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The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae

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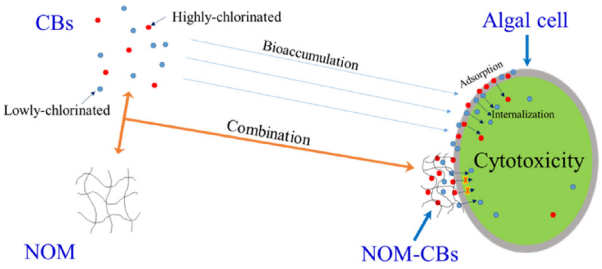
HIGHLIGHTS

- NOM-CB interaction and its effect on bioaccumulation and algal toxicity were studied.
- The NOM-CB interaction increased with increasing Cl-substitutions in CBs.
- NOM increased cell surface hydrophobicity of algae exposed to CBs.
- NOM increased BCFs and accumulations of CBs in algae.
- The effect of SRNOM on the algal toxicity of CBs varied with Cl-substitutions.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of natural organic matter (NOM) on toxicity and bioavailability of hydrophobic organic contaminants (HOCs) to aquatic organisms has been investigated with conflicting results and undefined mechanisms, and few studies have been conducted on volatile HOCs. In this study, six volatile chlorobenzenes (CBs) with 1–6 chlorine substitutions were investigated for their bioaccumulation in an acute toxicity to a green alga (*Chlorella pyrenoidosa*) in the presence/absence of Suwannee River NOM (SRNOM). The fluorescence quenching efficiency of SRNOM increased as the number of chlorine substitutions of CBs increased. SRNOM increased the cell-surface hydrophobicity of algae and decreased the release rates of algae-accumulated CBs, thus increasing the concentration factor (CF) and accumulation of the CBs in the algae. SRNOM increased the toxicity of monochlorobenzene and 1,2-dichlorobenzene, decreased the toxicity of pentachlorobenzene and hexachlorobenzene, and had no significant effect on the toxicity of 1,2,3-trichlorobenzene and 1,2,3,4-tetrachlorobenzene. Relationships between the 96 h CF/IC_{50} (i.e., the CB concentration leading to a 50% algal growth reduction compared with the control) and physicochemical properties of CBs with/without SRNOM were established, providing reasonable explanations for the experimental results. These findings will help with the accurate assessment of ecological risks of organic pollutants in the presence of NOM.

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1. Introduction

Natural organic matter (NOM) consists of humic substances, such as humic and fulvic acids, and nonhumic substances. Humic substances are macromolecules with complex three-dimensional molecular structures comprised of hydrophilic and hydrophobic monomers [1]. NOM can interact with hydrophobic organic contaminants (HOCs) [2–6] and thus alter the environmental behaviors and ecological effects of the HOCs [7,8]. Such changes should be included in environmental risk assessments of HOCs.

Many investigations have reported either positive or negative effects of NOM on the toxicity and bioavailability of HOCs to aquatic organisms. Some studies showed that NOM could decrease the bioavailability of organic contaminants and consequently their toxic effects. For example, high-aromaticity NOM decreased the uptake rate of pyrene by *Daphnia magna* [9]; low concentrations of Suwannee River NOM (SRNOM) and Svartberget NOM lowered the toxicity of cypermethrin to *D. magna* [10]; and humic acid inhibited the absorption of organo-chlorine pesticides by fish [11]. In contrast, Aldrich humic acid increased the concentration factor (CF) of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin to rainbow trout [12]; and lake NOM increased the CF of *p,p′* −DDT to rainbow trout [13]. Moreover, for some organic contaminants, the effects of NOM on their bioavailability and toxicity were generally not significant. The presence of NOM in natural river water did not significantly change the toxicity of atrazine, permethrin, or chlorothalonil to *Ceriodaphnia* cf. *dubia* [14]; the bioavailability and toxicity of permethrin to *D. magna* were not significantly affected by 12 surface-water NOMs [15]; and fluoranthene bioaccumulation in *D. magna* was not affected by the presence of NOM [16]. The effects of NOM on the toxicity and bioavailability of HOCs to aquatic organisms are diverse. The effects of NOM on volatile HOCs would be more complex compared with non-volatile HOCs, which have been studied rarely and the underlying mechanisms of effects have been undefined.

Chlorobenzenes (CBs) are volatile HOCs. They are toxic and highly persistent water pollutant, easily migratory owing to their volatility, resistance to degradation, and long history of used in industry and agriculture [17–19]. Because of their toxicity and potential risk, they have been ranked as prior pollutants by United States Environmental Protection Agency (USEPA) [20]. CBs have high octanol-water partition coefficients and thus are biomagnified easily in food chains [21]. The bioaccumulation and toxicity of CBs increase as chlorine substitutions increase [22,23]. However, the effects of NOM on the bioaccumulation and toxicity of CBs merit more investigations.

