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Ecological risks assessment of organophosphorus pesticides on bloom of *Microcystis wesenbergii*

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ABSTRACT

Hormesis induced by organophosphorus pesticides (OPPs) on *Microcystis wesenbergii* was studied to demonstrate the mechanism of water bloom. The dosage response of OPPs on *M. wesenbergii* was detected in the presence and absence of inorganic phosphorus. The results showed median inhibitory effect concentrations of phoxim, trichlorphon, dimethoate and glyphosate-isopropylammonium on fluorescence of chlorophyll *a* at 96 h were 13.48, 148.52, 382.34, 6.84 μmol l^{-1}, respectively. Fluorescence of chlorophyll *a* was stimulated significantly in low OPPs treatments, ranged among 1/10,000–1/20 EC_{50} concentrations. However, remarkable increase in the maximum photochemical efficiency was only detected under certain treatments and time points. Physiological traits showed no remarkable difference between OPPs as sole phosphorus treatments and non-phosphorus treatments. However, both decreased significantly when compared with inorganic phosphorus (PO_4^{3-}) treatments. The results indicated that *M. wesenbergii* was not capable of utilizing OPPs as phosphorus and the stimulatory effect caused by inert ingredients was negligible. Hormetic response induced by OPPs increased competitive advantage to overcome other species. Therefore, this study confirmed that abusive utilization of OPPs had stimulatory impact on population dynamic of *M. wesenbergii*.

1. Introduction

As primary producers in aquatic food chain, algae occupy a crucial role in maintaining the balance and stability of aquatic system. However, harmful algae bloom caused by certain species induced serious destruction to aquatic systems. Many researchers revealed that *Microcystis* was able to overcome other species, like *Aphanizomenon*, *Scenedesmus*, *Chlorella*, *Cryptomonas*, *Bacillaria paradoxa* (Lehman et al., 2010). Therefore, *Microcystis* blooms have been frequently detected in various environmental compartments such as lake (Li et al., 2007), estuary (Lehman et al., 2010), river (Rocha et al., 2002) and bay (Lehman et al., 2005). Decrease of water quality, reduction of species diversity and abundance in different trophic levels, even breakdown of the aquatic ecosystem were recorded (Lehman et al., 2010). Mechanism of *Microcystis* bloom was studied through interspecies competition on growth rate, nutrient uptake, tolerance, and so on. Some studies pointed out that efficiency of nitrogen and phosphorus utilization was the main factor in the outbreak of algae, while others suggested that trace elements were the inducers of algae bloom, such as heavy metals, amino acid and fatty acids (Li et al., 2007; Otten et al., 2012). However, investigations on content of phosphorus in surface waters showed that phosphorus limitation was more serious than other biogenic elements (Tian et al., 1997). Direct utilization of phosphorus was predominant in the form of orthophosphate in most algae, but the ratio of orthophosphate was less than 10% in the total phosphorus in most eutrophic surface waters (Subramanian et al., 1994; Zhou et al., 2004). Therefore, some researchers regarded phosphorus input as the key factor in water bloom.

Organophosphorus pesticides (OPPs), such as insecticides and herbicides, increased social and economic profits significantly. Taken California for example, more than 200 species of OPPs were utilized and the total amount was over 3.0 million kilogram in 2004, which only took 25% of total OPPs used around the world (Sparling and Fellers, 2007). However, the abusive usage of pesticides during decades caused serious hazards on environmental health (Mann et al., 2009; Burridge et al., 2010). OPPs that did not bind to the target were ultimately transported to surface waters via drifting, leaching and flushing (Sabater and Carrasco, 2001b). Therefore, residues of pesticides in surface waters became new

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stressors to aquatic organisms (Sabater and Carrasco, 2001a; Lewis et al., 2009). Moreover, contamination of OPPs in surface waters was complex, which made the total concentration reached to high levels and induced lethal or sublethal toxicity on aquatic organisms (Tankiewicz et al., 2010; Coelho et al., 2011). Due to the advantage of *Microcystis* on adaptation and competition in fresh water, it was considered to be able to evaluate the ecological risks of pollutants, like OPPs. Toxicity of OPPs on microalgae was reported mainly as cholinesterase inhibition at high levels, besides to chlorophyll destruction (Tahara et al., 2005; Paixão et al., 2008; Schweikert and Burritt, 2012), antioxidative disturbance, increase in ionic permeability and reduction in membrane integrity (Chen et al., 2007; Srivastava et al., 2012), photosynthetic inhibition (Sabater and Carrasco, 2001b; Jena et al., 2012).

Hormesis was recorded as non-linear dose response and characterized by low dose stimulation and high dose inhibition (Calabrese, 2005; Calabrese and Blain, 2005). Hormesis induced by low dose of OPPs has been detected by stimulating various physiological responses (Cedergreen, 2008; Schweikert and Burritt, 2012). However, some researchers suggested that mechanism of hormesis induced by OPPs might be caused by direct utilization of OPPs in algae as additional phosphorus (Tian et al., 1997; Zhou et al., 2004). *Microcystis wesenbergii* bloomed a few days after application of OPPs around the lake for several times, which was first recorded in 2009 (Li et al., 2010). The relationship between bloom of *M. wesenbergii* and application of OPPs is rather interesting for further exploration. Does hormetic phenomenon of tested OPPs exist in *M. wesenbergii*? Was stimulating effect limited to low levels just like others reports? Among the effects of OPPs on algae, which takes the dominant role in the formation of water bloom? The purposes of this study are (1) to investigate the dose response between OPPs concentration and physiological process of *M. wesenbergii*; (2) to evaluate the capacity of growth stimulation in the formation of water bloom; and (3) to clarify the relationship between toxicity of tested OPPs and chemical structure. Moreover, the results would make a clear understand of hormesis and the potential ecological risks of OPPs on algae bloom.

