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Effects of TiO_2 nanoparticles and sunscreens on coastal marine microalgae: Ultraviolet radiation is key variable for toxicity assessment

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ABSTRACT

Given the large numbers of sunbathers on beaches, sunscreen compounds are being released into the coastal aquatic environment in significant amounts. Until now the effect of these potential pollutants on microbiota has been not well-known. Phytoplankton is a key component of the microbiota community. It forms the basis of the aquatic trophic networks, and any change in the natural population of phytoplankton can affect the structure of aquatic biota. This paper describes an experiment performed outdoors (in natural sunlight conditions including ultraviolet radiation (UVR) and with UVR blocked) on mixed microalgae populations (four species from different key marine taxonomic groups, *Nannochloropsis gaditana*, *Chaetoceros gracilis*, *Pleurochrysis roscoffensis and Amphidinium carterae*), for three days, exposed to a range of concentrations of three commercial sunscreens (with variable TiO_2 concentrations: highest concentration for sunscreen C, followed by sunscreen A; and sunscreen B did not contain TiO_2 in its composition).

With regard to UVR effect, in the absence of sunscreens, the most sensitive species is the centric diatom, *Chaetoceros gracilis*, and the least is *Nannochloropsis gaditana;* this last species presented the same behavior in the absence of UVR and with high sunscreen concentrations. The toxicity gradient obtained for sunscreens and nanoparticles under UVR is: TiO_2 NPs > Sunscreen C > Sunscreen A > Sunscreen B. The differential sensitivity of microalgae to sunscreens and TiO_2 NPs can produce a change in the dynamics of phytoplankton populations and provoke undesirable ecological effects (such as giving dinoflagellates more prominence). The effects of UVR, commonly neglected in bioassays, could alter the results in important ways and should be considered when performing environmentally-relevant bioassays. The toxicity mediated by hydrogen peroxide production associated with the concentration of TiO_2 NPs cannot be considered the only factor responsible for the toxicity: the organic compounds in the sunscreens must also be taken into account.

1. Introduction

Sunscreen compounds applied to the human skin provide effective protection against the damage caused by exposure to ultraviolet radiation (UVR) (Diffey, 2005). However, recent studies show toxicity of some UV organic filters to humans (Kunisue et al., 2012). The use of these cosmetics has increased considerably in recent years; and, in the sun/skincare market, sunscreens are currently the product category with the highest level of sales (Hall, 2001; Honey and Krantz, 2007). UV filters incorporated in the formulation of sunscreens may be organic and/or inorganic. Metal oxide nanoparticles (NPs) are commonly used as inorganic UV filters; only two of these, ZnO and TiO_2, have been approved under the relevant product safety regulations (Chisvert and Salvador, 2007).

Recent studies have shown that sunscreen components are able to reach the marine environment after being released from human skin during swimming or washing, via wastewater treatment plants (Giokas et al., 2007; Gondikas et al., 2014; Tovar-Sánchez et al., 2013). Given their lipophilic properties, persistence and stability against biodegradation, organisms can accumulate the components from sunscreens and, in this way, they could reach food webs (Díaz-Cruz and Barceló, 2009; Fent et al., 2010; Santos et al., 2012). In fact, bioaccumulation, hormonal effects and genotoxicity caused by the chemicals from sunscreens have been detected in fish and molluscs (Fent et al., 2008; Gomez et al., 2012; Gonzalez et al., 2008). On the other hand, sunscreens may release high concentrations of inorganic nutrients capable of being used by phytoplankton and thus of enhancing microalgae growth (Tovar-Sánchez et al., 2013).

Due to the complexity and variety of sunscreen formulations, existing studies on the toxicity of these contaminants for phytoplankton have considered specific compounds, such as benzophenone-3 (BZ-3),

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octyl methoxycinnamate (OMC), 3-benzylidene camphor (3-BC), 4-methylbenzylidene camphor (4-MBC), isoamyl p-methoxycinnamate (IMC) or p-aminobenzoic acid (PABA), and mostly in freshwater species (Rodil et al., 2009; Sieratowicz et al., 2011). However, studies on the effect of sunscreen components in marine phytoplankton are still scarce (Paredes et al., 2014; Sánchez-Quiles and Tovar-Sánchez, 2014).

