Science of the Total Environment 610–611 (2018) 356–366

Contents lists available at ScienceDirect

Science of the Total Environment

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

Exposure of engineered nanoparticles to *Alexandrium tamarense* (Dinophyceae): Healthy impacts of nanoparticles via toxin-producing dinoflagellate

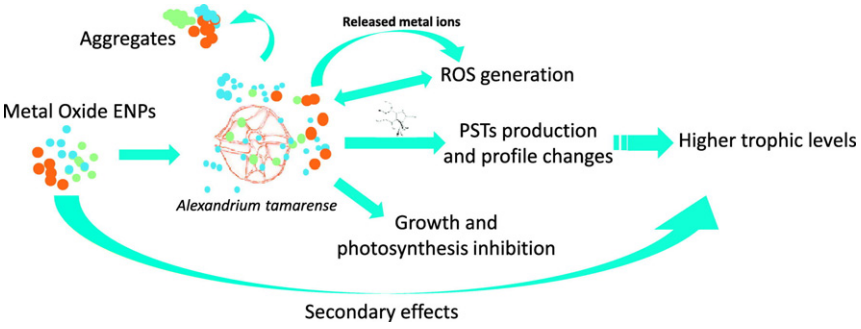
Manlu Li [a,b], Daoyi Chen [a,b], Yang Liu [c,d], Chia Ying Chuang [e], Fanzhou Kong [c,f], Paul J. Harrison [g], Xiaoshan Zhu [a,b], Yuelu Jiang [a,b,*]

[a] Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China
[b] School of Environment, Tsinghua University, Beijing 100084, China
[c] Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[d] University of Chinese Academy of Sciences, Beijing 100039, China
[e] Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan
[f] Laboratory of Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China
[g] Dept Earth & Ocean Sciences, University of British Columbia, Vancouver, BC, Canada

HIGHLIGHTS

- The PSTs production increased upon exposure to $_nTiO_2$ and $_nAl_2O_3$, but not to $_n$ZnO.
- All three selected metal oxide ENPs were proven to change the PSTs composition.
- The F_v/F_m was inhibited upon exposure to three ENPs with concentration ranging from 20 to 200 mg L^{-1}.
- $_n$ZnO showed the most toxic effect on cell growth among three selected ENPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 February 2017
Received in revised form 17 May 2017
Accepted 19 May 2017
Available online 11 August 2017

Editor: Henner Hollert

Keywords:
Engineered nanoparticles
Growth
Photosynthesis performance
Paralytic shellfish poisoning toxins
Reactive oxygen species

ABSTRACT

Human activities can enhance the frequency, intensity and occurrence of harmful algal blooms (HABs). Engineered nanoparticles (ENPs), contained in many materials, will inevitably enter coastal waters and thus cause unpredictable impacts on aquatic organisms. However, knowledge of the influence of ENPs on HAB species is still lacking. In this study, we examined the effects of titanium dioxide nanoparticles ($_nTiO_2$), zinc oxide nanoparticles ($_n$ZnO) and aluminum oxide nanoparticles ($_nAl_2O_3$) on physiological changes and paralytic shellfish poisoning toxins (PSTs) production of *Alexandrium tamarense*. We found a dose-dependent decrease in photosynthetic activity of *A. tamarense* under all three ENPs and a significant growth inhibition induced by $_n$ZnO. The largest reactive oxygen species (ROS) production was induced by $_nTiO_2$, followed by $_n$ZnO and $_nAl_2O_3$. Moreover, the PSTs production rate increased by 3.9-fold for $_nTiO_2$ ($p < 0.01$) and 4.5-fold for $_nAl_2O_3$ ($p < 0.01$) at a concentration of 200 mg L^{-1}. The major component, C2 was transformed to its epimer C1 and the proportion of decarbamoyl toxins increased under 200 mg L^{-1} of $_n$ZnO and $_nAl_2O_3$. In addition, the proportion of carbamate toxins increased upon exposure to 2 mg L^{-1} ENPs, while decreased upon exposure to

Abbreviations: ENPs, engineered nanoparticles; EPS, exopolymeric substances; HAB, harmful algal bloom; PSTs, paralytic shellfish poisoning toxins; ROS, reactive oxygen species.

