Science of the Total Environment 635 (2018) 1004–1011

Contents lists available at ScienceDirect

Science of the Total Environment

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

Causes and mechanisms on the toxicity of layered double hydroxide (LDH) to green algae *Scenedesmus quadricauda*

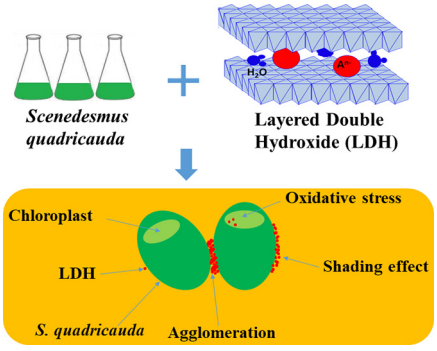
Tengda Ding [a,b], Kunde Lin [b], Jin Chen [c], Qi Hu [a], Bo Yang [a], Juying Li [a,*], Jay Gan [d]

[a] Shenzhen Key Laboratory of Environmental Chemistry and Ecological Remediation, College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China
[b] State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen 361005, China
[c] Core Facilities, School of Medicine, Zhejiang University, Hangzhou 310058, China
[d] Department of Environmental Sciences, University of California, Riverside, CA 92521, United States

HIGHLIGHTS

- The growth of *S. quadricauda* was significantly inhibited by LDH.
- The light and dark 24 h EC50s for algae by LDH was 10 and 25 mg L^{-1}, respectively.
- LDH induced changes in algal photosynthesis, oxidative stress and lipid peroxidation.
- Shading effect, agglomeration and oxidative stress mainly contributed such toxicity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 January 2018
Received in revised form 15 April 2018
Accepted 16 April 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Layered double hydroxide (LDH)
Toxicity
Scenedesmus quadricauda
Shading effect
Agglomeration

ABSTRACT

Layered double hydroxides (LDHs) are widely used nanomaterials in industrial catalysis, pharmaceuticals, and environmental remediation, and may pose potential negative effects in the aquatic environment. However, little information is available on their toxicity to aquatic organisms. In this study, toxicity of LDH to a typical freshwater green algae *Scenedesmus quadricauda* was systematically investigated and the underlying mechanisms were elucidated. The growth of *S. quadricauda* was significantly inhibited by LDH at 72 h with a half maximal effective concentration (EC_{50}) and lowest observed effect concentration (LOEC) of 10.0 and 1.5 mg L^{-1}, respectively. Shading effect was observed, and the photosynthetic activity and cellular chlorophyll production were also severely suppressed by LDH. LDH also enhanced the reactive oxygen species production from *S. quadricauda* and lipid peroxidation in algal cells. Such algal toxicity of LDH might be mainly induced by the shading effect, agglomeration and physical interactions, and oxidative stress. The agglomeration and physical interactions contributed more to the algal toxicity at 72 h-EC_{50} LDH concentrations. The results from the present study provided new insights and a better understanding of the environmental behavior and adverse effects of LDHs in the surface waters.

1. Introduction

Nanomaterials (NMs) have attracted a great deal of interest due to their unique optical, electronic and magnetic characteristics as compared

* Corresponding author.
E-mail address: jyli@szu.edu.cn. (J. Li).

https://doi.org/10.1016/j.scitotenv.2018.04.222
0048-9697/

to their bulk counterparts (Zhu et al. 2009). Hydrotalcite or hydrotalcite-like layered double hydroxide (LDH) nanoparticles are a family of inorganic lamellar materials with a general formula of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}[A_{x/n}]^{n-}\cdot mH_2O$, where M^{2+} is a divalent cation, M^{3+} a trivalent metal cation, x the molar ratio of the trivalent cation $[M^{3+}/(M^{2+}+M^{3+})]$, and A^{n-}a gallery anion with charge n (Sideris et al. 2008). Layered double hydroxides have received wide-spread attention in the application of catalysis (Zhao et al. 2007), polymer nanocomposites (Manzi-Nshuti et al. 2009), pharmaceuticals (Ladewig et al. 2009; Choi and Choy 2011), and sensors (Han et al. 2011). Additionally, as environmental pollution has emerged as an important issue in the recent decades, interests in using LDHs to remove environmental contaminants (e.g., heavy metal, pesticides and polycyclic aromatic hydrocarbons) are growing (Ambrogi et al. 2009; Koilraj et al. 2016; Li et al. 2016; Peligro et al. 2016). The rapidly increasing use of LDHs may lead to the occurrence of LDHs in the effluents and the freshwater systems receiving effluent discharge, which has not been reported so far. It may raise ecological and human health concerns as LDHs can pose toxic effects on non-target organisms. A considerable amount of literature has been published on the toxicity effects of organic NMs, such as fullerenes and carbon nanotubes (Sergio et al. 2013; Ging et al. 2014) to organisms including algae (Schwab et al. 2011; Long et al. 2012), zooplankton (Zhu et al. 2006; Zhu et al. 2009), fish (Kim et al. 2012; Myer et al. 2017) and human cells (Hu et al., 2010; Larner et al. 2017). However, studies on the toxicity of inorganic LDHs to aquatic organisms are few.

