Marine Pollution Bulletin 126 (2018) 241–249

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Impact of two plastic-derived chemicals, the Bisphenol A and the di-2-ethylhexyl phthalate, exposure on the marine toxic dinoflagellate *Alexandrium pacificum*

Charaf M'Rabet[a,b,*], Olivier Pringault[b], Habiba Zmerli-Triki[a], Héla Ben Gharbia[a,b], Douglas Couet[b], Ons Kéfi-Daly Yahia[a]

[a] Tunisian National Agronomic Institute (INAT), U.R 13ES36 - Marine Biology (El Manar I), IRESA - Carthage University, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia
[b] UMR 9190 MARBEC IRD-Ifremer-CNRS-Université de Montpellier, Place Eugène Bataillon, Case 093, 34095 Montpellier Cedex 5, France

ARTICLE INFO

Keywords:
A. pacificum
BPA
DEHP
Photosynthetic activity
Abundance
Recovery

ABSTRACT

The effects of two plastic-derived chemicals: Bisphenol A (BPA) and di-2-ethylhexyl phthalate (DEHP) were assessed on abundance and physiological responses of the marine toxic dinoflagellate *Alexandrim pacificum*. During 7 days experiment, *A. pacificum* was exposed to different levels of BPA and DEHP (separately and in mixture). The responses were evaluated and compared with controls. Results showed that *A. pacificum* was highly sensitive to this contaminants comparing to other phytoplankton species. BPA and DEHP caused the decrease of the biomass (1.2 to 50 times lower relative to the controls), as well as the perturbation of the photosystem and the photosynthetic activity. Nevertheless, our results show a recovery of contaminated cells activity depending on exposure time and BPA and DEHP contamination. This could be related to an adaptation to induced stress or a degradation of BPA and DEHP in the medium.

1. Introduction

Plastic pollution is one of the most important environmental issues in the aquatic ecosystem (Derraik, 2002; Eriksen et al., 2013; Vince and Hardesty, 2016). Due to their durability and persistence, plastic particles could create disposal problems, such as physical harm through the ingestion and the entanglement of aquatic organisms and the transport of toxic chemicals to living being (GESAMP, 2015; Lee et al., 2013). During their accumulation in the ecosystem, plastic components degrade and release plastic-derived chemicals, such as bisphenol A (BPA) and di-2-ethylhexyl phthalate (DEHP) (Kako et al., 2014; Law, 2010; Lusher et al., 2014). BPA and DEHP are used as plasticizers or additives for the production of different resins, such as polyester resins and polyacrylate resins (Chapin et al., 2008; Cooper et al., 2011; Fasano et al., 2012). With the industrialization and the demographic development, the use of plastic (including plastic derivatives) increased significantly (Dargnat et al., 2009; Eerkes-Medrano et al., 2015; Ivar Do Sul and Costa, 2014). These substances take a long period of time to degrade (hundreds to thousands of years) (Mansui et al., 2015). Even more, this process releases other ester components, which are becoming omnipresent and impacting marine food webs (Mansui et al., 2015; Oberbeckmann et al., 2014). Despite being listed as pollutants by many authorities (US-Environmental Protection Agency, European Union, Chinese waters list), BPA and DEHP substances are still being released into the aquatic and terrestrial environments alike (Barnes et al., 2009; Dargnat et al., 2009; Liu et al., 2010a). These substances are found in relatively low concentrations (about dozens of ng/L to hundreds of µg/L) in aquatic environment: coastal sea-waters (Basheer et al., 2004), bay (Paluselli et al., 2017), rivers and surface marine water (Huang et al., 2012; Liu et al., 2009, 2010b).

