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Mercury Methylation In A Tropical Macrophyte: Influence Of Abiotic Parameters

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Sediment has been considered to be one of the most important mercury methylation sites, but recent studies have demonstrated a new site that is relevant, i.e. the roots of floating aquatic macrophytes, where high methylation is observed. The effects of temperature, pH and electric conductivity on net mercury methylation were studied in the roots of the water-hyacinth *Eichhornia crassipes* of a freshwater coastal lagoon (Lagoinha, RJ, Brazil). Root samples were incubated, over three days, with $^{203}HgCl_2$ addition, at different temperatures (10–90 °C), pH values (3–8) and different electrolytic solutions ($KClO_4$, KCl and $CaCl_2$, at 1, 5, 10, 25 and 50 mM, ranging between 18 and 760 $\mu S\ cm^{-1}$). ^{203}Hg-labeled methylmercury ($Me^{203}Hg$) was extracted in toluene, after acid leaching, and measured by β-counting. Up to 35% of mercury added was converted to MeHg Methylation increased from 10 to 35 °C, and decreased thereafter. The process was completely inhibited at 90 °C. At pH values of 6 and 7 methylation was stimulated and a significant decrease was verified at pH 8. Increasing $KClO_4$ concentrations led to a significant decrease in the methylation rates, while for KCl and $CaCl_2$ solutions only a slight decrease was observed.

Keywords: mercury; methylmercury; methylation; macrophytes; *Eichhornia crassipes*; tropical lakes

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INTRODUCTION

The conversion of inorganic mercury into methylmercury (MeHg), a strong neurotoxin,[1] is a critical step in the environmental behavior of this metal. Mercury methylation is a very complex process which may be abiotic[2] or biotic, mainly mediated by sulfate-reducing bacteria.[3,4] The net environmental concentrations of methylmercury are a result of the opposite processes of methylation and demethylation, which are not completely understood. The most important factors influencing biological mercury methylation are inorganic mercury bioavailability and the nature of the microbial community present in an ecosystem. Both are influenced by physical and chemical parameters such as temperature, pH, salinity, organic carbon and redox potential.[5–8]

Most methylation studies have been carried out in temperate regions, where there are significant temperature variations during the year, and this parameter seems to be an important factor controlling the process, since it directly affects microbial activity and chemical reactions. Summer temperatures were associated with higher methylation rates in temperate lakes.[8–10]

The influence of pH on mercury methylation has been the subject of a variety of studies[7,8,11] because of the observed higher methylmercury concentrations in fish from acidified lakes in the northern hemisphere. $MeHg^+$ seems to be formed under moderately acid and neutral conditions (pH 5–7) and Me_2Hg is mostly formed under alkaline conditions.[8]

Studies regarding the influence of electrical conductivity (a measure of ionic strength) on mercury methylation are scarce. A decrease in mercury methylation rates in sediments with increasing conductivities, obtained by addition of NaCl, was reported.[12] The biota from black-water Amazonian rivers, which are characterized by low pH values and reduced electric conductivities, have

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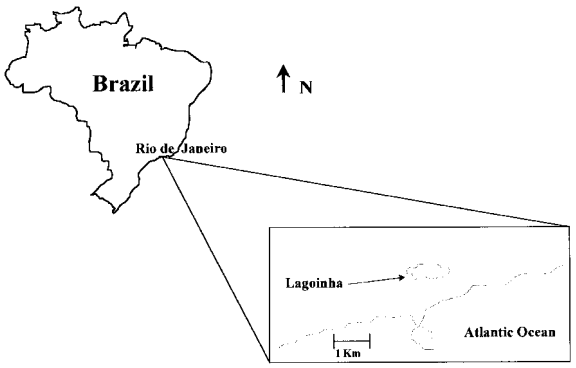

Figure 1 Location of study site: Lagoinha, RJ (23°4′S, 43 °32′W).

higher methylmercury concentrations than the organisms from white-water rivers, characterized by higher pH and electric conductivities.[13]

Mercury methylation is usually studied in compartments such as surface sediments, water and soils.[6,14] Recent studies demonstrated that roots of floating aquatic macrophytes are a very active mercury methylation site.[12,15] Several regions in the Brazilian Amazon and Pantanal floodplain areas are widely colonized by macrophytes, therefore, higher methylation rates in this compartment may have important ecological implications. MeHg produced in floating macrophyte roots is bioavailable due to rapid water diffusion and to the dense and diverse biota living near the macrophyte stands. Little is known about methylation in macrophyte roots, despite its relevance.

