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Differential Response of Four Cyanobacterial and Green Algal Species to Triazophos, Fentin Acetate, and Ethephon

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Pesticides are often used in agriculture to reduce or destroy pests. Pollution of aquatic systems by pesticides has been noticed by the public. Such pollution may be washed from leaves and soil and enter freshwater ecosystems and present risks for aquatic flora (Van den Brink and Ter Braak 1999). Alterations of the species composition of an aquatic community as a result of toxic stress may affect the structure and the functioning of the whole aquatic ecosystem (Verdisson et al. 2001). Algae and cyanobacteria (blue-green algae) are known to be comparatively sensitive to many chemicals (Real et al. 2003), as their ecological position at the base of most aquatic food webs and essential roles in the nutrient cycling and oxygen production are critical to all ecosystems (Sabater and Carrasco 2001). A lot of work has been published about the toxicological aspects of pesticides on green algae. However, little is known about that of pesticides on cyanobacteria (Abou-Waly et al. 1991; Sabater and Carrasco 2001). Cyanobacteria can produce algal toxins, which has important implications for humans and aquatic organisms (An and Kampbell 2003).

Tests on a certain species of algae are of limited applicability in assessing the effects of environmental contaminants on algal communities, which are composed of an array of species with different sensitivities (Sanchez and Tarazona 2002). Some reports have been published about the comparative sensitivity of pesticides toward various green algae (Ma et al. 2004a; b), yet there are few reports concerning the differential response of various cyanobacteria and green algae. In order to compare the differential sensitivity of the tested pesticides to cyanobacteria and green algae, an acute toxicity test has been devised. In the present work, 3 pesticides, which were widely used in China, were tested to examine their effects on 3 cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae*, *Mirocystis aeruginosa* and the green alga *Chlorella pyrenoidosa*.

MATERIALS AND METHODS

The tested pesticides, which were purchased from Anyang Chem-tech Group CO.,

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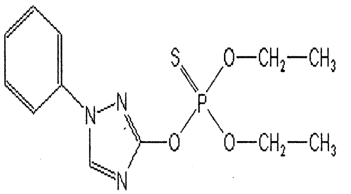
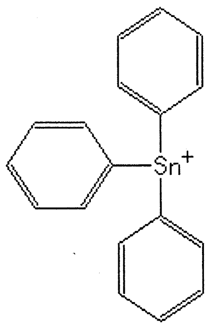
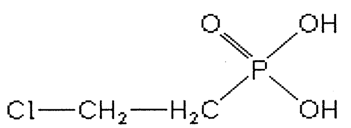
Ltd. in China and their structure, CAS NO. and formulation are shown in Table 1, were dissolved in acetone. Acetone concentration in the medium was less than 0.05%, it was not significant with regard to toxicity (Jay 1996). The toxicity tests were carried out with the freshwater cyanobacteria *A. flos-aquae*, *M. flos-aquae*, *M. aeruginosa*, and the green alga *C. pyrenoidosa*, obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Science. The media for cyanobacterial and green algal growth inhibition tests were HGZ and HB-4 medium, respectively (Ma et al. 2001; 2003). The culture media were sterilized at 121℃, 1.05 kg cm-2 for 30 min.

Table 1. Selected pesticides, their structure, formulation and activity.

Pesticides	Structure	CAS No.	Formulation*	Activity
Triazophos		24017-47-8	80%TC	Acaricides Insecticides Nematicides
Fentin acetate		900-95-8	95%TC	Algicides Antifeedants Fungicides
Ethephon		16672-87-0	85%TC	Plant growth regulators Defoliants

*TC stands for technical product

Cyanobacterial or green algal cells were propagated photoautotrophically in a 250 mL Erlenmeyer flask containing 100 mL liquid HGZ or HB-4 medium and kept on a rotator shaker (100 rpm) at 24℃, illuminated with cool-white fluorescent lights at a continuous light intensity of 450 μ mol $s^{-1}m^{-2}$ respectively (Ma 2002). 20 mL HGZ or HB-4 medium containing cyanobacterial or green algal cells (initial concentration OD680nm=0.008) were distributed to sterile 50 mL Erlenmeyer flasks individually. A wide range of concentrations was examined in a previous test in order to find the adequate range of toxicity for each pesticide. Then, adequate concentration were tested according to the results of the previous test (Moreno-Garrido et al. 2000). The adequate concentration range of toxicity for triazophos, ethephon, fentin acetate and acetone were 0.02-200 mg/L, 1-1000 mg/L, 0.0002-0.2 mg/L and 1000-200000 mg/L respectively. The media was then treated with various pesticide concentrations, and incubated for 96 h on an rotator shaker (100 rpm) at 24℃ and a continuous light intensity of 450 μ mol $s^{-1}m^{-2}$ (Ma et al. 2001). The most suitable wavelength for monitoring culture growth was 680 nm. Good linear relationships between dry weight concentration (DWC) or Chlorophyll-a (ChlaA) content of algal cultures and OD680nm are highly correlated, thus, algal biomass was calculated indirectly using OD680nm data (Verdisson et al. 2001). Three replicates were made for each pesticide concentration and control. In each experiment, percent inhibition values, relative to growth in control systems, were calculated using OD680nm data (Ma et al. 2002a; b). The EC_{50} values were calculated using linear regression analysis of transformed pesticidal concentration as natural logarithm data versus percent inhibition (Ma and Liang 2001). Weighted analysis of variance (ANOVA) was used, followed by a one-sided Dunnett's test using a 5% significance level to obtain the LOEC (lowest observable effect concentration). The NOEC (No observable effect concentration) was taken to be the test concentration immediately below the LOEC, CV (chronic value) was the geometric mean of the NOEC and LOEC (Saker and Neilan 2001).

