary have predominant mineralogical composition of quartz and kaolinite with lesser concentrations of smectite and illite. The levels of SiO₂, Al₂O₃, and Fe₂O₃ in these sediments were high, as were those of Na₂O, K₂O, MgO, and CaO. In general, the concentrations of trace elements were close to or below mean UCC (Upper Continental Crust) levels, with the exception of Cr and As (Table 1).

In the Pará estuary, the predominantly sand/silt sediments are primarily composed of quartz, and to a lesser extent, kaolinite, illite, goethite, and hematite. The concentrations of SiO₂ were high, with low concentrations of Al₂O₃ and Fe₂O₃, with Na₂O, K₂O, MgO, and CaO (< 0,5%). The concentrations of trace elements were below UCC levels (Table 1). The concentrations of the bioavailable elements were higher for Na, K, Ca, Mg, Fe, Al, and Ba, although these values are still extremely low (< 1%) when compared to the total chemical composition of the sediments (Table 1).

Surface waters

The waters of the Mocajuba estuary are slightly acid (pH 6.2-6.9) and strongly enriched with alkalis (Na⁺>Mg²⁺>Ca²⁺>K⁺), with high levels of Cl⁻ and Br⁻. Relatively high concentrations of most chemical elements, such as Fe, Zn, Se, Sr, Pb and Al, were recorded in the samples (Table 2).

The Pará estuary had a pH of between 5.8 and 7.8, Na⁺ (0.16-1,172 mmol L⁻¹) and low concentrations of Ca²⁺, Mg²⁺ and K⁺ (< 0.13 mmol L⁻¹). These waters were relatively rich in Fe and Al (Table 2). During the dry season (August-November), the mean concentrations of the chemical elements were higher than those recorded in the rainy season (January and April). The concentrations of the principal nutrients (phosphates, silicates, and nitrates) in the water were also higher during the dry season.

Table 1. Chemical composition (means and standard deviations in percentage weight) and trace elements (in $\mu\text{g g}^{-1}$) of the bottom sediments of the Pará river and Mocajuba river estuaries and Upper Continental Crust – UCC (Taylor and McLennan 1985)

	Pará River		Mocajuba River		UCC
	Total sample	Bioavailable fraction	Total sample	Bioavailable fraction	
SiO ₂	85.2±11.42	0.001	57.37±11	0.003	66
Al ₂ O ₃	5.46±5.06	0.001	15.26±2.85	0.002	15
Fe ₂ O ₃	2.4 ± 1.48	0.002	4.96±1.01	0.010	4.3
MnO	0.02±0.01	0.0001	0.02±0.00	0.001	0.07
Na ₂ O	0.13±0.09	0.0006	1.21±0.60	0.002	3.9
K ₂ O	0.45±0.37	0.0002	1.47±0.23	0.001	3.4
CaO	0.13±0.11	0.002	0.28±0.13	0.003	2.2
MgO	0.21±0.20	0.0002	1.4±0.40	0.003	4.2
P ₂ O ₅	0.13±0.01	0.0001	0.2±0.02	0.001	
Ni	20±0.00	0.01	22.4±3.15	0.02	20
Cr	30.2±19.3	0.01	50±30.05	0.03	35
Cu	4.2±3.65	0.02	7.6±2.11	0.05	25
Zn	14±11	0.56	28.4±7.78	0.81	71
As	1.5±0.71	0.01	9.33±2.53	0.05	1.5
Se	0.5±0.10	0.0005	0.5±0.10	0.00	
Sr	21±15	0.12	87.38±27	0.31	350
Cd	<0.10	0.0002	<0.10	0.00	0.09
Ba	133±93	2.19	210±54	0.72	550
Pb	4±2.58	0.01	12±2.75	0.10	20

Phytoplankton

The microphytoplankton community is more diverse in the Mocajuba estuary, with a total of 126 taxa, 85% of which belong to the Bacillariophyceae, 10% to the Cyanophyceae, 3% to the Chlorophyceae, 1% to the Dynophyceae, and 1% to the order Tintinnidae. *Coscinodiscus oculus iridis* and *C. janischii* were relatively abundant during the rainy season months, but rarer in May, June, and July. *Coscinodiscus* was common in most of the months analyzed.

A total of 60 species were found in the Pará estuary, which was dominated by diatoms, in terms of both the number of species and density, especially for the species *Actinocyclus normanii*, *Aulacoseira granulata*, *Coscinodiscus rothii* and *Polymyxus coronalis* (Figure 2), which are common in the brackish waters of the Amazon region (Paiva 2006). During the drier months, the diversity of algae increased and a number of different taxa became highly abundant, such as *Polymyxus coronalis*, and the genera *Aulacoseira* and *Coscinodiscus*. During

Table 2. Chemical composition (means and standard deviations) for cations and anions ^b(mmol L⁻¹); NO₃⁻, PO₄³⁻ and H₄SiO₄ ^a($\mu\text{mol L}^{-1}$) and trace elements ($\mu\text{g L}^{-1}$) of the surface water and the chemical composition of the phytoplankton (mean values in $\mu\text{g g}^{-1}$)

