



Tolerance of polar phytoplankton communities to metals



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ABSTRACT

Large amounts of pollutants reach polar regions, particularly the Arctic, impacting their communities. In this study we analyzed the toxic levels of Hg, Cd and Pb to natural phytoplankton communities of the Arctic and Southern Oceans, and compared their sensitivities with those observed on phytoplankton natural communities from temperate areas. Mercury was the most toxic metal for both Arctic and Antarctic communities, while both Cd and Pb were toxic only for the Antarctic phytoplankton. Total cell abundance of the populations forming the Arctic community increased under high Cd and Pb concentrations, probably due to a decrease of the grazing pressure or the increase of the most resistant species, although analysis of individual cells indicated that cell death was already induced at the highest levels. These results suggest that phytoplankton may have acquired adapting mechanisms to face high levels of Pb and Cd in the Arctic Ocean.

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1. Introduction

Polar oceans have been considered pristine areas far from industrial activities and other sources of anthropogenic pollutants. However, since the Industrial Revolution contaminants have reached both Arctic and Antarctic regions, mainly via atmospheric long-range transport (Duce et al., 1991; Macdonald et al., 2000), and specifically, from local mining activities in the Arctic (Bargagli, 2004; Braune et al., 2005; Lu et al., 2013; Presley, 1997). Among the major groups of contaminants reaching these areas, trace metals are of special interest due to their wide dispersal capacity and long-lived chemistry (Bargagli, 2004; Braune et al., 2005; Lu et al., 2013; Presley, 1997). Many metals are natural components of seawater and sediments (Bruland and Lohan, 2006), however some of them (i.e. Hg, Cd and Pb) have been transported and accumulated in the polar regions, presenting higher concentrations due to anthropogenic pollution (Bargagli, 2004; Braune et al., 2005; Lu et al., 2013; Presley, 1997). These toxic metals displace biogenic metals from their metabolic sites, entering into cells through the same transport systems (Bruland et al., 1991; Sunda and Huntsman, 1998) and denaturing protein molecules (Gadd and Griffiths, 1978). At very low concentrations, some toxic elements such as Cd can act as

nutrients due to their physical and chemical similarities with other Group 2 metals such as Zn (Price and Morel, 1990).

Combustion of fuels, particularly coal and gasoline, represent the main sources of anthropogenic emissions of Cd, Pb and Hg (Bargagli, 2004). In the Arctic, approximately 60–80 t of anthropogenic Hg is deposited annually, originated primarily from Eurasia and North America, and accumulating in Arctic biota (Braune et al., 2005). In Antarctica, no change in Hg concentrations has been observed in biota since the late 1980's (Bargagli, 2004). Anthropogenic emissions during last century have significantly altered the biogeochemical cycle of Cd and Pb in both Arctic and Antarctic regions (Bargagli, 2004; Braune et al., 2005; Lu et al., 2013; Presley, 1997).

Many biological parameters have been used to define the sublethal levels of trace metals in phytoplankton (Shaw, 1990), such as reduction of photosynthetic electron transport, inhibition of respiratory oxygen consumption or disruption of nutrient uptake processes, which may inhibit primary production in marine ecosystems (Davies, 1978; Thomas et al., 1980). Most of this knowledge has been attained through testing with cultured species, which often grow in mono-specific flasks, where competition for nutrient resources among species or grazing pressures are avoided (Shaw, 1990). Moreover, the long-term exposure to higher levels of pollutants in laboratories gives cultured species a higher resistance to pollution than natural communities (Echeveste et al., 2010a).

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The majority of studies on the toxicity of trace metals to natural marine phytoplankton have focused on the phytoplankton communities of temperate and cold areas of the Northern hemisphere (Echeveste et al., 2012; Knauer and Martin, 1972; Kuiper, 1981a, 1981b). These oligotrophic seawaters are generally dominated by pico-sized phytoplankton communities (Alonso-Laita and Agustí, 2006) and have a longer exposure history to pollution (Slaveykova et al., 2009; Zeri et al., 2000), which have derived in adapting processes in these communities (Echeveste et al., 2012). Until now there have not been records in polar communities, and one of the reasons of this lack could be due to the remoteness of these communities, which avoids an easy and economically affordable testing. Moreover, polar species are difficult to cultivate, decreasing drastically their survival after isolation.

