AMAZONIANA XVII (3/4): 513-523 Kiel, Dezember 2003

DOC photo-oxidation in clear water Amazonian aquatic ecosystems

by

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(Accepted for publication: October, 2002).

Abstract

This investigation evaluated dissolved inorganic carbon (DIC) photo-production and its correlation with dissolved organic carbon (DOC) concentration in several Amazonian clear-water ecosystems, and determined the changes in bulk DOC affected by solar radiation. The study was performed with samples from eight Amazonian clear-water aquatic ecosystems in the State of Pará, Brazil. We filtered four replicate samples through a 0.2-µm membrane and exposed them to sunlight in quartz tubes, with an equal number of dark controls, for six hours (from 9:00 to 15:00). After incubation, we measured DIC photo-production and changes in water colour (430 nm) and the absorbance ratio (250/365 nm). There was a weak significant ($p < 0.05$) correlation between DIC photo-production and DOC concentrations considering all data set. However, we observed a strong correlation between DIC photo-production and DOC concentrations, considering separately ecosystems with similar conditions of sunlight exposure, *e.g.*, separating large rivers and lakes from small streams (igarapés). We concluded that, besides the concentration and quality, the origin and the history of DOC in clear-water Amazonian ecosystems determine the rate of DOC photo-oxidation.

Keywords: **DOC, DIC, photo-oxidation, Amazonian aquatic ecosystems, sunlight, clear water.**

Resumo

Os objetivos do presente estudo foram avaliar a foto-produção de carbono inorgânico dissolvido (CID) em alguns ecosistemas aquáticos amazônicos de águas claras, correlacionar a fotoprodução de CID com as concentrações de carbono orgânico dissolvido (COD) e avaliar as mudanças nas moléculas de COD em função da ação da radiação solar. Essa pesquisa foi realizada em oito ecossistemas aquáticos amazônicos de águas claras no Estado do Pará (Brasil). As amostras filtradas em membrana 0,2 µm foram expostas à luz solar em tubos de quartzo (quatro réplicas com controles no escuro) durante seis horas. Após a incubação foram medidas a fotoprodução de CID, perda de coloração e alterações na razão de tamanho das moléculas de COD. Foi encontrada fraca correlação significativa ($p < 0{,}05$) entre a foto-produção de CID e as concentrações de COD considerando todos os resultados. Contudo, observa-se uma forte correlação entre os mesmos parâmetros quando considerados apenas ambientes com condições similares de exposição ao sol (separando rio e os lagos, dos igarapés). Concluímos que além da concentração e da qualidade, a origem, e o histórico do COD em ecossistemas aquáticos amazônicos de águas claras, determinam as taxas

ISSN 0065-6755/2003/513/ © MPI für Limnologie, AG Tropenökologie, Plön; INPA, Manaus

de fotooxidação.

Introduction

Human activities have led to depletion of ozone in the stratosphere, which has resulted in an increase in ultraviolet radiation reaching the earth's surface. Many recent studies have demonstrated the consequences for climate, ecosystems, and biota (ERICKSSON et al. 2000; WÄNGBERG et al. 2001). Solar radiation (Photosynthetic Active Radiation, PAR, plus Ultraviolet Radiation, UV-A and UV-B) directly affects the decomposition of dissolved organic matter (DOM) in aquatic ecosystems (MORAN & ZEPP 1997). Its high energy promotes the chemical decomposition of carbon macromolecules (e.g., humic acids) into low-molecular-weight compounds (LMW; acetaldehyde, citrate, acetate, formaldehyde, pyruvate, etc.; MORAN & ZEPP 1997), oxidation to dissolved inorganic carbon (DIC, e.g., CO and CO_2; MORAN & ZEPP 1997), and hydrogen peroxide (SCULLY et al. 1995), among others. This phenomenon is termed photo-oxidation or photo-degradation of dissolved organic carbon (DOC).

The photo-oxidation process has been recognized as an important pathway for carbon cycling, because of its effects both on bacterial growth substrates (MORAN & ZEPP 1997; FARJALLA et al. 2001), and on mineralization of dissolved organic matter (DOM) in surface waters (LINDELL et al. 1996; GRANÉLI et al. 1998). Because the earth is more than 70 % covered by water and research has shown an increase in the CO_2 concentration of the atmosphere (data from Mauna Loa Observatory, Hawaii; in: SHORT & NECKLES 1999), investigations of CO_2 formation via photo-oxidation of DOC are fundamental to understanding the greenhouse effect.

