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Ecotoxicity of the lipid-lowering drug bezafibrate on the bioenergetics and lipid metabolism of the diatom *Phaeodactylum tricornutum*

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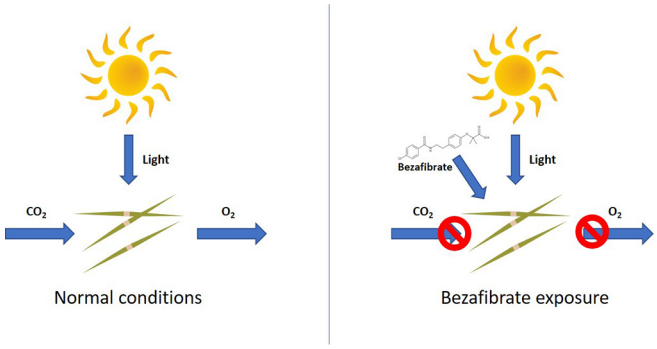
HIGHLIGHTS

- High Bezafibrate concentrations increased cell density, promoted by a shift from autotrophic to mixotrophic metabolism.
- Bezafibrate can be used by diatoms as carbon source, along with light-generated redox potential.
- The concentrations of plastidial marker fatty acids showed negative correlations with Bezafibrate exposure.
- This metabolic shift derived from bezafibrate exposure may reduce O_2 generation and CO_2 fixation.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical residues impose a new and emerging threat to the marine environment and its biota. In most countries, ecotoxicity tests are not required for all pharmaceutical residues classes and, even when mandatory, these tests are not performed using marine primary producers such as diatoms. These microalgae are among the most abundant class of primary producers in the marine realm and key players in the marine trophic web. Blood-lipid-lowering agents such as bezafibrate and its derivatives are among the most prescribed drugs and most frequently found human pharmaceuticals in aquatic environments. The present study aims to investigate the bezafibrate ecotoxicity and its effects on primary productivity and lipid metabolism, at environmentally relevant concentrations, using the model diatom *Phaeodactylum tricornutum*. Under controlled conditions, diatom cultures were exposed to bezafibrate at 0, 3, 6, 30 and 60 μg L^{-1}, representing concentrations that can be found in the vicinity of discharges of wastewater treatment plants. High bezafibrate concentrations increased cell density and are suggested to promote a shift from autotrophic to mixotrophic metabolism, with diatoms using light energy generated redox potential to breakdown bezafibrate as carbon source. This was supported by an evident increase in cell density coupled with an impairment of the thylakoid electron transport and consequent photosynthetic activity reduction. In agreement, the concentrations of plastidial marker fatty acids showed

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negative correlations and Canonical Analysis of Principal coordinates of the relative abundances of fatty acid and photochemical data allowed the separation of controls and cells exposed to bezafibrate with high classification efficiency, namely for photochemical traits, suggesting their validity as suitable biomarkers of bezafibrate exposure. Further evaluations of the occurrence of a metabolic shift in diatoms due to exposure to bezafibrate is paramount, as ultimately it may reduce O_2 generation and CO_2 fixation in aquatic ecosystems with ensuing consequences for neighboring heterotrophic organisms.

1. Introduction

Over the last decades significant attention has been directed to the effects of the so-called "classical contaminants" (e.g. trace metals) in marine biota and their metabolism (Thomaidis et al., 2012). Recently, there are suites of contaminants of emerging concern that present new challenges and risks to natural ecosystems, as the result of the continuous uncontrolled development of multiple human activities (Gavrilescu et al., 2015). Emerging pollutants (EPs) include a wide range of man-made chemicals used daily worldwide (such as pesticides, cosmetics, personal and household care products, pharmaceuticals) (Thomaidis et al., 2012). Between 2002 and 2011, over 50% of the total production of chemicals comprised environmentally harmful compounds, of which over 70% have significant environmental impact (EUROSTAT, 2017). The continued increase in the chemical synthesis industry, reflects the speed of synthetic chemical innovation (CAS, 2011), with several of these novel substances rising concerns regarding their ecotoxicological effects and how to assess them efficiently.

