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Differential Sensitivity to 30 Herbicides Among Populations of Two Green Algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*

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Today, environmental problems are multiple and complex, especially those arising from the toxic substances. Assessment of human exposure through biological monitoring to pesticides and other toxicants offers one means to evaluate the magnitude of potential health risk of these chemicals (Ma et al. 1999; Ghosp et al. 1997). Little is known about the sensitivity of algae to herbicides. Algae occupy an important position as the primary producers in aquatic ecosystems and they are the basis of many aquatic food chains. The action of herbicides on algae is therefore important not only for the organisms themselves, but also for the other parts of the food chains (Ferrando et al. 1996). Herbicides play an important role in agricultural practices and there are over 300 of them, their increased usage has elicited extensive research into herbicide effects on non target organisms such as algae. Algae are important to provide the energy that sustains invertebrates and fish in most aquatic ecosystems. Test on single species of algae are therefore of limited applicability to assess the effects of environmental contaminants on algal communities that are composed of an array of species with different sensitivities. Even at concentrations that are sublethal, toxicants can change the structure of algal communities (Boyle 1984; Abou-Waly et al. 1991; Saenz et al. 1997a; 1997b; Jay 1996; Tadros et al. 1994). Algae species vary widely in their response to toxic chemicals. Some works demonstrated that there was a differential photoinhibitory response to various herbicides among single species of algae and that the sensitivity of various species of algae in four different phyla exposed to a single herbicide varied by nearly two orders of magnitude, variation among species within plyla was lower, although there was still a tenfold difference from species to species (Boyle 1984). A few reports have been published on the comparative toxicity of solvents toward various test organisms (Abou-Waly et al. 1991; Jay 1996; Tadros et al. 1994). Fewer reports are involved with the differential response of various green algal species to pesticides (Saenz et al. 1997a; 1997b; Kasai et al. 1993). For the purposes of the assessmental comparative differential sensitivity among populations of green algae, a set of the acute toxicity tests have been devised. The work reported here was done to examine the effect of 30 herbicides, with 16 different chemical classes and 10 different specific mechanisms of action, on the green algae *Scenedesmus obliquus* and to compare differential sensitivity among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* to those herbicides.

MATERIALS AND METHODS

The Chinese National Environmental Protection Agency recommends the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* for acute tests as ecological indicators because of their high sensitivity to the compounds (The Chinese NEPA 1990). *Scenedesmus obliquus* 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 *Scenedesmus obliquus* were propagated photoautotrophically in a 250 mL Erlenmeyer flasks containing 100 mL liquid HB-4 medium (Ma et al. 2001) and kept on a rotator shaker (100 rpm) at 25℃,

Table 1. Selected herbicides, their chemical classes and putative mechanism

Herbicides	Formuations	Chemical class	Influenced mechanisms
Diclofop-p	99.5% TC[a]	Aryloxyphenox propionates	Acetyl-CoA carboxylase (ACCase)
Quizalofop-p	5% EC[b]		
Fenoxaprop	6.9% EC		
Haloxyfop-R	10.8% EC		
Fluazifop-p	53% EC		
Nicosulfuron	4% SC[c]	Sulfonylureas	Acetolactate synthase (ALS)
Metsulfron -methyl	90% TC		
Cyclosulfamuron	10% WP[d]		
Tribenuron	95% TC		
Ethametsulfuron	25% WP		
Bispyribac	10% SC	Dimethoxypyrimidinylsalicylic acids	
Anilofos	30% EC	Organophosphorus	Unknown
Pendimethalin	33% EC	Dinitrophenols	Microtubue process
Pretilachlor	93% TC	Chloroacetamides	Cell division
Butachlor	90% TC		
Atrazine	38% SC	Triazines	Photosynthetic process
Simazine	92% TC		
Ametryne	92% TC		
Prometryne	77% TC		
Isoproturon	95% TC	Ureas	
Chlorotoluron	95% TC		
Diuron	50% WP		
Paraquat	20% SL[e]	Bipyridyliums	
Fluroxypyr	11% EC	Pyridnecarboxylic	Hormones synthesis
Quinclorac	90% TC	Quinoline acids	
MCPA	10% SL	Phenoxycarboxylic acids	
Benazolin- ethyl	50% SC	Others	
Oxyfluorfen	81% TC	Diphenylethers	Protoporphyrinogn oxidase (Protox)
Glyphosate	95% TC	Glycines	EPSP synthase
Molinate	96% EC	Thiocarbamates	Lipid synthesis

