Environmental Pollution xxx (2017) 1–11

Contents lists available at ScienceDirect

Environmental Pollution

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

Ecological assessment of an algaecidal naphthoquinone derivate for the mitigation of *Stephanodiscus* within a mesocosm☆

Jae-Hyoung Joo [a, 1], Zhen Kuang [b, 1], Pengbin Wang [a], Bum Soo Park [a, c], Shailesh Kumar Patidar [a], Myung-Soo Han [a, b, c, *]

[a] Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, South Korea
[b] Department of Environment Science, College of Natural Sciences, Hanyang University, Seoul 133-791, South Korea
[c] Research Institute for Natural Sciences, Hanyang University, Seoul 133-791, South Korea

A R T I C L E I N F O

Article history:
Received 14 September 2016
Received in revised form
14 February 2017
Accepted 18 February 2017
Available online xxx

Keywords:
Stephanodiscus hantzschii
Novel algicidal substance naphthoquinone derivate
Mesocosm
Microbial community structure

A B S T R A C T

The novel eco-friendly algaecidal naphthoquinone derivate was used to control harmful algal bloom causing species *Stephanodiscus* and, its effect was assessed on other undesired and non-targeted microbial communities. We conducted a mesocosm experiment to investigate the effects of this novel algaecide on native microbial communities rearing in water collected from Nakdonggang River. Upon treatment of the mesocosm with the naphthoquinone derivate the concentration of Chl-*a* decreased from 20.4 μg L^{-1} to 9.5 μg L^{-1} after 2 days. The turbidity has also shown decrement (exhibited 15.5 NTU on the 7th day). The concentrations of DOC and phosphate in the treatment were slightly higher than those in the control due to the decomposition of dead *Stephanodiscus*, whereas the DO and pH in the treated condition were slightly lower than those in the control; which was due to increment of organic acids and higher degradation activity. Results showed that bacterial abundance were not significantly different but community composition were slightly different as revealed by NGS (Next generation sequencing). The variation in HNF (Heterotrophic nanoflagellates) revealed that the bacterial community composition changed following the change in bacterial abundance. During the treatment, the abundance of *Stephanodiscus* was significantly reduced by more than 80% after 6 days, and the abundance of ciliates and the dominant species, *Halteria grandinella*, had shown marked decline. The abundance of zooplankton sharply decreased to 5 ind. L^{-1}on the 8th day but increased again by the end of the study period. The Shannon-Wiener diversity index of phytoplankton, ciliates and zooplankton in the treated mesocosm increased significantly after 4, 7 and 8 days, respectively. The marked changes in the ecosystem structure were observed in treatment compare to control. However, the beneficial microalgal populations were not affected which indicated possibility of restoration of treated ecosystem and regain of healthy community structure after certain period.

1. Introduction

Stephanodiscus blooms adversely affect riverine ecology and water quality. These are occurring more frequently in eutrophic and shallow river–reservoir systems (Jung et al., 2009). The adverse effects of these blooms include an unpalatable taste and odour of the water, difficulties in purifying the water at treatment plants, and, so negative impact on the fishing industry (Ha et al., 2003). Blooms of *Stephanodiscus hantzschii* have been causing serious water quality problems in the Nakdonggang River in South Korea in particular and, are of serious concern (Jeong et al., 2006). The Nakdonggang River is the longest river in South Korea, with a main channel that is 526 km in length and having a total drainage area of 23,817 km^2. The water of the Nakdonggang River and its tributaries serve as a major source of drinking water for many South Korean cities (Ha et al., 2003). *Stephanodiscus* blooms have been reported for its consistence occurrence from winter to early spring, due to increase in domestic sewage, agricultural wastewater and, as a consequence, strong anthropogenic eutrophication prevails (Ha et al., 2003; Jung et al., 2008). If *Stephanodiscus* remained

☆ This paper has been recommended for acceptance by Klaus Kummerer.

* Corresponding author. Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, South Korea.
E-mail address: hanms@hanyang.ac.kr (M.-S. Han).

[1] These authors contributed equally as co-first authors.

http://dx.doi.org/10.1016/j.envpol.2017.02.045
0269-7491/© 2017 Published by Elsevier Ltd.

dominant in freshwater then the low species diversity was observed for the same time and site (Descy, 1993), hence, species diversity is positively correlated with the stability of the ecosystem. High species diversity reduces the risk of large changes in the ecosystem in response to the directional or stochastic variations in the environment, such as invasions of pathogens and other species (Chapin et al., 1998).

Many physical, chemical, and biological algaecidal techniques have been developed to combat *Stephanodiscus* blooms, of which the physical and chemical techniques employed for killing harmful algae are more stable than those of biological controls e.g. algaecidal bacteria, ciliates, and zooplankton (Jeong et al., 2006; Pierce et al., 2004). Additionally, chemically employed algicidal techniques are the most practical, economically attractive, facile and reproducible (Joo et al., 2016). Chemical algaecidal such as copper sulfate ($CuSO_4$) (Song and Wang, 2015), hydrogen peroxide (H_2O_2) (Barrington et al., 2013), Diuron ((3-[3,4-dichlorophenyl]-1,1-dimethylurea) (Schrader et al., 2003), and others have been widely used to control algal blooms. However, chemical algaecides are highly toxic, have other potentially detrimental effects and can cause large variations in the biotic and abiotic factors in freshwater ecosystems (Hanson and Stefan, 1984). Therefore, it is necessary to develop an eco-friendly algaecidal substance to combat *Stephanodiscus* blooms. Among the various newly developed algaecides, a novel algaecidal naphthoquinone derivate is a safe algaecidal substance; it is a typical secondary metabolite derived from living organisms (O'Brien, 1991; Monks et al., 1992; Rahmoun et al., 2012; Tangmouo et al., 2006) and has recently been used safely in home remedies and cosmetics. (Rostkowska et al., 1998)