The Amazon floodplain is the largest floodplain on earth, containing 20 % of all available freshwater on the planet (ESTEVES 2000). SIOLI (1950) suggested classifying the many Amazonian rivers and lakes as clear-water, white-water, and black-water systems. Clear-water systems are poor in humic substances and in suspended matter, so their water is only slightly colored (JUNK 1997). The DOM leached from the tropical rain forest is usually the main source of DOC for Amazonian aquatic systems (JUNK 1985).

Despite the abundance of water bodies we could not find out any research focusing on photo-oxidation of DOC in the Amazonian aquatic ecosystems. In this way, the present investigation can be considered pioneer in this kind of approach in the Amazon region. The aims of the present research were: (1) to evaluate DIC production as a function of solar radiation incidence in water samples from several Amazon clear-water aquatic ecosystems having small differences in DOC content, (2) to correlate DIC production with initial DOC concentration, and (3) to determine the changes in bulk DOC from the action of solar radiation.

Study area

The research was performed in the Municipality of Oriximiná, State of Pará, Brazil (10°27'04"S; 56°22'17"W). The sampling area is located in the Trombetas River floodplain, specifically in the Lake Batata basin, on the left bank of the Amazon River (Fig. 1). The Lake Batata basin covers an area of about 271.6 Km^2, with a 72.0 Km perimeter, and includes approximately 87 channels or streams, locally termed igarapés (PANOSSO 2000).

One sample was collected in the main channel of the Trombetas River. This is a clear-water river, with relatively low concentrations of nutrients. Two other samples were collected in Batata and Mussurá lakes, which are located near the right and left banks respectively of the Trombetas River, and are connected with the river during flood season (Fig. 1). Samples were also collected in five clear-water igarapés (streams): Caranã, Quatorze, Madeireiro, Periquito, and Saracá. These streams differ in size and flow rate, but all are small clear-water rainforest streams.

Annual precipitation in the region varies between 1,800 and 3,000 mm/yr (CALLISTO et al. 1998). In the lakes and the Trombetas River, the water level can vary as much as eightfold, mainly as a result of the Amazon River flood pulse (ESTEVES 2000). In the Amazon region, four water level phases are recognized: filling (March), high water (July), drawdown (September), and low water (December). However, the water level in the igarapés responds to the frequency of local rainfall, rather than to this general pattern (ESTEVES et al. 1990).

Sampling and experimental setup

Samples were collected during the filling period (March, 2001), in polyethylene flasks previously washed with 10 % HCl and rinsed with deionized water. The samples were filtered through WHATMAN GF/C glass fiber filter and stored at 4 °C to reduce bacterial activity until the experiments were set up (~3 days). In the Limnology Laboratory at Rio de Janeiro, the samples were filtered through 0.2 μm membranes to exclude bacteria, and divided among 8 quartz tubes of which 4 were wrapped with aluminum foil to block the light (controls). The tubes were previously washed with 10 % HCl, rinsed with deionized water and then milli-Q water, and sterilized by autoclaving for 30 minutes (120 °C, 1 atm).

All tubes were exposed to the sunlight for about six hours in midday (from 9:00 to 15:00). During the exposure, the tubes were immersed in water in small plastic pools, with running water to maintain constant temperature (about 30 °C), in full sunlight. PAR (400–700 nm) radiation was measured hourly with a radiometer (LI-COR : LI-185b; GRANÉLI et al. 1998).

After the 6-hour incubation, the mineralization of dissolved organic carbon (DOC) by photo-oxidation in the samples was measured by the increase in inorganic carbon (DIC), in at least three injections (sd <2 %), with a SHIMADZU 5000A TOC Analyzer. The photo-oxidation rates were calculated by the difference in DIC between the light-exposed and the dark samples (GRANÉLI et al. 1998). Initial measurements of the same parameters were made and compared to the control, to verify possible changes in those results.