Regarding inorganic UV-filters, some authors have suggested that the toxicity of TiO_2 NPs in freshwater organisms could be due to their photochemical properties (Hund-Rinke and Simon, 2006; Li et al., 2014; Mansfield et al., 2015). Sunscreen formulations generally mix the anatase and rutile forms of TiO_2, which are the most frequent and photoreactive (D, 1999; Shao and Schlossman, 1999). Under UVR, these NPs can generate very high concentrations of reactive oxygen species (ROS) which could inhibit the growth of freshwater and marine phytoplankton (Chaudhuri and Majewski, 1998; Handy et al., 2008; Wang et al., 2016). Moreover, some organic UV-filters (e.g. octocrylene (OCR), OMC and PABA) can also produce ROS (Aliwell et al., 1994; Allen et al., 1996; Cantrell et al., 2001; Hu et al., 1995). Elevated concentrations of hydrogen peroxide (H_2O_2) cause cell wall or membrane damage (Labille et al., 2010), lipid peroxidation, growth inhibition or a decrease in the percentage of healthy cells in microalgae populations (Chaudhuri and Majewski, 1998), even if NPs are not internalized in cells (Chaudhuri and Majewski, 1998; Handy et al., 2008). Additionally, NPs can be adsorbed onto algal cell surfaces, and this can also reduce growth by physical shading effects (Hund-Rinke and Simon, 2006; Wang et al., 2016) and the additional weight of the NPs can force the sedimentation of algae to non-photic zones (Huang, 2005; SS, 2005).

In the work reported here, we have hypothesized that succession in the phytoplankton community is adversely affected by sunscreens whose composition includes TiO_2 as the main inorganic UV-filter, under solar light radiation, and that this effect is mediated by H_2O_2 production.

2. Materials and methods

2.1. Experiments in which sunscreens and TiO_2 NPs have been added to seawater

Incubation experiments were performed to test the response of coastal planktonic communities to the addition of sunscreens and TiO_2 NPs, under a daily summer solar cycle. Microalgae species were chosen with a view to including the most important groups of planktonic algae found in marine environments. The four species selected were *Chaetoceros gracilis* (Bacillariophyceae); *Amphidinium carterae* (Dinophyceae); *Pleurochrysis roscoffensis* (Primnesiophycae); and *Nannochloropsis gaditana* (Eustigmatophyceae). Diatoms, dinoflagellates and coccolithophorids are the main components of phytoplankton in marine environments. Centric diatoms and coccolithophores are the most common microalgae in oceans, with the centric diatoms being the most obiquitous taxons, while coccolithophores play a key role in the 'biological pump" (Not and others 2012). Dinoflagellates are the second most important component of the phytoplankton community in oceans, this group is cosmopolitan in eutrophic coastal and continental shelf waters and contains species that have the capacity to form large blooms (Honer, 2002). In addition, Morán et al. (2010) have demonstrated that small cells, such as nanoplankton communities (2–20 μm), experiment gradual dominance in warmer oceans and need to be considering in ecological studies (Morán et al., 2010). We therefore consider that mixing of those four phytoplankton groups could provide a good proxy of ecological coastal scenario.

Microalgae were obtained from the ICMAN Marine Microalgae Culture Collection (MMCC). Cells were grown in filtered (0.2 μm) seawater enriched with f/2 medium (Guillard and Ryther, 1962) lacking EDTA, for two weeks prior to the experiment. An initial cellular density of 10^3 per mL (for each species) was chosen for the experiment. Cultures of the four species were mixed and added in a batch of 600 mL to 60 L of filtered 0.2 μm seawater with 150 mg L^{-1} of $NaNO_3$, 10 mg L^{-1} of $PO_4H_2Na \cdot 2H_20$ and 50 mg L^{-1} of SiO_2 as nutrients (Araujo et al., 2010).