Algae are one of the most common species in natural waters, and are commonly used as model organisms in regulatory testing and ecotoxicological studies (Zhang et al. 2013; Zhang et al. 2015; Ding et al. 2017a). As environmental concern over the presence of NMs in the environment and their potential subtle effects on non-target organisms is increasing, many studies have been conducted to examine the potential effects of NMs to algae and the associated mechanisms. For example, NMs (e.g., carbon nanotubes, silver nanoparticles) could generate reactive oxygen species (ROS) to pose a variety of interrelated effects on algae (e.g., *Pseudokirchneriella subcapitata*), such as lipid peroxidation and DNA damage (Nel et al. 2006; Ma et al. 2010). In addition, nanoparticle aggregates may attach to and/or entrap algal cells, reducing available light needed for photosynthesis and restricting other cellular functions. This phenomenon is defined as shading effect (Aruoja et al. 2009; Evers, 1991; Li et al. 2015; Navarro et al. 2008; Petersen et al. 2014; Wright et al. 2018). For example, Long et al. (2012) reported that multi-layered carbon nanotubes (MWCNTs) showed shading effects on algal growth and accounted for approximately 25% of algal growth inhibition to *Chlorella* sp. The agglomeration of NMs and algae could also lead to the algal growth inhibition. For instance, Zhang et al. (2015) reported that MWCNTs could agglomerate with a green algae (*Chlorella pyrenoidosa*) and lead to damage in the organelles. The electrostatic attraction between nanoparticle and the organisms could also lead to the heteroagglomeration (Thill et al. 2006). In addition to these toxic causes, the toxicity of NMs or NPs to algal cells could be caused by a number of other factors, such as physiological changes, the blocking of nutrient uptake, and the heavy metal release from NPs. For instance, Meyer et al. (2010) found that AgNPs exerted sublethal toxicity to *Caenorhabditis elegans* at low concentrations in the low mg L^{-1} levels, which was mediated by the release of ionic silver (Ag^+). However, to date no studies have focused on the toxicity of LDHs to algae, and the potential toxicity mechanism of LDHs to algae is still unclear.

The specific objectives of this study were (1) to evaluate the toxicity of Cu-Mg-Fe LDHs (LDH) to a typical freshwater green algae *S. quadricauda*, (2) to explore the possible toxicity mechanisms of LDH to algae, and (3) to provide information for better assessing biological behavior and ecological risks of LDHs in aquatic environments.

2. Materials and methods

2.1. Chemicals

All solutions were prepared with ultra-pure water (18.2 MΩ, Millipore system). All organic solvents used were of HPLC grade and purchased from Thermo Fisher Scientific Inc. (Shanghai, China). The LDH was synthesized and provided by the State Key Laboratory of Chemical Resource Engineering from the Beijing University of Chemical Technology. The LDH had a surface area of 114.5 $m^2\ g^{-1}$. The particle size and zeta potential of LDH was measured by the dynamic light scattering (DLS) (Zetasizer Nano Series, Malvern, Shanghai, China). The BG11 medium, prepared using the method described by Ding et al. 2017b, was used as algae culture. A stock solution of LDH was prepared by mixing the LDH in BG11 medium at 5 g L^{-1}, and was suspended by ultrasonication for 15 min before use. For algal cell agglomeration experiment, the stock solution was prepared by adding LDH to the BG11 medium to arrive at an initial concentration of 1000 mg L^{-1}, which was sonicated (100 W, 40 kHz, 25 °C) for 15 min and stored at 4 °C.

2.2. Algal growth assays

The green algae *S. quadricauda* used in this study was obtained from the Institute of Wuhan Hydrobiology, Chinese Academy of Sciences. The algal cells were inoculated into 50 mL sterile BG11 medium as described by Ding et al. (2017b). The flasks were kept on an incubator at 23 ± 1 °C with or without illumination by incandescent lights (4000 lx, light: dark cycle of 12:12 h). The medium was autoclaved at 0.1 MPa for 20 min and sonicated for 15 min at 25 °C. The LDH (0, 5, 10, 20, 50, and 100 mg L^{-1}) was then added to the medium before the inoculation of algal cells. The contents of all flasks were mixed manually three times a day.

Algal cell numbers were measured by the spectrophotometer method and the growth rate was calculated by fitting the cell numbers to an exponential function as described in our previous study (Ding et al. 2017a). The chlorophyll (Chl-a and Chl-b) content of *S. quadricauda* was determined by the hot methanol method as described in Xiong et al. (2016). Briefly, a 10 mL algal suspension was centrifuged at 3506*g* for 20 min. The pellet was extracted by a 10 mL 90% methanol solution in a water bath (60 °C) for 15 min, followed by centrifugation at 3506*g* for 10 min. The optical density of the supernatant was measured at 665, 652 and 470 nm wavelengths in a UV-2550 spectrophotometer (Shimadzu, Japan). The chlorophyll and carotenoid contents were calculated by the equations as follows:

$$\text{Chlorophyll a}\left(C_a, \text{mg L}^{-1}\right) = 16.82A_{665} - 9.28A_{652} \quad (1)$$

$$\text{Chlorophyll b}\left(C_b, \text{mg L}^{-1}\right) = 36.92A_{652} - 16.54A_{665} \quad (2)$$

$$C_{\text{carotenoid}}\left(\text{mg L}^{-1}\right) = (100A_{470} - 1.91C_a - 95.15C_b)/225 \quad (3)$$

The initial cell numbers of algae were counted using a counting chamber under a light microscope (×40, Nikon, E100, Japan). The cell numbers of *S. quadricauda* during the incubation were determined by the following equation:

$$\text{Cell numbers of } S.quadricauda\left(\times 10^4 \text{ cell mL}^{-1}\right) = 555.56 \times OD_{680} + 2.67$$

where OD_{680} is the optical density of algal suspensions at 680 nm, the numbers are calculated based on the standard curve of cell numbers and optical density.