Mercury methylation and bacterial activity associated to tropical phytoplankton

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Abstract

The methylated form of mercury (Hg), methylmercury (MeHg), is one of the most toxic pollutants. Biotic and/or abiotic methylation, often associated to sulfate-reducing bacteria metabolism, occurs in aquatic environments and in many tropical areas, mostly in the periphyton associated to floating macrophyte roots. Data about mercury methylation by phytoplankton are scarce and the aim of this study was to verify the biotic influence in the methylation process in Microcystis aeruginosa and Sinececcystis sp. laboratory strains and in natural populations of phytoplankton from two different aquatic systems, the mesotrophic Ribeirão das Lajes reservoir and hypereutrophic oligohaline Jacarepaguá lagoon, Rio de Janeiro state, Brazil. Adapted radiochemical techniques were used to measure sulfate-reduction, mercury methylation and bacterial activity in phytoplankton samples. Methyl-²⁰³Hg formation from added inorganic ²⁰³Hg and ³H-Leucine uptake were measured by liquid scintillation as well as sulfate-reduction, estimated as H₂¹⁵S produced from added Na₂¹⁵SO₄. There was no significant difference in low methylation potentials (0.37%) among the two cyanobacterium species studied in laboratory conditions. At Ribeirão das Lajes reservoir, there was no significant difference in methylation, bacterial activity and sulfate-reduction of surface sediment between the sampling points. Methylation in sediments (3–4%) was higher than in phytoplankton (1.5%), the opposite being true for bacterial activity (sediment mean 6.6 against 150.3 nmol gdw⁻¹ h⁻¹ for phytoplankton samples). At Jacarepaguá lagoon, an expressive bacterial activity (477.1 × 10³ nmol gdw⁻¹ h⁻¹ at a concentration of 1000 nM leucine) and sulfate-reduction (~21% H₂¹⁵S trapped) associated to phytoplankton (mostly cyanobacteria M. aeruginosa) was observed, but mercury methylation was not detected.

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1. Introduction

Mercury (Hg) is one of the most toxic elements and its methylated form, methylmercury (MeHg) despite its low concentration in the environment, is highly neurotoxic to humans (Gilbert and Grantwebster, 1995). The methylation of inorganic mercury occurs by abiotic and biotic processes. The more studied abiotic pathway involves the transfer of a methyl radical by methylcobalamine (Craig, 1986). In the biotic process, mercury methylation occurs by microbiological activity, where Hg\(^{2+}\) reacts with a functional enzyme system. In the environment, the biotic and abiotic routes are difficult to distinguish because some methyl donors are products of biological processes (Kelly et al., 1995). The biotic methylation of mercury seems to be more intense than the abiotic one in freshwater and estuarine ecosystems (Craig, 1986; Compeau and Bartha, 1985; Gilmour and Henry, 1991).

In temperate locations, the sulfate-reducing bacteria (SRB) were shown to be the most important microorganisms in the formation of methylmercury (MeHg), and a wide variety of SRB species are able to methylate Hg in laboratory cultures and sediments (Compeau and Bartha, 1985; Devereux et al., 1996; Benoit et al., 1999, 2001; King et al., 2000, 2001, 2002). It was observed that different phylogenetic groups of SRB methylate mercury at different rates (King et al., 2000) and that a quantitative relationship exists between mercury methylation and the community composition and activity of SRB in marine sediments (King et al., 2001).

In tropical freshwater environments, the periphyton associated to macrophyte roots presents the highest net mercury methylation potentials (Guimarães et al., 1998, 2000a,b; Mauro et al., 1999, 2000, 2001a,b, 2002; Lemos, 2001). Some authors (Guimarães et al., 1998, 2005; Mauro et al., 2000) showed that MeHg formation in the roots of freshwater macrophytes was stimulated by Na\(_2\)SO\(_4\) and inhibited by molybdate addition, suggesting an important role for SRB in Hg methylation. In floodplain lakes of the Beni river, Bolivian Amazon, Acha et al. (2004) found a diverse SRB community associated to the roots of different floating macrophytes including groups such as Desulfotomaculum, Desulfobulbus, Desulfovibrio, Desulfo bacterium, Desulfo bacter and Desul fococcus. Besides sulfate-reducing bacteria, a variety of others microorganisms are able to methylate Hg, including aerobic bacteria, fungi and seaweeds (Kelly et al., 1995), possibly acting as a Hg detoxification process, since MeHg is easily transferred along trophic chains (Craig, 1986). Hg methylation in the water column is usually low in tropical aquatic systems (Guimarães et al., 1998) and has not been studied in phytoplankton yet, despite the evidence that marine cyanobacteria produce Hg\(^0\) as a way of mercury detoxification (Mason, 2000). An association of bacteria with cyanobacteria mucilage is common (Paerl, 1982; Brunberg, 1999) including sulfate-reducing bacteria (Krekeler et al., 1998).

