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Differential Sensitivity of Three Cyanobacteria (*Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*) to 10 Pesticide Adjuvants

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Today chemicals are widely used and may enter aquatic ecosystems. Adverse effects of those pollutants on nontarget plants are particularly of concern due to the annual, increasing worldwide use of these chemicals (Van der Brink and Ter Braak 1999). Algae play an important role in the equilibrium of aquatic ecosystems, by being the first level of the trophic chain to produce organics and oxygen (Ma et al. 2003). Algal toxicity tests are extensively applied to assess the effects of hazardous substances in water. However, many of these tests are performed with single species in the laboratory under ecologically unrealistic circumstances (Junghans et al. 2003; Kuang et al. 2003).

A test organism's sensitivity to toxic substances is a complex issue. Some studies have shown that the sensitivities of plants and other groups of organisms vary widely among toxicants (Hughes and Erb 1989). Some reports have been published about the comparative toxicity of solvents toward various test organisms (Tadros et al. 1994). A few reports involved the differential response of various species to pesticides (Kasai et al. 1993; Ma et al. 2004a,b,c). However, few works involved the assessment of the toxicity of surfactant to cyanobacteria especially in pesticide adjuvants (Cardellini and Ometto 2001), and differential sensitivity of various species of cyanobacteria to pesticides. It is known that each pesticide contains an 'active ingredient' which is responsible for its pesticidal effect, but pesticides are rarely supplied as preparations of neat, concentrated or technical grade chemical. The active ingredient must be formulated with other non-pesticidal compounds before it is ready to use. In order to compare sensitivity of cyanobacteria to adjuvants, a toxicity test has been devised.

Little is known about the toxicological aspects of pesticides on cyanobacteria (Abou-Waly et al. 1991; An and Kampbell 2003; Sabater and Neilan 2001). In this study, 10 pesticide adjuvants, which have been widely used to manufacture various formulations in the pesticide industry, have been tested to examine their effects on *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*, and for comparison of sensitivity.

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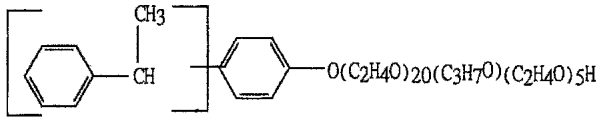
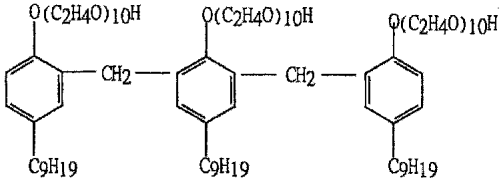
MATERIALS AND METHODS

All of the tested adjuvants were purchased from Hangzhou Electrochemical Group Auxiliary Chemical Co., Ltd.; Xingtai Lantian Fine Chemical Co., Ltd. and Jiangsu Tianyin Chemical Industry Co., Ltd. in China, and their chemical structures are shown in Figure 1. The purity of the tested adjuvants was >98%. The toxicity tests were carried out with the freshwater cyanobacteria *A. flos-aquae*, *M. flos-aquae* and *M. aeruginosa* obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Science. The cyanobacteria were kept on agar slants at approximately 4℃. The medium for the cyanobacterial growth inhibition test was HGZ medium; composed of distilled water and the following chemical ingredients (mg/L): $NaNO_3$ 1500, K_2HPO_4 39, $MgSO_4.7H_2O$ 75, Na_2CO_3 20, $CaCl_2$ 27, $Na_2SiO_3.9H_2O$ 58, EDTA 1, Citric acid 6, Fe-Citric 6, and an A_5 liquid 1 mL (ingredients of A_5 liquid are (mg/L): H_3BO_3 2860, $MnSO_4$ 2060, $ZnSO4.7H_2O$ 222, $Na_2MO_4.2H_2O$ 391 and $CuSO_4.5H_2O$ 79). The culture medium was sterilized at 121℃, 1.05 kg cm^{-2} for 30 min (Ma 2005).

