Original Research

Comparative Sensitivity of Eight Freshwater Phytoplankton Species to Isoprocarb, Propargite, Flumetralin and Propiconazol

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Abstract

This work compared the sensitivity of three cyanobacteria (*Anabaena flos-aquae, Microcystis flos-aquae* and *Mirocystis aeruginosa*) as well as five green algae (*Pseudokirchneriella subcapitata, Scenedesmus quadricauda, Scenedesmus obliquus, Chlorella vulgaris* and *Chlorella pyrenoidosa*) to four pesticides through 96h short-term chronic tests. The results showed that the toxicity of the pesticides to the organisms increased in the order: propiconazol > isoprocarb > flumetralin > propargite. A wide variation in toxicity response of the organisms was observed. The sensitivity of the organisms varied by over one order of magnitude for propargite, by over two orders of magnitude for isoprocarb and propiconazol, and by over three orders of maginitude for flumetralin. Compared to green algae, cyanobacteria were less sensitive. This may result in the alteration of green algae dominated species to those dominated by cyanobacteria, stimulating to cyanobcterial bloom during a certain period.

Keywords: pesticide, toxicity, sensitivity, cyanobacteria, green algae, aquatic ecosystem

Introduction

Pesticide pollution of aquatic ecosystems has been a serious concern for human health [1]. Pesticides enter aquatic ecosystems through activities such as spraying and drifting, soil leaching, surface runoff, and accidental spills. They pose potential risks to aquatic plants and vegetables. The alteration of the species composition of an aquatic community as a result of toxic stress may affect the structure and the function of the whole ecosystem [2]. Green algae are known to be comparatively sensitive to many chemicals, including pesticides [3]. Their ecological functions in aquatic food webs and essential roles in the nutrient cycling and photosynthesis are critical to all ecosystems [4]. While studies have shown the effects of pesticides on green algae, little is known about the toxicity of pesticides to cyanobacteria. This information is needed as cyanobacteria have important implications for humans and aquatic organisms [5].

A thorough understanding of the effects of environmental contaminants on algal communities requires various tests using the algal species specific to an ecosystem and subsequent analysis. Previous studies have reported the comparative sensitivity of various species of green algae to organotins and pyrethroid pesticides [6]. There are few reports on the differential responses between cyanobacteria and green algae to pesticides. In the present study, four pesticides were tested to examine their effects on three

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cyanobacteria (*Anabaena flos-aquae, Microcystis flos-aquae* and *Mirocystis aeruginosa*) and five green algae (*Pseudokirchneriella subcapitata, Scenedesmus quadricauda, Scenedesmus obliquus, Chlorella vulgaris* and *Chlorella pyrenoidosa*).

Experimental Procedures

Chemicals

Test pesticides were purchased from Hunan Chemical Industry Research Institute and Zhejiang Chem-Tech Group Co. Ltd, China. Their registration numbers, formulation and uses are listed in Table 1. Before use, the pesticides were dissolved in a small volume of acetone (99.5%). The acetone concentration in the media for the tests was < 0.05% [7].

Test Organisms and Nutrient Media

The toxicity tests were carried out with the freshwater cyanobacteria *A. flos-aquae, M. flos-aquae* and *M. aeruginosa*, and the green algae *P. subcapitata, S. quadricauda, S. obliquus, C. vulgaris* and *C. pyrenoidosa*, which were obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Science. HB-4 and HGZ were the respective growth media for green algae and cyanobacteria and their chemical compositions can be found elsewhere [6, 8]. The media were sterilized by heating at 121ºC for 30 min.

Test Methods

Cyanobacterial and green algal cells were incubated in 250-ml Erlenmeyer flasks containing 100 ml of either HGZ or HB-4. The flasks were continuously shaken on a rotary shaker at 100 rpm at 24ºC. During shaking, the flasks were illuminated with continuous fluorescent lights at an intensity of 5000 Lx. The media (20 ml) containing either cyanobacterial or green algal cells (with the initial OD680 nm= 0.008) were transferred into pre-sterilized 50-ml Erlenmeyer flasks [9]. To the flasks were added the pesticides and incubated for 96 h on a rotary shaker at 100 rpm at 24ºC and continuous fluorescent lights at an intensity of 5000 Lx [10]. Control flasks containing no pesticides were also prepared and incubated. At the end of incubation, the biomasses in the flasks were determined by absorbance at the wavelength of 680 nm on a Shimadzu UV-2401PC spectrophotometer. Good linear relationships between the concentration of biomass (expressed as dry weight or Chlorophyll-a content of algal culture) and the UV-visible absorbance were previously obtained [6, 7]. The percentages of growth inhibition on cells relative to controls were thus calculated using the A680nm data. All the tests were in triplicate. The tested pesticidal concentrations were used (Table 1). Absorbance (680 nm) were realized in each of the concentrations tested.