	Pará River		Mocajuba River	
	Freshwater	Phytoplankton	Saltwater	Phytoplankton
H ₄ SiO ₄	166±156 ^a		159±48 ^a	
Na	220±327 ^b	143 ± 80	1099±1149 ^b	2094±852
K	0.03±0.00 ^b	1250±445	3.29±1.51 ^b	2118±272
Mg	0.07±0.03 ^b	1452±651	18±9.51 ^b	2666±1831
Ca	0.01±0.04	2029±1872	3.57±1.79 ^b	3919±1732
P		775±424		2074±372
Cr	1±0.65	16.61±4.48	198±138	32
Mn	6±7.04	510±197	79±32	1064±662
Fe	196±151	21887±9605	5368±4810	33435±26437
Cu	2.26±0.27	16±3	43±37	17±3
Zn	11±6.81	38±25	164±140	102±55
Se	1±0.05	1.4±1.2	1638±1500	1.8±1.1
Sr	29±5.90	15±6.3	2685±1435	53
Al	124±219	8111±5820	373±298	4506±2661
Ni	1±0.06	7.5±4.8	183±147	6.5±2.7
As	1±0.13	6.5±1.6	1514±152	9.4±4
Cd	0.13±0.14	1.2±0.6	351±357	0.5±0.2
Ba	23±3.03	100±53	48±35	15.3±5
Pb	0.8±0.53	10±1.4	2002±2041	36±18
Cl ⁻	0.4±0.43 ^b		191±112 ^b	
Br ⁻	0.36±0.70 ^b		150	
PO ₄ ³⁻	5.77±3.05 ^a		3.1	
NO ₃ ⁻	45±7.36 ^a		6.9	

the rainy season, the diversity of algae remained high, with a growing predominance of *Coscinodiscus sp.*

The phytoplankton of the Mocajuba estuary contained the highest concentrations of Na, K, Ca, Mg, P, Mn, Fe, Zn, and Pb. In the Pará estuary, high concentrations were recorded only for Fe, Al, and Ba (Table 2). The high levels of Al, Fe, K, Ca, and P were confirmed by the energy dispersive spectroscopy (EDS) analyses of *Actinocyclus normanii*, *Polymyxus coronalis*, and *Aulacoseira granulata* (Table 3 and Figure 2).

In the Pará estuary (Figure 3A, C), significant positive correlations were found between the abundance of metals in the phytoplankton and the chemical composition of the sediments and the water ($r^2 = 0.77$ and 0.95 , respectively;