[a]TC (technical product); [b]EC (emulsible concentrate); [c]SC (suspension concentrate); [d]WP (wettable powder); [e]SL (soluble concentrate)

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 was about 4×10^5 cells/mL (their initial spectrophotometric data was OD_{680nm}=0.05)) were distributed to sterile 50 mL Erlenmeyer flasks. The media of *Scenedesmus obliquus* were then treated with various herbicides concentrations ranging from zero to 150 mg/L, and incubated for 96 hr on an orbital shaker (100 rpm) at a temperature of 25℃ with a continuous light intensity of 5000 lux/cm^2. Cell counts were correlated with absorbance over time for 96 hr on a Shimadzu UV-2401PC spectrophotometer. 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. In our previous work, a strong confirmed in the experiment, with coefficient correlation r values>0.99 and significance level P<0.01 (r=0.9970, P=0.0001) for *Scenedesmus obliquus* tested (Ma and Liang 2001). Thus, growth of algal cells was calculated indirectly using spectrophotometric data in this work. Each herbicide concentration was tested in triplicate. Appropriate control systems containing no herbicide were included in each experiment. Control and treated cultures were grown under the same conditions of temperature, photoperiod and shaking of the stock cultures. In each experiment, percent inhibition values, relative to growth in control systems, were calculated using spectrophotometric data (Ma et al. 2001). The EC_{50} values (herbicide concentration required to cause a 50% reduction in growth) were calculated using linear regression analysis of transformed herbicide concentration as natural logarithm data versus percent inhibition. All of tested herbicides were purchased from People's Republic of China and their chemical classes and influenced mechanisms (Ma et al. 2001) are shown in Table 1. The tested herbicides were dissolved with a little acetone, methanol or distilled water. The concentration of solvent in the medium was less than 0.05%. The US Environmental Protection Agency recommends maximum allowable limits of 0.05% solvent for acute tests and 0.01% for chronic tests (Jay 1996), This level was not significant with regard to toxicity (Ma and Liang 2001).

RESULTS AND DISCUSSION

The acute toxicity of the 30 herbicides to the green algae *Scenedesmus obliquus* is shown in Table 2 and 3. The 96 h EC_{50} values of herbicides which block the *de novo* sysnthesis of fatty acids by inhibiting the activity of acetyl-CoA carboxylase (ACCase) varied around 1-124 mg/L (10^{-4}-10^{-6}M), diclofop-p was 10^{-4} M, haloxyfop-R, fluazifop-p and quizalofop-p were about 10^{-5} M, fenoxaprop was about 10^{-6} M. The 96 h EC_{50} values of lipid synthesis inhibitor--molinate was 29mg/L (10^{-4} M). The 96 h EC_{50} values of acetolactate synthase (ALS) inhibitors which block the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine varied around 2-156 mg/L (10^{-4}-10^{-6} M), metsulfron and ethametsulfuron were approximately 10^{-4} M, nicosulfuron, tribenuron and

bispyribac were approximately 10^{-5} M level, cyclosulfamuron achieved 10^{-6} M. The average acute toxicity of ALS inhibiting-herbicides to the green algae *Scenedesmus obliquus* was lower than that of ACCase inhibiting herbicides and lipid synthesis inhibitor. The same results have also been obtained using *Chlorella pyrenoidosa* as a tested organism (Ma and Liang 2001). The 96 h EC_{50} values of protoporphyrinogen oxidase (Protox) inhibiting herbicides oxyfluorfen was around 5 mg/L (10-5 M). Protox inhibitors lead to the accumulation of its substrate protoporphyrinogen, which is readily oxidized to proto IX by oxidative enzymes. Proto IX is an effective photosensitizer, and in the light it transfers

Table 2. Dose response relationships of the 30 herbicides to *Scenedesmus obliquus*