Three cyanobacteria were used as test organisms. Cells of cyanobacteria were propagated photoautotrophically in a 250 mL Erlenmeyer flask containing 100 mL liquid HGZ medium and kept on a rotary shaker (100 rpm) at 24℃, and illuminated with cool-white fluorescent lights at a continuous light intensity of 5000 lux/cm^2 (Ma et al. 2004b). 20 mL aliquots of the HGZ medium containing cyanobacteria cells (initial concentration OD680nm=0.008) were distributed to sterile 50 mL Erlenmeyer flasks. In a previous test, a wide range of concentrations were used for determining an adequate range of concentrations for toxicity tests of each adjuvant. Then, adequate concentrations were tested owing to the results of the previous test (Moreno-Garrido et al. 2000). The cyanobacterial medium was then treated with various concentrations of adjuvants, and incubated for 96 h on an orbital shaker (100 rpm) at a temperature of 24℃ and a continuous light intensity of 5000 lux/cm^2 (Ma et al. 2003). Biomass was correlated with absorbance over time for 96 h on a Shimadzu UV-2401PC spectrophotometer. The most suitable wavelength for monitoring culture growth was 680 nm. There was a good linear relationship between dry weight concentration or chlorophyll-a content of the three cyanobacteria cultures and OD680nm. All had a correlation coefficient of $R>0.97$ and significance level $P<0.001$. Thus, the growth of cyanobacterial biomass was calculated indirectly using spectrophotometric data. Three replicates were made for each adjuvant concentration and control. Appropriate control systems containing no adjuvant were included in each experiment. Control and treated cultures grew under the same temperature, photoperiod and shaking conditions as the stock cultures. In each experiment, percent inhibition values, relative to growth in the control, were calculated using spectrophotometric data (Ma et al. 2004a, b).



Pesticide emulsifier #1601



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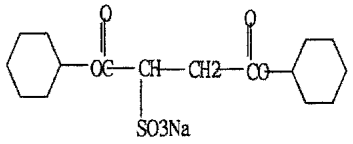

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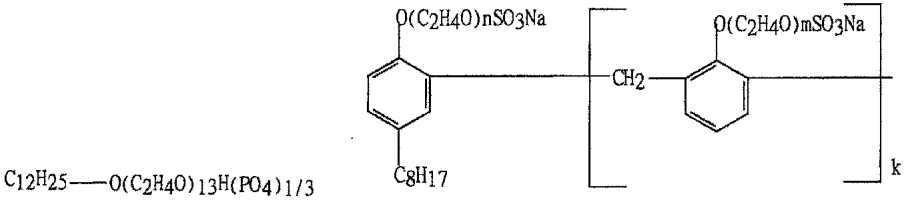
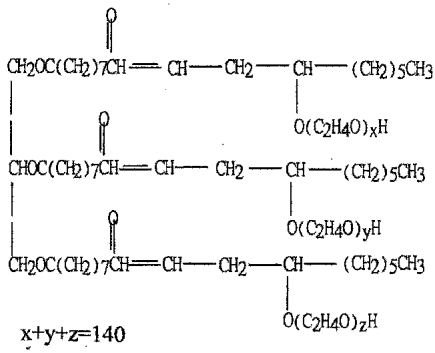
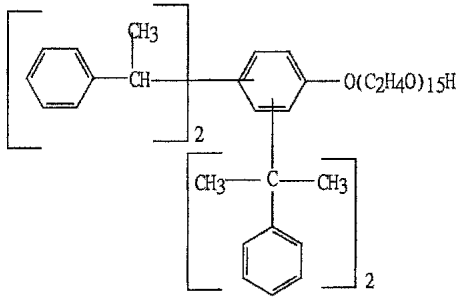


AEO-13 phosphate

SOPA



BY-140



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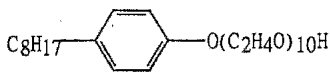
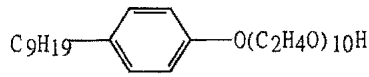
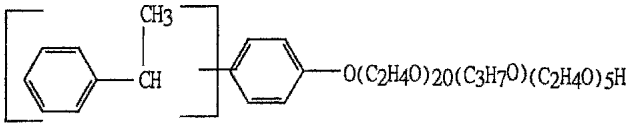