The traditional view of the role of bacteria in plankton communities has changed as new techniques revealed that heterotrophic bacteria in the plankton were much more abundant and productive than previously thought (Rothhaupt, 2000). Moreover, it became clear that bacteria are often not remineralizers of mineral nutrients but, rather compete with phytoplankton for the uptake of dissolved nutrients (Rothhaupt and Guède, 1992). Gilmour et al. (1990) presented evidences against incorporation of exogenous thymidine by sulfate-reducing bacteria while data about leucine incorporation by SRB are scarce.

Some authors showed that attached bacteria are metabolically more active than free-living bacteria (Kirchman and Mitchell, 1982; Pedrós-Alio and Brock, 1983; Worm and Sondergaard, 1998; Kamjunke and Mehner, 2001). Attached bacteria can directly take up exudates released by phytoplankton and these are rapidly used by bacteria associated to Microcystis (Sundh, 1992). In contrast, many free-living small bacteria are inactive and in a dormant-like state (Jürgens and Güde, 1994).

The attachment of bacteria (including methylators such as SRB) to phytoplankton is also relevant in view of the capacity of the latter to accumulate Hg. Palermo (2002) found low THg concentration in phytoplankton (66 ng/g) at Ribeirão das Lajes reservoir, one of the sites studied in the present work, and MeHg was 11% of THg. In contrast, in floodplain lakes of the Tapajós River, Brazilian Amazon, Pacheco-Peleja (2002) found high Hg concentrations in the plankton of lakes of the Negro river (452 ng/g in <40 µm plankton and 242 ng/g in >40 µm plankton) and of the Tapajós river (264 ng/g in <40 µm plankton and
150 ng/g in >40 μm plankton). At the same Tapajós river lakes studied by the latter author, Mauro et al. (2001b) reported no net MeHg formation in phytoplankton or suspended particulate material samples but a 0.76% methylation was found in floating filamentous green algae.

The diversity of microorganisms on living aquatic substrates such as macrophytes and phytoplankton, the complexity of their physiological interactions and their role in the cycle of many elements including Hg is becoming increasingly evident. Planas et al. (2004) and Guimaraes et al. (2005) showed respectively in boreal epilithon and epiphyton and in tropical macrophyte periphyton that Hg methylation was reduced not only when inhibiting SRB but also other physiological groups.

Craig and Moreton (1985) concluded that methylmercury production is limited by the supply of mercury available for methylation rather than by the rate of the methylation reaction and Regnell and Tunlid, 1991 found that both factors were dependent on microbial activity Mauro et al. (2002) affirmed that tropical and equatorial conditions favor continuous mercury methylation, because temperatures are constantly high, allowing a higher bacterial activity.

The aim of this study was to verify if cultures of Microcystis aeruginosa and Sineccocystis sp. and natural populations of freshwater phytoplankton are able to methylate mercury and infer the influence of bacteria associated to cyanobacteria mucilage on mercury methylation through measurements of bacterial production (3H-leucine uptake) and sulfate reduction activity (Na₂³⁵SO₄ reduction).

2. Methodology

2.1. Laboratory cultures

*M. aeruginosa* and *Sineccocystis* sp. colonies were obtained from isolates from Broa Reservoir (São Carlos/SP/Brazil). The cyanobacteria were grown on 2% agar in a Petri plaque and the colonies were cultivated in ASM–1 medium (Gorham et al., 1964) and stored in 4 °C under light. To manipulate the population growth more nutritive solution was gradually added, at 25 °C and constant luminosity of 300 μE m⁻² s⁻¹. The incubations with ²⁰³HgCl₂ were done in the light and in the dark while the cyanobacteria population was in exponential or in plateau phase. The growth phase of the cyanobacteria cells was followed by counting under an optic microscopic using a Fuchs–Rosenthal lamina (Table 1).

