



# Toxicity of natural mixtures of organic pollutants in temperate and polar marine phytoplankton



Pedro Echeveste <sup>a,\*</sup>, Cristóbal Galbán-Malagón <sup>b,c</sup>, Jordi Dachs <sup>c</sup>, Naiara Berrojalbiz <sup>c,d</sup>, Susana Agustí <sup>a,e</sup>

<sup>a</sup> Department of Global Change Research, Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Mallorca, Illes Balears, Spain

<sup>b</sup> Departamento de Ecología y Biodiversidad, Universidad Nacional Andrés Bello, Santiago, Chile

<sup>c</sup> Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Catalunya, Spain

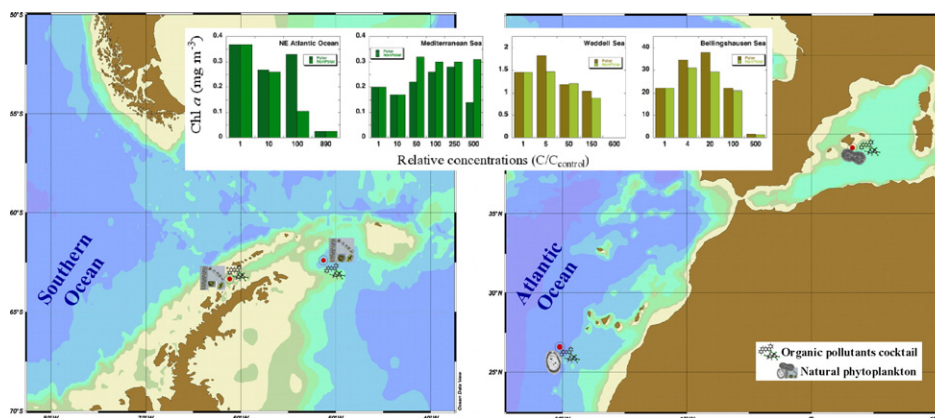
<sup>d</sup> Norges Geotekniske Institutt, Oslo, Norway

<sup>e</sup> Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

## HIGHLIGHTS

- Toxicity of naturally occurring complex mixtures of organic pollutants (OP) to temperate and polar phytoplankton communities from the Mediterranean Sea, North East Atlantic Ocean and Southern Ocean was tested.
- Toxicity was observed at significantly lower relative concentrations than simple OP mixtures, i.e., as low as 5 fold the oceanic concentrations.
- Overall, smaller sized picocyanobacteria (<2 μm diameter) of temperate seawaters were the most affected.
- In some cases, larger sized Antarctic phytoplankton (>20 μm diameter) showed higher sensitivity to OPS than Mediterranean picophytoplankton.
- Results suggest that current concentrations of OPs present in seawater are impacting natural marine phytoplankton communities.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Semivolatile and persistent organic pollutants (POPs) undergo atmospheric transport before being deposited to the oceans, where they partition to phytoplankton organic matter. The goal of this study was to determine the toxicity of naturally occurring complex mixtures of organic pollutants to temperate and polar phytoplankton communities from the Mediterranean Sea, the North East (NE) Atlantic, and Southern Oceans. The cell abundance of the different phytoplankton groups, chlorophyll *a* concentrations, viability of the cells, and growth and decay constants were monitored in response to addition of a range of concentrations of mixtures of organic pollutants obtained from seawater extracts. Almost all of the phytoplankton groups were significantly affected by the complex mixtures of non-polar and polar organic pollutants, with toxicity being greater for these mixtures than for

**Abbreviations:** POP, persistent organic pollutant; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; PBDE, polybrominated diphenylether; SALC, semivolatile aromatic-like compounds; GC-ECD, gas chromatography coupled to an electron capture detector; GC-MS, gas chromatography coupled to a mass spectrometer; CTD, Conductivity, Temperature, density; GFF, glass fiber filter; LC, lethal concentration.

\* Corresponding author at: Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile.

E-mail address: [echevestepedro@gmail.com](mailto:echevestepedro@gmail.com) (P. Echeveste).

<sup>1</sup> Present address: Millennium Institute of Oceanography, Chile.

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single POPs or simple POP mixtures. Cocktails' toxicity arose at concentrations as low as tenfold the field oceanic levels, probably due to a higher chemical activity of the mixture than of simple POPs mixtures. Overall, smaller cells were the most affected, although Mediterranean picophytoplankton was significantly more tolerant to non-polar POPs than picophytoplankton from the Atlantic Ocean or the Bellingshausen Sea microphytoplankton.

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## 1. Introduction

The last two centuries of social and economic development have resulted in the release of a myriad of synthetic organic compounds - from agricultural, industrial and household activities - into the environment (Stone et al., 2014). Due to their resistance to degradation and semi-volatility, persistent organic pollutants (POPs) can undergo long-range atmospheric transport before being deposited to remote regions, such as the global oceans (Dachs and Méjanelle, 2010; Iwata et al., 1993; Lohmann et al., 2007). POPs incorporate into planktonic food webs due to their chemical hydrophobicity (Berrojalbiz et al., 2011a; Galbán-Malagón et al., 2012, 2013; Nizzetto et al., 2012). Individual POPs impact marine phytoplankton by altering the carbon and silicate incorporation, the chlorophyll *a* content or by inhibiting photosystem I and interfering with the electron transport downstream from photosystem II (Marwood et al., 1999; Petersen et al., 2008). However, the influence of POPs mixtures on phytoplankton-mediated processes remains unclear and, therefore, needs to be explored via assessing POP effects on phytoplankton abundance and productivity.