Absorbance at 250, 365, and 430 nm was measured with a BECKMAN DU-520 spectrophotometer and quartz cubets (1 cm wide). Photo-bleaching (loss of water colour) of the samples was calculated from the difference in water colour between exposed and dark samples. Changes in the size of molecules were estimated by the difference in the ratio of 250/365 nm between dark and light-exposed samples (GRANÉLI et al. 1998; FARJALLA et al. 2001).

The results for the control and exposed samples were compared by the KRUSKAL-WALLIS non-parametric statistical methods, with DUNN's post-hoc test. The correlation between DIC photo-production and DOC content was tested by PEARSON's test.

Results

The samples were subject to sunlight intensity varying between 1,000 and 1,350 $\mu E.m^{-2}.s^{-1}$ during the three incubation periods. Because the samples were exposed at the same time of year (between March 21st and April 6th, 2001), the light intensities were similar (Fig. 2).

DOC concentrations in clear-water Amazonian ecosystems varied between 2.69 mg l^{-1} in Igarapé Periquito and 6.17 mg l^{-1} in Igarapé Caranã. The water color (430 nm) ranged from 0.005 in Lake Batata to 0.024 in Igarapé Caranã. The molecular size ratio ranged from 4.329 to 5.746, being highest in Batata and Mussurá lakes and the Trombetas River (Table 1).

There was no DIC production in the dark tubes, i.e., there was neither thermal influence nor bacterial activity. Abiotic production of DIC, via photo-oxidation, occurred in all the light-exposed samples ($p < 0.05$) (Table 2). Water from Igarapé Caranã showed the highest DIC production (1.67 mg l^{-1}), and Lake Batata the lowest (0.23 mg l^{-1}). There was significative ($p < 0.05$) correlation between DOC concentration and DIC production ($r^2 = 0.70$; Fig. 3). GRANÉLI et al. (1998) also found significative correlation between the same parameters studing 5 temperate lakes and five tropical aquatic bodies.

Only Mussurá and Batata lakes and Igarapé Periquito showed significant photo-bleaching ($p < 0.05$) after the 6-hour incubation, but there was no clear pattern (Table 2). In all but Batata and Mussurá lakes, we observed significant ($p < 0.05$) increases in the proportion of small to large organic molecules (250/365 ratio). Igarapé Caranã, which showed the highest DIC production, also showed the most change in the proportion of molecular sizes (Table 2).

Despite the experiments set up have been ran in Rio de Janeiro, we believe the results would be similar if the experiment was ran in the Amazon region. We can only suppose that changes could occur in a narrower scale because of less sunlight incidence in Rio de Janeiro.

Discussion

Photo-oxidation is the action of sunlight in cleaving bulk DOC into smaller DOC molecules and DIC. Increases in UV-B radiation that achieves the Earth's surface, which have been recorded by some authors (MANDRONICH et al. 1995; ORCE & HELBLING 1997), may lead to increases of emission of CO_2 to atmosphere, contributing to the greenhouse effect, and in profound changes in the carbon utilization by bacterioplankton.

An increase in DIC in freshwater aquatic ecosystems, as a measurement of photo-oxidation, has been recorded both in clear-water and humic lakes (SALONEN & VÄHÄTALO 1994; GRANÉLI et al. 1998). We also found that the method of measuring photo-oxidation from increases in DIC concentrations was efficient in Amazonian clear-water ecosystems, since we could detect these changes in all the exposed samples.

Amazonian clear-water ecosystems showed higher photo-oxidation rates compared to other waterbodies submitted to similar light exposures (Table 2 and 3; data from GRANÉLI et al. 1998). While Lake Klintsjön (DOC = 2.90 mg l^{-1}) had approximately 0.10 mg l^{-1} of photo-produced DIC, Lake Batata (DOC = 3.01 mg l^{-1}) had 0.23 mg l^{-1} of photo-produced DIC. While Lake Straken (DOC = 9.00 mg l^{-1}) had approximately 0.20 mg l^{-1} of photo-produced DIC, Igarapé Caranã (DOC = 6.17 mg l^{-1}) had 1.67 mg