2.2. Natural populations samples

The samples were obtained at two different sites in Rio de Janeiro state. The Ribeirão das Lajes reservoir supplies electric energy and potable water to some districts of Rio de Janeiro city and was sampled in summer (December/2001) by trawling 25 μm phytoplankton nets at the center of the reservoir during ten minutes. Bottom sediments were also sampled at four different points of the reservoir.

The other studied area, the hypereutrophic oligohaline Jacarepaguá lagoon, was sampled in autumn (April/2002) and winter (June/2002). This lagoon receives industrial and domestic effluents and presents frequent toxic cyanobacteria blooms (Magalhães and Azevedo, 1998). It is located in the South coast of Rio de Janeiro State, in the metropolitan zone of Rio de Janeiro City. It is a shallow lagoon (Zmean=1 m), connected with other two lagoons and with the Atlantic Ocean by means of a straight channel and has been investigated for the consequences of cyanobacterial blooms on its aquatic community (Ferrão-Filho et al., 2002a,b). Ferrão-Filho et al. (2002a) reported that microcystins in net phytoplankton ranged from 0.3 to 3.9 mg g⁻¹ dw from January to June 1997.

2.3. Mercury methylation assays

Methylmercury formation in phytoplankton and bottom sediments was assayed with the radiochemical

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Growth phase</th>
<th>Time (h)</th>
<th>Cells/mL</th>
</tr>
</thead>
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<tr>
<td>Light</td>
<td>Log</td>
<td>0</td>
<td>3.4 × 10⁷</td>
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<tr>
<td></td>
<td>Log</td>
<td>24</td>
<td>5.6 × 10⁷</td>
</tr>
<tr>
<td></td>
<td>Lag</td>
<td>0</td>
<td>8.6 × 10⁷</td>
</tr>
<tr>
<td>Dark</td>
<td>Log</td>
<td>0</td>
<td>1.5 × 10⁷</td>
</tr>
<tr>
<td></td>
<td>Log</td>
<td>24</td>
<td>4.3 × 10⁷</td>
</tr>
<tr>
<td></td>
<td>Log</td>
<td>48</td>
<td>2.0 × 10⁷</td>
</tr>
</tbody>
</table>
technique described by Furutani and Rudd (1980) and modified by Guimarães et al. (1995). Briefly, 20 mL samples of phytoplankton were incubated during 1 to 4 days with 3.3 KBq of $^{203}$HgCl$_2$ (equivalent to 35 ng of total Hg) obtained from Isotope Products Laboratories, USA, under constant shaking of 80 rpm in light and dark conditions. Bottom sediments from the Ribeirão das Lajes reservoir were incubated for 1 day only. Incubations were done in triplicate and included an acidified control. All incubations were done in the laboratory and started within 6 h after sampling. Methylation was stopped by addition of 1 mL of 4 N HCl. Me$_2$Hg was extracted, with 4 mL of 3M NaBr and 1 mL of CuSO$_4$ to form CH$_3$2HgBr$^+$, a compound with a high solubility in toluene (Horvat et al., 1990). The Me$_2$Hg from the sample supernatant was directly transferred to scintillation cocktail, 7 g L$^{-1}$ POP (2,5-diphenyloxazole) and 1 g L$^{-1}$ POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] by shaking for 15 min in glass separatory funnels and measured by liquid scintillation counting. Net mercury methylation was expressed as the proportion of Me$_2$Hg formed from the total $^{203}$Hg added for incubation. The specificity of this technique for MeHg was verified by thin-layer chromatography (Guimaraes et al., 1995) and the efficiency of the MeHg extraction procedure was 82% for sediment. Brito and Guimarães (1999) showed that for sediment and roots of aquatic macrophytes this technique performed as well as acid leaching followed by distillation (Horvat et al., 1993) or alkaline digestion followed by extraction in dithizone and TLC separation (Akagi and Nishimura, 1991).

2.4. Heterotrophic bacterial activity

The $^3$H-Leucine uptake method for determination of bacterial activity described by Kirchman et al., 1985; Simon and Azam, 1989 and Smith and Azam, 1992 was adapted to freshwater phytoplankton samples. Firstly, a pre-incubation was done by shaking bottles with 20 mL of natural phytoplankton (~0.02 g d w) for 1 to 2 days under light or dark conditions. Three drops of this phytoplankton and 3.3 KBq of $^3$H-Leucine purchased from Amersham (UK) were added to vials containing 5 mL of distilled water filtrated in 0.22 μm membranes. After 30 min of incubation of triplicate samples and a control with 0.5 mL of 37% formaldehyde, incubation was stopped with 0.5 mL of 37% formaldehyde. The extraction followed the filtration method using 50% TCA and the 0.22 μm membranes were measured by liquid scintillation to subsequently calculate leucine incorporation rate (mol. g d w$^{-1}$ h$^{-1}$). This rate was calculated considering DPM, with quenching curve, weight of the samples, leucine concentration and $^3$H-Leucine specific activity.