The main objectives of this study are: (i) to quantify the toxicity of Hg, Cd and Pb on natural marine photosynthetic plankton of the Arctic and Southern Oceans, (ii) to compare the results obtained in this study with previous ones obtained in temperate regions where adapting processes to pollution were observed (Echeveste et al., 2012), (iii) to analyze cell size's role in determining phytoplankton sensitivity to pollutants, as it has been observed that contaminants' toxicity in phytoplankton depends on cell size (Echeveste et al., 2010a, 2010b, 2011, 2012).

2. Methods

The experiments to analyze the toxic thresholds of Cd, Pb and Hg on natural communities of phytoplankton were performed with Arctic and Southern Oceans plankton communities, sampled during the oceanographic cruises ATOS-I and ATOS-II, on board the RV Hespérides. The cruise ATOS-I took place in the Arctic Ocean from June 27 to July 27, 2007 (Departure: 64°08'N 21°56'W – Arrival: 78°13'N 15°33'E), while the cruise ATOS-II took place in the Southern Ocean from January 24 to March 2, 2009 (Departure: 53°10'S 70°56'W – Arrival: 54°49'S 68°19'W; for experimental coordinates, see Table 1).

Surface water (5 m) used in the experiments was sampled using Niskin bottles attached to a rosette-CTD system. Experiments began with the distribution of 2 L of sampled water into acid clean polycarbonate bottles. Subsequently Hg, Cd and Pb were inoculated at different concentrations in duplicate bottles, and incubated under natural solar radiation in an incubator with seawater surface running system to maintain ambient temperature conditions. Metal solutions for experiment bottles were prepared with filtered (<0.22 µm) seawater from Pb, Cd and Hg standard solutions of 1000 mg L⁻¹ (Scharlau Chermie S.A). Bottles were covered with a neutral net to simulate light conditions at 5 m depth. The final gradient concentrations of each metal were 0.05, 0.5, 5, 50 and 500 µg L⁻¹. Duplicate bottles without chemical additions were also run as experimental controls. Daily sampling was performed in the experiments for a duration of 6–8 days. A total of 6 experiments were performed, with one experiment of Cd, Pb and Hg performed in each water mass (Table 1).

Changes in total phytoplankton abundance during the experiments were followed by analyzing Chlorophyll *a* concentration (Annex 1.1). The effect of the trace metals on the different groups forming the phytoplankton communities was also analyzed. The changes in the abundance of nano- and micro-phytoplankton communities were analyzed by using a FlowCam (FlowCAM, Fluid Imaging, Inc., Edgecomb, ME, USA), a submersible flow cytometer and microscope (Annex 1.2), with the exception of the Hg experiment in the Arctic Ocean, where an epifluorescence microscope was used (Annex 1.3). Eukaryotic picophytoplankton community was also analyzed for the experiments performed in the Southern Ocean through flow cytometry (Annex 1.4). The proportion of living vs. dead cells in the picophytoplankton communities throughout the experiments were also followed by applying a cell membrane permeability test; the cell digestion assay (Agustí and Sánchez,

2002; Llabrés and Agustí, 2008), which allows the counting and identification of living phytoplankton cells (Annex 1.5).

Trace metal concentrations in the field stations were studied by sampling surface seawater (1 m depth) collected from a Zodiac deployed from the research vessel using clean protocols (Tovar-Sánchez, 2012). Seawater was pumped through acid-cleaned Teflon tubing coupled to a C-flex tubing (for the Cole–Parmer peristaltic pump head), filtered through an acid-cleaned polypropylene cartridge filter (0.22 µm, MSI, Calyx[®]), and collected in a 0.5 L LDPE bottle. Samples were acidified on board to pH < 2 with Ultrapure-grade HCl (Merck) in a class-100 HEPA laminar flow hood, and stored for at least one month before extraction. Metals (Cd and Pb) were pre-concentrated by the APDC/DDDC organic extraction method of Bruland et al. (1979), and analyzed by ICP-AES (Perkin Elmer Optima 5300 DV).

