Inorganic mercury (Hg2+) uptake by different plankton fractions of Andean Patagonian lakes (Argentina)

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Abstract. The species composition and the size structure of natural planktonic food webs may provide essential information to understand the fate of mercury and, in particular, the bioaccumulation pattern of Hg2+ in the water column of lake ecosystems. Heterotrophic and autotrophic picoplankton and phytoplankton are the most important entry points for Hg in aquatic ecosystems since they concentrate Hg2+ and MeHg from ambient water, making them available to planktonic consumers at higher trophic levels of lake food webs. In this investigation we studied the uptake of 197Hg2+ in natural plankton assemblages from four Andean lakes (Nahuel Huapi National Park, Patagonia, Argentina), comprised in the size fractions 0.2-2.7 µm (picoplankton), 0.2-20 µm (pico and nanoplankton) and 20-50 µm (microplankton) through experiments using Hg2+ labeled with 197Hg2+. The experimental results showed that the uptake of Hg2+ was highest in the smallest plankton fractions (0.2-2.7 µm and 0.2-20 µm) compared to the larger fraction comprising microplankton (20-50 um). This pattern was consistent in all lakes, reinforcing the idea that among pelagic organisms, heterotrophic and autotrophic bacteria with the contribution of nanoflagellates and dinoflagellates constitute the main entry point of Hg2+ to the pelagic food web. Moreover, a significant direct relationship was found between the Hg2+ uptake and surface index of the planktonic fractions (SIf). Thus, the smaller planktonic fractions which bore the higher SI were the major contributors to the Hg2+ passing from the abiotic to the biotic pelagic compartments of these Andean lakes.

Keywords: Hg2+, bioaccumulation, plankton size fractions, Andean Patagonian

Introduction

In freshwater ecosystems, the circulation of mercury (Hg) in abiotic and biotic compartments is a concern worldwide. This metal is dangerous to organisms since it accumulates affecting their behavior, reproduction and their fitness. Mercury is transferred to different levels of the aquatic food webs through natural trophic pathways up to the top predator's level. This level ultimately reflects the potential and magnitude of the biomagnification of this metal in a given ecosystem. In lakes, the picoplankton and phytoplankton, at the base of the pelagic food webs, typically have total Hg concentrations several times higher than natural water and therefore, they represent the first and greatest bioaccumulation step for this metal. Algae concentrate both Hg2+ and monomethylmercury (MeHg), and this last species readily biomagnifies beyond the base of the food web (Mason et al. 1997, Pickhardt and Fisher, 2007).

Environmental factors such as the concentration of dissolved organic matter, Fe, chloride, pH, among others, can regulate the bioavailability and/or bioaccumulation of Hg in natural systems. Ecological features such as species-specific patterns in Hg accumulation at the base of food webs may regulate its trophic transport. Thus, the species composition and the size structure of planktonic food webs may provide essential information to understand the Hg fate and bioaccumulation in aquatic ecosystems. Hg^{2+} burdens in algal cells and bacteria are known to be related to the greater surface to volume ratio (S:V), as far as the process involved is sorption.

In this investigation we analyzed experimentally the uptake of Hg^{2+} by different plankton fractions in natural water (NW) from four pristine, oligotrophic lakes of Northwestern Patagonia (Argentina). The objectives were: i - to explore the uptake of Hg^{2+} by three planktonic size fractions: picoplankton (0.2 - 2.7 µm), pico

and nanoplankton (0.2-20 µm) and microplankton (20-50 µm) within each of the four lakes, ii - to compare the uptake of Hg^{2+} of the natural plankton fractions among the four lakes and, iii - to relate the Hg^{2+} uptakes from the different plankton assemblages with the particular potential for absorption through the application of a surface index for the studied plankton size fractions (SI_f) of all lakes. Our main hypothesis is that the Hg^{2+} uptake of the different planktonic size fractions in the four lakes is tightly related with the size structure and abundance reflected as a certain SI_f which determines the potential for Hg^{2+} absorption.