l^{-1} of photo-produced DIC (Tables 1-3). The amount of photo-produced DIC in Igarapé Caranã is comparable only to the amount of photo-oxidized DIC in Comprida Lagoon, a very humic lagoon of Northern Rio de Janeiro (Table 3), although the percentage of photo-oxidized DOC in Igarapé Caranã was much higher (Table 2). We can also compare Igarapé Quatorze and Cabiúnas Lagoon; both showed the same DIC photo-production, however the DOC concentration in Cabiúnas Lagoon was approximately 5 times higher. The DOC in Cabiúnas Lagoon is more refractory, possibly because in the coastal lagoon, water retention time is higher and the bulk DOC mostly comes from leaching of humic substances in the restinga (coastal dune vegetation). Alternatively, the higher absorbance at 430 nm of the dark water of Cabiúnas Lagoon may attenuate the sunlight, reducing its action on the bulk DOC more than in Igarapé Quatorze (Table 2).

The highest photo-produced DIC, recorded in the sample from Igarapé Caranã, may be related to the high content of DOC in this system (Table 2). According to GRANÉLI et al. (1998), at higher DOC contents, proportionally more DIC is photo-produced. Therefore we would expect that Igarapé Periquito, which had the lowest DOC concentration, would have the least amount of photo-produced DIC. However, the minimum was actually recorded in the sample from Lake Batata (Table 2). This supports the hypothesis that the correlation between DIC production and DOC concentration (Fig. 3) is quite weak and that there probably has any other factor acting either.

Excluding the samples from larger ecosystems (Batata and Mussurá lakes and the Trombetas River) from the analysis, we found a strong significant correlation between DIC photo-production and DOC content in the igarapés ($r^2 = 0.96$; Fig. 4). The igarapés are less exposed to sunlight because they are narrower than the large lakes and rivers, and the forest canopy covers them almost completely. Furthermore, the organic matter in the forest soils is continually leached by equatorial rains into the small igarapés, so these bulk DOC molecules are less degraded and more sensitive to sunlight effects, showing higher rates of photo-oxidation. On the other hand, lakes Batata and Mussurá and the Trombetas River are fully exposed to sunlight, so their bulk DOC must have been previously photo-oxidized. We suggest the separation of those Amazonian clear-water ecosystems into two groups, lakes and large rivers (Trombetas River), and the small channels (igarapés). THOMAZ et al. (1998), studying bacterial activity and limnological parameters, proposed the same separation in some of these ecosystems. According to ROLAND & ESTEVES (1998), the euphotic zone in Lake Batata reaches a depth of 3.5 m, i.e., most of the water column is subjected to the effects of sunlight. Consequently the DOC molecules of these ecosystems are more refractory to the action of sunlight, which is reflected in the lower rates of photo-oxidation. THOMAZ et al. (1998) suggested that bulk DOC in the same ecosystems is also refractory to bacterial consumption. AMON & BENNER (1996) reported that LMW compounds that had been previously degraded is more refractory to bacterial consumption than the (less degraded) high molecular weight (HMW) compounds in Amazonian aquatic ecosystems. HONGVE (1994) noted that larger molecules are more easily photo-oxidized than smaller molecules. SALONEN & VÄHÄTALO (1994) found that hypolimnetic water has higher photo-oxidation rates than epilimnetic water in Lake Skjervatjern, because the hypolimnetic water in a stratified lake receives much less radiation than epilimnetic water and therefore contains more moleculres susceptible to photo-oxidation.

The ratio between absorvances at 250 nm and 365 nm express an estimation of proportion of large and small organic molecules. Increases in the 250/365 nm ratio indicates that the proportion of smaller to larger DOC molecules increased. In our study, the higher ratios (Table 1) founded to lakes Batata and Mussurá (Table 2) indicate that this bulk DOC is composed by a higher proportion of smaller molecules (and more refractory) when compared to the other studied ecosystems. FARJALLA et al. (2001) recorded that the ratio increased as the DOC was exposed to UV radiation and became more refractory to bacterial consumption. This fact and the lack of increase in this ratio seen in lakes Batata and Mussurá (Table 2), corroborate the hypothesis that these ecosystems have previously been photo-oxidized. The larger changes in 250/365 ratio to the other samples also corroborates the hypothesis that the canopy covered ecosystems have a higher quality bulk DOC different from lakes.