The aim of this work was to investigate the influence of temperature, pH and electrical conductivity on net mercury methylation in the roots of the floating macrophyte *Eichhornia crassipes*.

STUDY SITE

Water and *E. crassipes* root samples were obtained from a freshwater coastal lagoon, the Lagoinha, situated in the Parque Ecológico Municipal Chico Mendes in Rio de Janeiro city (Brazil) (Fig. 1). Despite being located in an ecological park, this is an eutrophicated ecosystem, receiving a considerable amount of raw sewage, which provides suitable conditions for colonization by the opportunistic water-hyacinth species, *E. crassipes*. This species, widely distributed in tropical regions, accumulates several pollutants from the water, including mercury and, as such, is used in industrial and domestic effluent treatment.[16]

METHODS

Water samples were obtained at the surface and bottom of the water column (approx. 2.0 m deep) with a van Dorn bottle and transported to the laboratory in acid-washed polyethylene containers. Temperature, pH and dissolved oxygen were measured *in situ*. The pH of the waters was measured by an Analion PM 603 pH-meter and dissolved oxygen was determined using a YSI portable oximeter, model 57. In the laboratory, salinity was analyzed by argentometry[17] and the electrical conductivity was analysed with a Cole Parmer Co. 1481-90 instrument. Orthophosphate PO_4^{3-} was determined by the phosphomolybdic method with ascorbic acid reduction,[17] total phosphorus by acid digestion with potassium persulfate followed by PO_4^{3-} analysis[17] and ammonia nitrogen by the indophenol method.[17] Root samples were separated manually from individual *E. crassipes* specimens and transported in sealed plastic bags. In the laboratory, coarse debris was removed and roots were chopped and homogenized, forming composite samples. Samples of 15 g wet weight (corresponding to 0.5 g dry weight) and 30 ml of solution were incubated for three days with $^{203}HgCl_2$ in 50-ml Teflon© lined screw-cap borosilicate tubes. A control, acidified with 1 ml of 4 N HCl, and triplicate samples were subjected to different temperatures, pH values and electrical conductivity regimes. Different incubation temperatures (10, 22, 25, 32, 42, 50 and 90 °C) were obtained by placing samples in a refrigerator, an oven and a refrigerated room. For this particular experiment, incubations were performed with Lagoinha lake water. Solutions with different pH values (3, 4, 5, 6, 7 and 8) were prepared with addition of HNO_3, HCl or NaOH to Milli-Q water in two separate sets of experiments. Stock solutions of $KClO_4$, KCl and $CaCl_2$ were diluted with Milli-Q water to concentrations of 1, 5, 10, 25 and 50 mM, resulting in increasing electrical conductivities, ranging from 15 to 761 $\mu S\ cm^{-1}$. These solutions were used in order to compare the influence of the chloride and perchlorate anions on methylation, since chloride affects mercury speciation.[6] $KClO_4$ was included because, in contrast with KCl and $CaCl_2$, it will not result in free Cl^-. Approximately 135 ng of Hg was added as $^{203}HgCl_2$ (supplied by

Table 1 Physical and chemical parameters of the Lagoinha water

Parameter	Surface	Bottom
Temperature (°C)	24.5–28.5	24.0–26.0
pH	7.01–7.48	6.79
Electric conductivity ($\mu S\ cm^{-1}$)	123–137	—
Dissolved oxygen ($mg\ l^{-1}$)	4.9–12.0	0.40–1.70
Salinity (S)	0.15–0.35	0.20–0.41
Orthophosphate (μM P as PO_4^{3-})	18.80	12.23
Total phosphorus (μM P)	27.30	24.91
Ammonia (μM N as NH_3/NH_4^+)	2.20	2.70