Statistical Evaluation

The median effective concentration (EC_{50}) was obtained by linear regression between percent inhibition and pesticide concentration (natural logarithm). The no-observed-effect concentration (NOEC) was determined as the test concentration immediately below the lowest significant concentration where a statistically significant reduction ($P < 0.05$) was observed when compared with the control. The lowest-observed-effect concentration (LOEC) was obtained by weighted analysis of variance, followed by a one-sided Dunnett's test at the 5% significance level. The chronic value (CV) was the geometric mean of the NOEC and LOEC [6]. Statistical analysis was performed using SPSS version 11.0; all the other calculations were performed using Microsoft Excel 2003.

Results

Toxicity of Tested Pesticides

The short-term chronic toxicity of four pesticides to three cyanobacteria and five green algae is shown in Table 2. With isoprocarb, the respective EC_{50} and CV values varied within 7.2-66.7 mg/l and 0.7-14.2 mg/l for cyanobacteria and within 2.1-24.1 mg/l and 0.3-1.4 mg/l for green algae. With propargite, the respective values of EC_{50} and CV varied within 19.9-210.3 mg/l and 7.0-31.7 mg/l for cyanobacteria and within 38.8-736.4 mg/l and 3.1-141.5 mg/l for green algae. With flumetralin, the respective values of EC_{50} and CV varied within 47.0-2411.3 mg/l and 7.0-141.3 mg/l for cyanobacteria and within 2.7-8.6 mg/l and 0.1-1.5 mg/l for green algae. By comparison, the respective values of EC_{50} and CV with propiconazol varied within 8.1-28.0 mg/l and 3.1-7.1 mg/l for cyanobacteria and within 1.2-3.6 mg/l and 0.07-0.15 mg/l for green algae. It is apparent that propiconazol was the most toxic to both cyanobacteria and green algae. The toxicity decreased in the order (owing to EC_{50} values): propiconazol > flumetralin > isoprocarb > propargite.

Sensitivity of Cyanobacteria and Green Algae

A wide variation was observed in the response of the test organisms to the test pesticides, indicating that the organisms differed significantly in their sensitivity to the pesticides. With isoprocarb, the EC_{50} values indicated that the sensitivity decreased in the order: *C. vulgaris* > *P. subcapitata* > *S. quadricauda* > *S. obliquus* > *M. aeruginosa* > *C. pyrenoidosa* > *M. flos-aquae* > *A. flos-aquae*. In particular, *C. vulgaris* was over one order of magnitude more sensitive than *A. flos-aquae* and *M. flos-aquae*. Similarly, both *S. quadricauda* and *S. obliquus* were over one order of magnitude more sensitive than *A. flos-aquae*. The values of CV indicated a slightly different sensitivity of the organisms to isoprocarb, which decreased in the order:

Table 1. Registration numbers, formulation, and tested concentrations.

Pesticide	Registration number	Formulation[a]	Use	Tested concentrations (mg/L) [b]
Isoprocarb	2631-40-5	96%TC	Insecticide	0.05-200
Propargite	2312-35-8	90%TC	Acaricide	1-2000
Flumetralin	62924-70-3	98%TC	Herbicide	0.1-5000
Propiconazol	60207-90-1	92%TC	Fungicide	0.02-100

[a]TC: technical grade, [b] Tested concentrations denote min-max concentrations, such as 0.05-200 mg/L denote 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 mg/L.

S. obliquus > *C. vulgaris* ≈ *P. subcapitata* > *M. aeruginosa* > *S. quadricauda* ≈ *C. pyrenoidosa* > *M. flos-aquae* > *A. flos-aquae*. The sensitivity of *P. subcapitata* and *C. vulgaris* was over one order of magnitude higher than that of *A. flos-aquae* and *M. flos-aquae*. *S. quadricauda* was also over one order of magnitude more sensitive than *A. flos-aquae.* The difference in sensitivity between *S. obliquus* and *A. flos-aquae* was increased to over two orders of magnitude. In general, green algae were much more sensitive to isoprocarb than cyanobacteria.