Conclusions

Measuring the photo-production of DIC is an efficient method for studying the photo-oxidation of DOC in clear-water Amazonian aquatic ecosystems. In these ecosystems, not only the DOC concentration, but also the quality and probably the history of exposure of the molecules (water retention time) determine DIC photo-production. These three parameters determine the fraction of DOC that can be photo-degraded, and the susceptibility of the bulk DOC to DIC photo-production.

Seasonal studies should be undertaken to better understand the photo-oxidation processes in clear-water Amazonian aquatic ecosystems. For instance, during the filling phase, there is input of dissolved organic matter (DOM) into the system; and during drawdown, the "old" DOM is probably pre-photo-oxidized.

Acknowledgments

We gratefully acknowledge financial support from the company Mineradora Rio do Norte, CNPq, and CAPES; Rodrigo S. Coutinho for drawing Figure 1, as well as João J. F. Leal and Marcos P.F. Barros for assistance with sampling.

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Table 1: Caracterization of studied ecosystems. Initial results of DOC, water color and proportion of smaller and larger molecules (250/365).

Samples	**DOC (mg l^{-1})**	**Watercolor (430 nm)**	**250/365 ratio**
Lake Batata	3.01	0.005	5.476
Lake Mussurá	4.20	0.013	5.326
Igarapé Caranã	6.17	0.024	4.329
Igarapé Quatorze	2.97	0.007	4.625
Igarapé Madeireiro	3.09	0.009	4.517
Igarapé Saracá	4.60	0.013	4.683
Igarapé Periquito	2.69	0.009	4.690
Trombetas river	4.68	0.012	5.073

Table 2: Photo-production of inorganic carbon, photo-bleaching and changes in molecular size (250/365) after six hour incubation. The asterisks (*) mark the significant changes ($p < 0.05$).

Samples	IC produced ($mg\ l^{-1}$)	DOC photo-oxidized (%)	photo-bleaching (430 nm)	Increase of 250/365 ratio
Lake Batata	0.23*	7.64	0.0010*	0.401
Lake Mussurá	0.60*	14.29	0.0003*	0.161
Igarapé Caranã	1.67*	27.07	0.0045*	3.862*
Igarapé Quatorze	0.48*	16.16	0.0035*	2.042*
Igarapé Madeireiro	0.52*	16.83	0.0035*	1.511*
Igarapé Saracá	1.16*	25.22	0.0053*	1.210*
Igarapé Periquito	0.60*	22.30	0.0030*	4.671*
Trombetas river	0.54*	11.54	0.0015*	0.945*

Table 3: Photo-oxidation rates from different aquatic freshwater ecosystems. [1]Data extracted from GRANÉLI et al. 1998 (five Sweden lakes, one north Brazilian river and four southeast coastal lagoons). [2]Data extracted from SALONEN & VÄHÄTALO 1994 (lake from Finland).

Samples	IC photo-produced ($mg\ l^{-1}$)	DOC ($mg\ l^{-1}$)	DOC photo-oxidized (%)	Water color (430 nm)
Lake Klintsjön[1]	~ 0.10	2.90	3.45	0.001
Lake Fiolen[1]	~ 0.10	6.00	1.67	0.005
Lake Straken[1]	~ 0.20	9.00	2.22	0.018
Lake Skärshultsjön[1]	~ 0.40	13.10	3.05	0.039
Lake Lindhultsgöl[1]	~ 0.30	15.30	1.96	0.063
Negro river[1]	~ 0.70	9.90	7.07	0.049
Carapebus lagoon[1]	~ 0.60	16.80	3.57	0.024
Cabiúnas lagoon[1]	~ 0.40	15.50	2.58	0.092
Coca Cola lagoon[1]	~ 0.90	27.00	3.33	0.213
Comprida lagoon[1]	~ 1.70	41.80	4.07	0.223
Lake Skjervatjern[2]	~ 0.44	6.20	7.10	

