Aquatic Toxicology 181 (2016) 22–28

Contents lists available at ScienceDirect

Aquatic Toxicology

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

The influence of urea and nitrate nutrients on the bioavailability and toxicity of nickel to *Prorocentrum donghaiense* (Dinophyta) and *Skeletonema costatum* (Bacillariophyta)

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ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 24 October 2016
Accepted 26 October 2016
Available online 27 October 2016

Keywords:
Nickel
Urea
Bioavailability
Toxicity
Prorocentrum donghaiense
Skeletonema costatum

ABSTRACT

Nitrogen nutrients and nickel(Ni) are ubiquitous in aquatic environments, and they are important for primary production of ocean ecosystem. This study examined the interaction of nitrogen nutrients (specifically urea and nitrate) and Ni on chlorophyll (Chl *a*) concentration and photosynthesis parameters values of *Prorocentrum donghaiense* and *Skeletonema costatum*. The data presented here indicate that low concentration of Ni for *P. donghaiense* and *S. costatum* can enhance both Chl *a* concentration and photosynthesis parameters values when grown in urea containing environment. Despite this increase there was also an observed depression in both species tested when incubated in high concentration of Ni for *P. donghaiense* and *S. costatum* regardless of incubating in urea or nitrate. Additionally, EC_{50} values of Chl *a* and Fv/Fm for Ni at different time intervals were calculated in this study. These observations indicated that the Ni tolerance was higher in *P. donghaiense* as compared to *S. costatum*. The Ni tolerance of *P. donghaiense* incubated in urea was higher than that incubating in nitrate. The same phenomenon was not observed in *S. costatum*, which indicated that the influence of urea was dependent on the species investigated. Thus, urea input could impact Ni bioavailability and toxicity, and then affect the biodynamics thereafter.

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1. Introduction

Eutrophication is common in many estuarine and coastal waters. It is mainly due to an increase in agricultural development in recent years. This increase has become a serious environmental problem (De Jong, 2006). It has been reported that nutrient enrichment, accompanied by a variation in nutrient ratio (due to disproportionate inputs of nutrients) has been shown to profoundly affect the phytoplankton species composition and production (Smith et al., 1999). Nutrient enrichment mainly refers to an increase in nitrogen and phosphorus concentrations. Nitrogen nutrients include dissolved inorganic nitrogen (i.e., nitrite, nitrate, and ammonia) and dissolved organic nitrogen (DON) (i.e. urea, amino acid and so on) in the ocean (Peers et al., 2000). Nitrate and urea are often the predominant components of dissolved nitrogen in estuarine and coastal waters due to riverine input (Dyhrman and Anderson, 2003; Liu et al., 2011). Phytoplankton use inorganic nitrogen and can also take up and assimilate urea. It has been demonstrated that the bioavailability of urea to dinoflagellate enhances the intensity of dinoflagellate bloom (Bronk et al., 2007; Dyhrman and Anderson, 2003). There are numerous lines of evidences that indicate most phytoplankton (Antia et al., 1991), except chlorophytes, use the Ni-containing enzyme urease to hydrolyze urea to ammonium and carbon dioxide (Worms et al., 2007). Consequently, these phytoplankton require Ni for growth in urea containing environment, an important nitrogen source that can support 5–50% of oceanic primary production (Dupont et al., 2010). Thus, the potential interactions between phytoplankton community composition, nitrogen and Ni can significantly influence the ocean ecosystems (Dupont et al., 2010).

Ni is a necessary metal for organisms, but it is toxic at elevated concentrations. It is important in the functioning of urease; an important enzyme in the hydrolysis of urea to produce ammonia and carbamate (Smyj, 1997). Because Ni plays such an important

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http://dx.doi.org/10.1016/j.aquatox.2016.10.027
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role in metabolism of urea, it is essential to study the function and fate of Ni in the context of marine biogeochemical cycles. Numerous studies have been conducted to investigate this role of Ni. However, current reported data focused on Ni accumulation in, or uptake from water, the dissolved phase by marine biota and its transfer in food chain (Mehta et al., 2000; Singh et al., 1992; Wang et al., 2007; Wang and Dei, 2001). The major contribution to net primary production in coastal and estuarine waters could be impacted by metal pollution including Ni. Despite these studies there has been a lack of study, especially in urea incubation, on the influence of Ni on growth and photosynthesis parameters. Therefore the data presented here addresses this important issue.

Prorocentrum donghaiense and *Skeletonema costatum* are 2 key species that contribute to harmful algal blooms in the coastal waters of China (Ou et al., 2008). In the past few years, they had formed extensive blooms in East China Sea (Ou et al., 2008); a region that coexisted with an abundance of urea. These observations suggest urea might affect the bioavailability of Ni in coastal waters, thus influencing the growth and photosynthesis parameters of the phytoplankton that was examined. However, no work has been done to investigate this possibility. This study investigates the influence of different nitrogen resource (specifically nitrate and urea) and Ni on the growth and photosynthesis parameters of *P. donghaiense* and *S. costatum*. The data reported here aims to determine the impacts and mechanisms of urea on Ni bioavailability and risk assessment in the phytoplankton.

