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Toxicity of Linear Alkylbenzene Sulfonate and Alkylethoxylate to Aquatic Plants

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Shortage of water resources due to pollution is a very serious environmental problem in the world. Because of various contaminants poured down into water bodies, water quality decreases and lots of aquatic plants are killed. Aquatic plants are major producers in the aquatic ecosystem and play a very important role in the course of water self-purification. When aquatic plants are injured, water eutrophication will be accelerated. More and more studies show that surfactants, mostly coming from various detergents, are an ignored contaminant in the aquatic environment, and result in harmful effects on aquatic plants (Patoczka and Pulliam 1990; Bubenheim et al. 1997; Bussotti et al. 1997) and aquatic ecosystems (Dorn et al. 1997; Maki et al. 1998; Fujita et al. 2000). Toxicities of surfactants to aquatic animals have been summarized in the study of Lewis (1991). Although previous studies have provided useful insights, they usually were focused on the toxicity of anionic surfactant LAS to aquatic animals and planktons. It is necessary to know the impacts of other surfactants such as nonionic surfactants, for example AE, on aquatic ecosystems. In this paper we report the toxic effects of two most important surfactants, linear alkylbenzene sulfonate (LAS) and alkylethoxylate (AE), on aquatic plants, including *Pistia stratiotes* L, *Lemna paucicostata* L, *Azolla imbricata*, and *Spirogyra* sp., by observing injury symptoms using a transmission electron microscopy and measuring changes of the activities of protective enzymes surperoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

MATERIALS AND METHODS

Pistia stratiotes L, *Lemna paucicostata* L, *Azolla imbricata*, *Spirogyra* sp., representing different evolutional levels, and LAS, AE, representing anionic surfactants and nonionic surfactants, respectively, were selected in this experiment.

Spirogyra sp. was treated with 6.0 mg/L LAS or AE for 30 minutes, observed with Olympus microscope, and then photos were taken. After treated with 50.0 mg/L

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LAS or AE for 24 hours, the leaf cells of *Pistia stratiotes* L were cut into thin pieces, then dyed and observed with transmission electronic microscope H600, and photos were taken.