The toxicity of organic pollutants to marine phytoplankton has been studied previously with both laboratory and field experiments, and with individual contaminants and simple mixtures of pollutants (Arrhenius et al., 2004; Carrera-Martínez et al., 2010; DeLorenzo and Serrano, 2003; Echeveste et al., 2010a; Faust et al., 2003; Fernández-Pinos et al., 2015; Magnusson et al., 2008; Pomati and Nizzetto, 2013; Walter et al., 2002). Some studies have assessed the effects of multi-chemical mixtures on phytoplankton, but they mostly focus on hydrocarbons from crude or refined fuel oils (Ansari et al., 1997; El-Sheekh et al., 2000; O'Brien and Dixon, 1976; Podkuiko, 2013; Sikkema et al., 1995; Taş et al., 2010). Only recently have researchers used complex mixtures of organic pollutants - reflecting marine environment background levels - to assess current impacts of POPs on biota (Echeveste et al., 2010b).

Oceanic regions differ in their nutrient levels, which determine the phytoplankton community composition. Oligotrophic seawaters are dominated by picophytoplanktonic organisms (Partensky et al., 1999; Scanlan and West, 2002), accounting for quarter of the photosynthetic carbon fixation in global oceans (Field et al., 1998; Flombaum et al., 2013). These ecosystems are expanding (Polovina et al., 2008), with the smallest algae thriving as a consequence of ongoing global change (Flombaum et al., 2013; Li et al., 2009). On the contrary, eutrophic seawaters are generally dominated by larger sized phytoplankton, mainly diatoms (Arrigo et al., 1999; Boyd et al., 2000), which account for >20% of the photosynthesis occurring in global oceans (Falkowski and Woodhead, 1992; Field et al., 1998). In the Southern Ocean, one of the relatively last pristine ecosystems on Earth, the phytoplankton communities may be less adapted to face anthropogenic pollution due to a shorter historical exposure, although this assumption merits further research (Kosek et al., 2016).

The objective of this study was to further elucidate the potential toxic effect of complex mixtures of organic pollutants in seawater on native phytoplankton communities. We analyzed this effect, at varying mixture concentrations, on the population abundance and cell death of phytoplankton communities from the Mediterranean Sea and Southern Ocean, and compared it to previous results with Atlantic Ocean communities (Echeveste et al., 2010b).

## 2. Materials and methods

### 2.1. Location of experiments and preparation of complex mixtures of POPs

To analyze the toxic levels of complex mixtures of organic pollutants on natural phytoplankton communities, three sets of experiments were performed: one in the coastal Mediterranean Sea (Faro'09 campaign, September 2009), and two in the Southern Ocean, one in the Weddell Sea and one in the Bellingshausen Sea (ATOS-II cruise, February 2009); both on board the RV Hespérides (ancillary data in Table A.1). Data from a similar experiment, performed previously in the NE Atlantic Ocean, was included in the discussion (Echeveste et al., 2010b).

The cocktails of organic pollutants in seawater were previously obtained by concentrating between 120 and 300 L of seawater using XAD-2 as an adsorbent. Seawater was taken from the continuous seawater pumping system of the ship, and was filtered with pre-baked glass fiber filters (GFF, 0.7  $\mu\text{m}$  nominal pore) before going through a stainless-steel column containing 60 g of XAD-2. The organic pollutants were extracted from the XAD-2 column as described elsewhere (Galbán-Malagón et al., 2013; Berrojalbiz et al., 2011a; González-Gaya et al., 2016). The extract was concentrated to 0.5 mL, and fractionated on an alumina column (3% deactivated) in three fractions. During the fractionation, the chemicals with large molecular weight and polarity are removed, as these chemicals are biogenic and interfere with the analysis of POPs and non-persistent organic pollutants such as PAHs. For example, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) cannot be analyzed properly if polar compounds (containing OH functional groups) are present in the fraction. Thus, a first fraction was eluted with hexane, followed by a second fraction eluted with hexane:dichloromethane (2:1). Finally, polar compounds were recovered with a third fraction eluted with dichloromethane:methanol (1:1). After concentrating these fractions to 50  $\mu\text{L}$ , PCBs, hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCBs), and PAHs were analyzed in the first and second fractions by gas chromatography coupled to an electron capture detector (GC-ECD) and gas chromatography coupled to a mass spectrometer (GC-MS), respectively. Details on the methods used have been described previously (Annex 1) (Galbán-Malagón et al., 2013; Berrojalbiz et al., 2011a, 2011b). The average recoveries for the four sampling cruises ranged from 62 to 75% for PCB65 and PCB 200 (surrogates of organochlorine compounds), and between 67 and 85% for d10-Phenanthrene and d12-Chrysene (surrogates of PAHs). The field blanks of XAD-2, processed using the same methodology as for field samples, showed concentrations of some 3–4 ring PAHs and lighter PCB congeners, but at concentrations much lower than field samples (Table 1 and Table A.2 for concentrations in field samples and blanks).

After performing the analysis of the organic pollutants, the first and second fractions were pooled and the solvent was transferred to acetone. An aliquot of the fraction corresponding to ~100 L of seawater was transferred to a vial and concentrated to 100  $\mu\text{L}$ . This "non-polar" fraction contained aliphatic and aromatic hydrocarbons, including PAHs and the aliphatic and aromatic unresolved complex mixtures, polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), and any other known or yet unidentified pollutants with similar polarity. The third fraction collected from the fractionation using methanol/dichloromethane as eluting solvents was referred to as

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