Materials and Methods

Study sites and sampling. The study combined field sampling and laboratory experiments. Four Andean Patagonian lakes, the deep lake Moreno and the shallow lakes El Trébol, Morenito and Escondido (Nahuel Huapi National Park, North Patagonia, Argentina) were visited to obtain natural water (NW) samples. These lakes are ultra to oligotrophic systems of glacial origin and encompass a gradient in dissolved organic carbon and nutrient concentration (Table 1).

Table 1: Limnological features of the Andean lakes studied. References: Z_{max} = maximum depth (m); Cond = Conductivity ($\mu S\ cm^{-1}$); Chl$a$ = Chlorophyll a ($\mu g\ L^{-1}$); DOC = dissolved organic carbon (mg L^{-1}); TP = total phosphorus ($\mu g\ L^{-1}$); TN = total nitrogen ($\mu g\ L^{-1}$).

Feature	Lakes			
	Moreno	Trébol	Morenito	Escondido
Z_{max}	90	12	12	8
Cond.	40-42	65-78	57-75	50-65
pH	6.8-7.5	7.3-7.6	6.8-7.8	6.7-7.8
Chl a	1.0-1.2	2.4-5.5	0.8-5.5	1.0-1.9
DOC	0.6	2.5	2.3	5.4
TP	1.5-4.1	8.6-11.4	8.9-17.1	5.7-17.5
TN	120-200	n.a.	280-420	190-420

Water samples from each lake were collected using a 12 L Schindler-Patalas trap, poured in acid-clean carboys and transported insulated to the laboratory.

Laboratory procedures and experimental set up. In the laboratory, a serial filtration was performed to each NW sample through 50 µm, 20 µm, 2.7 µm and 0.2 µm membranes, to concentrate the organisms present in the size fractions: 20-50 µm, 0.2-20 µm and 0.2–2.7 µm. A volume of 1.2 L of NW from each lake was sterilized by filtration (Gamafil nitrocellulose, 0.2 µm) to be used as culture medium for the plankton fractions and also for the control in the experiment (without addition of the planktonic fractions). The concentrates were resuspended at a final volume of 400-500 mL in sterile NW. For each lake NW, the experiments were set up with a treatment (with organisms) which had three levels (size fractions: 0.2–2.7 µm, 0.2-20 µm and 20-50 µm) and a control consisting in sterile NW. A total of 9 replicates were set up with organisms (3 for each size fraction) and 3 consisting in sterile NW of each lake. Each replicate contained a volume of 100 mL of the specific culture medium (NW from each of the four lakes). Once mounted, each replicate was amended with Hg^{2+} labeled with the radioisotope ^{197}Hg to a final concentration ranging from 10 to 12 ng L^{-1}. After incubation (22-24 h), each replicate was filtered through 0.2 µm Millipore PVDF membranes. The remaining solution was sampled (4 mL) to determine the labeled Hg^{2+} in solution after incubation. The same filtration protocol was applied to the replicates belonging to the control. All filters coming from the treatment and control replicates were folded and placed separately in glass tubes in order to evaluate the retention of labeled Hg^{2+}. The net retention or uptake in the organisms was corrected by subtracting the retention of labeled Hg^{2+} in the control filters. The isotope $^{197}Hg^{2+}$ (T½=2.673 d) amended to evaluate Hg^{2+} uptake by the plankton fractions 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 (ϕth $=1\times1013$ 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. Total $^{197}Hg^{2+}$ concentrations in water, organisms and filtrates were measured through the activity of labeled Hg^{2+} in the samples, comparing them to the standards measured in the same geometry; evaluating X-ray and γ-ray emissions associated with $^{197}Hg^{2+}$ decay, using a well type High Purity Germanium (HPGe) detector.

Plankton characterization. Subsamples for bacterial enumeration (heterotrophic picoplankton) were fixed and stained with 4',6-diamidino-2-phenylindole (DAPI, final concentration 0.2% w/v). Subsamples for autotrophic picoplankton enumeration were fixed with formaldehyde–cacodylate. Both bacteria and autotrophic picoplankton were quantified on 0.2 µm black membrane filters (Poretics) at 1000×

magnification on an Olympus BX50 epifluorescence microscope, using UV light (U-MWU filter) and blue light (U-MWB filter), respectively. The enumeration of nano and microplankton was performed following the Utermöhl technique.