2. Materials and methods

2.1. Culture conditions

Seawater was collected from the Zhangzhou Bay (23.62°N, 117.61°E), which is in the Fujian province in China. Samples were stored at 20 °C for 6 months, and filtered through acid-washed Pall Acropak Supor capsule 0.22 μm filters before use. A flow injection analyzer (FIA) was used to determine the background nutrient concentration in the seawater. The background concentration of total dissolve nitrogen (TDN) was measured using a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). Urea concentration was measured using the diacetylmonoxime reagent method for seawater at room temperature (Goeyens et al., 1998). The seawater was added to a closed vessel with mixed acid (HNO_3:H_2O_2, v:v = 2:1). It was then microwave digested for 10 min at 10 atm, then used for determining the background concentrations of Ni in the seawater using inductively coupled plasma mass spectrometry (ICP-MS).The amount of Ni was 1.23 μmol L^{-1}, and the relative standard deviation was 1.3%. This coastal seawater, with both Ni and nutrient enrichment, could be used for Ni bioavailability and risk assessment by phytoplankton experiments.

Unialgal cultures of *P. donghaiense* and *S. costatum* were obtained from the State Key Laboratory for Marine Environmental Science (Xiamen University), the samples were collected from Yongtze River estuary in 2003. They were maintained in seawater, f/10 levels of phosphate and Si for *S. costatum* only, at 140 μmol photons m^{-2} s^{-1} by a light: dark cycle as 14:10 h. Additionally, the samples were fortified with vitamins, but without trace metals. Different N (added as $NaNO_3$, urea) concentrations at 20 °C.The cells were transferred to a new medium every 3–6 days, to ensure that the cells were acclimated to these nutrient conditions.

2.2. Algal growth assays

During the growth experiments, the algal cells were cultured in the filtered seawater in a media containing f/10 levels of phosphate, silicate (only for *S. costatum*) and vitamins without trace metals. Concentrations of 172 μmol L^{-1} N (f/10 levels, added as $NaNO_3$, urea) were also added. Incubation was at 20 °C. Stock solutions of Ni were added to the algal medium for treatments at different concentrations (0.17, 1.7, 17 μmol L^{-1} and 0.17, 0.85, 1.7 mmol L^{-1}). The algal biomass measurements were performed from 0 to 96 h, and all treatments were performed in triplicate. Chl *a* was used as an indicator for the concentration of *P. donghaiense* and *S. costatum* during the experiments, using the method of Juneau P et al. (Juneau et al., 2002).

2.3. Photosynthesis parameters

Photosynthetic parameters were measured daily up to 4 days, treated samples using a pulse amplitude modulated fluorometer (PHYTO-PAM, Heinz Walz, Germany). The measurement principle of PAMF is based on changes in the Chl *a* fluorescence level after application of a saturated light pulse from which the photosynthetic yield as well as quenching was calculated. These parameters were used to reflect the impact of certain stress factors on photosynthetic pathway. The maximum photochemical efficiency of PSII was calculated as Fv/Fm. Chl *a* content was estimated by photosynthesis parameters (Chl *a*) and were also performed to account for phytoplankton biomass changes (Ahmed and Häder, 2010).

Light-response curves were determined for all treated samples each day. Cells were first dark-adapted for 15 min before measurement. Cells were then exposed to increasing illumination intensity in 15 steps from 0 to 3500 μmol m^{-2} s^{-1}. After 20 s of each illumination, a saturating pulse was applied, photosynthetic yield and rETR (electron transport rate) were measured automatically. Then, the maximum relative electron transport rate (rETRmax) was calculated from the light-response curves. A minimum of four independent samples for all photosynthesis parameters were measured for the same treatment (Ahmed and Häder, 2010).

2.4. Data analysis and statistical analysis

Principal response curve (PRC) analyses were performed with in vivo Chl *a* fluorescence parameters ($rETR_{max}$ and Fv/Fm) and Chl *a* in order to obtain a comprehensive overview of different nitrogen nutrients on Ni toxicity during the experiment.

Dose response curves were constructed for photosynthetic yields (Fv/Fm) and Chl *a* using a 3-parameter log-logistic model following the equation (Eq. (1)). The EC curve has a sigmoidal shape, from which many toxicity values such as EC_{50} were determined.

$$y = \frac{d}{1 + (c/EC_{50})^b}$$

Where *y* is the response variable (inhibition percentage), *c* is Ni concentration, *d* is the response when the concentration tends to infinity, EC_{50} of Ni concentration that results in 50% inhibition of parameters and *b* is a scale factor.

For all parameters, the effects of exposure time, different nitrogen nutrients and Ni concentration were tested by repeated measure ANOVA. The effects of different nitrogen nutrient on Ni exposure were then tested at each exposure time by one-way ANOVA. The ANOVA was followed by a Tukey-HSD test by SPSS statistics 19.0. A *p* value of 0.05 levels were considered significant,

3. Results

3.1. Effects of Ni and nitrogen nutrients on the Chl a content of P. donghaiense and S. costatum

During the 96 h exposure, compared to the control treatment, the Chl *a* of *P. donghaiense* content at 0.17 μmol L^{-1}, 1.7 μmol L^{-1},

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