A leucine saturation curve was done with 10, 20, 50, 100 and 1.000 nM. Our experiments were done with 1.000 nM leucine, as used by Mauro et al. (2001a) and Coelho-Souza (2003) in experiments with periphyton of macrophyte roots. To verify how much of the bacterial activity was associated to phytoplankton cells, the culture tubes were left still for 4 h until cyanobacteria formed a mat at the surface of the culture and bacterial activity was measured before its condensation, on this surface mat and in unfiltered water taken at the bottom of the tubes.

Previous tests on the influence of added mercury on bacterial activity showed that the addition of 150 ng/g of unlabelled mercury caused no decrease in bacterial production in natural populations of cyanobacteria from Jacarepaguá lagoon (Coelho-Souza, 2003). The same was observed in periphyton associated to floating macrophyte roots (Mauro et al., 2001a).

2.5. Sulfate-reduction assays

Sulfate reduction was measured in Ribeirão das Lajes reservoir sediments following the radiochemical technique described by Rosser and Hamilton (1983) and Ulrich et al. (1997). It was first applied to Ribeirão das Lajes superficial sediment and then adapted to natural phytoplankton population of Jacarepaguá lagoon. In spite of the O$_2$ production of phytoplankton, the samples were maintained in an inert medium under a constant N$_2$ flux in a glove box. The amount of O$_2$ and H$_2$O$_2$ secreted by cyanobacterium via photosyntetic reaction in lake water is not known (Takenaka and Watanabe, 1997). Bottles containing 20 mL of natural phytoplankton were incubated during 6 and 48 h in the light or dark and kept under constant shaking (80 rpm). The experiments were done in triplicate and with a control with 2 mL of 20% zinc acetate.
Sample incubation was stopped with 2 mL of 20% zinc acetate. The samples were fitted with test tubes containing 2 mL of 10% zinc acetate to capture the $^{35}$S-sulfides produced from added $^{35}$SO$_4$. The extraction followed the acidic method and was done during two days under constant shaking (80 rpm). Passive extraction was carried out in the same incubation flasks (60 mL) and under a constant N$_2$ flux in a glove box. To extract total reduced inorganic sulphur (Tris), 4 mL of 12 N HCl and 8 mL of 1 M CrCl$_2$ (in 0.5 M HCl) were added. The $^{35}$S–H$_2$S was trapped in the same trap that captured the $^{35}$S-sulfides from incubation. Sulfate-reduction was estimated as the percentage of total added activity found in the traps, once the sample sulfate concentration were not measured.

2.6. Statistical treatment

Data from different sites were compared with the Tukey test and considered statistically different when $p \leq 0.05$. Pearson correlation coefficient ($r^2 \geq 0.5$) was used to compare data obtained with different techniques in a same site (mercury methylation, bacterial activity and sulfate reduction).

3. Results

3.1. Jacarepaguá lagoon

The Fig. 1 shows the leucine saturation curve obtained by natural phytoplankton samples from the hypereutrophic tropical Jacarepaguá lagoon. Though bacterial activity was detected from 20 nM, no saturation was reached up to 1.000 nM leucine, which was the leucine concentration used in all our experiments as well as in those by Mauro et al. (2001a) to measure bacterial activity in the periphyton associated to the roots of floating macrophytes.

$\text{Me}^{203}\text{Hg}$ formation was not detected in light incubations of natural phytoplankton samples from two different sampling dates though relevant bacterial production (Fig. 2a) and sulfate-reduction (Fig. 2b) were measured on the same samples. Both heterotrophic production and sulfate-reduction were higher after 48 h of pre-incubation, and leucine incorporation was higher under light than in the dark (Fig. 2).

