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Uptake of Hg^{2+} by picocyanobacteria in natural water from four Andean lakes

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Abstract. In lake food webs, planktonic bacteria and algae represent the greatest bioconcentration step for Hg^{2+} and monomethyl-Hg (MeHg). As they are the most abundant organisms in planktonic trophic webs and also the main food resource for herbivorous plankton, they can mobilize large amounts of Hg to higher trophic levels. In Andean Patagonian lakes (Argentina), dissolved organic matter (DOM) concentration and character, coupled with photo-reactions, play a central role in the complexation of Hg^{2+} in the water column and can even regulate the uptake of Hg^{2+} by planktonic algae. In this investigation we evaluated the DOM character of natural waters (NW) from four Andean lakes and studied its influence on the uptake of $^{197}Hg^{2+}$ in a strain of the picocyanobacteria *Synechococcus* by using Hg^{2+} labeled with $^{197}Hg^{2+}$. The uptake of radiolabeled Hg^{2+} by *Synechococcus* showed different magnitude in NW of lakes Moreno, El Trébol, Morenito and Escondido. Increasing lake DOM concentration reduced the bioavailability of Hg^{2+} as indicated by the lower uptakes rates found in NW with higher complexity and concentration of the DOM pool. Uptakes of Hg^{2+} by this picocyanobacteria contrasted among NW from pelagic (surface and bottom) and littoral compartments of Lake Escondido which suggest that the entry of this metal may be highly variable even in the same environment. The study of the uptake of radiolabeled Hg^{2+} in a set of dilutions of NW from Lake Escondido demonstrated that the bioavailability of Hg^{2+} decrease with increasing DOM concentration.

Keywords: Hg^{2+} bioaccumulation, picocyanobacteria, natural water, Andean-Patagonian lakes

Introduction

Mercury is a global pollutant, atmospherically transported, that affects the atmosphere and also the terrestrial and aquatic environments (Horvat, 2002). Its inorganic divalent form (Hg^{2+}) enters into aquatic ecosystems from the watershed through run off and also by atmospheric deposition (Driscoll et al., 2007), and readily forms complexes with natural dissolved organic matter (DOM) (Lamborg et al., 2007). Ligands available in the DOM are involved in the speciation of Hg, and therefore can regulate the bioavailability of this metal in aquatic ecosystems. In fact, several studies have reported positive Hg-DOM correlations in natural water (Ravichandran 2004, Chen et al., 2005). However, due to the complex character of DOM in natural water (NW), it is still unclear up to what extent DOM may exert a control in the availability of Hg compounds to aquatic organisms (Gorski et al., 2008). Within aquatic foodwebs, bacteria and phytoplankton represent the greatest bioconcentration step for Hg^{2+} and monomethyl-Hg (MeHg) and, due to their role as a trophic base in the pelagic compartment, they can be considered the main source of this element for higher trophic levels (Mason et al., 1996, Pickhardt and Fisher, 2007). Thus, the relationship between the accumulation of Hg by the biota and the amount and character of DOM appear to be of central importance for understanding the Hg cycle in freshwater.

In Andean lakes of Northwestern Patagonia (Argentina) high levels of total Hg (THg) have been reported at different trophic levels, including top predators, herbivorous zooplankton, small mixotrophic ciliates and phytoplankton at the base of the pelagic food web (Rizzo et al. 2011). In these lakes, DOM coupled with photo-reactions play a central role in the complexation of Hg^{2+} in the water column (Ribeiro Guevara et al., 2008).

In this investigation we evaluated the influence of DOM character and concentration on the uptake of Hg^{2+} in the picocyanobacteria *Synechococcus*. We studied the fluorescence and UV-visible

absorbance features of natural waters from four Andean lakes and performed laboratory incubations exposing the picocyanobacteria to NW from these Andean lakes amended with Hg^{2+} radiolabeled with ^{197}Hg. We hypothesized that in Andean lakes, the bioaccumulation of $^{197}Hg^{2+}$ by picoplankton is influenced by the natural DOM and therefore, that the incorporation of this metal at the base of the pelagic food webs differs among lakes according to their DOM pools. In addition, we propose that in-lake DOM heterogeneity across depths and between littoral and pelagic zones may influence as well the uptake of Hg^{+2} by picocyanobacteria.

