



Enantioselective changes in oxidative stress and toxin release in *Microcystis aeruginosa* exposed to chiral herbicide diclofop acid



Jing Ye^{a,b}, Ying Zhang^c, Shengwen Chen^d, Chaonan Liu^a, Yongqiang Zhu^a, Weiping Liu^{b,*}

^a School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai 201418, China

^b MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Natural Research and Environmental Sciences, Zhejiang University, Hangzhou 310058, China

^c Department of Environmental Science, East China Normal University, Shanghai 200241, China

^d School of Urban Development and Environment Engineering, Shanghai Second Polytechnic University, Shanghai 201209, China

ARTICLE INFO

Article history:

Received 4 September 2013

Received in revised form 17 October 2013

Accepted 23 October 2013

Keywords:

Diclofop acid

Environmental toxicology

Enantioselectivity

Toxin release

ABSTRACT

Enantioselective oxidative stress and toxin release from *Microcystis aeruginosa* after exposure to the chiral herbicide diclofop acid were investigated. *Racemic* diclofop acid, *R*-diclofop acid and *S*-diclofop acid induced reactive oxygen species (ROS) generation, increased the concentration of malondialdehyde (MDA), enhanced the activity of superoxide dismutase (SOD) and triggered toxin release in *M. aeruginosa* to varying degrees. The increase in MDA concentration and SOD activity in *M. aeruginosa* occurred sooner after exposure to diclofop acid than when the cyanobacteria was exposed to either the *R*- and the *S*-enantiomer. In addition, enantioselective toxicity of the enantiomers was observed. The *R*-enantiomer triggered more ROS generation, more SOD activity and more toxin synthesis and release in *M. aeruginosa* cells than the *S*-enantiomer. Diclofop acid and its *R*-enantiomer may collapse the transmembrane proton gradient and destroy the cell membrane through lipid peroxidation and free radical oxidation, whereas the *S*-enantiomer did not demonstrate such action. *R*-diclofop acid inhibits the growth of *M. aeruginosa* in the early stage, but ultimately induced greater toxin release, which has a deleterious effect on the water column. These results indicate that more comprehensive study is needed to determine the environmental safety of the enantiomers, and application of chiral pesticides requires more direct supervision and training. Additionally, lifecycle analysis of chiral pollutants in aquatic system needs more attention to aid in the environmental assessment of chiral pesticides.

© 2013 Published by Elsevier B.V.

1. Introduction

Cyanobacterial blooms can form in aquatic systems eutrophied by abiotic natural sources such as surface or ground water emerging from sediments naturally rich in nutrients (Phlips et al., 2002). However, recently increasing anthropogenic input of nutrients such as phosphorus and nitrogen from urbanization and agricultural expansion has led to increases in nutrient effluxes into aquatic systems. Cyanobacteria have been quite opportunistic in exploiting these available nutrients which has resulted in more frequent and extensive blooms (Ross et al., 2006). Such blooms of cyanobacteria often cause serious environmental, esthetic, and economic problems such as decreased recreational value of waterways, fish kills, and increased cost of water treatment (Dokulil and Teubner, 2000). Furthermore, causative cyanobacteria can produce microcystins (MCs) or other cyclic hepatotoxins which are toxic to domestic livestock and wildlife around the world. They also pose a serious

health hazard to humans exposed to them when they use contaminated water for drinking, cooking, or recreation (Dittmann and Wiegand, 2006). Among the toxic cyanobacteria *Microcystis aeruginosa* is the most frequently observed in highly eutrophic lakes (Dai et al., 2009).

Certain environmental factors such as pH, temperature, and the amount of phosphorus and nitrogen in the water have been found to affect the production of MCs and have been extensively studied in both batch and continuous cultures (Downing et al., 2005; Ame and Wunderlin, 2005). But other factors, such as the concentration of environmental pollutants including pesticide residues which may be prevalent in aquatic ecosystems, have not been studied in sufficient detail.