Emphasizing the relationship between cyanobacteria and the bacteria associated to its mucilage, the bacterial production was measured in separate portions as described in the methodology. The top (concentrated natural phytoplankton cells) presented higher $^3$H-leucine incorporation rate than the dissolved bloom and no detectable heterotrophic activity was observed in unfiltered water sampled from the bottom fraction (Fig. 3).
3.2. Laboratory strains

Data on mercury methylation by the cultivated cyanobacteria *M. aeruginosa* are shown in Fig. 4a, obtained during the log growth phase during 24 and 48 h in presence of light and in the dark. A slight Me$_{203}$Hg production (0.37%) was observed only after two days of incubation under light and was not detectable in dark conditions. As seen in Fig. 4a, there was no significant ($p \leq 0.05$) difference in methylation upon 2 days of incubation of samples from the log phase culture or 72 and 96 h incubation of samples from the same *M. aeruginosa* culture but taken in the lag phase, 60 days after the start of cultivation under light. Table 1 shows the cell counts during the experiments. Fig. 4b shows methylation measurements obtained with log-phase *Sineccocystis* sp. cultures incubated for 4 days under light. It presents the same pattern and no significant ($p \leq 0.05$) difference with *M. aeruginosa* cultures.

Fig. 3. Leucine incorporation rate by different portions of phytoplankton from Jacarepaguá lagoon after 4 h for condensation. nd=not detectable (detection limit=5.89 $\times$ 10$^{-9}$).

Fig. 4. a. Me$_{203}$Hg formation by *Microcystis aeruginosa* cultivated in laboratory conditions. b. Me$_{203}$Hg formation by *Sineccocystis* sp. cultivated in laboratory conditions.

Fig. 5. a. Me$_{203}$Hg production by sediments samples from different sites in Ribeirão das Lajes reservoir and by phytoplankton sampled at the centre of the reservoir. *$p=0.005$. b. Leucine incorporation in sediments samples from different sites of Ribeirão das Lajes reservoir and in phytoplankton sampled at the centre of the reservoir. *$p<0.001$, **$p<0.0001$. c. Sulfate-reduction potential in sediment samples from different sites of Ribeirão das Lajes reservoir. *$p=0.008$. 
3.3. Ribeirão das Lajes reservoir

In Ribeirão das Lajes reservoir Me²⁰³Hg formation was higher in the top layer of bottom sediment than in phytoplankton (Fig. 5a), though bacterial activity was significantly \( p \leq 0.05 \) higher in phytoplankton samples (Fig. 5b). Despite the relatively low values of Me²⁰³Hg formation by phytoplankton, it is important to stress that these microorganisms are found in the water column resulting in a higher bioavailability of MeHg compared to MeHg produced in sediments. There was no significant difference \( p \leq 0.05 \) in bacterial activity among the superficial sediments from the studied sites of the reservoir (Fig. 5b). The sediment from site 2 presents a lower sulfate-reduction percentage (Fig. 5c) and is characterized by a lower percentage of fine particulate matter and lower MeHg concentrations in water column (Palermo, 2002).

4. Discussion

4.1. Mercury methylation

The results (Fig. 4) showed that besides the production of toxic microcystins (Carmichael, 1992), cyanobacteria like Microcystis and Sineccocystis are associated with the toxification of mercury through methylmercury production. The M. aeruginosa cultures used in this study were not axenic, permitting the growth of other bacteria and their association with M. aeruginosa mucilage, therefore our data allow no inference on the relative role of cyanobacteria and their associated bacteria in mercury methylation. Microcystis colonies possess a mucus layer that allows a more intense bacterial colonization (Kamjunke and Mehner, 2001) but Watanabe et al. (1992) affirmed that the species and activity of the bacteria associated to Microcystis cells would change depending on the characteristics of the dissolved organic matter and some environmental factors.

There was no significant difference in Me²⁰³Hg production by growth stage of M. aeruginosa colonies. Low methylation was observed once a small number of cells, compatible with cell density in a cyanobacteria bloom, was incubated, and a similar pattern was found for the other investigated hepatotoxic cyanobacteria, Sineccocystis sp.

A non-linear relation of mercury methylation with time such as found herein was also found by Mauro et al. (2002), on Little Rock Lake (Wisconsin, USA), where MeHg production peaked after 2–4 days, and by Catán et al. (2004) who observed a burst of MeHg production in sediments only after a few days of incubation. On the other hand, reduced methylation rate under illuminated conditions in other environmental compartments (Cleckner et al., 1999; Mauro et al., 2002) contrasts with the data shown here. It is important to point that several processes as photode-methylation (Sellers et al., 1996; Morel et al., 1998), photosynthetic microbial sulfide oxidation, or inhibition of sulfate-reducing bacteria, could potentially reduce the net methylmercury production under light (Mauro et al., 2002). Cyanobacteria are photo autotrophic organisms and their metabolism must influence associated bacterial activity and mercury methylation.