Herbicides	Regression Equation	Significa-nce level	Coefficient correlcation
Diclofop-P	P[a]=145.7064+ 40.6057lnC[b]	0.0764	0.9236
Quizalofop-p	P= 8.0870+ 32.9421lnC	0.0403	0.9597
Fenoxaprop	P= 34.5180+ 45.1904 lnC	0.0778	0.9222
haloxyfop-R	P=-2.6006+16.3095lnC	0.0 421	0.9579
Fluazifop-p	P=-93.2483+ 43.6103 lnC	0.0274	0.9726
Nicosulfuron	P= -4.8573+ 35.7979 lnC	0.0895	0.9105
Metsulfron methyl	P=-27.2677+ 18.0162lnC	0.0656	0.9344
Cyclosulfamuron	P= 5.1747 + 44.3417 lnC	0.0278	0.9722
Tribenuron	P= -4.9364 +15.2827lnC	0.0458	0.9556
Ethametsulfuron	P=-186.9394+46.9389lnC	0.0198	0.9802
Bispyribac	P= -24.2566+ 42.1710lnC	0.0436	0.9564
Anilofos	P= -20.2522+ 30.8043lnC	0.0941	0.9059
Pendimethalin	P= 72.5180+ 31.5499 lnC	0.0045	0.9955
Pretilachlor	P=-26.1823+34.1714 lnC	0.0363	0.9638
Butachlor	P=-15.6529+33.6502lnC	0.0647	0.9353
Mefenacet	P=-45.4786+24.4348lnC	0.0485	0.9515
Molinate	P=-56.3405+31.4403lnC	0.0716	0.9284
Atrazine	P=106.9248+27.2433lnC	0.0219	0.9781
Simazine	P= 76.2855+ 19.3478lnC	0.0059	0.9707
Ametryne	P=163.3969+25.4958lnC	0.0105	0.9895
Prometryne	P= 95.8744+ 7.1636 lnC	0.0361	0.9639
Isoproturon	P=151.6531+27.2283lnC	0.0452	0.9548
Chlorotoluron	P=124.2616+30.0660lnC	0.0058	0.9942
Diuron	P=211.6845+29.3988lnC	0.0350	0.9650
Paraquat	P= 70.2756+ 5.3465 lnC	0.0907	0.9093
Fluroxypyr	P=-82.9648+40.5507lnC	0.0242	0.9758
Quinclorac	P= 29.0331+11.1770lnC	0.0330	0.9670
MCPA	P=-84.9695+37.8145lnC	0.0628	0.9372
Benazolin- ethyl	P=-85.9927+37.0714lnC	0.0483	0.9517
Glyphosate	P=-111.2677+40.0882lnC	0.0190	0.9876
Oxyfluorfen	P= 9.4829+ 25.0620 lnC	0.0294	0.9706

[a] P (percent inhibition); [b]C (herbicide concentration)

absorbed energy to molecular oxygen to form singlet oxygen. The singlet oxygen peroxidizes lipids leading to the destruction of cellular membranes (Ma et al. 2001). The 96 h EC_{50} values of herbicides, such as pretilachlor and butachlor which influence cell division varied around 7-9 mg/L (10-5 M). Acute toxicity