Materials and Methods

Study location. Natural water (NW) was collected from four Andean Patagonian lakes, the ultraoligotrophic deep lake Moreno West and the oligotrophic shallow lakes El Trébol, Morenito and Escondido (800 m.a.s.l; Nahuel Huapi National Park, North Patagonia, Argentina). These selected lakes represent a gradient in DOC concentrations, ranging from 0.4 mg L^{-1} in Lake Moreno to 5.4 mg L^{-1} in Lake Escondido (Morris et al., 1995; Pérez et al., 2007). The water samples taken using a 12 L Schindler-Patalas trap were poured into acid-washed 20 L polycarbonate carboys and transported insulated to the laboratory within 1 h of sampling. In Lake Escondido, water samples were obtained from a central point at 0.7 and 6 m deep and also in one littoral location inside the *Potamogeton* belt.

Laboratory procedures and experimental set up. Water samples from each of the four lakes and/or lake sites were pre-filtered through 0.7 µm glass-fiber filters (Whatman GF/F) and filter-sterilized through 0.22 µm PVDF (Millipore). These filtrates were used for the experimental culture of a strain of the picocyanobacteria *Synechococcus* (GALD ~ 1-1.5 µm). This picocyanobacteria was obtained from laboratory cultures grown in Marine Biological Laboratory (MBL) medium, in a photoperiod (14hLight: 10hDark).

Two series of experiments were performed to analyze the uptake of Hg^{2+} by *Synechococcus* in relation to the DOM. The first experiment compared the uptake of radiolabeled Hg^{2+} in a gradient of DOM conditions and consisted in the incubation of *Synechoccocus* in treatments with sterile NW from lakes Moreno, El Trébol, Morenito and Escondido. A second experiment explored the uptake of radiolabeled Hg^{+2} by *Synechococcus* in NW from different spatial compartments of Lake Escondido, pelagic (surface and bottom) and littoral (Experiment 2a). Additionally, the effect of a concentration factor on the uptake of Hg^{2+} by *Synechococcus´*s was studied by diluting surface and bottom NW with ASTM1 water (Experiment 2b). In general, the experiments consisted in the incubation of the picocyanobacteria at a density of ~$1x10^6$ cells mL^{-1} in 100 mL of the different NW/culture media amended with a solution of radiolabeled Hg^{2+}. Two conditions were tested: filter sterilized NW amended with sterile culture medium in the same proportion than added in the replicates with the picocyanobacteria served as control (2-4 replicates) and filtered sterilized NW with the addition of $1x10^6$ cells mL^{-1} of *Synechococcus* (3-4 replicates). All replicates of the experiments were amended with radiolabeled Hg^{2+} at a final concentration of 7-11 ng L^{-1}, corresponding to natural Hg^{2+} levels found in precipitation water. After incubation (20-23 h), *Synechococcus* was recovered by filtration through 0.22 PVDF membranes and $^{197}Hg^{2+}$ uptake was calculated for each experimental unit based upon the initial $^{197}Hg^{2+}$ amendment compared to the final $^{197}Hg^{2+}$ activity measured in the organisms retained in the filters, after correction with the retention in the control filters. The high specific activity $^{197}Hg^{2+}$ (T½=2.673 d) was produced by irradiation of a 2% HNO_3 solution of Hg^{2+} enriched to 51.6 % in the $^{196}Hg^{2+}$ isotope in the nuclear research reactor RA-6 (ϕ_{th} =1×10^{13} n cm^{-2} s^{-1}), Centro Atómico Bariloche, Argentina. The final concentration of $^{196}Hg^{2+}$ concentration was 57 µg mL^{-1} and the isotope amendments used in the experiments are relative to this value. The radiolabeled Hg^{2+} concentration in water, organisms and filtrates were measured through the activity of $^{197}Hg^{2+}$ in the samples, comparing them to the standards measured in the same geometry; evaluating the X-ray and γ-ray emissions associated with $^{197}Hg^{2+}$ decay, using a well type High Purity Germanium (HPGe) detector. DOC concentration in NW was estimated using the absorbance scans (HP UV-Vis 8453) of filtered (0.22 µm, PVDF) NW, following Morris et al. (1995). Synchronous fluorescence spectra (SFS) were recorded to characterize NW of the four lakes at natural pH and 20°C. ASTM-1 water blanks and NW were analyzed over the excitation wavelength range 250-550 nm (Perkin Elmer LS55 spectrofluorometer in a 1 cm quartz cell, with an offset value of 30 nm between excitation and emission wavelengths with a slit width of 10 nm). The SFS were processed with the software FL-WinLab, corrected for Raman spectral overlap and for the inner-filter effect. The SFS were used as proxies for fluorophores revealed by individual peaks (I, II, and III).