2. Material and methods

2.1. Algal strain and culture medium

M. wesenbergii was isolated from a hypereutrophic environment (Ming Lake, Guangzhou, China). The lake is about 7500 m^2 in area and 1.5 m in depth. The mean values of transparency, pH, TP and TN were 0.47 m, 7.04, 0.094 mg l^{-1} and 1.87 mg l^{-1}, respectively. The phytoplankton community in this lake was dominated by some small species such as *Scenedesmus*, *Chlorella*. However, *Microcystis* bloom appeared frequently in recent years, we obtained the alga through repeated washing using capillary pipettes when it bloomed and recorded by Li et al. (2010). BG-11 medium was used as the growth medium for the algal species (Chen et al., 2007). The stock cultures were maintained at 24 ± 1 °C and the light intensity of 90 μmol m^{-2} s^{-1} with 12 h:12 h light–dark cycle.

2.2. Organophosphorus pesticides

Four OPPs, glyphosate-isopropylammonium (41%, Roundup, Monsanto Company, Missouri), phoxim (40%, Lianyungang Liben Agro-Chemical Co., Ltd., Jiangsu Province), trichlorphon (30%, Hainan Daxing pesticide factory, Hainan Province) and dimethoate (50%, Guangzhou pesticide factory, Guangdong Province) were chosen according to the species used around the lake. Concentrations of OPPs were set by direct dilution of commercial formulation in distilled water with a high stock solution. The quantity of OPPs in this experiment was calculated by amount of phosphorus regard to the active ingredients.

2.3. Experimental setup

2.3.1. Acute toxicity test

In the first step, the algae in exponential phase were inoculated to fresh medium and divided to 150 ml flasks. Then, 10 concentrations calculated by equal space between logarithm values were added for each OPPs. After homogenized by shaking, 40 ml aliquots were taken from flasks into 50 ml grass tubes (Schott Duran, Germany) to start the culture, while the initial fluorescence of chlorophyll *a* was 45.7–58.4 μg l^{-1}. The concentration range of phoxim, glyphosate-isopropylammonium, trichlorphon and dimethoate were 1.34–134.10, 1.80–539.47, 23.35–583.66 and 65.63–1310.04 μmol l^{-1}, respectively. Treatment without adding OPPs was taken as control. The cultures were maintained at 24 ± 1 °C and the light intensity was 90 μmol m^{-2} s^{-1} with 12 h: 12 h light–dark cycle.

2.3.2. The hormetic effect test

The algae in exponential phase were inoculated to fresh medium and taken 400 ml aliquots into 500 ml flasks. Then, series of OPPs concentrations were set according to the median inhibitory effect concentration, that is, from 1/20 EC_{50} to 1/10,000 EC_{50}. After homogenized by shaking, 100 ml test solutions were taken into 150 ml flasks to start experiment. The initial fluorescence of chlorophyll *a* was 57.6 μg l^{-1} and the cultural conditions were just the same as above (Section 2.3.1). 2 ml cultures diluted in 30 ml distilled water in 50 ml grass tube (Schott Duran, Germany) were used to detect fluorescence of chlorophyll *a*. Another 2 ml cultures were taken to measure the maximum photochemical efficiency (F_v/F_m) of algae everyday at the same time.

2.3.3. The compensatory effect test

The algae in exponential phase were inoculated to fresh medium without phosphorus for 3 days. After phosphorus starvation, phosphorus in algae were consumed (Tian et al., 1997). Then, the pre-cultured algae were inoculated to fresh medium without phosphorus and taken 400 ml aliquots into 500 ml flasks. In the next step, series of OPPs were set lower than that could induce observable stimulating effect. Equivalent amount of phosphorus either in the form of OPPs or inorganic phosphorus (PO_4^{3-}) was added, while the control treatment did not add any phosphorus. After that, 100 ml test solutions were taken from flasks into 150 ml flasks. The initial fluorescence of chlorophyll *a* was 41.3 μg l^{-1} and the cultural conditions were just the same as mentioned above (Section 2.3.1). Fluorescence of chlorophyll *a* and the maximum photochemical efficiency were measured everyday at the same time.

2.4. Analytical methods

2.4.1. Determination on fluorescence of chlorophyll a

Fluorescence of chlorophyll *a* was measured by TD-700 fluorometer (Turner Design, USA). A 600 s countdown timer was selected before measurement to warm up the instrument. The tubes were treated with dark adaption for 20 min at room temperature (Chalifour et al., 2009). Prior to detection, test solutions in tubes were shaken homogeneously for several times. Fluorescence of chlorophyll *a* was measured continuously and the average value of sample was displayed at 5 s, which was taken as the algal growth indicator.

2.4.2. Determination of the maximum photochemical efficiency

The maximum photochemical efficiency was the key parameter of OJIP chlorophyll *a* fluorescence transient detected by Plant