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Effect of Triphenyltin on Duckweed *Lemna minor*

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Organotin compounds such as tributyltin (TBT) and triphenyltin (TPT) have been used in industries as biocides, heat stabilizers for polyvinyl chloride and catalysts in a variety of chemical reactions (Blunden and Chapman 1986). In particular, the use of TBT and TPT as antifouling agents in paints applied to boat hulls has been widespread because of their superior effectiveness over copper oxide paints.

However, there are few reports about the effect of TPT on plants. Plants provide habitats for aquatic animals and also serve as food for the animals. Plants play as an important role in aquatic ecology. Thus, it is necessary to study the effect of TPT on aquatic plants. This study is the first time to report the TPT effect on chloroplast activity and other physiological parameters (nitrate reductase activity and peroxidase activity) of duckweed.
The research presented here evaluates the effect of TPT on the growth rate, chlorophyll content, sugar content, chloroplast activity, preoxidase and nitrate reductase activity, and the bioconcentration of duckweed *Lemna minor*.

The results show that the IC_{50} of TPT on *Lemna minor* was 15.80 μgSn/L, and the enzymes activity increased when the TPT concentration was 2 and 5 μgSn/L, However, when TPT was 5 μg/L, the chloroplast activity decreased. It was determined that TPT bioconcentrationed in duckweed, and the bioconcentration factor (BCF) was calculated.

METHODS AND MATERIALS

Duckweed *Lemna minor* was collected from the Weijin River in Tianjin City. Duckweed was cultured in the Hoagland solution. The healthy plants were used in the tests after one week cultured.

The IC_{50} (median inhibitory concentration) tests were conducted in a series of 100 mL beakers. Each beaker contained 80 mL of test water. Five concentrations of TPT (0, 5.0, 10.0, 25.0, 50.0 μg/L, Sn as calculated) were used as experimental treatments, each replicated 3 times. The number of fronds in each beaker was counted every two days. The test solution in each beaker was renewed every two

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days. Whole light photoperiod (24 hr light)was used at 25 ±1℃.

The bioconcentration tests were conducted in 250 mL beakers. Each beaker contained 200 mL of test water. Three concentrations of TPT (0, 2.0, 5.0 μg/L, Sn as calculated) were used, each replicated 3 times. The number of fronds and nitrate reductase activity in each beaker was determined every two days. The test solution in each beaker was renewed every two days. Whole light photoperiod was used at 25 ±1℃. After 8 days, TPT concentrations in each beaker and in duckweed, the chlorophyll content, sugar content, chloroplast activity and peroxidase activity were determined.

The method of determining chlorophyll content and IC_{50} was published (Song and Chen 1998). At the end of the tests, a certain amount of duckweed (5, 10 or more)was weighed and dried. The dried duckweed was homogenized with 5 mL 80% ethanol to extract sugar. The extraction solution was centrifuged at 3000 × g for 15 minutes. One mL clear solution was added to 5 mL concentrated H_2SO_4, containing 0.2% anthrone and 1% thiurea and incubated at 100℃ for 10 minutes. The solution absorbance was determined after 30 min at 620 nm. The standard curve of sugar content vs. absorbance at 620nm was made with standard glucose solution.

Nitrate reductase catalyzes nitrate to convert into nitrite, and the nitrite is released from the cells to the test solution. The concentration of nitrite reveals the nitrate reductase activity. The method of Zhu (1990) was used to determine the nitrite concentration in the test solution. One mL solution of each test was added to 2 mL 15% HCl solution containing 1% sulfanilamide; then this 3 mL solution was added to 2 mL 15% HCl solution containing 0.02% N-1-naphthylethylenediamine dehydrochloride. Absorbance of the above solution was measured after 30 min at 520 nm. The standard curve was made with sodium nitrite solution. The nitrate reductase activity was calculated as follows:

$$N_t = (A_{520t}-0.015)/0.083 \cdot t \cdot U_t$$
$$N_{rt} = N_t / N_{0t}$$

Where t was the time (days), N_t was nitrate reductase activity at t time, A_{520t} was the absorbance at t time at 520 nm, U_t was the number of duckweed fronds at t time, N_{rt} was relative nitrate reductase acitivity at t time, N_{0t} was the nitrate reductase activity of control tests at t time.

At the end of these tests, a certain amount of duckweed (5, 10 or moer)was weighed and homogenized with 5 mL of 0.05 mol/L phosphate buffer solution (pH 5.7) to extract the peroxidase. The extraction solution was centrifuged at 3000 × g for 15 minutes. The above, clear centrifuged solution was stored in refrigerator at 4℃. The peroxidase activity was determined with 0.2 mL peroxidase extraction solution and 2.8 mL of 0.05 mol/L phosphate buffer solution (pH 5.7) containing 0.056% guaiacol and 0.012 % H_2O_2 in 10 mm cell. The reaction solution was put immediately into the spectrophotometer and absorbance was determined per minute at 470 nm. The peroxidase activity (N_O)

was calculated as follows:

$$N_O = (A_{470\text{-}5} - A_{470\text{-}0}) / G$$

Where $A_{470\text{-}5}$ was the absorbance at 5 minutes at 470 nm, $A_{470\text{-}0}$ was the initial absorbance at 470 nm.