Pesticide emulsifier OP-10



Pesticide emulsifier NP-10

Figure 1. Selected pesticide adjuvants and their chemical structure



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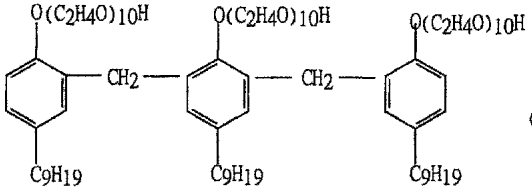

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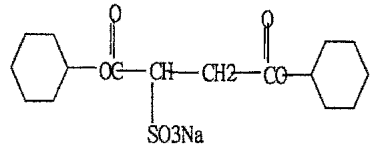
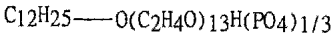
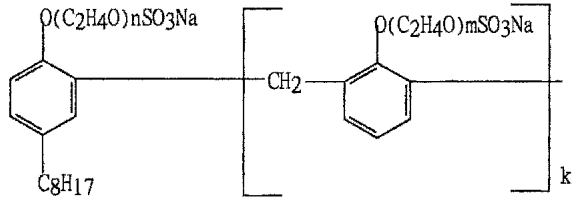


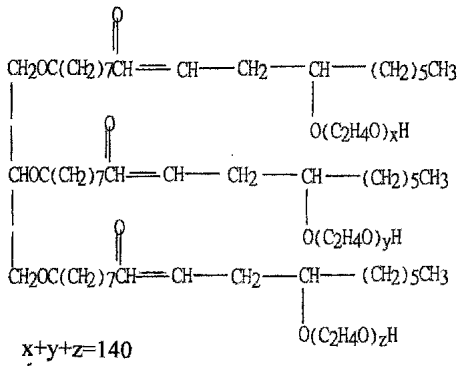
OT



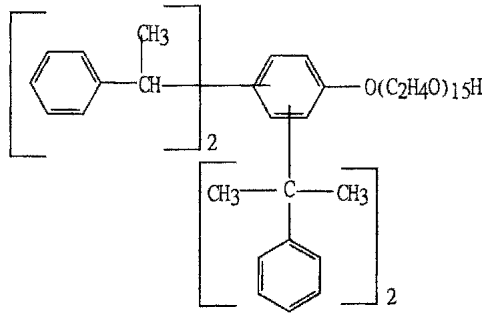
AEO-13 phosphate



SOPA



BY-140



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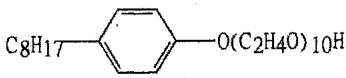
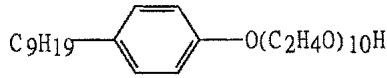


Pesticide emulsifier OP-10



Pesticide emulsifier NP-10

Figure 1. Selected pesticide adjuvants and their chemical structure

For the growth inhibition tests with cyanobacteria, the EC_{50} were computed using linear regression analysis of transformed adjuvant concentration as natural logarithm data versus percent inhibition (Ma et al. 2003). Statistical analysis of the data was performed using SPSS version 11.0 (Saker and Neilan 2001).

RESULTS AND DISCUSSION

Table 1. Dose response relationship of 10 adjuvants to *M. aeruginosa* (1), *M. flos-aqua* (2) and *A. flos-aquae*(3)