In comparison with classical contaminants, and in large part due to the analytical procedures required, only more recently has there been a commitment to study the release and effects of pharmaceuticals from sewage and other land-base sources into coastal and marine environments (Gaw et al., 2014), in particular near the outfalls of treated and untreated sewage outfalls where pharmaceutical residues can appear in particularly high concentrations (namely at $\mu g/L^{-1}$ concentrations) (Reis-Santos et al., 2018; Thomas and Hilton, 2004). Blood-lipid-lowering agents such as bezafibrate and the metabolization production of different fibrates, are among the most commonly prescribed drugs and also among the most frequently detected human pharmaceuticals in the aquatic environment (Weston et al., 2009). Fibrates are widely used to treat lipidemic diseases such as hypercholesterolemia and to prevent heart attack (Weston et al., 2009). In the specific case of bezafibrate, maximal concentrations of up to 4.6 and 3.1 $\mu g\ L^{-1}$ have been found in wastewater and surface waters, respectively (Fent et al., 2006; Reis-Santos et al., 2018; Thomas and Hilton, 2004). Yet, most of the available ecotoxicity data for these compounds has been obtained from freshwater organisms (Minguez et al., 2016) with the risk posed by these pharmaceutical compounds to coastal and marine environments and biota still poorly documented. Ecotoxicological assays with bezafibrate have shown immobilization EC50 (half maximal effective concentrations) for *Daphnia magna* ranging from 30.3 to 240.4 mg L^{-1}, while for *Thamnocephalus platyurus* and *Anabaena* sp. EC50 were 39.69 and 7.62 mg L^{-1}, respectively (Han et al., 2006; Isidori et al., 2007; Rosal et al., 2010). Claessens et al. (2013) determined an EC50 dose of bezafibrate for *P. tricornutum* of 0.355 mg L^{-1}. Ultimately, baseline ecotoxicological data on marine organisms is paramount to delineate adequate measures to safeguard marine environments (Marchand and Tissier, 2007; Minguez et al., 2016).

At the base of every marine system are the phototrophs, cycling the energy of the sun, soaking carbon and fueling the trophic web. Any disturbance at this level has inevitable impacts throughout the marine ecosystems. Emerging pollutants are known to impair the photosynthetic metabolism of phototrophic organisms (Anjum et al., 2016; Cabrita et al., 2016; Santos et al., 2014). Although these impacts are well described at sub-cellular level for some contaminants, like metals and metalloids (see Anjum et al., 2016, and references herein), to the best of our knowledge, no photochemical-based ecotoxicity studies have yet been done regarding pharmaceuticals. Remote sensing techniques such as Pulse amplitude modulated (PAM) fluorometry arise as potential non-invasive high-throughput screening (HTS) tools (Cabrita et al., 2017; Santos et al., 2014) to evaluate ecotoxicity in phototrophic organisms. These techniques evaluate the photonic energy harvest and transformation processes into electronic energy, using the involved pigments, their fluorescence and spectral signatures signals as proxy (Anjum et al., 2016; Cabrita et al., 2016; Santos et al., 2014). Any change at the primary productivity level can be efficiently assessed by these techniques (Duarte et al., 2017b; Feijão et al., 2017), and have proved to evaluate contaminants' effects at a physiological level with a dose-related response (Anjum et al., 2016; Cabrita et al., 2017; Santos et al., 2014).

Marine microalgae are promising biomonitor organisms, having simultaneously a high ecological importance as base of marine food webs (Guschina and Harwood, 2009a), and have been shown to act as bioindicator of disturbance under natural conditions and extreme contamination events (Cabrita et al., 2017, 2016, 2014). Specifically, phytoplankton is probably the first compartment to be affected by contaminants. As small (0.2–200 μm) single or chain-forming cells suspended in water, phytoplankton have very high surface-to-volume ratios, respond quickly to suspended toxicants with high uptake rates, and therefore can provide sensitive and effective biomarkers of contaminant stress (Cabrita, 2014; Cabrita et al., 2017, 2016; Gameiro et al., 2016). Additionally phytoplankton are the major marine producers of many complex biomolecules, including fatty acids present in diverse lipid classes (Guschina and Harwood, 2009b). Photosynthetic organisms can synthetize linoleic- and linolenic acids, which belong to omega-6 (ω-6) and omega-3 (ω-3) classes, respectively, and are essential fatty acids (EFA) for vertebrates. EFA are precursors of long chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play key roles in various biological functions, including heart health, immune and inflammatory responses, visual acuity, as well as being major components of neurological tissues (see review from Wiktorowska-Owczarek et al. (2015)). Since most organisms in the higher trophic levels of marine food webs, such as fish and humans, have limited ability to produce LC-PUFA from EFA, they obtain them through diet, relying on their de novo production by aquatic algae (Arts et al., 2001).

Due to their widespread presence in the marine environment, it is paramount to evaluate the effects of pharmaceuticals at the primary productivity level. In the present study, we aim to assess the ecotoxicological effects of bezafibrate exposure, at environmentally relevant concentrations such as the ones found in the vicinity of wastewater outfalls, in the primary productivity and fatty acid composition of a cosmopolitan model diatom *Phaeodactylum tricornutum*, and its potential impact on coastal marine or estuarine ecosystems.

2. Material and methods

2.1. Experimental setup

Monoclonal cultures of model diatom *P. tricornutum* Bohlin (Bacillariophyceae) (IO 108–01, IPMA) were grown in 250 ml of f/2 medium (Guillard and Ryther, 1962) under controlled conditions for 6 days