During the past few decades, chiral pesticides have become an important class of environmental pollutants. It has been estimated that over 40% of pesticides used currently in China have chiral components (Ye et al., 2009a). Large quantities of chiral pesticides can be released into aquatic ecosystem during application or after heavy rainfall and exhibit severe toxicity toward nontarget organisms, including invertebrates (Zhou et al., 2007, 2009; Zhao et al., 2009), fishes (Xu et al., 2008), plants (Zhang et al., 2012; Ye et al.,

* Corresponding author. Tel.: +86 571 88982341; fax: +86 571 88982341.
E-mail address: wliu@zju.edu.cn (W. Liu).

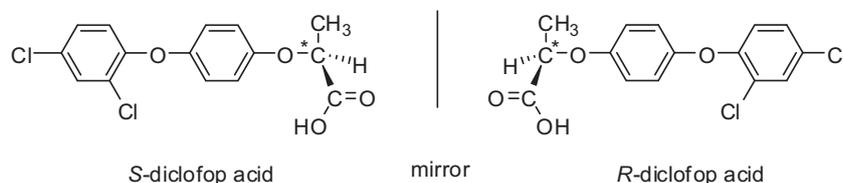


Fig. 1. Chemical structures of the diclofop acid enantiomers (* indicates chiral position).

2009b; Qian et al., 2009), and algae (Cai et al., 2008). The enantiomers of a chiral compound usually have different ecotoxicities and the environmental fates of these chiral pollutants have also been found to be enantioselective (Ye et al., 2010). Chiral pollutants also impact cyanobacteria in waters. Recently, it has been reported that chiral herbicides pose enantioselective toxicity to *M. aeruginosa*, but did not quantify the release of the intracellular toxins or identify the mechanism responsible for cell mortality (Ye et al., 2013). Therefore, better understanding about the effects of these chiral pollutants on cyanobacteria is needed. Further, the toxin release caused by this class of compounds also needs to be investigated.

Diclofop acid ((*R,S*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid) is the active form of the herbicide diclofop methyl used on wheat, barley, and golf courses (turf). The total annual domestic usage of diclofop methyl between 1987 and 1996 in the USA was approximately 340,200 kg of active ingredient (a.i.) (RED, 2000a). In China, the usage was between 1 and 5 million kilograms in 2006 (Ye et al., 2009b). Up to 73% of the active ingredient of diclofop methyl falls onto soil surfaces during application rather than on the target weeds (Smith et al., 1986). Under alkaline aquatic conditions, diclofop methyl rapidly hydrolyzes to diclofop acid which has a relatively high solubility in water compared to diclofop methyl making it more prone to enter aquatic systems after precipitation events (RED, 2000b). Therefore, diclofop acid is likely to be present in surface water in significant amounts (Liu et al., 1991).

Diclofop acid is a chiral herbicide with one stereogenic center (Fig. 1). A study of the enantioselective herbicidal activity of diclofop acid revealed that the *R*-enantiomer is approximately twice as active as the racemic mixture against millets and oats (Kurihara et al., 1997). It has been reported that the two enantiomers of diclofop acid posed different ecotoxicities to three freshwater algae and that their degradation in alga cultures was enantioselective (Cai et al., 2008). Diclofop acid was also found to present enantioselective ecotoxicity on rice *xiushui* 63 seedlings (Ye et al., 2009b), *Arabidopsis thaliana* (Zhang et al., 2012), and *M. aeruginosa* (Ye et al., 2013). The results demonstrated that herbicidal activity and environmental safety are not always consistent. Therefore, considering the target (herbicidal activity) and nontarget (environmental safety) bioactivity of chiral pesticides simultaneously is necessary when designing an herbicide application regimen.