Incubations were carried out in 2 L Teflon (PTFE) bags submerged in 400 L PVC tanks located outdoor, under direct solar exposure. A saline well-water recirculation system was designed in order to keep the water temperature constant (20 ± 0.5 °C). pH was measured at the beginning (8.1) and at the end (7.1) of the experiment. Salinity, recorded with a seawater refractometer HI 96822 (HANNA), was constant over the experiments (i.e. 36 psu). Organic matter measured as TOC with a TOC analyzer Shimadzu TOC-V CHS was 1.5 mg L^{-1}. For treatments with UVR blocked, tanks were covered with a visible-light transparent plastic UV filter (Lee brand, model 226). All treatments were inoculated with 500 mL of microalgae cultures and exposed to the experimental conditions for 75 h in the tanks. Double controls (with microalgae culture and with seawater without algae) were run, as well as four treatments (3 commercial sunscreens and another with TiO_2 NPs). Both the treatments and controls were carried out in triplicate. Treatments were inoculated with four different concentration of sunscreens (1, 10, 100 and 200 mg L^{-1}) or TiO_2 NPs (1, 2, 5 and 10 mg L^{-1}). Lowest concentrations were within environmentally-relevant concentrations (Diffey, 2001; Tovar-Sánchez et al., 2013). Three different commercial sunscreens were used (details of the sunscreens selected are included in Table S1 and S4 (supporting material). TiO_2 NPs used were a mixture of anatase/rutile which is the most common mineral used in the sunscreen formulations (Barker and Branch, 2008). TiO_2 NPs were obtained from Sigma Aldrich (634662-25G-nano-powder, Anatase/Rutile: 79/21%, particle size < 100 nm). UVR levels in incubation tanks were monitored using an UV radiometer (PCE-UV34, PCE Instruments).

2.2. Characterization of TiO_2 NPs

Textural characterization of samples was carried out by measuring the absorption/desorption of N_2 at 196 °C, employing a Micromeritics ASAP 2010 automatic device. Before measurements, samples were submitted to a surface cleaning pre-treatment under high vacuum at 200 °C during 2 h. The obtained isotherms were used to calculate the specific surface area (S_{BET}) as well as the micro- and meso-porosity features of studied samples.

Initial particle size of TiO_2 NPs, as well as zeta potential of TiO_2 was studied in ultrapure water and artificial marine water through Dynamic Light Scattering (Zetasizer nano ZS90, Malvern, and its software version 7.10) at 1 mg·L^{-1}. TiO_2 NPs dispersion was prepared in ultrapure water, following the Standard protocol CEINT/NIST 1200-3 and 1200-4 (Taurozzi et al., 2012a; Taurozzi et al., 2012b). In artificial marine water polydispersity index (PdI) was higher than 0.3 so particle size was measured by a Master Sizer 2000, Malvern with the software version 5.61. Particle size, shape and structure were confirmed by Transmission Electron microscopy (TEM) for 130 particles. Table S2 and Fig. S1 (supporting material).

2.3. Cell abundance and H_2O_2 analysis

Aliquots of 5 mL were taken at 0, 8, 24, 48 and 72 h and fixed with glutaraldehyde + formaldehyde (10%) for cellular density determination. A flow cytometer (FACScalibur, Beckton-Dickinson) equipped with a 488-nm excitation argon laser was used for this purpose. The trigger was set on the red fluorescence (chlorophyll auto-fluorescence, FL3). Samples were analyzed in high flow for 300 s or 10^6 events.

Samples (5 mL) were also collected every 6 h, filtered through a 0.22 μm polypropylene filter and immediately analyzed after collection for H_2O_2 measurements. H_2O_2 levels were determined following (Price et al., 1994): Briefly, a flow injection system with chemi-luminiscence detection (Felume System Waterville, ME) was used to monitor the oxidation of luminol at pH 10.8 in the presence of H_2O_2 using cobalt (II) as the catalyst. The concentration of the stock H_2O_2 solution was