Amersham International, UK) resulting in a concentration of 0.27 $\mu g\ Hg\ g^{-1}$ dry weight. Activity varied from 1.8×10^2 to 9.3×10^2 Bq. All samples remained in darkness and those used in the pH and conductivity experiments were incubated at room temperature (approx. 25 °C). Incubation was terminated by addition of 1 ml of HCl and samples were kept frozen until undergoing the $Me^{203}Hg$ extraction procedure. The extraction was made by addition of 4 ml of 3 M NaBr in 11% H_2SO_4 and 1 ml of 0.5 M $CuSO_4$, shaking for 1 min and centrifuging for 10 min at 3000 rpm. The supernatant was transferred to 125-ml glass separatory funnels and shaken for 15 min with 15 ml of a scintillation cocktail, prepared with POP (2,5-diphenyloxazole) and POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] dissolved in toluene, at concentrations of 7.0 and 1.0 $g.l^{-1}$, respectively. The cocktail samples were shaken with sodium sulfate, to remove any trace of water, and were transferred to another vial for β-spectrometry using an LKB-Wallac Rackbeta 1214 liquid scintillation counter.[18] Results were submitted to ANOVA and Tukey tests.

RESULTS

Water temperature was relatively high, with similar values at both depths, pH values were about neutral and salinity was low, characterizing this lagoon as a freshwater system (Table 1). Electrical conductivity was relatively high and most of the dissolved oxygen concentrations were low, especially at the bottom. One sample presented an exceptional high oxygen concentration (12 $mg.l^{-1}$) on the surface, which is attributed to intense productivity in a thin superficial and well aerated water layer, resulting in oversaturation of the dissolved oxygen (Table 1). Except for ammonia, nutrient concentrations were high, indicating the eutrophic condition of this system.

Temperature assay

Results showed an increase in $MeHg^+$ production up to 32 °C. Methylation decreased thereafter, and the process was completely inhibited at 90 °C, probably due to suppression of bacterial activity (Fig. 2). The Tukey test showed that $MeHg^+$ production obtained at the lowest (10 °C) and the highest temperatures (50 and 90 °C) were significantly lower ($P < 0.05$) than those obtained at the other temperatures.

These data are in agreement with similar tests on the influence of temperature on methylmercury production in sediments of the Amazon region, showing increased methylation in the 35–45 °C range.[12] Higher mercury methylation observed in this temperature range is relevant especially for

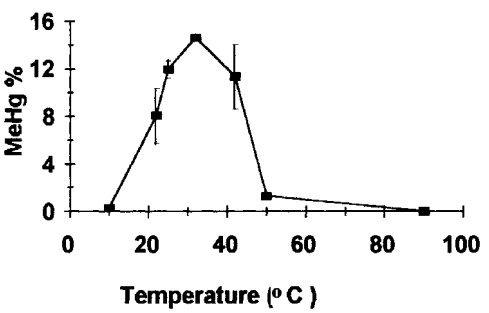

Figure 2 Methylmercury production in samples of *Eichhornia crassipes* roots, as a function of the incubation temperature. Vertical bars represent the 95% confidence interval (triplicate samples).

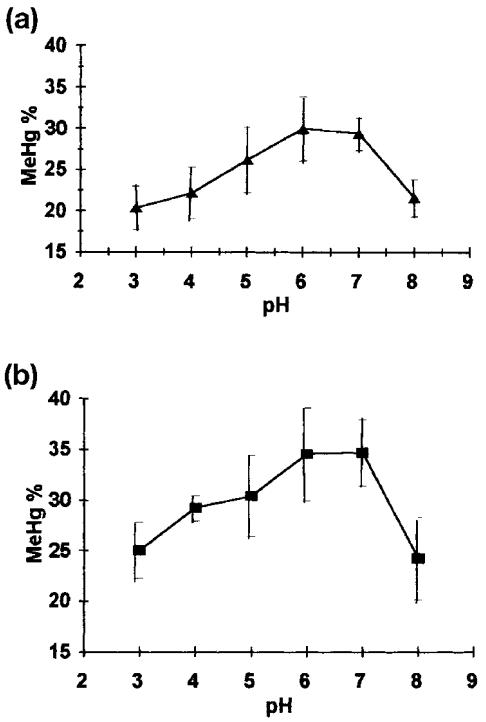

Figure 3 Methylmercury production in samples of *Eichhornia crassipes* roots, as a function of pH, adjusted using HNO_3 (a) or HCl (b). Vertical bars represent the 95% confidence interval (triplicate samples).

equatorial and tropical water systems, where temperatures up to 40 °C are frequently reached.