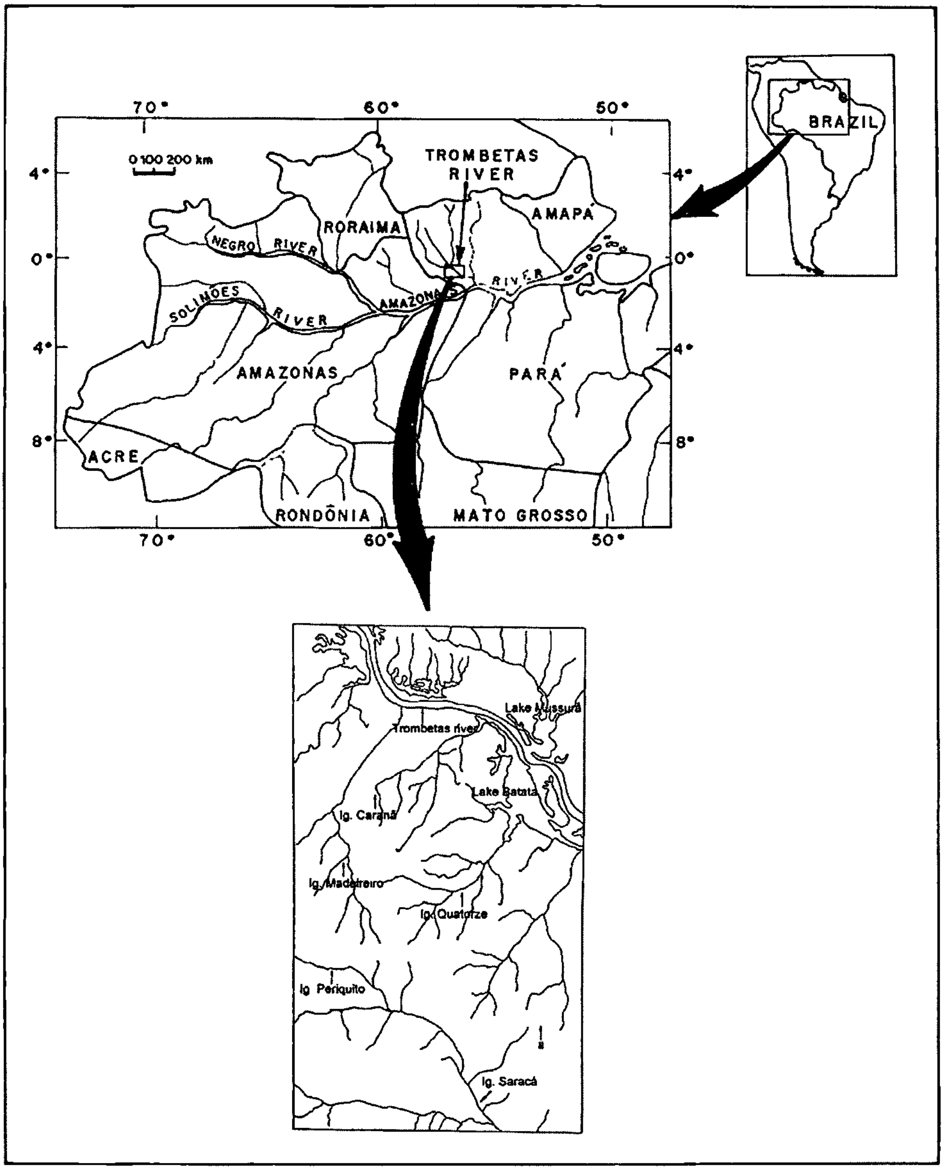

Fig. 1:
Sampling site localization. Municipality of Oriximiná, Pará State - Brazil. The ecosystems names are placed in the map.