As to propargite, the EC_{50} values indicated that the sensitivity decreased in the order: *A. flos-aquae* > *S. obliquus* > *M. flos-aquae* > *P. subcapitata* > *C. vulgaris* > *S. quadricauda* > *M. aeruginosa* > *C. pyrenoidosa*. The sensitivity of *A. flos-aquae* was over one order of magnitude higher than *M. aeruginosa, S. quadricauda* and *C. pyrenoidosa*. The CV-based sensitivity decreased in the order: *P. subcapitata* ≈ *S. obliquus* > *A. flos-aquae* > *C. vulgari* > *M. aeruginosa* ≈ *M. flos-aquae* ≈ *S. quadricauda* > *C. pyrenoidosa*. The sensitivity of *P. subcapitata* and *S. obliquus* was over one order of magnitude higher than *M. aeruginosa, M. flos-aquae, S. quadricauda* and *C. pyrenoidosa.* There was no distinguishable difference in sensitivity to propargite between cyanobacteria and green algae.

With respect to flumetralin, the EC_{50} values indicated that the sensitivity decreased in the order: *S. quadricauda* > *C. vulgaris / P. subcapitata* > *C. pyrenoidosa* > *S. obliquus* > *A. flos-aquae* > *M. aeruginosa* > *M. flos-aquae*. Not only was the sensitivity of all the green algae (except *S. obliquus*) over one order of magnitude higher than that of all the cyanobacteria, but also even two orders of magnitude higher than that of *M. flos-aquae*, in particular. Similar results were obtained from the CV values, which indicated that the sensitivity decreased in the order: *S. quadricauda* > *P. subcapitata / C. pyrenoidosa* > *C. vulgaris* > *S. obliquus* > *A. flos-aquae* > *M. aeruginosa* > *M. flos-aquae*. Not to mention at least one to two orders of magnitude higher sensitivity of all the green algae than that of all the cyanobacteria, the difference in sensitivity of *S. quadricauda* and *M. flos-aquae* was even over three orders of magnitude.

Finally, with respect to propiconazol, all the green algae were much more sensitive than cyanobacteria. The EC_{50} values indicated that the sensitivity decreased in the order: *P. subcapitata* ≈ *S. quadricauda* ≈ *C. vulgaris* ≈ *C. pyrenoidosa* > *S. obliquus* > *M. flos-aquae* > *M. aeruginosa* > *A. flos-aquae*. The largest difference in sensitivity was over one order of magnitude. The CV values indicated that the sensitivity decreased in the order: *S. obliquus* > *P. subcapitata* ≈ *S. quadricauda* ≈ *C. vulgaris* ≈ *C. pyrenoidosa* > *M. aeruginosa* > *A. flos-aquae* ≈ *M. flos-aquae*. The sensitivity of *P. subcapitata, S. quadricauda, C. vulgaris* and *C. pyrenoidosa* was over one order of magnitude higher than that of *A. flos-aquae, M. aeruginosa* and *M. flos-aquae*. The sensitivity of *S. obliquus* was even over two orders of magnitude higher than that of *A. flos-aquae* and *M. flos-aquae*.

Discussion of Results

Previous studies have shown that aquatic algae bloom is due primarily to algal overgrowth [10] and the gradual shift of cyanobacterial and green algal community structure [11]. Under appropriate growing conditions (e.g., light and temperature), the algal bloom is facilitated by excess nitrogen and phosphorus in water [12]. In contrast to the clear understanding of the role of aqueous nutrients, there is a paucity of data on the potential effects of pesticides on green algae and cyanobacteria. Pesticides may alter the community structure of green algae and cyanobacteria. In particular, the species dominated by green algae may be altered to those dominated by cyanobacteria, resulting in sustaining cyanobcterial blooms during a certain period. An alteration may occur when cyanobacteria are less sensitive to pesticides than green algae. Cyanobacteria can produce a variety of toxins including hepatotoxins and neurotoxins [6]. In addition, cyanobacteria can fix atmospheric nitrogen to further contribute to the blooms [12]. The pesticide contamination would thus pose additional ecosystem hazards when the cyanobacterial population increases. Understanding the potential green algae-to-cyanobacteria alteration requires information about the differential sensitivity between the cyanobacteria and green algae to pesticides.

The results showed that the decreasing order of the average toxicity to eight phytoplancton species of four tested pesticides was: propiconazol > isoprocarb > flumetralin > propargite. On the other hand, the results in Table 2 suggested that the sensitivity of the algae to the pesticides, the decreasing order was (owing to EC_{50} values): flumetralin > propiconazol > isoprocarb > propargite. There was a various order between their toxicity and ecosystem risk.

Table 2. Toxicity of pesticides to cyanobacteria and green algae.