Data analysis. The experimental results were tested for normality (Kolmogorov-Smirnoff test) and homoscedasticity before performing the

Analysis of Variance to examine responses among treatments. Non-linear regression analysis was applied to fit the response in Hg^{2+} uptake by *Synechococcus* under different dilutions of NW.

Results and Discussion

The estimated DOC concentration resulted highest in Lake Escondido and decreased from lakes Morenito towards El Trébol and Moreno (Table 1). The synchronous fluorescence spectra (SFS) of NW from these lakes were characterized by the intensities of three fluorescence maxima: Peak I (270-285 nm), Peak II (305-315 nm) and Peak III (330-375 nm). Peak I exhibited the largest intensity in Lake El Trébol followed by Morenito and Moreno (Table 1). Peak II was present in NW from lakes Moreno and Escondido. Peak III was measured in all lakes, with the highest intensity in Lake Escondido, followed by Morenito, El Trébol and Moreno (Table 1). These results indicate a difference in the molecular composition of the natural DOM among lakes.

Table 1. Characterization of NW from four Andean Patagonian Lakes: 1-sterile NW and 2-sterile NW with $1x10^6$ cells mL^{-1} of *Synechococcus*, *DOC = estimated dissolved organic carbon concentration, SFS=Synchronous Fluorescence Spectra. Intensities of SFS peaks are expressed in quinine sulfate units (QSU).

Lake	Treatment	DOC*	SFS analysis		
		(mg L^{-1})	Peak I	Peak II	Peak III
Moreno	1	0.87	2.73	6.30	3.19
	2	0.92	3.97	6.94	3.11
El Trébol	1	1.42	21.78	0.00	10.16
	2	1.45	15.11	11.51	9.51
Morenito	1	2.06	11.54	0.00	16.40
	2	2.01	17.15	17.59	16.44
Escondido	1	3.07	0.00	24,64	41.84
	2	2.96	0.00	20.30	37.54

Experiment 1 showed that the uptake of labeled Hg^{2+} by *Synechococcus* was significantly different among NW of the four lakes ($F=56.32$, $p<0.05$, $n=15$). The highest mean uptake of Hg^{2+} was found in NW of Lake Moreno, followed by similar and comparatively lower uptakes in NW from lakes El Trébol and Morenito. The lowest mean uptake of Hg^{2+} of *Synechococcus* was found in cultures with NW from Lake Escondido (Fig. 1). Moreover, a significant negative correlation was found between the mean uptake of Hg^{2+} in *Synechococcus* and the intensity of Peak III (Pearson correlation, $r=-0.976$, $p<0.05$). This peak was positively correlated with the estimated DOC concentration (Pearson correlation, $r=0.980$, $p<0.05$). Noteworthy, the retention of labeled Hg^{2+} in the control filters was positively correlated with the intensity of Peak III (Pearson correlation, $r=0.978$, $p<0.05$). These two patterns likely suggest that increasing concentrations of DOM reduce the availability of Hg^{2+} for *Synechococcus* since the Hg^{2+} is readily retained in the dissolved phase likely bound to organic ligands.

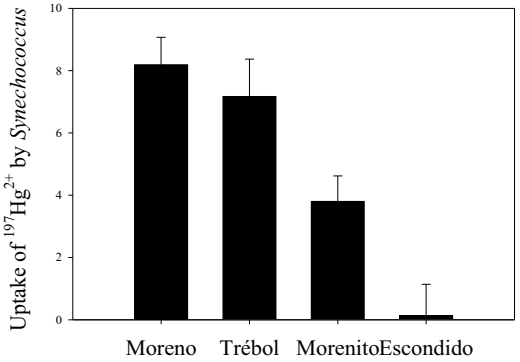

Fig. 1. Uptake of radiolabeled Hg^{2+} (% of spike) in NW from four Andean Patagonian lakes.