Data analysis. The surface of each species (S_i, μm^2 cell^{-1}) was calculated using the geometric models proposed by Sun and Liu (2003). S_i was multiplied by the specific abundance (cell mL^{-1}) to obtain the total surface of each species. Then, the surface index of each fraction (SI_f, μm^2 mL^{-1}) was calculated as: $SI_f = \sum S_i$.

The uptakes of labeled Hg^{2+} of the different fractions obtained from the experiment were tested for normality (Kolmogorov-Smirnoff test) and homoscedasticity before performing the Two Way Analysis of Variance to examine the influence of lake and of the different plankton fractions in the uptake of labeled Hg^{2+}. Linear regression analysis was applied to fit the response in the uptake of Hg^{2+} by the different plankton fractions to their surface index (SI_f).

Results and Discussion

The analysis of the plankton showed up that the size fraction 0.2-2.7 μm (picoplankton) was similar among lakes and composed in ~95% by heterotrophic bacteria and ~5% of autotrophic bacteria. The fraction 0.2-20 μm was also composed by ~99% of picoplankton and just ~1% of nanoplankton, with the prymnesiophyceaen *Chrysochromulina parva* prevailing in lakes Moreno, El Trébol and Escondido. In Lake Morenito this fraction was dominated also by picoplankton but in combination with the cryptophycean *Plagioselmis lacustris*. Within the fraction 20-50 μm, the diatom *Fragilaria* in assemblages with other species were characteristic in most lakes except in Lake El Trébol in which *Dinobryon sertularia* and *Cryptomonas* spp. were co-dominant. The co-dominant species accompanying the diatom *Fragilaria* were *Gymnodinium paradoxum* in Lake Moreno, *Cryptomonas* spp. in Lake Escondido, and other diatoms, *Synedra* spp, in Lake Morenito. In lakes Moreno, El Trébol and Morenito the highest cell abundance was found in the fraction 0.2-20 μm followed by the fraction 0.2-2.7 μm. In contrast, in Lake Escondido the fraction 0.2-2.7 μm was more abundant than the fraction 0.2-20 μm. In the four lakes, the cell abundance of the fraction 20-50 μm was around three orders of magnitude lower than the preceding fractions (Fig. 1a).

The results of the experiment using different plankton fractions showed up significant differences in the uptake of labeled Hg^{2+} among lakes (F=125.15, p<0.05). In general, the Hg^{2+} uptake pooling the plankton fractions were higher in Lake Morenito followed by El Trébol, Moreno and at last by Lake Escondido (p<0.05). Also, there were significant differences in the Hg^{2+} uptake among fractions regardless of the lake (F=415.16, p<0.05). The significant interaction between the main indicated different behaviors in the uptake by different planktonic size fractions among lakes (F=40.8, p<0.05). The pos-hoc contrasts showed significantly higher uptakes of Hg^{2+} in the fraction 0.2-20 μm followed by the fraction 0.2-2.7 μm (p<0.05), except in Lake Moreno, in which the uptake of the fractions 0.2-2.7 μm and 20-50 μm were similar (p>0.05). Overall, these results indicate that picoplanktonic and nanoplankonic species represent the main entry points of Hg^{2+} in the different lakes, such as has been evidenced by other authors (Mason et al. 1997; Pickhardt and Fisher, 2007).
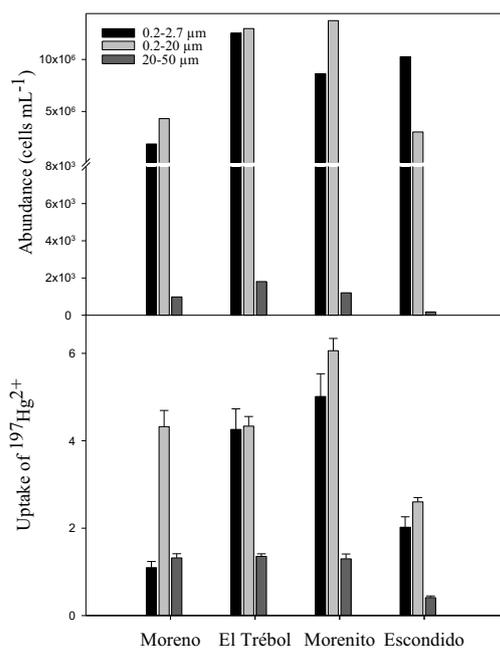
Fig. 1. a- Total cell abundance in the different plankton fractions (cell mL^{-1}) and, b-Uptake of labeled Hg^{2+} (% of amended Hg) by planktonic fractions in NW from Andean Patagonian lakes.