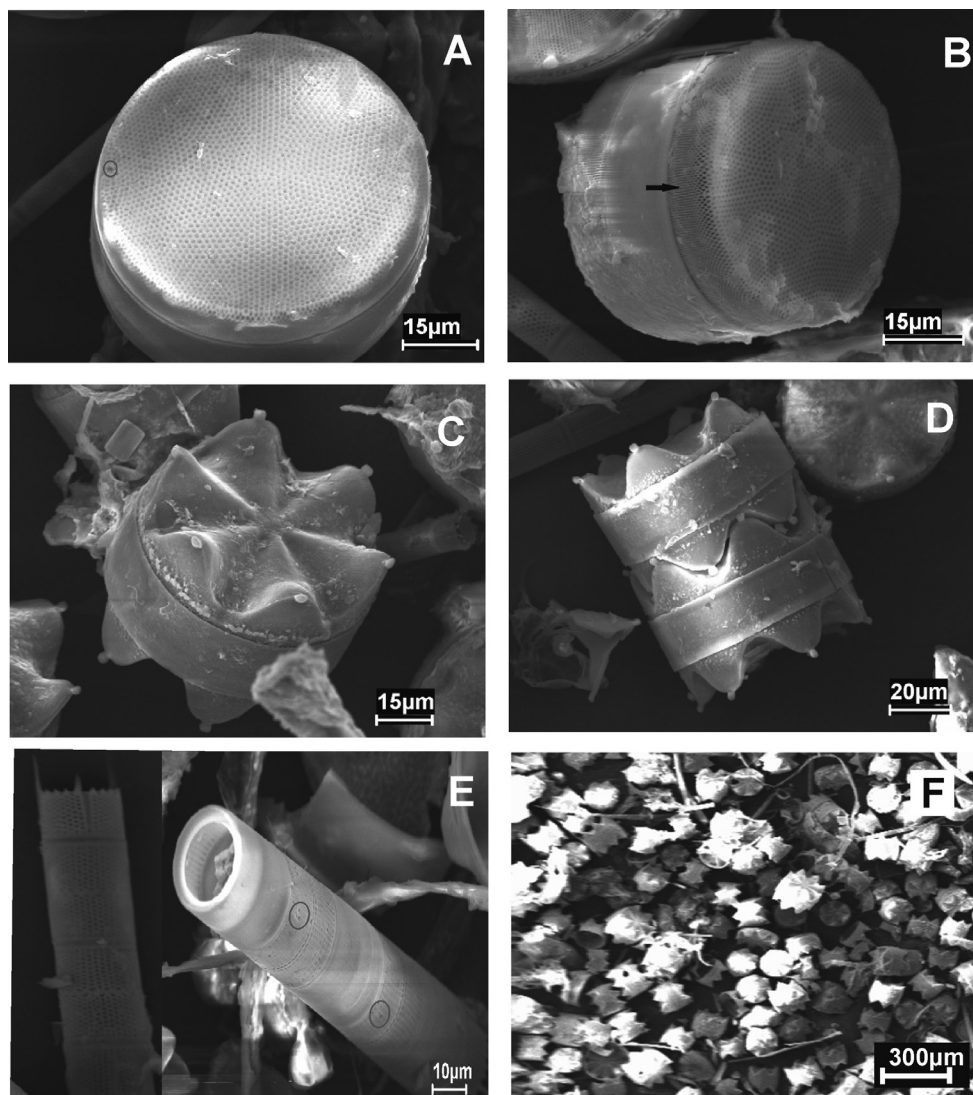


Figure 2. SEM image: *Actinocyclus normanii* (Gregory) Hustedt: (A) valvar view showing the pseudonodules and (B) valves with a distinct ring between the mantle and the valvar face; *Polymyxus coronalis* Bailey: (C) valvar view showing six elevated points and (D) valves agglomerated in colonies; *Aulacoseira granulata* (Ehrenberg) Simonsen: (E) lateral view of the colony showing the rimoportulae adjacent to the valvar face: (F) predominance of *Polymyxus coronalis* in the samples from the Pará estuary

Table 3. Chemical concentrations (means and standard deviations) of the elements Na, Mg, K, Ca, P, Fe, Al and Si ($\mu\text{g g}^{-1}$) analyzed in the diatoms of the Pará estuary by X-ray dispersion spectrometry (SED 500 DP)

Species	Na	Mg	K	Ca	P	Fe	Al	Si
<i>A. normanii</i>	1766	382	610	732	1372	2070	6918	402332
	± 333	± 102	± 385	± 128	± 199	± 502	± 1846	± 22173
<i>P. coronalis</i>	1967	291	2357	1377	1510	7700	10759	535113
	± 1755	± 177	± 1102	± 1085	± 438	± 4436	± 9348	± 144911
<i>A. granulata</i>	773	298	2903	1931	1430	5006	6277	620058
	± 731	± 143	± 1485	± 758	± 395	± 2872	± 2614	± 143274