Table 3. Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*

Herbicides	[a] *Sce* EC_{50} (mg/L)	[b] *Chl*EC_{50} (mg/L)	[a] *Sce* EC_{50} (M)	[b] *Chl* EC_{50} (M)	Ratio of *Sce* / *Chl*	[c]Orders
Diclofop-P	123.9252	0.690	3.63×10^{-4}	2.02×10^{-6}	179.6	+++
Quizalofop-p	3.5691	5.628	1.04×10^{-5}	1.53×10^{-5}	0.6	_
Fenoxaprop	1.4086	1.001	4.22×10^{-6}	2.76×10^{-6}	1.4	+
haloxyfop-R	25.1574	5.340	5.80×10^{-5}	1.23×10^{-5}	4.7	+
Fluazifop-p	26.7019	15.660	8.16×10^{-5}	4.78×10^{-5}	1.7	+
Nicosulfuron	4.6294	2.200	1.13×10^{-5}	5.36×10^{-6}	2.1	+
Metsulfron methyl	72.8782	14.220	1.13×10^{-4}	3.73×10^{-5}	5.1	+
Cyclosulfamuron	2.7481	0.141	6.52×10^{-6}	3.35×10^{-7}	19.5	++
Tribenuron	36.4040	26.544	9.54×10^{-5}	6.71×10^{-5}	1.4	+
Ethametsulfuron	155.6836	1.827	3.79×10^{-4}	4.45×10^{-6}	85.2	++
Bispyribac	5.8174	2.760	1.35×10^{-6}	6.42×10^{-6}	2.1	+
Anilofos	9.7825	7.320	2.66×10^{-5}	1.99×10^{-5}	1.3	+
Pendimethalin	0.4898	0.394	1.74×10^{-6}	1.40×10^{-6}	1.2	+
Pretilachlor	9.2944	2.560	2.98×10^{-5}	8.21×10^{-6}	3.6	+
Butachlor	7.0360	3.630	2.26×10^{-5}	1.16×10^{-5}	1.9	+
Molinate	29.4384	5.038	1.57×10^{-4}	2.69×10^{-5}	5.8	+
Atrazine	0.1237	0.145	5.73×10^{-7}	6.72×10^{-7}	0.9	_
Simazine	0.2570	0.082	1.27×10^{-6}	4.09×10^{-7}	3.1	+
Ametryne	1.17×10^{-2}	3×10^{-4}	5.15×10^{-8}	1.41×10^{-9}	39.0	+
Prometryne	1.65×10^{-3}	1.2×10^{-2}	6.90×10^{-9}	4.93×10^{-8}	0.1	_
Isoproturon	2.39×10^{-2}	0.5×10^{-2}	1.16×10^{-7}	2.42×10^{-8}	4.8	+
Chlorotoluron	8.46×10^{-2}	1.490	3.98×10^{-7}	7.01×10^{-7}	0.06	_ _
Diuron	4.09×10^{-3}	1.3×10^{-3}	1.75×10^{-8}	5.59×10^{-9}	3.1	+
Paraquat	2.25×10^{-2}	1.0×10^{-4}	9.67×10^{-8}	5.36×10^{-10}	225	+++
Fluroxypyr	26.5486	3.044	7.19×10^{-5}	8.24×10^{-6}	8.7	+
Quinclorac	6.5267	1.267	2.70×10^{-5}	5.23×10^{-6}	5.2	+
MCPA	35.4901	21.960	1.77×10^{-4}	7.30×10^{-5}	1.6	+
Benazolin-ethyl	39.1891	37.260	1.44×10^{-4}	1.15×10^{-4}	1.1	+
Glyphosate	55.8585	3.530	3.30×10^{-4}	2.09×10^{-5}	15.8	++
Oxyfluorfen	5.0363	4.008	1.39×10^{-5}	1.11×10^{-5}	1.3	+

[a] *Sce* denotes *Scenedesmus obliquus.*
[b] *Chl* denotes *Chlorella pyrenoidosa*, the two columns data that are marked with the letter b came from our previous published data in BECT66.
[c]orders denotes orders of ratio of EC_{50} vaule between *Scenedesmus obliquus* and *Chlorella pyrenoidosa.*

of protox inhibitors and cell division inhibitors was higher than that of ALS inhibitors, ACCase inhibitors and lipid synthesis inhibitors to the green algae *Scenedesmus obliquus*. Similar results have also been obtained using *Chlorella pyrenoidosa* as a tested organism (Ma et al. 2001). The 96 h EC_{50} values of pendimethalin which influenced the microtubule process was 0.5 mg/L (1.7×10^{-6} M). Auxin herbicides such as quinclorac, fluroxypyr varied around 6-27 mg/L (10^{-5} M), MCPA and benazolin-ethyl varied around 20-40 mg/L (10^{-5} M). Auxin herbicides stimulate ethylene biosynthesis by inducing the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. In susceptible dicots, increased levels of ethylene trigger an accumulation of abscisic acid (ABA). In susceptible grasses, the levels of tissue cyanide (HCN), a co-product formed during ethylene biosynthesis, increases. These increases in ethylene, ABA, and HCN cause epinasty of leaves, growth retardation, and senescence. However, not all researchers are in agreement about the association between plant sensitivity to the auxin type herbicides and the increase in ethylene production. Their acute toxicity to the green algae *Scenedesmus obliquus* was lower than others. The same results have also been obtained using *Chlorella pyrenoidosa* as a tested organism (Ma et al. 2001). The 96 h EC_{50} values of 5-enolpyruvyl- shikimate-3-phosphate synthase (EPSP synthase) inhibitors glyphosate was 56 mg/L (10^{-4} M). It causes the concentration of glyoxylate to increase which inhibits RuBP carboxylase, the first enzyme involved in carbon fixation. The 96 h EC_{50} values of the photosynthesis-inhibiting herbicides were the lowest among the tested herbicides. The 96 h EC_{50} values of simazine and atrazine were 10^{-1} mg/L, chlorotoluron, isoproturon, ametryne and paraquat were 10^{-2} mg/L, prometryne and diuron were 10^{-3} mg/L, most of their molar concentration were 10^{-7}-10^{-8} M, diuron and prometryne were at 10^{-9} M. The acute toxicity of this typal herbicides to the green algae *Scenedesmus obliquus* was the highest among all of tested herbicides. The same results have also been obtained using *Chlorella pyrenoidosa* as a tested organism (Ma et al. 2001).