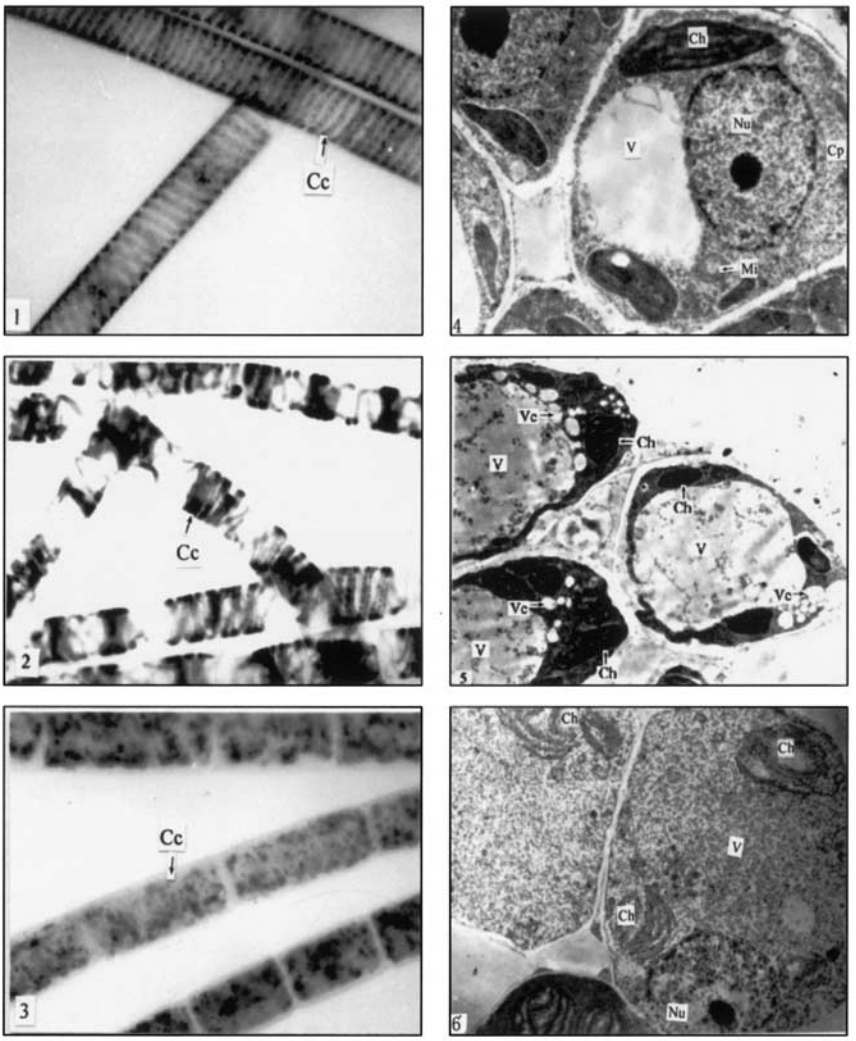

Figure 1. Photos of *Spirogyra* sp. cells and *Pistia stratiotes* L leaf cells treated with LAS or AE (1. Normal *Spirogyra* sp., ×200; 2. *Spirogyra* sp. treated with 6.0 mg/L LAS, ×200; 3. *Spirogyra* sp. treated with 6.0 mg/L AE, ×200; 4. Normal *Pistia stratiotes* L, ×200; 5. *Pistia stratiotes* L treated with LAS, ×12000; 6. *Pistia stratiotes* L treated with AE, ×16000. Abbreviations: Cc: Chlorophyll carrier; Ch: Chloroplast; Cp: Cytoplasm; Mi: Mitochondria; Nu: Nucleus; V: Vacuole; Vc: Vacancy)

To determinate the effects on the protective enzyme activities due to surfactant pollution, *Lemna paucicostata* L, *Azolla imbricata*, and *Spirogyra* sp. were treated with different concentrations of LAS or AE, and then the protective enzymes (SOD, CAT, and POD) of plant cells were determined every 24 hours during a 144-hour period. Determination of SOD, CAT, and POD activities was followed the procedures in Methods of Plant Bio-Chemistry Analysis (Jin and Ding 1981).

RESULTS AND DISCUSSION

Injury symptoms of the cell structures of *Spirogyra* sp. and *Pistia stratiotes* L were presented in Figure 1. For the *Spirogyra* sp. cells treated with LAS, the regular spiral of chlorophyll carriers was disturbed and assembled together. Treated with AE, the chlorophyll carriers in the *Spirogyra* sp. cells disintegrated and spread among the whole cells (Figure 1(1, 2, and 3)). As to *Pistia stratiotes* L, treated with LAS, many vacancies appeared in the cytoplasm and vacuoles enlarged with cytoplasm disintegrated gradually. Chloroplasts were injured seriously with their shape deforming and disintegrating. Treated with AE, vacuoles were fully filled with cytoplasm because of their membrane broken; the chloroplasts expanded and their membrane spoiled with increasing damage degree, and all the internal materials dispersed in the cytoplasm (Figure 1 (4, 5, and 6)). According to the injury symptoms of *Spirogyra* sp. and *Pistia stratiotes* L, we deduce that the toxic mechanisms of LAS and AE to aquatic plants are different. For AE, dissolution is the main cause; but for LAS, change in shape of protein due to the charges carried by LAS is another main cause besides dissolution.

The effects of AE treatments on SOD activity in the leaf cells of *Pistia stratiotes* L were showed in Table 1. SOD activities decreased with increasing AE concentrations, meaning that at higher concentrations of AE, *Pistia stratiotes* L were severely injured and a large amount of SOD was consumed in the process of cleaning free radicals produced in the plants. With increasing treatment time, SOD activities generally increased to a certain maximum for each AE concentration treatment and then decreased, implying that the resistant system of *Pistia stratiotes* L leaf cells worked well at the early stage of AE treatment, but at the later stage this system gradually lost its function due to a long term pollution of AE. The injury degrees were different at different AE concentrations. When AE concentration was lower than 1.0 mg/L, only slight effects took place on the SOD activities; when AE concentration was about 10.0 mg/L, the physiological activities of this plant were affected, but the plant could recover because of SOD activities increasing and cleaning free radicals; when AE concentration was higher than 10.0 mg/L, however, this surfactant caused an acute toxicity to *Pistia stratiotes* L, resulting in the SOD activities decreasing rapidly, and the plant died.

Table 1. Changes of superoxide dismutase activities ($\times 10^3$ NU/g) in the leaf cells of *Pistia stratiotes* L treated with different time and different AE concentrations

AE concentration (mg/L)	Treatment time (h)					
	24	48	72	96	120	144
0.0	3.59	3.67	3.53	3.56	3.38	3.56
0.1	3.39	3.49	3.44	3.48	3.03	3.27
1.0	3.21	3.32	3.36	3.48	3.14	3.26
10.0	2.67	2.99	3.10	3.44	3.27	3.30
20.0	2.53	2.95	3.06	3.89	2.46	2.54
50.0	1.96	2.38	2.98	3.58	2.31	2.22

Table 2. Relative activities* of three protective enzymes in the leaf cells of *Pistia stratiotes* L treated with 10.0 mg/L of linear alkylbenzene sulfonate (LAS) or alkylethoxylate (AE)

Indexes		Treatment time (h)					
		24	48	72	96	120	144
LAS	SOD	0.803	0.851	1.034	1.186	0.802	0.789
	CAT	0.953	0.896	0.892	0.842	0.943	0.856
	POD	1.281	1.272	1.202	1.118	1.139	1.206
AE	SOD	0.745	0.815	0.877	0.966	0.967	0.928
	CAT	0.864	1.191	1.023	1.055	0.831	0.986
	POD	1.212	1.352	1.332	1.357	1.196	1.237

*The relative activity here is the ratio of the enzyme activity treated with 10.0 mg/L surfactant solutions to the enzyme activity treated with no surfactant solution.