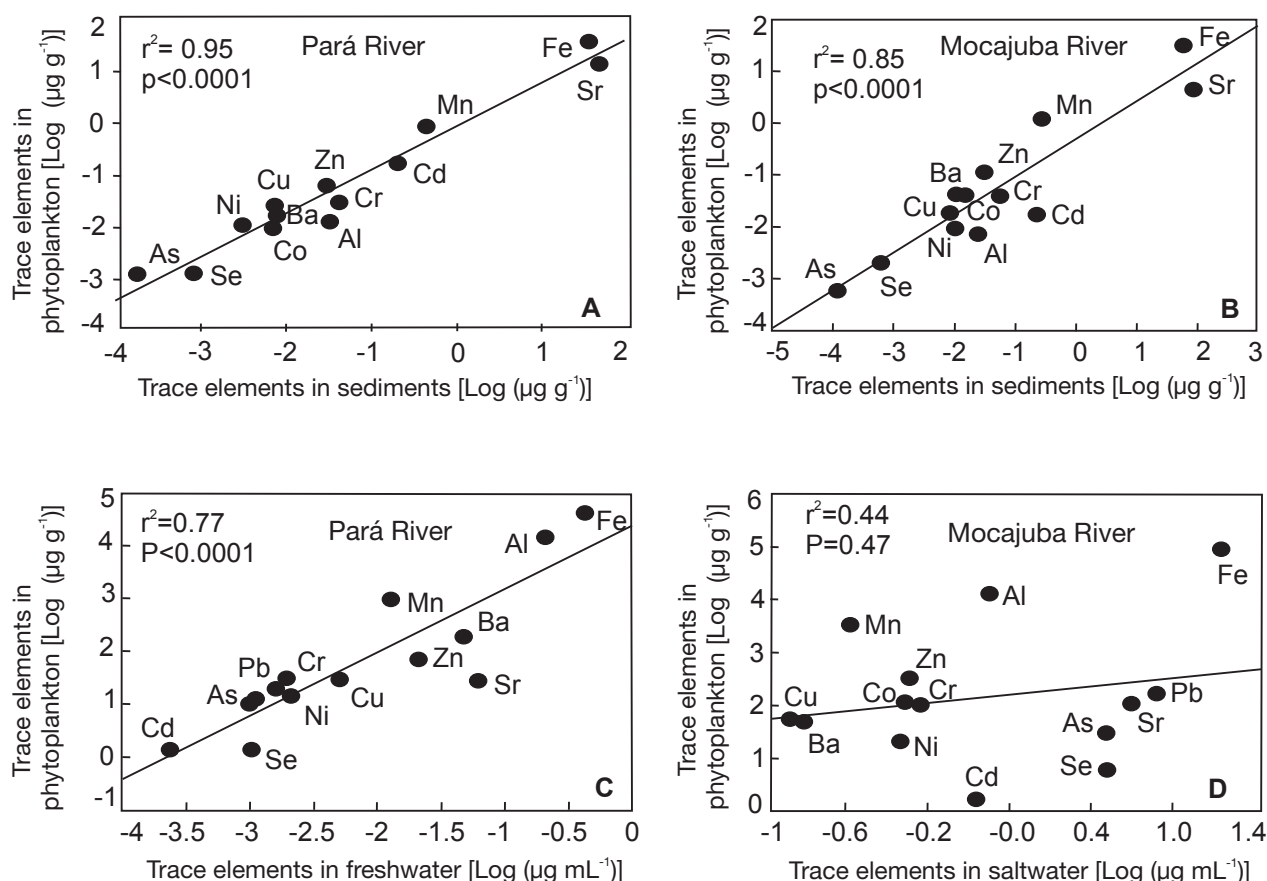


Figure 3. Correlations between the trace elements ($\mu\text{g L}^{-1}$) recorded in the phytoplankton and the water (values in $\mu\text{g L}^{-1}$ normalized by the phosphates). Chemical composition of the phytoplankton and sediments ($\mu\text{g g}^{-1}$) normalized by P in the Pará (A and C) and Mocajuba (B and D) estuaries

Table 4. Synthesis of the main components PCoA of the chemical elements

	Axis 1	Axis 2
Auto value	0.18	0.43
Percentage of variance explained	66.24	15.29
Percentage of cumulative variance	66.24	81.52
Results of randomization "p"	0.001	0.001

$p < 0.001$) - as found by Quigg *et al.* (2003) and Libes (2009). In the Mocajuba estuary (Figure 3B, D), by contrast, the contribution of the water to the chemical composition of the phytoplankton appears to be much less ($r^2 = 0.44$, $p = 0.47$), with a strong correlation being found only with the chemical composition of the sediments ($r^2 = 0.85$, $p < 0.001$).

The principal coordinates analysis summarized approximately 81% of the combined variability of the data on the first two axes (Figure 4, Tables 4 and 5). The positive side

of axis 1 contains all the values for the chemical composition of the water of the Pará estuary, while the negative side encompasses the remaining values (chemical composition of the phytoplankton, water, and sediment) associated primarily with the concentrations of K, Mg, Ca, Mn, Co, Se, Ni, As, Cd, Cr, Cu, Pb, and Al ($r \geq 0.05$). The positive side of axis 2 groups the chemical concentrations of the water of the Mocajuba estuary, associated primarily with Se, Sr, As, and Cd ($r \geq 0.03$), and separating the phytoplankton and sediment samples.

Bioconcentration factor (BCF)

The highest BCF values were recorded in the phytoplankton from the Pará estuary for most chemical elements except Zn and Pb, for which higher concentrations were recorded in the Mocajuba estuary (Table 6). The elements P, Mn, Fe, Al, and Ba generated the highest BCF values, although Al and Ba were found in low concentrations in the phytoplankton from the Mocajuba estuary.

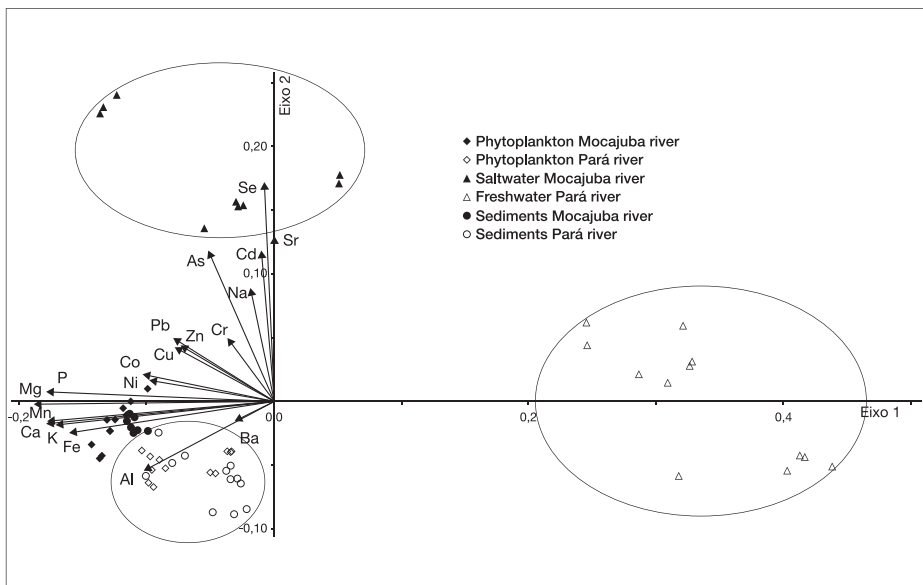


Figure 4. Principal coordinates analysis of the chemical elements in the Mocajuba and Pará Rivers (sediments, surface water and phytoplankton)