Phytoplankton plays a major role in the aquatic ecosystems. It is the base of aquatic ecosystems, as a primary producer which is the major element in food web structuration and dynamics (Cloern, 1996). Phytoplankton includes a large variety of micro-organisms responsible for biogeochemical cycle, such as the metabolism of a large part of the atmospheric oxygen through the photosynthetic activity (Hays et al., 2005; Pauly and Christensen, 1995). Within phytoplankton community, dinoflagellates are one of the most common classes. They are responsible for the marine harmful algal blooms (HABs). Among the involved species, there is *Alexandrium* genus, particularly *Alexandrium catenella*, which is one of the most common blooming species (Laabir et al., 2013; Rolland et al., 2012; Zmerli-Triki et al., 2015). *Alexandrium*

* Corresponding author at: Tunisian National Agronomic Institute (INAT), U.R 13ES36 - Marine Biology (El Manar I), IRESA - Carthage University, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia.
E-mail address: charaf.mrabet@gmail.com (C. M'Rabet).

https://doi.org/10.1016/j.marpolbul.2017.10.090
Received 5 July 2017; Received in revised form 30 October 2017; Accepted 30 October 2017

genus is known for the saxitoxins production, which impacts not only aquatic ecosystems, but also human health through the contamination with paralytic shellfish poisoning (PSP). The PSP causes human intoxications and death due to the consumption of contaminated shellfish, as well as the deterioration of trophic levels and the restriction of recreational and touristic activities (Anderson et al., 2012). Literature related to *Alexandrium*, focused on analyzing and identifying toxins and their repercussions on marine organisms (Armi et al., 2012; Laabir et al., 2013; Turki et al., 2014), on monitoring bloom activity (Laanaia et al., 2013; Lilly et al., 2002) and on studying the interaction between this dinoflagellate and toxic chemicals, such as the inhibition of cell growth by copper and the fluorescence perturbation by polycyclic aromatic hydrocarbons (Ben Othman et al., 2012; Herzi et al., 2013; Laabir et al., 2011).

Even at smaller sizes and lower concentrations, plastic litters and derivatives chemicals impact negatively phytoplankton. Liu et al. (2010a) showed that the contents protein, polysaccharides and lipid were declined by pollutants exposure (Nonylphenols and Bisphenol A). Also, according to Perron and Juneau (2011), the photosynthetic activity of phytoplankton is greatly sensitive to pollutants. They showed that the alkyl-phenol "4-nonylphenol (4-NP)" perturb and block the electron transport chain in photosystem II (*PSII*) of the green algae *Chlamydomonas reinhardtii* and the cyanobacteria *Microcystis aeruginosa*. Plastic derived-chemicals may provoke also dramatic effects as other compounds. For example, polychlorinated biphenyls (PCBs) and polycyclic aromatic carbons (PAHs) perturb the abundance and the physiology of vegetative cells of phytoplankton (Echeveste et al., 2010; Leitão et al., 2003). Despite its broad presence and serious toxicity on phytoplankton, the impact of plastic-derived chemicals is still poorly studied (Castañeda and Avlijas, 2014; Lusher et al., 2014). Essentially, the studies carried out were, about the ability of some phytoplankton species to accumulate and degrade plastic-derivatives (Chi et al., 2007; Kang et al., 2006; Staniszewska et al., 2015) or about the eco-toxicological answer of some phytoplankton species to high concentration of BPA (up to 3 mg/L) (Ebenezer and Ki, 2012; Li et al., 2009; Liu et al., 2010a).

Nowadays, and to our knowledge, no research attempted to study the eco-toxicological answer of *Alexandrium* to a contamination with plastic-derived chemicals. Considering the occurrence of this toxic dinoflagellate in ecosystems (where plastic pollution is huge), and the important ecological and economical role of *Alexandrium*'s blooms, it is essential to study the consequences of these plastic-derived chemicals on this phytoplankton species. Therefore, the present work aimed to evaluate the effects of two plastic derived chemicals: the BPA and the DEHP, both separately and combined, on the growth and the photosynthetic activity of the toxic marine dinoflagellate: *Alexandrium pacificum*. For that purpose, culture of *A. pacificum* was performed at concentrations simulating critical cases of strong chronic contamination in coastal marine ecosystems.

2. Materials and methods

2.1. A. pacificum culture establishment

A mixotrophic planktonic toxic dinoflagellate *Alexandrium pacificum* Litaker sp. nov (Group IV) (ABZ1) (former *Alexandrium catenella* Anderson et al., 2012; John et al., 2014) was isolated from Bizerte Lagoon (Northern Tunisia, 37.198°N, 9.863°E) (Fertouna-Bellakhal et al., 2015). The strain was employed as non-axenic culture in the present study. Different glass Erlenmeyer flasks capped with cotton plugs and filled with 250 mL Enriched Natural Sea Water medium (ESNW) were inoculated with 300·vegetative·cell·mL^{-1} of *A. pacificum*. All cultures were maintained at stable conditions of salinity of 36, 21 °C and an irradiance of 100 µmol photons·m^{-2}·s^{-1} on a 12:12 light: dark cycle (Harrison et al., 1980).