RESULTS AND DISCUSSION

The acute toxicity of acetone to three cyanobacteria *A. flos-aquae*, *M. flos-aquae*, *M. aeruginosa* are shown in Table 2. The 96 h EC_{50}, LOEC, and NOEC values of acetone to cyanobacteria varied around 11000-32000 mg/L, 5000-50000 mg/L and 2000-20000 mg/L, respectively. The US Environmental Protection Agency recommends the allowable maximal limits of 0.05% solvent for acute tests and 0.01% for chronic tests (Jay 1996), this may be fit to green algae. However, as to *A. flos-aquae*, *M. flos-aquae* and *M. aeruginosa*, the concentration of the acetone in the medium was less than 2%, 0.2% and 1%, respectively. Interaction between reagent and acetone was not significant with regard to toxicity in the study.

The acute toxicity of 3 pesticides to three cyanobacteria *A. flos-aquae*, *M. flos-aquae*, *M. aeruginosa*, and green alga *C. pyrenoidosa* are shown in Table 2. The acute toxicity of triazophos to cyanobacteria and green algae was higher than that of ethephon, but lower than that of fentin acetate. The decreasing order of the toxicity of 3 dissimilar pesticides was: fentin acetate > triazophos > ethephon.

Wide variations were found occurring in response to the tested pesticides among

Table 2. The effect of various pesticides to cyanobacteria and green algae.

Pesticides	Regression equation*	Coefficient correlation	Significance level	EC_{50} (mg/L)	LOEC (mg/L)	NOEC (mg/L)
Triazophos	(1) Y=4.2281+0.3308X	0.9545	0.0031	12.7446	5	2
	(2) Y=4.8856+0.3909X	0.9953	0.0047	13.4032	10	5
	(3) Y=4.2269+0.3107X	0.9911	0.0001	6.1637	2	1
	(4) Y=3.5336+0.2914X	0.9659	0.0340	30.1166	5	2
Fentin acetate	(1) Y=3.4574+0.1650X	0.9252	0.0240	0.0164	0.002	0.001
	(2) Y=5.1283+0.2638X	0.9685	0.0010	0.0240	0.005	0.002
	(3) Y=3.8118+0.1776X	0.9706	0.0010	0.0080	0.001	0.0005
	(4) Y=7.4094+0.4031X	0.9747	0.0250	0.0360	0.01	0.005
Ethephon	(1) Y=4.5853+0.4379X	0.9618	0.0380	88.7845	50	20
	(2) Y=30.0579+3.4139X	0.9803	0.0010	173.7134	100	50
	(3) Y=5.0093+0.4720X	0.9597	0.0400	70.9453	50	20
	(4) Y=2.2323+0.1625X	0.9539	0.0010	23.4578	5	2
Acetone	(1) Y=1.9657+0.4227X	0.9859	0.0400	31195	50000	20000
	(2) Y=1.8510+0.3034X	0.9824	0.0028	11642	5000	2000
	(3) Y=2.1595+0.4479X	0.9843	0.0157	24605	20000	10000

*Y and X stand for percent inhibition and natural logarithm of concentration respectively.

(1) *A. flos-aquae*; (2) *M. aeruginosa*; (3) *M. flos-aquae*; (4) *C. pyrenoidosa.*

individual species of four algae. For triazophos, according to magnitude of the EC_{50} values, the decreasing order of the sensitivity to cyanobacteria and green algae was *M. flos-aquae* > *A. flos-aquae* > *M. aeruginosa* > *C. pyrenoidosa*, the sensitivity of various algal species between *M. flos-aquae* and *C. pyrenoidosa,* when exposed to triazophos varied five-fold. According to the CV, the decreasing order of the sensitivity was: *M. flos-aquae* > *A. flos-aquae* / *C. pyrenoidosa* > *M. aeruginosa*, the sensitivity of various species of algae — between *M. flos-aquae* and *M. aeruginosa,* when exposed to triazophos varied five-fold (see Fig 1). i.e. the sensitivity of green algae is lower than that of all 3 cyanobacteria.