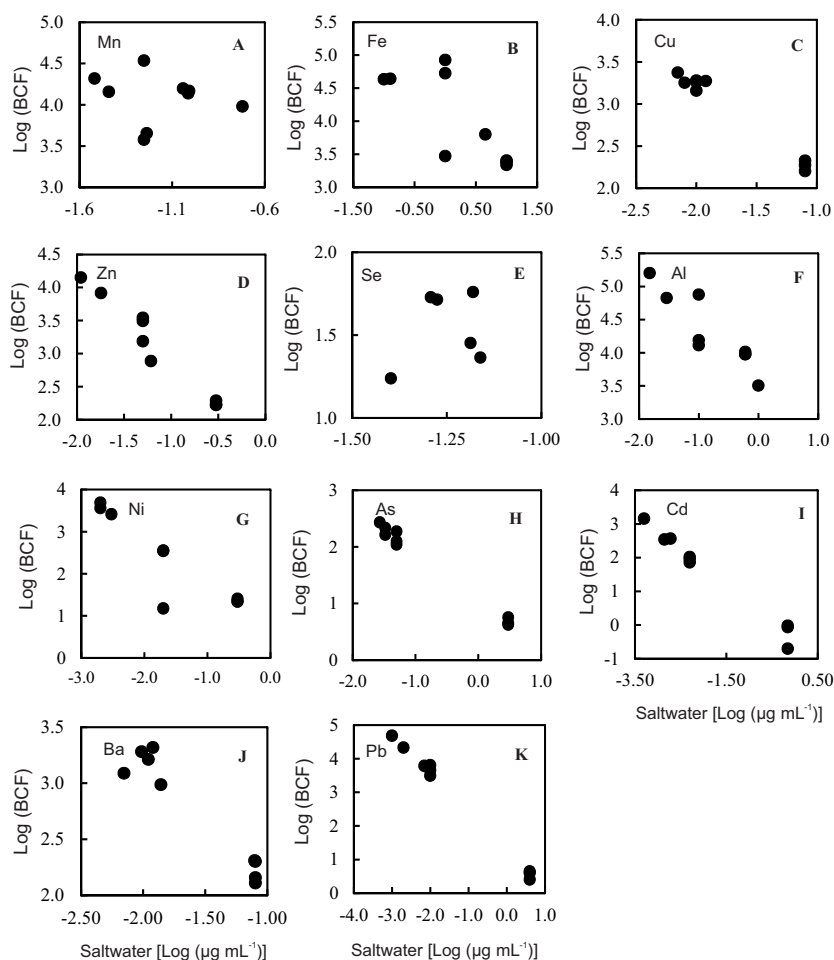


Figure 5. Correlations between the bioconcentration factor (BCF) of the phytoplankton and the trace elements ($\mu\text{g mL}^{-1}$) in the waters of the Mocajuba estuary

Table 5. Principal coordinates analysis (bold values are significant correlations)

Variable	Axis 1	Axis 2	Variable	Axis 1	Axis 2
Na	-0.01672	0.01727	Zn	-0.03195	0.01186
K	-0.07226	-0.1153	Se	-0.05086	0.11193
Mg	-0.06917	-0.00425	Sr	0.00523	0.03959
Ca	-0.06189	-0.00948	Al	-0.04152	-0.01451
P	-0.03699	0.00391	Ni	-0.06256	0.01250
Cr	-0.04924	0.02734	As	-0.06880	0.04987
Mn	-0.05842	-0.00850	Cd	-0.08319	0.13183
Fe	-0.03744	-0.00718	Ba	-0.01739	-0.00587
Co	-0.09531	0.02109	Pb	-0.07662	0.02909
Cu	-0.04350	0.01534			