* Corresponding author at: Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China.
E-mail address: jiang.yuelu@sz.tsinghua.edu.cn (Y. Jiang).

http://dx.doi.org/10.1016/j.scitotenv.2017.05.170

200 mg L^{-1} ENPs. The changes in PSTs production and composition might be an adaptive response for *A. tamarense* to overcome the stress of ENPs exposure. This work brings the first evidence that ENP would affect PSTs production and profiles.

1. Introduction

Among all the dinoflagellate harmful algal bloom (HAB) species, *Alexandrium* is one of the major HAB genera because of its wide diversity, widespread distribution, impacts from its blooms and synthesis of neurotoxins that are associated with paralytic shellfish poisoning toxins (PSTs) (Anderson et al., 2012). The neurotoxic PSTs, which comprise saxitoxin and about two dozen naturally occurring analogues (Soto-Liebe et al., 2012), can cause serious hazard in fishery, public health, and marine ecosystems via the ingestion of clams, oysters, and mussels (Deeds et al., 2008). It was estimated that there were about 2000 cases of paralytic shellfish poisoning with 15% human mortality every year all over the world, excluding the unreported or misdiagnosed cases (Hallegraeff, 1993). Therefore, the blooms and toxin dynamics of *Alexandrium* have aroused concerns worldwide. Algal blooms can be affected by many factors including nutrients availability, light intensity, physical and hydrographic conditions etc. The population dynamics and PSTs production of this noxious species can be affected by environmental and nutritional factors in a complex manner, such as salinity (Parkhill and Cembella, 1999), temperature (Navarro et al., 2006), irradiance (Etheridge and Roesler, 2005), nutrients (Anderson et al., 1990; Xu et al., 2012), and pCO_2 (Van de Waal et al., 2014). Emerging contaminants might make the problem more complex.

Possible causes behind the increase in observed HAB events in coastal regions range from natural mechanisms (such as species dispersal and climate change) to human activities (such as transport of HABs via ship ballast water and pollutant discharge) (Anderson, 2009). Engineered nanoparticles (ENPs) are emerging materials that have been widely applied and they pose increased risks to the environment, especially the increased contamination risks to aquatic ecosystems (Klaine et al., 2008). Direct and indirect toxic effects of ENPs on aquatic organisms (ranging from bacteria (Jiang et al., 2009), phytoplankton (Miller et al., 2010; Pakrashi et al., 2013; Qian et al., 2016), protozoa (Khare et al., 2011) to shellfish (Libralato et al., 2013)) have already been reported, including i) oxidative stress and inflammation induced by reactive oxygen species (ROS) production (Ma et al., 2014); ii) impacts on photosynthetic activity in phytoplankton and plants (Sadiq et al., 2011; Castro-Bugallo et al., 2014); and iii) membrane damage, protein denaturation, and DNA damage (Nel et al., 2006). It is believed that the toxicity of ENPs is a combined outcome of multiple mechanisms, such as the physiochemical properties of ENPs (Vinopal et al., 2007), physical inhibition (Schwab et al., 2011), release of metal ions (Lee and An, 2013), and oxidative stress (Li et al., 2015). The toxicity and the underlying mechanisms of ENPs varies among different types, sizes, and concentrations of ENPs (Miller et al., 2010; Clément et al., 2013). Of all the ENPs, metal oxide ENPs are most widely used, e.g. titanium dioxide nanoparticles ($_nTiO_2$) in sunscreen lotion, zinc oxide nanoparticles ($_nZnO$) in cosmetics and paints, and aluminum oxide nanoparticles ($_nAl_2O_3$) in alloys, explosives, and coatings (Bielmyer-Fraser et al., 2014). It has been estimated that >800 ENPs products are available in the market so far (Rizwan et al., 2017).