In the current study oxidative damage and toxin release from *M. aeruginosa* caused by the chiral pesticide diclofop acid were studied. Diclofop methyl was proposed to induce oxidative stress in susceptible plant tissues and to collapse the transmembrane proton gradient. Cells can develop a set of cellular defense systems via enzymatic and non-enzymatic antioxidants to counteract the toxicity of reactive oxygen species (ROS) like H_2O_2 , $\text{O}_2^{\bullet-}$ and HO^\bullet . In the enzymatic pathways, superoxide dismutase (SOD) is important to scavenge ROS (Shimabukuro et al., 1999). Previously, we studied the physiological effects of diclofop methyl and two enantiomers of diclofop acid on *M. aeruginosa* and found that the *R*-diclofop acid and *S*-diclofop acid pose different toxicity indicated by biomass, protein content and ultrastructural characteristics (Ye et al., 2013). Based on the proposed toxicity mechanism of diclofop methyl on

susceptible plants and the physiological effects on *M. aeruginosa* studied before, in this study, we speculate that oxidative stress may also be induced by diclofop acid in *M. aeruginosa* and that the release of toxins will also be increased as a result of cell death. The purpose of this study was to determine whether the chiral pollutant diclofop acid induces enantioselective oxidative stress on *M. aeruginosa* cells and leads to a significant release of soluble toxin into the surrounding water column. Further, our results will aid in developing a model that can predict outbreaks of water blooms and that ultimately protects our environment and human health.

2. Materials and methods

2.1. Chemicals and cell cultures

Diclofop acid {(*R,S*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate acid} was prepared from diclofop methyl according to Smith (1976) and identified by HPLC. Diclofop methyl with purity $\geq 97\%$ was generously provided by Iprochem Co., Ltd. (Shenzhen, China). *R*- and *S*-diclofop acid (purity $\geq 99.0\%$, optical purity $\geq 94.0\%$) were synthesized in our laboratory (Cai et al., 2008). Standard MC-LR with purity $\geq 95\%$ was purchased from Express Technology Co., Ltd. (Beijing, China).

The cyanobacteria *M. aeruginosa* was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, China. The unialgal inoculant was cultured in sterile BG11 medium under an irradiance of $40 \mu\text{mol}/\text{m}^2 \text{ s}$ with wavelength range from 400 to 750 nm and a photoperiod of 12 h light/12 h dark at $28 \pm 1^\circ \text{C}$. The algal cultures were shaken three times per day. Based on our previous results (Ye et al., 2013), a maximum concentration of 5 mg/L was selected for the present study. The oxidative stress tests were carried out using 0, 1, 2, and 5 mg/L of diclofop acid, *R*-diclofop acid and *S*-diclofop acid. Three replicates of each concentration were prepared in Erlenmeyer flasks (100 mL) containing 5 mL of algal inoculant and 45 mL of culture medium. The initial algal density in each flask was $(5.5\text{--}6.7) \times 10^6$ cells/mL.

2.2. Analysis of exposure concentrations

After the chemicals added into culture medium, the triplicate culture samples were filtered through a $0.45\text{-}\mu\text{m}$ filter and analyzed by HPLC. The analyses were performed on a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) with a PU-2089 quaternary gradient pump, a CO-2060 column temperature control compartment, and a UV-2075 plus UV/vis detector. The operation conditions were a Kromstar C18 column ($4.6 \text{ mm} \times 250 \text{ mm}$, Dai-CEL Chemical Industries, Tokyo, Japan), a flow rate of $0.8 \text{ mL}/\text{min}$, a mobile phase of methanol/water (50:50, v/v), a detection wavelength of 254 nm, an injection volume of $10 \mu\text{L}$, and an oven temperature of 40°C .

2.3. Detection of intracellular ROS generation

Oxidative stress or overproduction of ROS induced by diclofop acid was assessed with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is converted to DCFH by esterases during up

Download English Version:

<https://daneshyari.com/en/article/6382593>

Download Persian Version:

<https://daneshyari.com/article/6382593>

[Daneshyari.com](https://daneshyari.com)