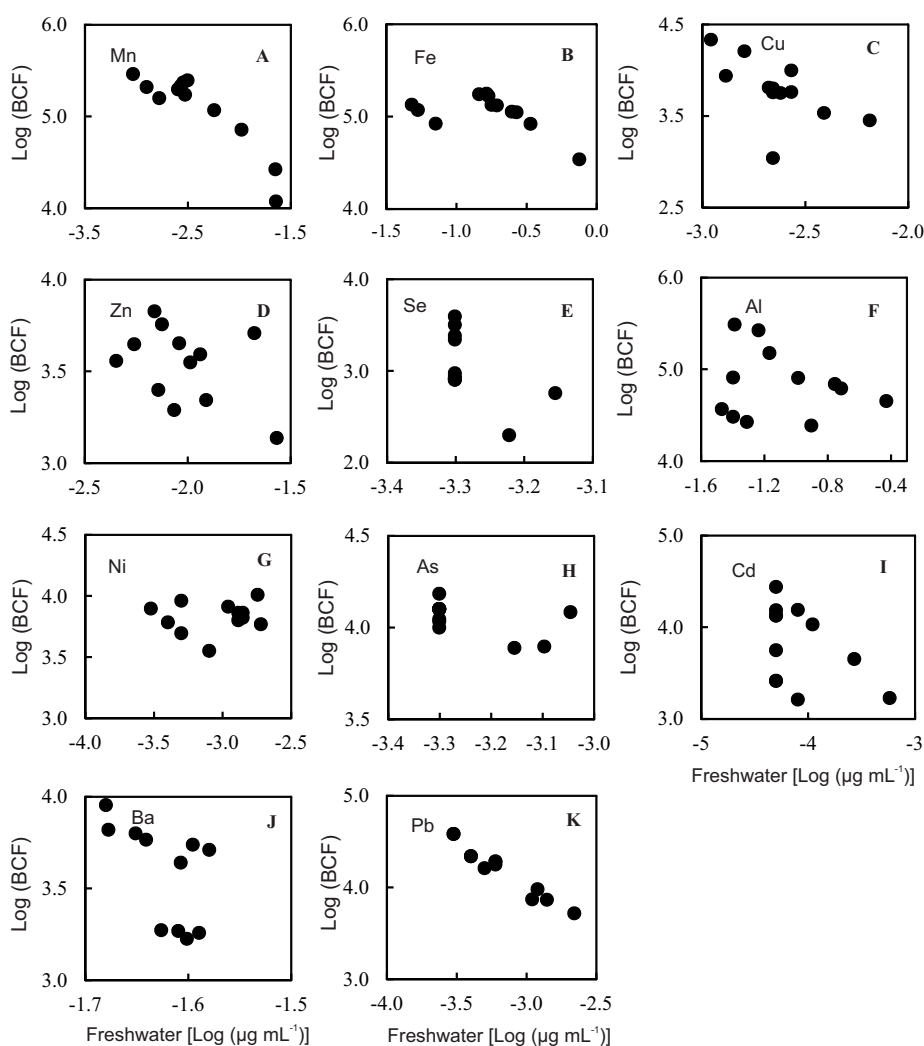


Figure 6. Correlations between the bioconcentration factor (BCF) of the phytoplankton and the trace elements ($\mu\text{g mL}^{-1}$) in the waters of the Pará estuary

Table 6. Regression analyses of the concentrations of chemical elements log BCF values and the chemical concentrations of the water ($\mu\text{g mL}^{-1}$) and the minimum, maximum and mean BCF values for the Pará and Mocajuba estuaries (BCF denotes bioconcentration factor). FW (denotes freshwater); SW (denotes saltwater); n=12 (phytoplankton Pará River), n=10 (phytoplankton Mocajuba River); r^2 , coefficient determination; r^2 to the level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

	Water type	Regression coefficients for log-linear relationship between BCF water conc. (log:log scale).				Empirical BCFs		
		Slope	Intercept	R ²	p-Valor	Minimum	Mean	Máximo
K	FW	2.925	-0.194	0.004	0.82 ns	366	847	1,297
	SW	3.428	-1.050	0.79	0.001***	10	17.04	30
Mg	FW	2.551	-0.146	0.003	0.85 ns	318	699	1,107
	SW	5.338	-1.489	0.65	0.008 **	2	6	16
Ca	FW	3.015	-0.326	0.04	0.516 ns	126	479	1,786
	SW	2.704	-0.938	0.08	0.45	17	31	88
P	FW	3.156	-0.761	0.43	0.01 *	6,217	16,2527	289,818
	SW	3.321	-0.943	0.98	>0.001***	521	3,488	11,712
Mn	FW	3.00	-0.864	0.82	>0.001***	11,843	162,527	289,818
	SW	3.893	-0.161	0.017	0.73 ns	3,785	14,618	34,230
Fe	FW	4.818	-0.301	0.27	0.07	34,493	121,908	177,108
	SW	4.168	-0.669	0.59	0.01 *	2,189	26,814	84,750
Cu	FW	1.120	-1.009	0.36	0.03 *	1,097	7,802	21,581
	SW	1.115	-1.058	0.98	>0.001***	159	1,306	2,361
Zn	FW	2.774	-0.378	0.17	0.183	1,369	3,796	6,718
	SW	1.533	-1.358	0.95	0.003 ***	166	3,538	14,187
Se	FW	-10.84	-4.229	0.28	0.07	199	1,460	3,921
	SW	0.070	-0.172	0.94	>0.001***	0.18	26	58
Al	FW	4.623	-0.195	0.03	0.581 ns	24,392	98,156	306,788
	SW	3.706	-0.75	0.80	0.001**	3,503	40,390	159,660
Ni	FW	4.000	0.57	0.01	0.69 ns	3,552	6,948	10,224
	SW	0.685	-1.018	0.80	>0.001***	15	1,318	4,854
As	FW	2.461	-0.489	0.27	0.07	8,327	10,455	18,220
	SW	1.070	-0.827	0.98	>0.001***	4	121	271
Cd	FW	1.619	-0.536	0.19	0.151 ns	7,756	11,497	15,272
	SW	-0.439	-1.063	0.97	>0.001***	0.39	269	1,442
Ba	FW	1.085	-975	0.46	0.01 *	1,326	4,273	8,999
	SW	1.075	-1.055	0.92	>0.001***	129	944	2,082
Pb	FW	1.085	-0.975	0.94	>0.001***	5,223	18,524	38,300
	SW	1.268	-1.162	0.99	>0.001***	2.6	10,000	48,338