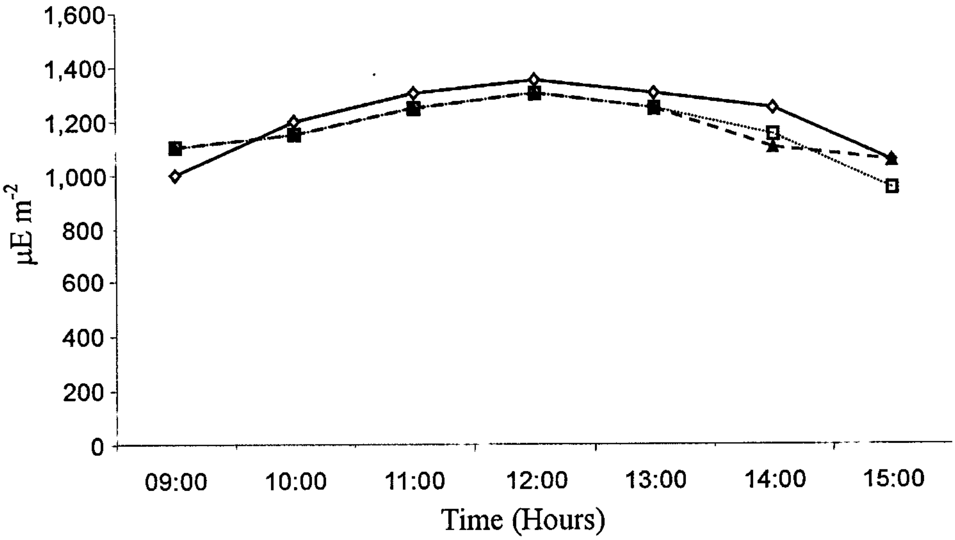

Fig. 2:
Sunlight intensity recorded during the March 21st incubation (rhombus), March 23rd incubation (square) and April 6th incubation (triangle).

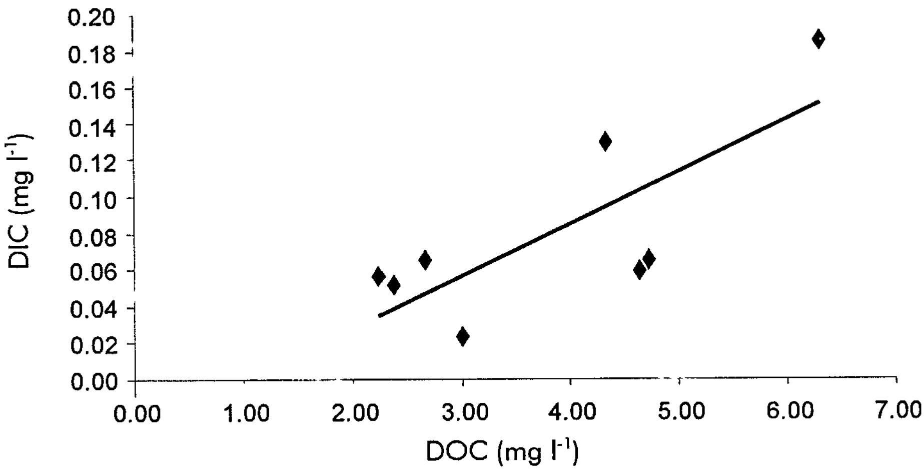

Fig. 3:
Pearsons Correlation between DOC contents and IC producted by light unit and time from the eight studied ecosystems ($r^2 = 0.70$; $p < 0.05$).

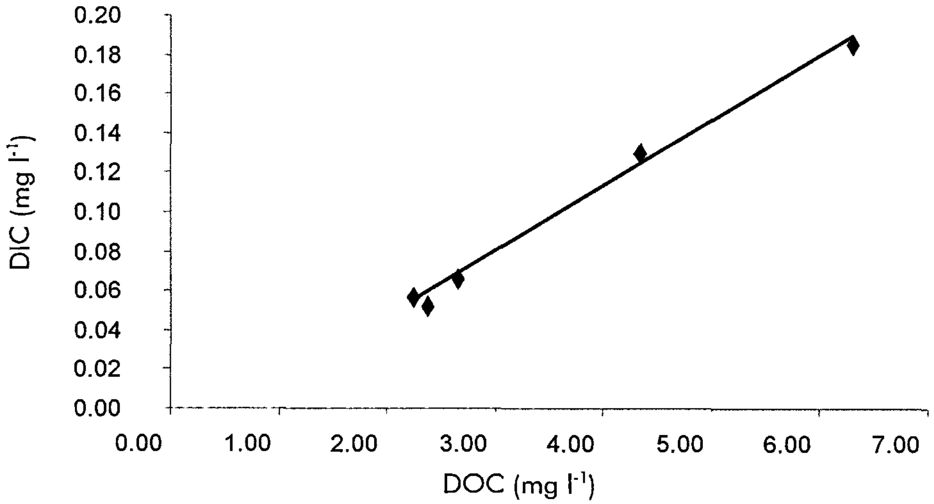

Fig. 4:
Pearsons Correlation between DOC contents and IC producted by light unit and time from the five igarapés studied ($r^2 = 0.96$; $p < 0.05$).