Contrasting with the Ribeirão das Lajes reservoir and with cyanobacteria cultivated under laboratory conditions, Me²⁰³Hg formation was not detected in samples from Jacarepaguá lagoon, in spite of their relevant bacterial activity. The phytoplankton from Jacarepaguá lagoon, characterized by intense eutrophication and contamination, is basically made of cyanobacteria, specially M. aeruginosa (Table 2), while the samples from Ribeirão das Lajes reservoir present, in the same site and season as studied here, a much higher diversity (Palermo, 2002).

4.2. Heterotrophic bacterial activity

The use of leucine or thymidine as substrates to measure bacterial activity associated to phytoplankton samples is relatively recent. Brunberg (1999) used 30 nM of thymidine concentration to measure bacterial activity associated to Microcystis spp. in a temperate hypereutrophic lake in Sweden contrasting with Worm and Sondergaard (1998) measurements using 600 nM leucine in an eutrophic lake in Denmark. Kamjunke and Jähnichen (2000) showed a leucine incorporation by M. aeruginosa and, Hietanen et al. (2002) reported that the cyanobacteria Nodularia spp. incorporates leucine but not thymidine. The latter authors studied axenic strains that showed high 212 nM leucine incorporation, whereas 121 nM thymidine was either taken up at a very low rate or not at all.
In the present study, the aim was to study the association between these two microorganisms groups and leucine at concentrations of up to 100 nM had a notably lower incorporation rate than found in a *M. aeruginosa* bloom with 1000 nM leucine. The uptake of leucine by freshwater bacterioplankton typically saturates at a concentration of about 100 nM in temperate ecosystems (Jorgensen, 1992a,b). Sediments and epilithic microhabitats typically have higher saturation requirements than planktonic habitats (Ward and Johnson, 1996). Besides substrate choice and its concentration, it is difficult to compare results of bacterial activity from different studies because the data are expressed in different units such as mM of substrate or C per unit time per gram (Mauro et al., 2001a), cell (Brunberg, 1999), area (Thomaz and Esteves, 1997), percentual (Worm and Sondergaard, 1998), chlorophyll a (Hietanen et al., 2002) or volume (Andrade et al., 2003).

The high substrate concentration used here can be explained because *M. aeruginosa* is able to incorporate leucine (Kamjunke and Jähnichen, 2000) and certain cyanobacteria show a distinct ability for heterotrophic nutrition (Fay, 1965). Furthermore, at the water surface the rate of photosynthesis of the colonies is high and the cells store large quantities of carbohydrates and essential nutrients and metabolites within their cytoplasm (Mur et al., 1999).

Brunberg (1999) showed that $^3$H-thymidine incorporation rate was nine times higher in total planktonic bacteria than in planktonic mucilage bacteria but this ratio was the opposite in sediment bacteria. Furthermore, the *Microcystis* colonies from sediment incorporated eight times more $^3$H-thymidine than pelagic *Microcystis* colonies. In the present study, the abundance of *Microcystis* and bacteria were not measured and it is not possible to compare the leucine incorporation per cell between planktonic mucilage bacteria and sediment bacteria. Therefore, the results showed here are in gram of dry weight of phytoplankton and not per cell of bacteria and the bacterial activity measured in phytoplankton is associated to phytoplankton cells and to planktonic mucilage bacteria once both, bacteria and phytoplankton without associated bacteria, are able to incorporate leucine (Kamjunke and Jähnichen, 2000).

However, Fig. 5b supports Brunberg (1999) studies, once substrate incorporation rates in total planktonic samples are higher than in total sediment bacteria but this ratio was the opposite in sediment bacteria. Furthermore, the *Microcystis* colonies from sediment incorporated eight times more $^3$H-thymidine than pelagic *Microcystis* colonies. In the present study, the abundance of *Microcystis* and bacteria were not measured and it is not possible to compare the leucine incorporation per cell between planktonic mucilage bacteria and sediment bacteria. Therefore, the results showed here are in gram of dry weight of phytoplankton and not per cell of bacteria and the bacterial activity measured in phytoplankton is associated to phytoplankton cells and to planktonic mucilage bacteria once both, bacteria and phytoplankton without associated bacteria, are able to incorporate leucine (Kamjunke and Jähnichen, 2000).