Significant inverse relationships ($p < 0.005$) were found between the BCF values and the concentrations of most chemicals in the waters of the Mocajuba estuary (Table 6), except for Mn (Figure 5). In the Pará estuary, significant inverse relationships ($p < 0.05$) were obtained only for Mn, Cu, Ba, and Pb (Figure 6). Previous studies of a number of

different organisms (Kenaga 1980; McGeer *et al.* 2003) have also recorded an inverse relationship between the BCF and the chemical composition of the water.

The bioconcentration factors - obtained for the sediments (BSCF) by dividing the total sample by the bio-available fraction - were high for all the trace elements in both estuaries.

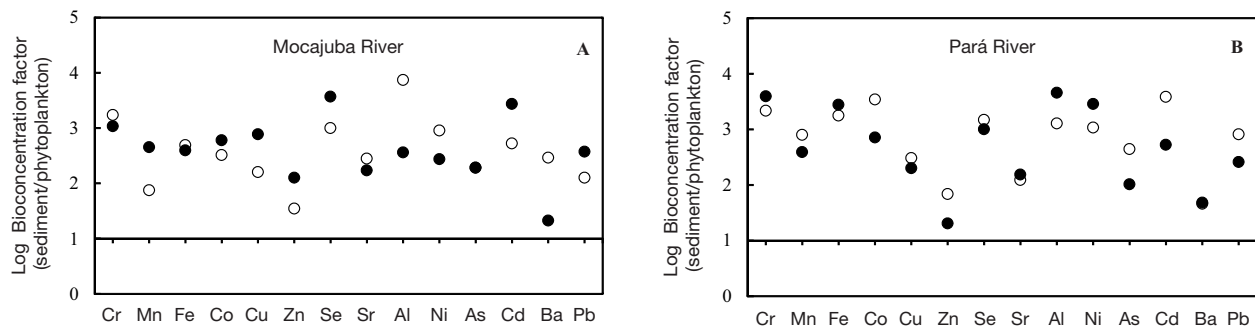


Figure 7. Bioaccumulation factors (total sediment/bioavailable fraction) for the bottom sediments (O) and the phytoplankton (●) (phytoplankton/bioavailable fraction)

The variation in Al and Ba was highest in the Mocajuba estuary, and variation in Cd was highest in the Pará estuary (Figure 7). The phytoplankton:bioavailable fraction ratio was highest for Se and Cd in the Mocajuba estuary, and for Cr, Fe, Al, and Ni in the Pará estuary. Similar relationships were observed between Cr, Fe, Co, Cu, Se, and Sr and the sediment ratios and the phytoplankton:bioavailable ratio, with some degree of variation. It seems likely that these elements were absorbed in more or less the same proportions. However, the phytoplankton in the Mocajuba estuary concentrated Al, Ni, and Ba at low rates, and Mn, Cu, Zn, Se, Cd, and Pb in excess.

DISCUSSION

The high concentrations of Na, K, Ca, Mg, Fe, Zn, Al, Ba, and Pb in the chemical composition of the phytoplankton collected from the two estuaries probably reflects the availability of these elements in the water and the sediments, in addition to the influence of oceanic waters. Fe and Al, the most concentrated elements in the Pará estuary, are strong indicators of the contribution of matter derived from the soil around the margins of the estuary, which are dominated by sediments rich in iron oxy-hydroxides (goethite and hematite) as well as aluminosilicates. The high levels of AL may also be influenced by aluminum of anthropogenic origin.

These elements are generally concentrated at high levels in the phytoplankton in comparison to the results of previous studies (Admiral *et al.* 1995; Fitzwater *et al.* 2000; Bu-Olayana *et al.* 2001), with the exception of As and Pb. Our results are similar to those of Ho *et al.* (2003) and Martins and Knauer (1973) for phytoplankton from natural environments. The occurrence of *Aulacoseira granulata* is associated with eutrophized environments, and *Aulacoseira* is one of the 60 genera most tolerant of organic or industrial pollution (Palmer 1969; Reynolds 1998). However, while this species predominates in the Pará estuary, the high concentrations of $H_4SiO_4 > NO_3^- > PO_4^{3-}$ in the water reflect a lack of major alterations of the environment.