Considering that one of the factors controlling Hg^{2+} uptake in organisms is their actual exposure to the chemical environment determined by their surface, we calculated a surface index for each plankton species, size fraction and lake. Higher SI corresponded to the smaller plankton fractions, 0.2-2.7 μm and 0.2-20

µm. The study of the relationship between the SI and the Hg^{2+} uptake considering all plankton fractions of the four lakes resulted in a significant direct pattern between these variables. The uptake of Hg^{2+} increased with the surface index (r^2=0.68, F=21.26, p<0.001).
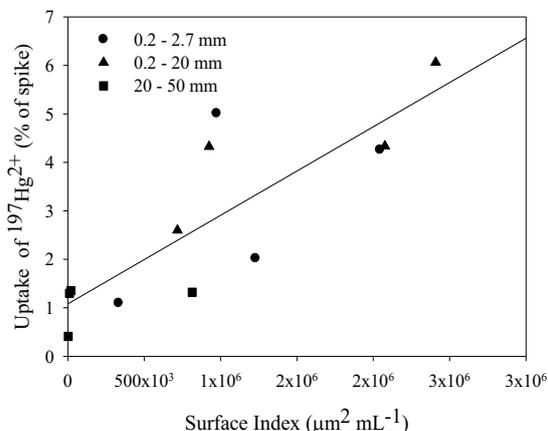
Figure 2. Relationship between the uptake of labeled Hg^{2+} and the surface index (SI_f) of the different plankton fractions.

This relationship between the SI and Hg^{2+} uptake suggest indirectly that the species composition and the relative importance of the different size fractions in the community determine partly the entry of Hg^{2+} in the food web. However, other factors may contribute also to explain the observed differences in the uptake of Hg^{2+} among lakes. For example, water chemistry may control the actual bioavailability of Hg^{2+}. In this line, differences in natural DOM concentration and quality between lakes may also influence the Hg^{2+} uptake by organisms, independently of the relative importance of the different plankton fractions. The DOM is a complex mixture of compounds that includes molecules with specific metal-binding properties due to the presence of functional groups such as carboxyl, phenol and thiol (Tipping, 2002; Ravinchandran, 2004). Thus, Hg complexation in the dissolved phase leads to a decrease the uptake of this metal or, what is the same, to a decrease of its bioavailability (Gorski et al., 2008). DOM itself can adsorb to surfaces modifying the membrane permeability and their surface potential (Vigneault et al., 2000; Knauer and Buffle, 2001).

Moreover, environmental conditions such as metal or radiation stress may induce organisms to produce molecules that may act as a defense or originate from molecular damage due to the stress (Sarkar et al., 2006). These metabolites may create a particular microenvironment in the membrane for Hg^{2+} binding and their production could be particular to each environment.

Conclusion

In Andean Patagonian lakes, heterotrophic and autotrophic bacteria, nanoflagellates and a few small phytoplanktonic algae, comprised between 0.2 and 20 µm, constitute the main entry points of Hg^{2+} in the pelagic compartment. Their high surface indexes determine a great potential for Hg^{2+} absorption. Nevertheless, the uptake of Hg^{2+} by these organisms is ultimately the result of Hg^{2+} bioavailability which is controlled by several aspects of the water chemistry, such as the concentration and quality of DOM, among other factors.

Acknowledgements

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