Effect of pH

Net mercury methylation was highest ($P < 0.05$) in both treatments at pH 6 and 7. Less methylation was observed at more acidic and alkaline pHs (Fig. 3). A decrease in mercury methylation at pH 8 possibly ocurred due to increasing demethylmercury formation in alkaline waters, reducing net methylmercury concentrations.[8] Methylation was significantly higher ($P < 0.05$) in the samples incubated with hydrochloric acid solutions, but this may be attributed to the fact that experiments with HCl and HNO_3 solutions were made with root samples obtained on different sampling dates.

Effect of electrical conductivity

Increasing $KClO_4$ concentrations led to a significant ($P < 0.05$) decrease in mercury methylation, while for increasing concentrations of KCl and $CaCl_2$ solutions only a slight decrease was observed (Fig. 4). At the lowest concentrations (1 mM), $MeHg^+$ formation in the $KClO_4$ system (23.5%) was higher than in the other systems (18.6% for KCl and 18.4% for $CaCl_2$). Significant differences ($P < 0.05$) in mercury methylation were observed between $KClO_4$ and the other electrolytes used, but no differences were verified between KCl and $CaCl_2$ ($P < 0.05$).

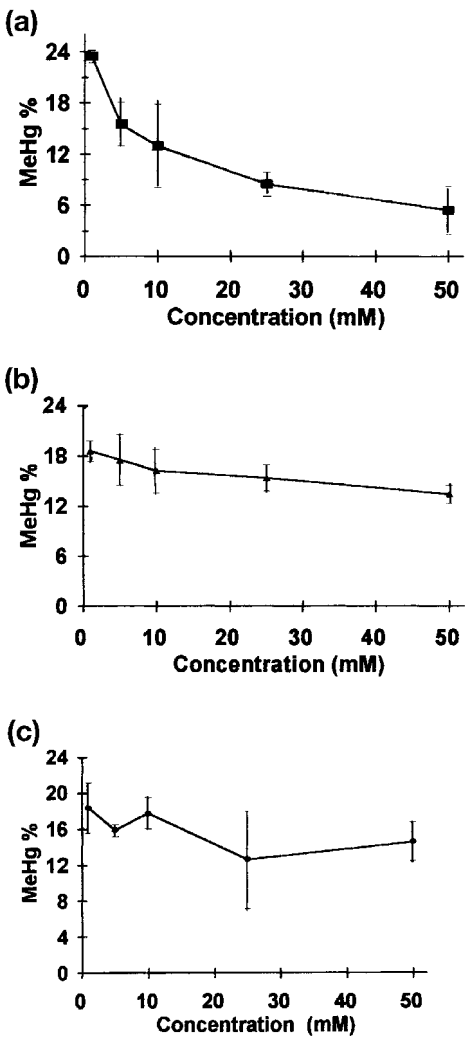

Figure 4 Methylmercury production in samples of *Eichhornia crassipes* roots, as a function of electrical conductivity, modified using $KClO_4$ (a), KCl (b) or $CaCl_2$ (c). Vertical bars represent the 95% confidence interval (triplicate samples).

DISCUSSION

Studies regarding the interaction of physical and chemical conditions with mercury methylation in freshwater sediments have suggested that higher temperatures, slightly acid pH and low electrical conductivities are favorable conditions,[7–9,12] increasing inorganic mercury bioavailability and/or stimulating the growth of methylating microorganisms. Sulfate-reducing bacteria seem to be the main mercury methylators in *E. crassipes* roots.[19] The large, compact stands formed by floating aquatic macrophyte roots are suitable environments for the development of sulfate-reducing bacteria communities, therefore the conditions for methylation in this compartment are expected to be similar to those in sediments. Our results demonstrated that temperatures in the range 30–35 °C, pH values between 6 and 7 and reduced electrical conductivities seem to increase mercury methylation in *E. crassipes* roots.