In this work, wide variations occurred in response to herbicides among individual species of the green algae *Scenedesmus* proved to be the more tolerant genera while, *Chlorella* was more sensitive to herbicides. Among the 30 herbicides, *Chlorella pyrenoidosa* was more sensitive to 26 herbicides than *Scenedesmus obliquus*. Similar results have also been obtained using the other 12 herbicides (Ma and Liang 2001). Meanwhile, algal species vary widely in their response to toxic chemicals. The results demonstrated that there was a differential response to various herbicides among two species of algae and that the sensitivity of various species of algae exposed to two herbicides— diclofop-P and paraquat varied by over two orders of magnitude, four herbicides—cyclosulfamuron, ethametsulfuron, ametryne and chlorotoluron varied by over one order. Variation among species within plyla was lower, although there was still a low tenfold difference from species to species among 23 herbicides. 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).

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REFERENCES

Abou-Waly H, Abou-Setta MM, Nigg HN, Mallory LL (1991) Growth response of freshwater algae, *Anabaena flos-aquae* and *Selenastrum capricornutum* to atrazine and hexazinone herbicides. Bull Environ Contam Toxicol 46:223-229

Boyle TP (1984) The effect of environmental contaminants on aquatic algae. In: Shubert LE (ed.) Algae as ecological indicators. Academic press, New York p237-256

Ferrando MD, Sancho E, Andreu-Moliner E (1996) Chronic toxicity of fenitrothion to an algae (*Nannochloris oculata*), a rotifer (*Brachionus calyciflorus*), and the cladoceran (*Daphnia magna*). Ecotoxicol Environ Saf 35:112-120

Ghosp SK, Doctor PB, Bhatnager VK, Yadav S, Derasan A, Kulkarni PK, Kashyap SK (1997) Response of three microbial test systems to pesticides. Bull Environ Contam Toxicol 58:482-488

Jay AE (1996) Toxic effect of organic solvents on the growth of *Chlorella vulgaris* and *Selenastrum capricornutum*. Bull Environ Contam Toxicol 57:191-198

Kasai F, Takamura N, Hatakeyama S (1993) Effect of simetryn on growth of various freshwater algal taxa. Environ Pollut 79: 77-83

Kong FX, Sang WL, Hu W, Li JJ (1999) Physiological and biochemical response of *Scenedsmus obliquus* to combined effects of Al, Ca, and low pH. Bull Environ Contam Toxicol 62:179-186

Ma J, Liang W, Xu L, Wang S, Wei Y, Lu J (2001) Acute Toxicity of 33 Herbicides to the Green Alga *Chlorella pyrenoidosa* . Bull Environ Contam Toxicol 66:536-541

Ma J, Liang W (2001) Acute toxicity of 12 herbicides to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Bull Environ Contam Toxicol 67:347-351

Ma M, Tong Z, Wang Z, Zhu W (1999) Acute toxicity bioassay using the freshwater luminescent bacterium *Vibrio-qinghaiensis sp.nov.-q67*. Bull Environ Contam Toxicol 62: 247-253

Saenz ME, Alberdi JL, Marzio DD, Accorinti J, Tortorelli MC (1997a) Paraquat toxicity to different green algae. Bull Environ Contam Toxicol 58:922-928

Saenz ME, Di Marzio WD, Alberdi JL, del Carmen Tortorefllli M (1997b) Effects of technical grade and a commercial formulation of glyphosate on algal population growth. Bull Environ Contam Toxicol 59:638-644

Tadros MG, Philips J, Patel H, Pandiripally V (1994) Differential response of green algal species to solvents. Bull Environ Contam Toxicol 52:332-337

The Chinese NEPA (1990) Algal growth inhibiting test, p.168-178. In: Guidelines for testing of chemicals. The Chinese Chemical Industry Press, Beijing.