2.2. Chemicals analyses

Analysis of BPA was performed by LC/MSMS in a negative ionization mode (UPLC Acquity; MSMS-Quattro Premier XE, Waters). The cartridge used was an Acquity UPLC BEH C18 (50 mm × 2,1 mm ID × 1,7 µm of granulomere, Waters). Direct injection volume was set at 40 µL. For DEHP, Solid-Phase Micro-Extraction (SPME-PDMS 100 µm fibre-Supelco) was used for the extraction of water samples. Then, the analysis was performed with GC/MS working in electro-ionization impact mode (GC-7890A; MSD-5975C, Agilent Technologies) using a HP5MS-UI column (5% phenyl methyl siloxane, 30 m × 0,25 mm I.D × 0,25 µm of phase thickness - Agilent Technologies).

2.3. Experimental design

Experiments were conducted to study growth and physiological response of *A. pacificum* to two different plastic contaminants BPA and DEHP. BPA and DEHP were added to each treatment flask separately (BPA and DEHP) and then mixed (BPA + DEHP), at different nominal concentration levels: BPA at 2 µg/L^{-1} and 20µg/L^{-1}, DEHP at 1 µg/L^{-1} and 10 µg/L^{-1} and finally BPA 2 µg/L^{-1} with DEHP 1 µg/L^{-1}, BPA 20 µg/L^{-1} with DEHP 10 µg/L^{-1}. These concentrations were chosen to mimic as much as possible a chronic or an accidental contamination from rivers to coastal marine environment. These concentrations are of course ten times higher to what it is observed in coastal marine ecosystem (where concentrations of BPA and DEHP are about dozens to hundreds of ng/L) but they are to much lower than river's concentration (where concentrations of BPA and DEHP could reach dozens of mg/L). Triplicates were used for each experimental contaminant level and also control flask with no contaminant addition (21 experimental Erlenmeyer flasks).

2.4. Physiological response of A. pacificum to different contaminants

The experiment began when BPA and DEHP were added simultaneously in all treatment flasks with 300 cells of *A. pacificum*/mL. Algal growth and physiological parameters monitoring was conducted for a week with 24 h intervals (0 h, 24 h, 48 h, 72 h, 96 h and 7 d). Factors monitored during experiments were cell density, growth rate, Chlorophyll *a*, photosynthetic performance (F_V/F_M, light response curves, α, E_K and P_{MAX}) and oxygen (O_2) metabolism.

2.4.1. Cell density, growth rate and Chlorophyll a

Cell number was counted with a Sedgewick rafter cell under an inverted microscope (Leica 521,234). Growth rates were calculated using the following equation: $GR = (LnN_2 - LnN_1) / (T_2 - T_1)$ where GR is the growth rate per day, N_2 and N_1 represent the cell density (cells·mL^{-1}) at the beginning of the culture (T_1) and the end (T_2) of the exponential phase (Guillard et al., 1973). To evaluate the influence of the plastic derivatives (BPA and DEHP) on cell pigmentation, five milliliters from each culture were filtered using a 25 mm GF/F filter. Chlorophyll *a* was extracted using 5 mL of 90% acetone after cooling for 24 h at 4 °C. A subsample was analyzed using Trilogy Turner Fluorometer (Model #7200-000) and Chl *a* concentration was expressed as µg Chl *a*·cell^{-1}(Neveux, 1976).

2.4.2. Photosynthetic performance measurement

2.4.2.1. Maximum quantum yield of photosystem II (FV/FM). In vivo chlorophyll fluorescence was measured using Aquapen AP-100 (Photon Systems Instruments). Samples were dark-adapted for at least 30 min in order to disable the electron transfer between photosystems and so the photosynthesis activity according to the manufacturer's operating manual. The OJIP-transient protocol was used to estimate photosynthetic efficiency via the maximum quantum yield of photosynthesis for stable charge separation of *PSII*: F_V/F_M. This ratio is a recognized cell stress indicator (Goiris et al., 2015). It was