The strong association observed between the chemical composition of the phytoplankton and the water and sediments of the Pará estuary may be related to the adsorption of metals by the iron oxy-hydroxides precipitated on the surface of the cells of the phytoplankton, particularly diatoms (Sunda and Huntsman 1995; 2000). In the Mocajuba estuary, the biogeochemical processes of oxide-reduction that control the environment (Sunda and Huntsman 1998) are probably responsible for the low correlation found between the chemistry of the phytoplankton and that of the water. The significant association found with the chemical composition of the sediments may be related to the precipitation of sulfides on the surface of the phytoplankton, which adsorb metals, in particular Fe (Maldonado and Price 1999).

The adsorption process probably resulted in the overestimation of the concentrations of a number of trace metals, in addition to Fe (Takeda 1998; Haritonidis and Malea 1999; Hutchins *et al.* 1999). The adsorption mechanism may account for the enrichment of Mn, Co, Zn, As, Cd, and Pb recorded in the phytoplankton from the Mocajuba estuary, and that of Mg, Fe, and Al in the Pará.

There was a strong relationship between the high levels of Mn, Fe, Cu, Zn, Se, Sr, Cd, and Pb found in the phytoplankton from the Mocajuba and the bioavailable fraction, with a similar pattern being found in the Pará for Cr, Fe, Al, and Ni. The interactions among the chemical composition of the sediment, the bioavailable fraction, and the water play an essential role in phytoplankton productivity (Valenta *et al.* 1986; Jensen *et al.* 1982; Wang and Dei 2001).

The multivariate analysis confirmed that the waters of the Mocajuba and Pará estuaries constitute two chemically distinct environments, with a clear separation of the chemical composition of the sediments and phytoplankton. The phytoplankton and sediments had the greatest chemical affinity, in particular with respect to Al, which was strongly correlated with the sediment and phytoplankton samples from the Pará estuary. Aluminum is transported by physical or chemical processes and is rapidly absorbed by phytoplankton

due to its large surface area and volume, reaching extremely high concentration factors (Fisher and Reinfelder 1995). It does not appear, however, that the high levels of Al present in the phytoplankton of the Pará estuary are derived from the sediments because of the low levels of clay minerals (< 7%) in this region. In this case, the high Al concentrations are probably associated with the discharge of domestic effluents and industrial waste into the estuary.

The high BCF values recorded for the elements Mn, Fe, Al, Ni, As, Cd, and Pb emphasize the capacity of the phytoplankton to bioaccumulate metals (Martin and Knauer 1973). The adsorption of metals is well understood in phytoplankton (French and Evans 1988; Franklin *et al.* 2002), and has been demonstrated in a number of studies (Moreno-Garrido *et al.* 2000; Nayar *et al.* 2004; Tien 2004). Diatoms, in particular, may be tolerant of heavy metals (Seidl *et al.* 1998; French and Evans 1988), especially copper, an excess of which may cause teratological formations in the frustule, in addition to a reduction in the production of phytoplankton and chlorophyll (Balczon and Pratt 1994; Viana and Rocha 2005). High Al concentrations inhibit the adsorption and physiological processing of phosphorus in the phytoplankton (Barabasz *et al.* 2002).

The observed BCF values are higher than those recorded in diatoms for Zn (Harvey and Patrick 1967), and those recorded in green algae for Pb, Cd, Cr, and Zn (Becker 1983). Three significant inverse relationships ($P < 0.005$) were found between the BCF and the chemical composition of the waters of the Mocajuba and Pará estuaries, indicating that the phytoplankton is capable of removing and detoxifying these elements in order to maintain low intracellular levels even when the environmental concentrations are high (Wood and Wang 1983). Similar results were obtained by Sigee *et al.* (1988) in a comparison of BCF and the chemical composition of the water. The lack of an inverse relationship between Ni and Al, and the reduced relationships recorded for most other elements in the Pará estuary (Figure 6) may reflect the capacity of phytoplankton to bioaccumulate and store these elements at a rate proportional to their adsorption (Trollope and Evans 1976).

The BSCF values recorded for Al and Ba indicate that these elements are poorly assimilated by the phytoplankton of the Mocajuba estuary (Figure 7A). However, even low BCF and BSCF values indicate that the phytoplankton has an enhanced capacity for the accumulation of bioavailable elements, and may therefore be good indicators of contamination (Franklin *et al.* 2002).

CONCLUSIONS

The phytoplankton of the study areas concentrate high levels of Ca, P, Mn, Fe, Zn, Al, and Ba, reflecting both

the natural characteristics of the environment and the anthropogenic inputs. In particular, the high Al levels found in the phytoplankton of the more polluted Pará estuary. The high concentrations of the chemical elements, and the high bioconcentration values (BCF and BSCF) between the phytoplankton, water, and the bioavailable fraction indicate that the phytoplankton have an enormous capacity for the accumulation of trace metals, and are thus potentially excellent bioindicators for the evaluation of the contamination of Amazonian ecosystems by metals.

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