Adjuvant	Regression equation [a]	Rsq [b]	Sigf [c]	EC_{50} (mg/L)
#602	(1)Y=3.4142+0.3363X	0.996	0.002	172.540
	(2)Y=1.9551+0.1313X	0.967	0.000	15.383
	(3)Y=6.9993+0.7610X	0.909	0.012	195.398
#700	(1)Y=4.0329+0.4719X	0.902	0.050	560.577
	(2)Y=2.2526+0.1498X	0.970	0.002	8.297
	(3)Y=2.3570+0.2523X	0.925	0.009	636.017
#1601	(1)Y=2.0149+0.2006X	0.980	0.010	525.147
	(2)Y=1.7481+0.0891X	0.956	0.022	0.083
	(3)Y=1.7424+0.1892X	0.949	0.005	1406.577
BY-140	(1)Y=3.1501+0.3539X	0.943	0.003	559.608
	(2)Y=1.8247+0.1131X	0.941	0.001	8.190
	(3)Y=1.6144+X0.1135	0.954	0.001	54.435
SOPA	(1)Y=1.1140+0.0560X	0.964	0.018	17.309
	(2)Y=1.5768+0.2703X	0.950	0.005	18616.220
	(3)Y=1.2837+0.1598X	0.953	0.024	7414.963
NP-10	(1)Y=2.1587+0.1320X	0.943	0.006	3.489
	(2)Y=3.5323+0.2577X	0.986	0.000	7.758
	(3)Y=5.6196+0.5013X	0.955	0.004	36.701
NP-10	(1)Y=4.0417+0.5360X	0.918	0.042	1350.002
phosphate	(2)Y=1.6924+0.1252X	0.949	0.005	73.080
	(3)Y=2.5340+0.2824X	0.914	0.011	744.685
OT	(1)Y=3.1640+0.2591X	0.999	0.000	34.253
	(2)Y=3.7101+0.3255X	0.931	0.0035	52.115
	(3)Y=3.3575+0.3078X	0.894	0.050	92.936
AEO-13	(1)Y=7.2287+0.7761X	0.906	0.048	171.679
phosphate	(2)Y=4.2334+0.3293X	0998	0.001	11.919
	(3)Y=4.4965+0.4281X	0.947	0.005	88.241
OP-10	(1)Y=3.7550+0.2867X	0.997	0.001	11.730
	(2)Y=2.9717+0.2210X	0.911	0.003	13.892
	(3)Y=3.3318+0.2621X	0.968	0.016	20.313

[a] Y and X stand for percent inhibition and adjuvant concentration respectively.
[b]Rsq and [c]Sigf denote correlation coefficient and significance level respectively.

Acute toxicity of 10 adjuvants to three cyanobacteria is shown in Table 1. Comparing the toxicity of 10 adjuvants to *M. aeruginosa*, the order from high to low was: NP-10 > OP-10 > SOPA > OT > AEO-13 phosphate > #602 > #1601 > By-140 > #700 > NP-10 phosphate, and to *M. flos-aquae*, the order was: # 1601> NP-10 > By-140 > #700 >AEO-13 phosphate > OP-10 > #602 > OT > NP-10 phosphate > SOPA, and to *A. flos-aquae*, the order was: OP-10 > NP-10 > By-140 > AEO-13 phosphate > OT > #602 > #700 > NP-10 phosphate > #1601 > SOPA.

Wide variations occurred in response to the tested adjuvants among the three individual species of cyanobacteria (Table 2). Compared with *M. aeruginosa*, *M. flos-aquae* was more sensitive to 6 adjuvants—#602, #700, #1601, By-140, NP-10 phosphate, and AEO-13 phosphate and was less sensitive to 4 adjuvants SOPA, NP-10, OT, OP-10. The sensitivity of cyanobacteria exposed to #602, #700, By-140, NP-10 phosphate, and AEO-13 phosphate varied by over one order of magnitude, and for #1601 and SOPA varied by over two order of magnitude.

However, compared with *M. aeruginosa*, *A. flos-aquae* was less sensitive to 7 adjuvants—#602, #700, #1601, SOPA, NP-10, OT and OP-10, and was more sensitive to 3 adjuvants—By-140, NP-10 phosphate and AEO-13 phosphate. The sensitivity of cyanobacteria exposed to By-140 and NP-10 varied by over one order of magnitude, and for SOPA varied by over two orders of magnitude. The decreasing order for the average sensitivity of 3 dissimilar cyanobacteria to the selected adjuvants was as follows: *M. flos-aquae*> *M. aeruginosa*> *A. flos-aquae*.

Standard aquatic ecotoxicity tests (single-species toxicity test) have historically been the source of biological data for hazard assessment, however, it has been discussed as to whether information from these tests alone is suitable to predict effects at the ecosystem level (Boxall et al. 2002; Cairns et al. 1996). Microcosm and field tests (multiple-species toxicity tests), enable observation of the indirect

