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Acute Toxicity of 12 Herbicides to the Green Algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*

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Effects of herbicides in the ecosystem do not remain restricted to target organisms but rather extend to non-target organisms such as algae which play an important role in the primary production of the aquatic ecosystem (Mohapatra and Mohanty 1992). Algae have been considered to be indicators of the bioactivity of industrial wastes. Unicellular algae vary in their response to a variety of toxicants (Tadros et al. 1994). Little is known, however, about the toxicity of new herbicides to freshwater green algae (Fargasova 1996). Up to now, only few herbicides' acute toxity to few algal species has been studied, such as trazine, paraquat, glyphosate, diquat, isoproturon, mecoprop, simetryn, etc (Kasai 1993; Abou-Waly et al. 1991; Saenz et al. 1997a; 1997b; Phlips et al. 1992; Kirby and Shahan 1994). Differential sensitivity of the green species to the compounds could induce species shifts within communities and could affect the structure and function of aquatic communities by altering the species composition of an algal community (Boyle 1984). The work reported here was done to examine the effect of 12 herbicides on the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus.*

MATERIALS AND METHODS

The Chinese National Environmental Protection Agency recommends green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus* for acute tests as ecological indicators because of their high sensitivity to the compounds (The Chinese NEPA 1990). They were used as the test organisms in this work and were obtained from the Institute of Wuhan Hydrobiology, the Chinese Academic of Science. Cells of *Chlorella pyrenoidosa* and *Scenedesmus obliqnus* were propagated photoautotrophically in a 250 mL Erlenmeyer flask containing 100 mL liquid HB-4 medium (Ma et al. 2001) and kept on a rotator shaker (100 rpm) at 25℃, and illuminated with cool-white fluorescent lights at a continuous light intensity of 5000 lux/cm^2. The culture medium was sterilized at 121℃, 1.05 kg cm^{-2} for 30 min (Kong et al. 1999). For cell experiments, 15 mL aliquots of the HB-4 medium containing green algal cells (initial cell concentration: *Chlorella pyrenoidosa* =2×10^5 cells/L, *Scenedesmus obliqnus*=3×10^5 cells/L, their initial spectrophotometric data was the same, OD_{680nm}=0.05) were distributed to sterile 50 mL Erlenmeyer flasks. The media of *Chlorella pyrenoidosa* and *Scenedesmus obliqnus* were then treated with various herbicides concentrations ranging from zero to 150 mg/L,

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RESULTS AND DISCUSSION

The acute toxicity of 12 herbicides to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus* is shown in Table 2. The 96 hr EC_{50} values of clethodim which blocks the *de novo* sysnthesis of fatty acids by inhibiting the activity of acetyl-CoA carboxylase (ACCase) (Ma et al. 2001) varied around 56-91 mg/L (10^{-4} M level) to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus*. The 96 hr EC_{50} values of acetolactate synthase (ALS) inhibitors which block the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine(Ma et al. 2001) varied around 11-270 mg/L (10^{-4}-10^{-5} M), The average acute toxicity of ALS inhibiting-herbicides to the green algae *Chlorella pyrenoidosa* and

Table 2. Dose response relationship of 12 herbicides to *Chlorella Pyrenoidosa* and *Scenedesmus obliqnus*

Herbicides	Regression Equation*	SL**	CC***	EC_{50} (mg/L)	EC_{50} (M)
Clethodim	①Y=-62.1574+24.9034X	0.0656	0.9344	90.3507	2.51×10^{-4}
	②Y=-61.1308+27.5101X	0.0756	0.9244	56.8057	1.58×10^{-4}
Bensulfur-	①Y=-0.8204+17.1602X	0.0075	0.9925	19.3275	4.71×10^{-5}
onmethyl	②Y=-43.5897+35.9583X	0.0055	0.9945	13.5005	3.29×10^{-5}
Chlorimur-	①Y=-7.0626+20.9143X	0.0035	0.9965	15.3084	3.69×10^{-5}
on-ethyl	②Y=-52.1663+41.3495X	0.0464	0.9536	11.8319	2.85×10^{-5}
Pyrazonsul-	①Y=21.3888+10.5973X	0.0465	0.9535	14.8776	3.60×10^{-5}
furon-ethyl	②Y=-33.3817+33.5548X	0.0003	0.9976	12.0004	2.90×10^{-5}
Flumetsul-	①Y=-6.0988+12.9377X	0.0097	0.9597	76.4068	2.35×10^{-5}
am	②Y=-86.7199+24.4350X	0.0844	0.9156	269.145	8.27×10^{-4}
Trifluralin	①Y=37.2911+21.3519X	0.0800	0.9200	1.8134	5.41×10^{-5}
	②Y=13.6978+21.3971X	0.0316	0.9684	5.4553	1.63×10^{-5}
Metolachlor	①Y=10.6096+15.4900X	0.0138	0.9487	12.7172	4.48×10^{-5}
	②Y=-44.8424+31.9949X	0.0138	0.9862	19.3811	6.83×10^{-5}
Acetochlor	①Y=-1.9811+27.1940X	0.0984	0.9016	6.7632	2.51×10^{-5}
	②Y=-78.0181+36.3351X	0.0143	0.9857	33.8948	1.26×10^{-4}
Cyanazine	①Y=80.1222+20.5876X	0.0111	0.9556	0.2315	9.62×10^{-7}
	②Y=109.2295+29.6919X	0.0670	0.9330	0.1360	5.65×10^{-7}
Methabenz-	①Y=36.8000+22.9904X	0.0460	0.9540	1.8162	8.21×10^{-6}
thiazuron	②Y=85.8535+33.6941X	0.0157	0.9843	0.3450	1.56×10^{-6}
Bromoxynil	①Y=40.1757+6.6244X	0.0022	0.9851	4.4064	2.37×10^{-5}
	②Y=-5.3765+13.9190X	0.0135	0.9865	53.4359	1.70×10^{-4}
Glufosinate	①Y=22.4254+26.7533X	0.0020	0.9723	2.8030	1.41×10^{-5}
	②Y=-23.5068+37.6714X	0.0861	0.9139	7.0376	3.55×10^{-5}