| Cyanobacteria | 17.255 |
| Cyanobacteria | 5.126 |
| Cyanobacteria | 1.398 |
| Cyanobacteria | 6.900 |
| Cyanobacteria | 2.200 |
| Cyanobacteria | 1.631 |
| Chlorophyceae | 699 |
| Chlorophyceae | 233 |
| Chlorophyceae | 233 |
| Bacillariophyceae | 2.796 |
| Bacillariophyceae | 1.165 |
| Bacillariophyceae | 1.631 |
| Cryptophyceae | 466 |
| Cryptophyceae | 699 |
| Cryptophyceae | 466 |
| Cryptophyceae | 233 |
| Total | 21.615 |

| Nostocophyceae | 3.500 |
| Nostocophyceae | 5.000 |
| Zygnemaphyceae | 6.500 |
| Xanthophyceae | 500 |
| Dinophyceae | 1.000 |
| Dinophyceae | 16.500 |

*Palermo (2002) studied the same site and verified highest density at this same location (center of the reservoir).*
The contrasting behaviour of mercury methylation and heterotrophic activity in superficial sediment and natural phytoplankton from a same place, as seen in Fig. 5, may be caused by a different microorganism community structure. Brunberg (1999) observed a different taxonomic composition in the bacterial community of the mucilage of *Microcystis* colonies, compared to the water column and sediments from the temperate lake that she studied.

The bacteria and cyanobacteria mucilage association is considered adaptive once it involves nutrient and organic compounds transport among them (Kirchman, 1993) and shelter against predation (Güde, 1989). Bacteria in sediments have numerous particles in their environment, and large part of these microorganisms is more or less tightly associated with these particles (Brunberg, 1999). Cyanobacteria represent a relatively unexplored and potentially rich source of bioactive secondary metabolites (Moore, 1996; Neilan et al., 1999) and some strains can use microcystin as a carbon and energy source (Park et al., 2001).

Samples of *M. aeruginosa* blooms had higher $^3$H-leucine incorporation under light presence, suggesting a correlation between cyanobacteria photosynthetic metabolism and bacterial activity (Fig. 5a). Heterotrophic activity had higher values after 48 h of pre-incubation, same incubation period as used in methylation essays. Additionally, sulfate-reduction tended to be higher in 48 than in 6 h incubations under light conditions but was possibly inhibited due to $O_2$ production by cyanobacteria. Krekeler et al. (1998) quantified sulfate-reducing bacteria in a cyanobacteria bloom and found they were 20 times more abundant during the day than during the night, and suggested that SRB present some strategies to cope with the changes in cyanobacterial $O_2$ concentration (Sass et al., 2002). Furthermore, they showed a SRB species gradient in cyanobacterial blooms where the different responses to $O_2$ concentration must result in heterogeneity in the distribution of these microorganisms. Such abundance of SRB in aerobic environments and, more surprisingly, the coupling of their activity with photosynthesis by the host organisms may explain the findings of Desrosiers (2004) and Guimarães et al. (2005) of a significant reduction of Hg methylation in periphyton when photosynthesis was blocked with a specific inhibitor.

5. Conclusions

Studies about methylmercury production, bacterial activity and sulfate-reduction rate in phytoplankton mucilage are scarce. The aim of this work was to gather preliminary data on these parameters and verify any relation between them in samples from tropical freshwater systems. Though no correlation between the parameters was observed and considerable variation among study sites was found, the data suggest that different heterotrophic microorganisms are associated to tropical freshwater phytoplankton and may play a role in Hg methylation.

Despite the low methylation potentials measured herein, it is important to point out that methylmercury, when produced in the water column, will be more bioavailable and more easily transferred along the trophic chain than methylmercury produced in sediments. Studies of mercury methylation by phytoplankton were not reported yet but we suggest that this group should be considered in mercury balances in different freshwater ecosystems. Hg concentrations in phytoplankton are $10^4$–$10^6$ times higher than in water (Miles et al., 2001) and phytoplankton is one of the main Hg pathways into the food web.

The few data on phytoplankton, periphyton, epilithon, and the different microorganisms they host suggest intricate interactions between them. The study of these interactions and of their effect on the cycle of Hg and other pollutants is a challenge that requires a multidisciplinary approach as well as further technical developments, but may contribute to a better understanding of the Hg cycle.

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