Additionally, *E. crassipes* roots methylmercury production obtained under natural (unchanged) conditions of temperature, pH and electrical conductivities in this study were at least one order of magnitude higher than those observed in sediments by other authors.[7,12,20] We hypothesize that larger contact surfaces (in comparison with the sediment–water interface) favor the growth of microbial populations, and that physical and chemical conditions among the macrophyte stands, such as suitable redox potentials, may contribute to increased mercury methylation. Tropical flooded areas are usually colonized by a number of aquatic macrophyte species and aquatic food chains are based on macrophyte herbivory and, mainly, on macrophyte detritus.[21] Therefore, a great number of organisms use the surroundings of floating macrophyte roots as shelter and a food source, thus increasing the bioavailability of MeHg produced at this site. Environments with these characteristics, when contaminated by mercury, are important potential methylmercury sources for the biota.[22]

Artifact formation of low methylmercury concentrations (up to 0.1% of added Hg) was reported by Bloom and co-workers[23] when using aqueous distillation as part of the procedure of methylmercury extraction from water and sediment samples. Possible artifact methylmercury formation in our samples would be expected to represent only a very small fraction of the total methylmercury formed. In addition, Brito[24] and Guimarães compared aqueous distillation, a method based on thin-layer chromatography after extraction using dithizone in benzene and the $Me^{203}Hg$ extraction procedure used in the present work, and obtained similar methylmercury extraction efficiencies for sediment and macrophyte root samples, suggesting that the formation of artifactual methylmercury in our samples is unlikely.

Among the factors studied, temperature seems to impose the strongest influence on mercury methylation, as shown by the impressive variation observed in the 10–90 °C range. Seasonal studies made in temperate and equatorial regions also showed that higher temperatures stimulate methylation,[8,9] which is attributed to increased bacterial activity. Tropical and equatorial areas present higher and less variable temperatures than temperate zones, potentially allowing the methylation process to be intense and continuous. In tropical regions, surface water temperatures are usually quite elevated (up to 40 °C) among large macrophyte stands, especially in shallow waters.

Acidified mercury-contaminated lakes in the Northern Hemisphere are known to present high MeHg concentrations in fish. Among several hypotheses to explain this correlation, increased methylation rates under lower pH conditions have been suggested.[7] Studies investigating the influence of this parameter on mercury methylation have shown that methylation seems to be increased under slightly acid pH conditions and that demethylation seems to be more intense under alkaline conditions. In our experiments, increasing methylation was observed from pH 3 up to 6 and 7 and a significant decrease occurred at pH 8. A decrease in pH may reduce bacterial activity, since most bacteria grow at pH 4–9, with an optimal pH, for aquatic bacteria, in the range 6.5–8.5[25] A decrease in methylation under more acidic conditions may also be a result of increasing inorganic mercury precipitation as insoluble mercuric sulfides, due to acid-mobilized H_2S and/or to increased mercury(II) adsorption to sediment particles.[26,27]

Methylation was significantly decreased as $KClO_4$ concentration was increased. However, the effect was less pronounced with increased concentrations of KCl and $CaCl_2$, showing that ClO_4^- concentrations interfered with methylation more effectively than chloride, although a slight decrease was also observed when the concentration of the two chloride salts was increased. Higher chloride concentrations, increasing the concentrations of the negatively charged $HgCl_3^-$ and $HgCl_4^{2-}$, could reduce the transfer of mercury through cell membranes, leading to a lower incorporation by the microbiota.[5] Therefore, it was expected that

higher chloride concentrations would reduce mercury methylation significantly. Environmental implications of the influence of the electrical conductivity and pH on mercury methylation may be related to the presence of higher methylmercury concentrations in biota from black-water Amazon rivers.[28] These are waters characterized by, among other factors, reduced electrical conductivity, a condition that might lead to higher methylation rates. The influence of the electrical conductivity on methylation induced by a single salt cannot be directly extrapolated, especially in environmental conditions, where several ions interact simultaneously. However, the influence of pH and conductivity on mercury methylation observed in this study could potentially explain the higher mercury concentrations found in the biota of black-water rivers of the Amazon basin.

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