*Y (percent inhibition); X (natural logarithm of herbicide concentration); ① (*Chlorella pyrenoidosa*); ② (*Scenedesmus obliqnus*); **SL (significance level); ***CC (coefficient correlcation).

Scenedesmus obliqnus was higher than that of ACCase inhibiting herbicides. The 96 hr EC_{50} values of herbicides, such as metolachlor and acetochlor which influence cells division varied around 6-34 mg/L (10^{-4}-10^{-5} M). Its acute toxicity was higher than that of ACCase inhibitors, but was close to that of ALS inhibitors to the green alga *Chlorella pyrenoidosa* and *Scenedesmus obliqnus*. The 96 hr EC_{50} values of trifluralin which influenced the microtubule process varied around 1-6 mg/L (10^{-5} M level). The 96 hr EC_{50} values of glutamine synthase inhibitors--glufosinate was 2-7 mg/L (10^{-5} M level). It causes the concentration of glyoxylate to be increased that inhibits RuBP carboxylase, the first enzyme involved in carbon fixation (Ma et al. 2001). Its acute toxicity was close to that of microtuble process inhibiting herbicides to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus.* The 96 hr EC_{50} values of the photosynthesis-inhibiting herbicide was the lowest among of tested herbicides. The 96 hr EC_{50} values of cyanazine was 0.1-0.3 mg/L (10^{-7} M level), methabenzthiazuron 0.3-1.8 mg/L (10^{-6} M level), bromoxynil 4-54 mg/L (10^{-4}-10^{-5} M), most of their molar concentration was 10^{-6}-10^{-7} M level. The acute toxicity of this type of herbicide to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliqnus* was the highest among all of tested herbicides.

In this work, wide variations occurred in response to herbicides among individual species of the green algae *Scenedesmus* proved to be more tolerant genera while, *Chlorella* was more sensitive to herbicides. Among the 12 herbicides, *Chlorella pyrenoidosa* was more sensitive to the 5 herbicides—flumetsulam, trifluralin, acetochlor, bromoxynil and glufosinate—than *Scenedesmus obliqnus*; And the same sensitivity to the 4 herbicides—bensulfuronm-ethyl, chlorimuron-ethyl, pyrazonsulfuron-ethyl and metolachlor; Yet, to the rest 3 herbicides—clethodim, cyanazine and methabenzthiazuron—*Chlorella pyrenoidosa* was less sensitive than *Scenedesmus obliqnus*. Investigations using different algal species as test organisms have shown that algae vary greatly in their response to chemicals. Differential sensitivity of the green species to the compounds could induce species shifts within communities (Tadros et al. 1994; Boyle 1984).

The most suitable wavelength to use for monitoring culture growth was 680 nm, the study of Kasai (1993) reported that cell numbers and OD_{680nm} were highly correlated. A strong confirmed in this experiment, with coefficient correlation r values>0.99 and significance level $P<0.01$ ($r=0.9970$, $P=0.0001$) for *Scenedesmus obliqnus* tested. In our previous work (Ma et al. 2001), The count of *Chlorella pyrenoidosa* cells was well proportioned to the absorbance at 680 nm, coefficient of correlation r values>0.99 and significance level $P<0.01$. Thus, growth of algal cells was calculated indirectly using spectrophotometric data in this work.

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