Phytoplankton as the major producer in aquatic ecosystems has been pervasively used as the testing organisms to evaluate the toxicity of ENPs, such as diatoms (Miller et al., 2010; Li et al., 2015), chlorophytes (Lee et al., 2012; Bhuvaneshwari et al., 2015; Manzo et al., 2013), and prymnesiophytes (Miller et al., 2010). However, the effects of metal oxide ENPs on the growth and toxin dynamics of dinoflagellate HAB species have not been reported yet. Moreover, the unique physiochemical properties of ENPs may not only induce the toxic effects on the growth and photosynthetic performance of *Alexandrium* sp., but also cause possible secondary effects mediated by the changes in PSTs production and composition. In the present study, we investigated the effects of three metal oxide ENPs on the growth, physiological changes, and PSTs dynamics of *A. tamarense*, in order to provide further information on public health and environmental risks in terms of both ENPs and PSTs.

2. Materials and methods

2.1. ENPs characterization and stability

Three ENPs tested were purchased from Sigma-Aldrich: $_nTiO_2$ (anatase, Ø < 25 nm, 637,254), $_nZnO$ (Ø < 50 nm, 677,450), and $_nAl_2O_3$ (Ø < 13 nm, 718,475). The ENPs characterization including morphology and size was analyzed using a ZEISS SUPRA®55 Scanning Electron Microscopy. For each ENPs, over 150 particles were measured in random to calculate mean particle size.

The mean hydrodynamic diameters (MHDs) and zeta potential of ENPs in seawater were measured in duplicate using Malvern Zetasizer Nano-ZS (Malvern Instruments, UK). Dissolution of $_nZnO$ and $_nAl_2O_3$ in seawater were conducted in duplicate using inductively coupled plasma-mass spectrometry (ICP-MS, NexION 300×, PerkinElmer, the USA) at pH 8.2 after the solution passing through ∅0.22 μm filters (Millipore, the USA). The total dissolved Zn/Al in this study represented the sum of free Zn/Al ions, labile inorganic Zn/Al complexes, Zn/Al-organic complexes and ZnO/Al_2O_3 particles/aggregates with the diameter <220 nm. The dissolution of $_nTiO_2$ was not measured because of its insoluble property in water at nearly neutral conditions (Wang et al., 2008). For each ENPs, the stock solution (10 g L^{-1}) was prepared with Milli-Q water after sonication at 40 kHz for 40 min, and dispersed in seawater to the final concentrations of 2, 20, and 200 mg L^{-1}. Both MHDs and concentrations of dissolved metal ions were measured at 2 h and 72 h under cultural temperature.

2.2. Culture

Alexandrium tamarense (CCMP 1598) was maintained in L1 medium (Guillard and Hargraves, 1993) at pH of 8.2 and a salinity of 30‰ with natural seawater from Daya Bay (Shenzhen, P.R. China). The culture was grown under the optimal condition recommended by Bigelow Laboratory, with a 14 h: 10 h light: dark cycle and 30 μmol photons m^{-2} s^{-1} irradiance provided by cool white fluorescence lights at 20 ± 1 °C. The medium was sterilized by double filtering (Ø0.22 μm) and autoclaved.

Experiments were started with 1–2 × 10^4 cells mL^{-1} and the ENPs concentrations tested were 0, 2, 20, and 200 mg L^{-1} for each of the three ENPs. Cultures were grown in biological triplicate and shaken twice a day.

2.3. Growth rate and photosynthesis performance (F_v/F_m)

Cells were counted under a light microscope (Nikon Ci-L, Japan) after being fixed by Lugol's solution. The specific growth rate (μ, d^{-1}) was calculated following Stein et al. (Stein et al., 1973).

Initial fluorescence (F_0) and maximum fluorescence (F_m) after a saturating light pulse that closed photosystem II (PSII) reaction centers were determined by pulse amplitude modulated fluorescence system (PHYTO-PAM, Walz, Germany) after a 15-min dark adaptation. F_v/F_m was defined as $(F_m - F_0) / F_m$ (Pan et al., 2009). To exclude the biases