Pesticide	Regression equation*	CC*	SL*	EC_{50}	LO EC	NO EC	CV
Isoprocarb	(1)Y=3.4204+0.3037X	0.9879	0.0002	66.65	20	10	14.1
	(2)Y=2.4112+0.1614X	0.9798	0.0200	7.20	1	0.5	0.7
	(3)Y=4.4513+0.3752X	0.9834	0.0000	26.69	10	5	7.1
	(4)Y=2.9769+0.1924X	0.9943	0.0000	2.56	0.5	0.2	0.3
	(5)Y=3.2730+0.2221X	0.9912	0.0000	3.79	2	1	1.4
	(6)Y=2.1887+0.1384X	0.9844	0.0000	5.02	0.2	0.1	0.1
	(7)Y=2.9707+0.1890X	0.9659	0.0000	2.10	0.5	0.2	0.3
	(8)Y=3.7938+0.3097X	0.9737	0.0260	24.05	2	1	1.4
Propargite	(1)Y=3.3773+0.2658X	0.9927	0.0001	19.90	10	5	7.1
	(2)Y=2.0351+0.1813X	0.9850	0.0150	210.23	50	20	31.6
	(3)Y=3.8500+0.3475X	0.9804	0.0196	65.14	50	20	31.6
	(4)Y=1.8032+0.1379X	0.9734	0.0011	78.84	5	2	3. 2
	(5)Y=1.9800+0.1800X	0.9854	0.0000	268.62	50	20	31.6
	(6)Y=2.0871+0.1563X	0.9721	0.0001	38.86	5	2	3.2
	(7)Y=1.9021+0.1574X	0.9844	0.0000	135.32	20	10	14.1
	(8)Y=2.9022+0.3330X	0.9915	0.0000	736.34	200	100	141.4
Flumtralin	(1)Y=2.6488+0.2156X	0.9781	0.0007	47.00	10	5	7. 1
	(2)Y=4.0756+0.3633X	0.9707	0.0155	53.17	20	10	14.1
	(3)Y=1.4180+0.1523X	0.9995	0.0010	2411.33	200	100	141.4
	(4)Y=3.6215+0.2502X	0.9899	0.0100	3.82	0.5	0.2	0.4
	(5)Y=3.5879+0.2415X	0.9747	0.0050	2.80	0.2	0.1	0.1
	(6)Y=2.8401+0.2006X	0.9726	0.0010	8.58	2	1	1.4
	(7)Y=3.4820+0.2388X	0.9747	0.0010	3.77	1	0.5	0.7
	(8)Y=2.4188+0.1548X	0.9460	0.0010	4.14	0.5	0.2	0.3
Propiconazol	(1)Y=7.7593+0.6924X	0.9762	0.0240	27.97	10	5	7.1
	(2)Y=2.8218+0.2159X	0.9343	0.0060	21.36	5	2	3.2
	(3)Y=5.0118+0.3851X	0.9900	0.0100	8.16	5	2	3.2
	(4)Y=3.1714+0.1975X	0.9907	0.0000	1.34	0.2	0.1	0.1
	(5)Y=3.1953+0.1989X	0.9930	0.0000	1.30	0.2	0.1	0.1
	(6)Y=2.7154+0.1767X	0.9919	0.0009	3.58	0.1	0.05	0.1
	(7)Y=2.9125+0.1779X	0.9834	0.0000	1.29	0.2	0.1	0.1
	(8)Y=4.1986+0.2735X	0.9576	0.0100	1.34	0.2	0.1	0.1

[a]Y, X, CC and SL stand for percent inhibition, natural logarithm of concentration, coefficient correlation significance level, respectively; (1) *A. flos-aquae*; (2) *M. aeruginosa*; (3) *M. flos-aquae*; (4) *P. subcapitata*; (5) *S. quadricauda*; (6) *S. obliquus;* (7) *C. vulgaris*; (8) *C. pyrenoidosa*.

It is often true that a contaminant with a "low environmental toxicity" does not necessarily have a "low ecosystem hazard", However, the aquatic ecological system is complicated. In addition, whether there exists a strong positive relativity between the effect on the respective cultivation of certain alga in the laboratory and the effect on the actual mixed growth of multiple algae in the field, it begs to be studied further and proved by more experimental data. The laboratory tests may thus provide useful preliminary information on the effects of pesticides on the growth of various species of aquatic organisms in the natural environment. Further studies both in the laboratory and in the field are needed to understand the toxicological impact of pesticides on aquatic habitats. The species tolerant of pesticides but harmful to the water body are of particular importance for assessing the potential ecological risk of pesticides. The results showed that:

1) The toxicity of the pesticides to the organisms decreasing in the order: propiconazol > isoprocarb > flumetralin > propargite;
2) Compared to green algae, cyanobacteria were less sensitive;
3) The sensitivity of the organisms varied by over one order of magnitude for propargite, by over two orders of magnitude for isoprocarb and propiconazol, and by over three orders of maginitude for flumetralin.

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