The interactions among the biotic and abiotic factors in any aquatic ecosystem are very complex. For instance, the DOM released from many dead organisms can affect a bacterial community (Thomas, 1997), and the altered bacterial community can then indirectly influence the microbes at higher trophic levels via a microbial loop (Azam et al., 1983; Sigee, 2005; Pérez and Sommarugo, 2006; Park et al., 2015). Hanson and Stefan (1984) reported that phosphorous recycling and the distribution of algal species can also change with the use of chemical algaecides, which have been used previously. Recently, several studies have linked chemical algaecide use with changes in the bacterial community composition (Song and Wang, 2015). Based on the observed changes in the bacterial communities, some studies have predicted that the water quality could deteriorate (Paerl et al., 2003) and may alter the composition of aquatic environment (Son et al., 2015). Therefore, although chemical algaecides can control algal blooms, they may also cause secondary pollution of the aquatic environment (Xing et al., 2010).

In our previous study, the novel algaecidal naphthoquinone derivate was demonstrated to be an effective algaecide against the diatom *S. hantzschii* in a 60 L microcosm experiment with low ecotoxicity and, no significant toxic effects on any other organisms observed (Joo et al., 2016). However, the understanding of the mechanism by which this naphthoquinone derivate imparts its algaecidal effects in the mesocosm, as well as the potential effects it has on microbial ecosystems, is still limited. Therefore, before this novel algaecidal substance is used in field, its effects on the microbial community structure and populations such as phytoplankton, ciliates, zooplankton, bacteria, and heterotrophic nanoflagellates (HNF), must be understood. In this study, we carried out a mesocosm experiment with this novel algaecidal naphthoquinone derivate near the Nakdonggang River. Herein, we report the results of an ecological assessment of the microbial community structure and the interactions between the observed changes in the community and physico-chemical factors.

2. Materials and methods

2.1. Production of naphthoquinone derivate 6-((4-chlorobenzothiazol-2-ylamino)methyl-5-8-dimethoxynaphthalene-1,4- dione

To a stirred solution of 4-chloro-*N*-((1,4,5,8-tetramethoxynaphthalene-2-yl)methyl) benzothiazol-2-amine 861.1 mg (1.95 mmol) in acetone (20 mL), at room temperature were added H_2SO_4 164 μL (2.93 mmol), H_2O 4 mL, CrO_3 203.2 mg (1.95 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from acetone and diethyl ether. The yield of the product was 75% (600 mg) (Fig. 1). The purity and identify of the derivate was assessed by nuclear magnetic resonance (NMR). NMR data are as follows: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11 (d, $J = 7.2$ Hz, 1H), 8.04 (d, $J = 7.2$ Hz, 1H), 7.97 (s, 1H), 7.74–7.70 (m, 1H), 7.63–7.59 (m, 1H), 5.68 (s, 1H), 3.21–3.17 (m, 2H), 2.79–2.76 (m, 2H), 2.61 (q, $J = 6.8$ Hz, 4H), 1.07 (t, $J = 6.8$ Hz, 6H).

2.2. Experimental design

The experiment was conducted near the Nakdonggang River (Fig. 2) with two polyethylene pool mesocosms (6 × 7 × 1.5 m^3: having 10 tons of volume capacity) (Pastel co., South Korea) located at south-eastern South Korea (35°14′25.62″N and 128°59′47.95″E). The two mesocosms were filled with lake water sampled from Nakdonggang River which was experiencing bloom caused by *S. hantzschii*. One mesocosm was treated with 1 μM of the novel algaecidal naphthoquinone derivate, and the other served as a control. After addition of the algaecide into mesocosm, the gentle mixing was done using the water pump.

2.3. Sampling strategy and analysis of physicochemical factors

The sampling was carried out from February 14 to March 1 of 2015 and, samples were taken daily for 15 days.

Before sampling, a Horiba U-52 Water Quality Checker™ was calibrated using a standard solution and used to measure the water temperature, pH, turbidity, conductivity, and DO in each of the pools.

Water samples were filtered through a glass microfiber filter (47 mm diameter, Whatman, UK) and the filtered water was collected in a 50 mL conical tube and stored at −20 °C before analysis of silicate (SiO_2-Si), phosphate (PO_4-P), nitrite (NO_2-N), nitrate (NO_3-N), and ammonium (NH_4-N) (APHA, 1995). The concentrations of the nutrients were measured using a spectrophotometer with different absorption values. The molybdenum blue method was used to measure the concentration of silicate; the diazotization method was used to measure the concentrations of phosphate, nitrite, and nitrate; and the indophenol method was used to measure the concentration of ammonium (Rand et al., 1976).

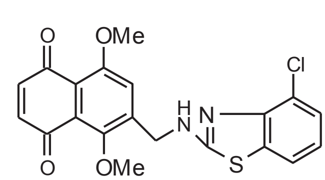

Fig. 1. Common chemical structure of naphthoquinone derivate.

Please cite this article in press as: Joo, J.-H., et al., Ecological assessment of an algaecidal naphthoquinone derivate for the mitigation of *Stephanodiscus* within a mesocosm, Environmental Pollution (2017), http://dx.doi.org/10.1016/j.envpol.2017.02.045