For total Pb cellular content analysis in field samples and end of incubation experiments, a volume of 50 ml seawater were collected at a depth of ~10 m using an acid-cleaned all-plastic 50 micron mesh plankton net deployed from the zodiac. In both, field and end of incubation experiments, samples were filtered through 0.22 µm acid clean polycarbonate filters under a class-100 laminar flow hood, stored frozen in Teflon microcentrifuge vials and transported back to the laboratory for acid digestion and analysis. Intracellular Pb levels were determined after washing a sub-sample of the collected phytoplankton with the oxalate reagent described by Tovar-Sánchez et al. (2003). Lead concentrations were determined by ICP-AES (Perkin Elmer Optima 5300 DV) after an acid-digestion (Tovar-Sánchez and Sañudo-Wilhelmy, 2011).

2.1. Statistical analysis and calculations

The dynamics of the communities in the different metal treatments were followed by analyzing the changes in the population abundance, the half lives ($t_{1/2}$) of the different species and size groups in each treatment were determined by observing the population decay and applying the following formula:

$$t_{1/2} = \ln 2/\mu \quad (1)$$

where μ is the slope of the line of the decay of cell abundance with time in days.

The 50% and 10% inhibition concentrations (IC50 and IC10, respectively) of Cd, Hg and Pb for each species tested were defined as the trace metal concentration at which the cell population will be inhibited by a half and 10%, respectively, and calculated using the following equations:

$$\text{IC50} = -\ln 0.5/\Omega \quad (2)$$

$$\text{IC10} = -\ln 0.9/\Omega \quad (3)$$

where Ω is the slope of the relationship between the natural logarithm of the decay of cell abundance and the trace metal concentration (µg L⁻¹) reached at the end of the experimental treatments.

Statistically significant differences among treatments and seawaters were determined by the analysis of variance (ANOVA) of data, complemented by the Dunnett Test, and performed at a 5% of confidence level ($p < 0.05$).

3. Results

Chlorophyll *a* concentrations in the surface waters of the Arctic and Southern Ocean waters were relatively high for oceanic values, ranging from 1.13 to 4.02 mg m⁻³ in the Arctic and from 0.57 to 3.44 mg m⁻³ in Antarctica (Table 1). In both seawaters, large nano- and microphytoplankton groups such as diatoms and flagellates dominated the phytoplankton community. In the Arctic Ocean, *Phaeocystis pouchetii* was also found, being dominant in the stations between the Fram strait and Svalbard Islands, while in the Southern Ocean picoeukaryotes and small nanoplankton were also present in the water masses. Cadmium and Pb concentrations in the Arctic Ocean were, on average, 0.034 and 0.006 µg L⁻¹, respectively, while Hg concentrations reported in these seawaters varied from <0.001 µg L⁻¹ to 0.004 µg L⁻¹ (Outridge et al., 2008; Schmidt and Freimann, 1984). In the Southern Ocean, Cd and Pb concentrations were 0.058 and 0.016 µg L⁻¹, respectively.

The addition of high concentrations of trace metals had a different effect on the phytoplankton communities depending on the metal and on the community studied, as observed by the changes in Chlorophyll *a* concentrations (indicative of phytoplankton biomass) among the experiments (Fig. 1). In the Southern Ocean, the addition of the lowest metal concentrations did not

Table 1
Date of sampling, position of the stations, metal used and abundance of phytoplankton (as Chlorophyll *a* concentration, Chl *a*, in mg m⁻³) of the experiments.

	Experiment	Coordinates	Date	Chl <i>a</i>
Arctic Ocean	Cd	80° 46' N–13° 26' E	07/18/2007	1.13
	Pb	72° 58' N–12° 39' W	07/03/2007	4.02
	Hg	78° 44' N–2° 58' E	07/07/2007	2.83
Southern Ocean	Cd	69° 02' S–75° 06' W	02/14/2009	0.57
	Pb	62° 39' S–59° 0' W	01/28/2009	2.72
	Hg	62° 10' S–57° 14' W	02/06/2009	3.44

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