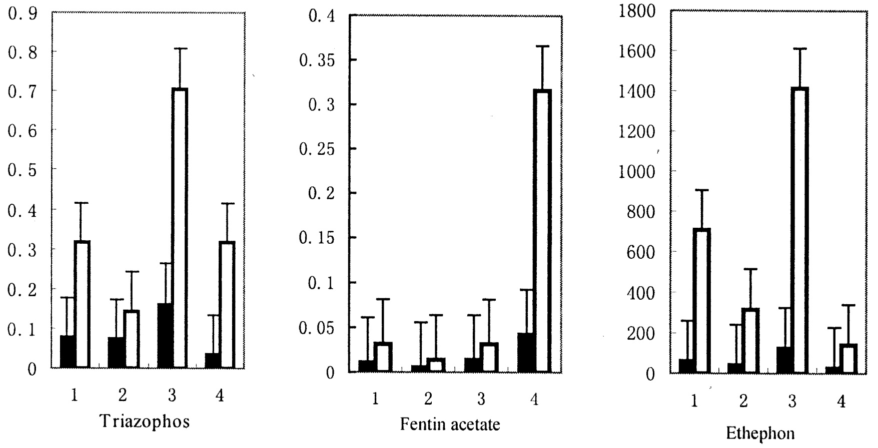


Figure 1. Differential sensitivities to triazophos, fentin acetate and ethephon. black and white stand for $1/EC_{50}$ and 1/CV respectively; ordinate and abscissa axis stand for sensitivity (L/mg) and cyanobacterial (1, 2, 3) , green algal (4) species respectively.

For fentin acetate, from EC_{50} values, the decreasing order of sensitivity to cyanobacteria and green algae was: *M. flos-aquae* > *A. flos-aquae* > *M. aeruginosa* > *C. pyrenoidosa*, the sensitivity of various species of algae was small. According to CV values, the decreasing order of the sensitivity also was: *M. flos-aquae* > *A. flos-aquae* > *M. aeruginosa* > *C. pyrenoidosa*, the sensitivity of various species of algae—between *A. flos-aquae* and *C. pyrenoidosa*, between *M. flos-aquae* and *C. pyrenoidosa,* when exposed to fentin acetate varied over five-fold and ten-fold respectively. (see Fig 1), i.e. the sensitivity of green algae is lower than that of 3 cyanobacteria.

As to ethephon, according to EC_{50} values, the decreasing order of the sensitivity to cyanobacteria and green algae was as follows: *C. pyrenoidosa* > *M. flos-aquae* > *A. flos-aquae* > *M. aeruginosa*, the sensitivity of various species of algae—

between *C. pyrenoidosa* and *M. aeruginosa*, when exposed to ethephon varied five-folds of magnitude. According to CV values, the decreasing order of sensitivity was: *C. pyrenoidosa* > *M. flos-aquae* / *A. flos-aquae* > *M. aeruginosa*, the sensitivity of various species of algae—between *C. pyrenoidosa* and *A. flos-aquae* / *M. flos-aquae*, between *C. pyrenoidosa* and *M. aeruginosa*, when exposed to ethephon varied 10-fold and 25-fold respectively (see Fig 1). The sensitivity of green algae is much higher than that of 3 cyanobacteria.

In the aquatic ecosystem, the formation of algal bloom is attributed to the overabundance of algal growth and the gradual shift of algal community structure (Boutin and Rogers 2000; Siegel et al. 2002). The latter usually indicates a gradual shift from dominance by green algae to dominance by cyanobacteria. It is owing to physical, nutritional and biological factors (Pei and Ma 2002). However, there are few reports involved in other factors, such as pollutants, in which the green algae and cyanobacteria have greater differential sensitivity. It may also be important for sustaining cyanobacterial blooms during the special period. Cyanobacteria can produce a variety of toxins including hepatotoxins (Best et al. 2002). Thus, research comparing the various sensitivity of cyanobacteria and green algae is of important scientific significance and value. If the green algae and cyanobacteria have greater differential sensitivity, the contamination may result in a shift from dominance by green algae to dominance by cyanobacteria, and sustain cyanobacterial blooms during the special period, thus, the contamination will exist more ecosystem hazard. The results indicate that decreasing order of the average toxicity to four algae of 3 dissimilar pesticides was: fentin acetate > triazophos > ethephon. However, according to magnitude of ecosystem hazard, the decreasing order of the ecosystem risk was: ethephon > triazophos > fentin acetate. There was a strong variance between toxicity and ecosystem hazard, i.e. "low toxicity" does not imply "low ecosystem hazard".

In this research, owing to the fact that HGZ medium is fit for cyanobacteria's growth and HB-4 medium is fit for green algae's growth, HGZ medium was selected for incubating cyanobacteria *A. flos-aquae*, *M. flos-aquae* or *M. aeruginosa*, and HB-4 medium for incubating green alga *C. pyrenoidosa*. In general, the choice of culture medium is based on whether it is suitable for the growth of the organism to be incubated. The EC_{50}, LOEC and NOEC values were calculated by referring respectively to the control systems, both the control and treatment systems using the same medium. Thus, it may cause no influence on the result. Also there is no uniform standard about medium selection in the testing guidelines for chemical toxicity. Furthermore, we have not found any media influence on toxicity data in previous literature. Therefore, in this work, the comparison of differential sensitivity is based on the orders of magnitude level. The result is not affected by the use of different media.

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