The results of experiment 2a showed significantly higher uptakes of radiolabeled Hg^{+2} by *Synechococcus* in NW from the littoral and bottom compartments of Lake Escondido compared to pelagic surface NW ($F=24.62$, $p<0.05$; Fig. 2).

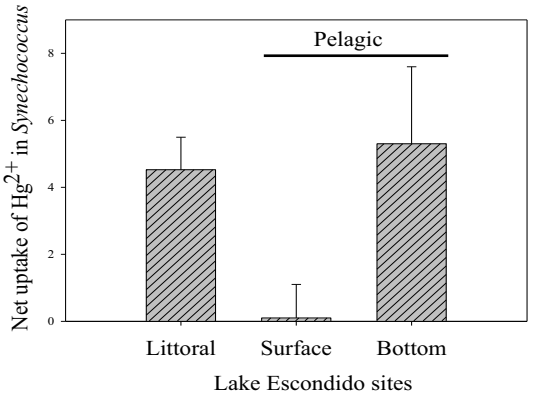

Fig. 2. Uptake of radiolabeled Hg^{2+} (% of spike) in NW from a littoral site and two pelagic (surface and bottom) sites of Lake Escondido.

In Lake Escondido the littoral vegetation *Schoenoplectus californicus* and *Potamogeton linguatus*) and the vegetation covering the bottom (*Nitella* sp.) appear to create particular conditions. However the SFS analyses did not reveal any stricking differences in the DOM among NW from the different compartments, perhaps

suggesting that other factors may be controlling the observed differential uptakes of Hg^{2+} measured in *Synechococcus* cells.

Experiment 2b indicated that the uptake of Hg^{2+} in *Synechococcus* increased with the dilution of NW both from the surface and bottom of Lake Escondido (Fig. 3). The non-linear model adjusted to the relationship between the dilution of NW and the uptake of Hg^{2+} by *Synechococcus* was highly significant in the case of the surface NW (r^2=0.988, F=247.05, $p<0.05$). In contrast, this relationship was not significant in bottom NW ($p>0.05$). The first relationship suggests that the concentration of DOM by itself can influence the bioavailability of Hg^{2+}in natural waters, as has been also suggested in other investigations (Pickhardt and Fisher, 2007; Gorski et al. 2008).

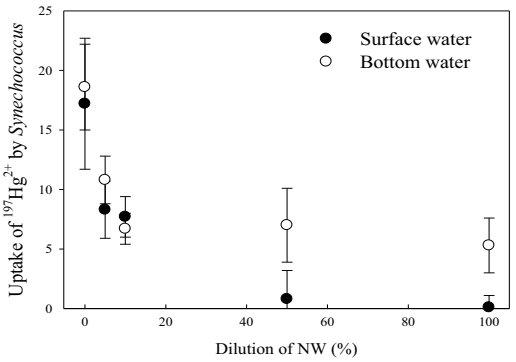

Fig. 3. Uptake of $^{197}Hg^{2+}$ by *Synechococcus* (% of the spike) pelagic NW (surface and bottom) of Lake Escondido diluted with ASTM1.

Conclusion

Increasing lake DOM concentrations present in NW reduced the bioavailability of Hg^{2+} for *Synechococcus´* bioaccumulation. In addition, the uptake of Hg^{2+} appears to be variable among lake spatial compartments as indicated by the contrasting uptake patterns showed by *Synechococcus* in littoral and pelagic (surface and bottom) water of the same lake. In addition, the uptake of Hg^{2+} by *Synechococcus* increased exponentially with the dilution of NW, strengthening the idea that the concentration of DOM regulates the uptake of Hg^{2+} in these picocyanobacteria. Overall, organic ligands present in NW appear to dampen the entry of Hg^{2+} in planktonic food webs by competing for this metal with basal organisms such as bacteria and algae.

Acknowledgements

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