Algae are important indicator organisms of environmental pollution and the model organisms of many aquatic toxicity tests. The aim of this study was to determine the effect of NOM on the CBs' bioaccumulation in and toxicity to a green alga and to address the underlying mechanisms of the effect. Six CBs with 1–6 chlorine substitutions and distinct physicochemical properties were selected for investigation. SRNOM was used as a model NOM because of its wide application in related environmental researches. The CBs' bioaccumulation in an acute toxicity to the green alga in the absence and presence of SRNOM were investigated. The results will increase knowledge on environmental risks of volatile HOCs in the presence of NOM.

2. Materials and methods

2.1. Chemicals and organisms

The unicellular green alga, *Chlorella pyrenoidosa*, was purchased from the Institute of Wuhan Hydrobiology, Chinese Academy of Sciences, China. The SRNOM was obtained from the International Humic Substances Society (IHSS, USA). The purities, manufacturers, and some physicochemical properties of monochlorobenzene (MCB), 1,2-dichlorobenzene (1,2-DCB), 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), pentachlorobenzene (PeCB), and hexachlorobenzene (HCB) are shown in Table S1 (in Supporting information).

2.2. Effects of SRNOM on toxicity of CBs to algae

The culture medium, the composition of which is detailed in Supporting information, for the algal growth assay was recommended by the Organisation for Economic Co-operation and Development (OECD). The algal cells were cultured in 100 mL of the OECD medium in 250 mL Erlenmeyer flasks with different concentrations of CBs in the presence or absence of SRNOM. The initial concentration of the SRNOM was 10 mg L^{-1}, for a total organic carbon (TOC) concentration of 4.56 mg C L^{-1} as determined with a TOC analyzer (TOC-VCPH, Shimadzu, Japan), which is a realistic and typical TOC concentration in natural rivers [24–26]. Preliminary experiments showed that there was no significant effect on the growth of algae when the concentration of the SRNOM was below 15 mg L^{-1} (Fig. S1A in Supporting information) and 5 and 10 mg L^{-1} but not 1 mg L^{-1} SRNOM had similar and significant effect on the 96 h algal growth inhibition of CBs (Fig. S2). The initial algae density was 2.5×10^6 cells mL^{-1}. The flasks were kept in an artificial climate cabinet at 25 ± 0.5 °C, with or without illumination by white incandescent lights (100 ± 5 μEm^{-2} s^{-1}, light:dark of 14:10 h). The flasks were shaken manually three times per day. The initial concentrations of MCB, 1,2-DCB, 1,2,3-TCB, 1,2,3,4-TeCB, PeCB, and HCB ranged from 0 to 150, 25, 10, 5, 0.66, and 0.0085 mg L^{-1}, respectively. Supplemental CBs were added every day.

Algal cells were counted using a counting chamber under a light microscope (LM, Olympus, CX21, Japan). The half-inhibition concentrations of CBs at 96 h (96 h-IC_{50}), (i.e., the concentration of CB leading to a 50% reduction in algal growth compared with the control) were determined by nonlinear regression, using the Four-Parameter Logistic function of the statistical software package SPSS Statistics 22.0. The concentrations at which there were no observed effects after 96 h (96 h-NOECs) were calculated using Dunnett's Test in SPSS Statistics 22.0. The growth curve of algae in the OECD medium is shown in Fig. S1B.

2.3. Fluorescence quenching experiments

Quantifying the interaction of HOCs with dissolved organic matter by using fluorescence quenching has been applied and reported elsewhere [27,28]. To elucidate the SRNOM-CB interaction, excitation-emission matrices (EEMs) of the SRNOM were collected in the absence and presence of the CBs. The SRNOM (10 mg L^{-1}) was dissolved in the OECD medium in 20 mL vials, followed by the addition of different concentrations of CBs. The initial concentrations of CBs were the same as those in Section 2.2. The vials were sealed and shaken (150 rpm, 25 °C) for 24 h. EEMs were obtained using a fluorescence spectrophotometer (F-4600, Hitachi, Japan). The excitation and emission wavelengths were increased from 200 to 400 nm in 5-nm steps and 250–550 nm in 2-nm steps, respectively. A 10-nm slit width was used for excitation and emission. The background fluorescence was deducted from the final EEMs.

The EEMs of the NOM were divided into five regions (Fig. 1A): tyrosine-like material (Region I), tryptophan-like material (Region II), fulvic acid-like material (Region III), microbial byproduct-like material (Region IV), and humic acid-like material (Region V) [29]. The peaks of the SRNOM EEMs were distributed mainly in Regions III and V. There were no peaks in the CB EEMs (Fig. S1D), except for MCB (which had peaks in Regions I and IV, see Fig. S1C). The