Table 2. Sensitivity of three cyanobacteria and two green algae to adjuvants

Adjuvants	MA/MF	AF/MF	MA/ AF	MA/SQ
#602	11.216	12.702	0.883	5.643
#700	67.563	76.655	0.881	3.106
#1601	636.534	1704.942	0.373	0.395
BY-140	68.332	6.647	10.280	7.165
SOPA	0.001	0.398	0.0024	0.001
NP-10	0.450	4.731	0.095	1.035
NP-10 phosphate	18.473	10.190	1.813	12.736
OT	0.657	1.783	0.369	3.639
AEO-13 phosphate	14.404	7.403	1.946	3.440
OP-10	0.844	1.462	0.577	1.741

Table 2. Continued

Adjuvants	MA/CV	AF/SQ	AF/ CV
#602	4.686	6.390	5.307
#700	0.145	3.524	0.165
#1601	0.052	1.058	0.139
BY-140	0.473	0.697	0.046
SOPA	0.003	0.636	1.373
NP-10	0.317	10.883	3.337
NP-10 phosphate	4.967	7.025	2.740
OT	0.561	9.872	1.522
AEO-13 phosphate	1.167	1.768	0.600
OP-10	0.714	3.015	1.237

MA, MF, AF, SQ and CV stand for *M. flos-aquae*, *M. aeruginosa*, *A. flos-aquae*, *S. quadricauda* and *C. vulgaris* respectively. Data was obtained from EC_{50} ratio between cyanobacteria and green algae. EC_{50} data of two green algae may refer to Ma et al. (2004c).

effects of chemicals caused by interactions among species. However, conducting mesocosm tests to assess the impact of chemicals on ecosystem involves skilled labor and high cost (Cairns et al. 1996). Approaches that are intermediate between standard toxicity tests and microcosm (or field) studies can provide valuable data for use in the assessment of pesticides.

From our previous works (Ma et al. 2004c), wide variation occurred in response to the tested adjuvants among individual species of green algae and cyanobacteria (Table 2). Compared with the sensitivity of the green alga *S. quadricauda*, *M. aeruginosa* was less sensitive to 8 adjuvants—#602, #700, By-140, NP-10, NP-10 phosphate, OT, AEO-13 phosphate and OP-10. The sensitivity of *A. flos-aquae* and *S. quadricauda* exposed to NP-10 phosphate and SOPA varied by over one and three orders of magnitude, respectively. However, compared with the sensitivity of *C. vulgaris*, *M. aeruginosa* was more sensitive to 8 adjuvants—#700, #1601, By-140, SOPA, NP-10, OT and OP-10. The sensitivity of *A. flos-aquae* and *C. vulgaris* exposed to #1601 and SOPA varied by one and two orders of magnitude, respectively. Compared with the sensitivity of *S. quadricauda*, *A. flos-aquae* was less sensitive to 8 adjuvants—#602, #700, #1601, NP-10, NP-10 phosphate, OT, AEO-13 phosphate and OP-10. The sensitivity of *A. flos-aquae* and *S. quadricauda* exposed to NP-10 varied by over one order of magnitude. Sensitivity of green alga *C. vulgaris*, *A. flos-aquae* was less sensitive to the 8 adjuvants—#700, #1601, By-140 and AEO-13 phosphate, the sensitivity of *A. flos-aquae* and *C. vulgaris* exposed to By-140 varied by one order of magnitude.

The formation of an algal bloom is attributed to the overabundance of algal

growth and the gradual shift of algal community structure in an aquatic ecosystem (Boutin and Rogers 2000). This indicates that a gradual shift from dominance by green algae to dominance by cyanobacteria, or a gradual shift within the cyanobacterial population from dominance by the species to dominance by anther species, owing to light and temperature factors, nitrogen and phosphorus in the water, or to the food chain and food web (Pei and Ma 2002). However, there are few reports referring to whether there exist other factors (e.g. pollutants) to which green algae and cyanobacteria have greater differential sensitivity. Pollutants may result in a shift of green algal and cyanobacterial group structure, especially in a shift from dominance by green algae to dominance by cyanobacteria. It may also be important for sustaining cyanobacterial blooms during the special period.

Cyanobacteria can produce a variety of toxins including hepatotoxins e.g. microcystins, and endotoxins (Bhaskar et al. 2004), but can fixate atmospheric nitrogen, which has important implications for humans and aquatic organisms (An and Kampbell 2003). Thus, research comparing the differential sensitivity of cyanobacteria and green algae is of important scientific significance.

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