Table 2 showed changes of the relative activity of three protective enzymes (SOD, CAT, and POD) of *Pistia stratiotes* L leaf cells in 10.0 mg/L LAS or AE solution. When *Pistia stratiotes* L plants were treated for 24 hours, the activities of SOD and CAT declined (their relative activities less than 1). This means that LAS or

AE could induce much more free radicals in the leaf cells. After that the leaf cells were stimulated to produce more enzymes, the activities of these protective enzymes increased gradually. With increasing treatment time, the injury effects accumulated with increasing free radicals, SOD and CAT activities decreased again, but POD activities kept high levels all the time. POD can decompose peroxides; in the meantime, it is a catalyst that can speed up protoplasm carbonization and protect the deep layer cells from injury. From the above discussion, we realize POD being the major enzyme to protect *Pistia stratiotes* L from LAS or AE injury. The same patterns were found in the other aquatic plants treated with LAS or AE.

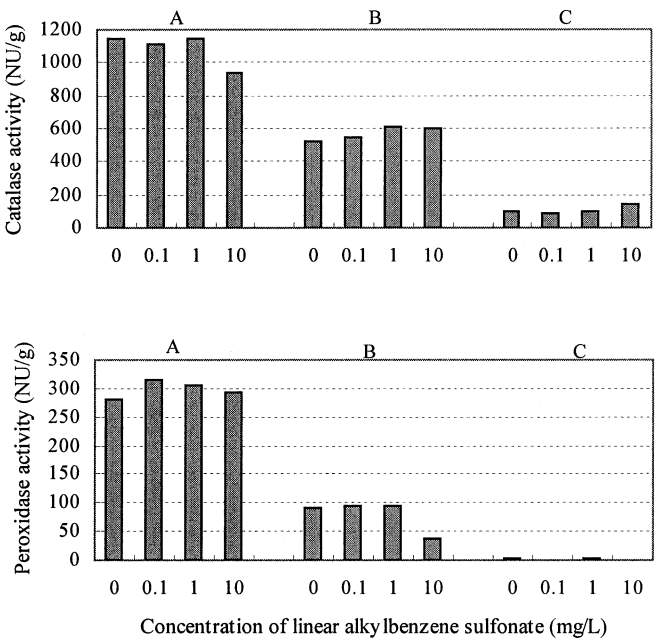

Figure 2. Catalase and peroxidase activities of three aquatic plants treated with different linear alkylbenzene sulfonate concentrations for 72 h (A: *Lemna paucicostata* L; B: *Azolla imbricate*; C: *Spirogyra* sp.)

Experimental results demonstrated that in the range 0.0~10.0 mg/L of LAS concentrations, *Lemna paucicostata* L had the highest enzyme activities, followed by *Azolla imbricata*, and *Spirogyra* sp. had the lowest (Figure 2). This sequence was the same as their evolutionary levels and their resistances to surfactant pollution. The trends of CAT and POD activities treated with AE solutions were the same. So we could conclude that aquatic plants with high evolutionary level have high protective enzyme activities and high resistances to surfactant pollution.

Injury symptoms of *Pistia stratiotes* L, *Lemna paucicostata* L, *Azolla imbricata*,

and *Spirogyra* sp. treated with a serial concentration of LAS or AE solutions were summarized in Table 3. Combining the effects of SOD, CAT, and POD activities, it is obvious that high concentrations of LAS or AE (≥ 10.0 mg/L) would lead to a serious injuries or death to the tested aquatic plants. Among the four plant species, the sequence of resistance to LAS and/or AE pollution was *Pistia stratiotes* L > *Lemna paucicostata* L ~ *Azolla imbricata* > *Spirogyra* sp. LAS seemed more toxicity to the plants than AE.

Table 3. Toxicological effects of surfactants linear alkylbenzene sulfonate (LAS) or alkylethoxylate (AE) on aquatic plants

Plant species	AE Concentration (mg /L)						LAS Concentration (mg /L)					
	0.1	1.0	10	20	50	100	0.1	1.0	10	20	50	100
Pistia stratiotes L	A	B	C	D	D	—	A	B	C	D	D	—
Lemna paucicostata L	A	B	CD	D	D	D	A	B	D	D	D	D
Azolla imbricata	A	B	BC	—	D	D	A	B	CD	—	D	—
Spirogyra sp.	A	B	D	—	D	D	A	C	D	—	D	—

A: no apparent effects; B: affected lightly and recoverable; C: affected and unrecoverable; D: lethal; CD: between C and D; —: not tested.

Parent nonylphenol polyethoxylate (a nonionic surfactant) was detected at a concentration range of 0.051~7.035 mg/L with an average value of 0.296 mg/L in the primary effluents from sewage treatment plants in Japan (Fujita et al. 2000). So, usually a light effect would take place to the experimental aquatic plants according to our experimental results. In some waste waters (for example, domestic sewage), LAS or AE concentrations could reach to several hundred mg/L. Under this condition, almost all the aquatic plants would be killed if these surfactants are not removed or diluted.

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