At the end of the tests, a certain amount of duckweed (5, 10 or moere) was weighed and homogenized with cold 3 mL of 0.35 M NaCl solution and 2 mL of 10 mM Tris – HCl buffer solution (pH 7.8) to extract chloroplast. The extraction solution was centrifuged at 500 × g for 5 minutes. The clear solution was centrifuged at 3000 × g for 15 minutes. The deposit was dissolved with 1 mL of 0.35 M NaCl solution. The chloroplast extraction solution was stored in the refrigerator at 4 ℃. The chloroplast activity was determined with 1 mL chloroplast extraction solution, 1.5 mL of phosphate buffer solution (pH 7.3) and 0.5 mL 0.3 mM 2,6-dichlorophenolindophenol solution in 10 mm cell. The reaction solution was quickly mixed and immediately put into the spectrophotometer and absorbance was determined at 620 nm. Then the reaction solution was illuminated for 10 min at 12000 Lux and the absorbance was determined per minute at 620 nm.. The chloroplast activity (Np) was calculated as follows:

$$Np = (A_{620\text{-}0} - A_{620\text{-}5}) / G_{chl}$$

Where $A_{620\text{-}5}$ was the absorbance at 5 minutes at 620 nm and $A_{620\text{-}0}$ was the absorbance at the initial time at 620 nm, G_{chl} was the chlorophyll weight, absorbance at initial time at 470 nm, G was the wet weight of the duckweed. which can refer to the Song's method (Song and Chen 1998) to calculate. .

Every two days, 100 mL of each test solution was put into a 250 mL separating funnel and was added to 5 mL n-hexane and 2 mL HCl. The funnel was shaken 300 times to extract TPT and was allowed to stand for 30 min. The organic phase was put into a color comparison tube. The organic solution was purged with N_2 near to dry and stored in the refrigerator at 4℃.

At the end of these tests, a certain amount of duckweed (5, 10 or more) was weighed and homogenized with water and digested with tetramethylammonium-hydroxide solution for 1 hr at 100℃. The digested solution was treated as test solution to extract the TPT from the duckweed.

The TPT concentration measurements were made with an LS-5 luminescence spectrometer (Perkin-Elmer) equipped with 10 mm silica cells. The slit widths of excitation and emission were 10 and 5 nm, respectively. The fix scale was 0.1. The TPT extraction solution was added to 0.5 mL phthalandionehydrogen-potassium buffer solution (pH 4.0), 1.0 mL 10% Triton X-100 solution and 0.2 mL of 0.0025% morin methanol solution. The final volume of reaction solution was 5 mL. After 10 minutes, the fluorescent intensity of reaction solution was determined by scanning emission wavelength from 490 to 540 nm at 412 nm

excitation wavelength. The observed values of the fluorescent intensity of TPT peak were recorded to calculate the TPT concentration. The standard curve was made with a standard TPT solution.

The t-test statistical method (Song and Chen 1998) was used to study the effect of each TPT treatment.

RESULTS AND DISCUSSION

The tests results are demonstrated in Fig. 1 to Fig. 6 and Table. 1. Figure 1 shows that the toxicity of TPT to growth of *Lemna minor* increased with the increase concentration of TPT. The 8 days IC_{50} of TPT to *Lemna minor* was 15.80 μg/L and 50 μg/L. There appears to be a small growth in the curve. At 8 days, the number of *Lemna minor* fronds at 10 μg/L is 60.8 percent of the control test, while at the same TPT concentration, the number of *Scenedesmus quadricauda* coenobia was 37.1 percent of the control test (Fargašová 1997). The EC_{50} of TPT on growth rate of *Scenedesmus quadricauda* is about 0.04 μg/L (Fargašová 1998). This means that duckweed is less sensitive to TPT than green algae. The 96 hr IC_{50} of TPT to *Lemna minor* growth rate was 96.17 μg/L, and the 96 hr IC_{50} of TBT to *Lemna minor* growth rate was 30.83 μg/L (Song and Chen 1998). This suggests that the toxicity of TPT is less than that of TBT. Figure 2 shows that there were small differences in the growth rate of duckweed between control and treatment tests at 2 and 5 μg/L TPT.

Sugar content shows the activity of anabolism of duckweed. The data in table 1. shows that the sugar content decreased with TPT. This suggests that TPT may obstruct duckweed anabolism.

One can see in Table 1 that when TPT is 2 μg/L, the values of the chlorophyll content, nitrate reductase and peroxidase activity were greater than in the control plants. These results mean that at low concentration, there was a small stimulative effect of TPT on *Lemna minor*. A similar phenomenon was presented in a previous paper (Gao et al. 1994).

At 5 μg/L, the effect of TPT on *Lemna minor* is different from the control plants. The peroxidase and nitrate reductase activity are higher than that of control tests. Peroxidase acts as the detoxicating factor. High activity of peroxidase indicates that the organism increases the ability of detoxication. However, abnormal high level of enzyme activity will disorder the average physiological process and finally cause the organism to die.

The chloroplast is an important plant body in the photosynthesis system. The light energy is fixed, which is the basal function of plant and vital to all organisms. The activity of decreasing chloroplast leads to low photosynthesis efficiency. Data in Table 1 show that at 5 μg/L TPT, chloroplast activity was much lower than the control test. TPT affected not only the chlorophyll content but also the chloroplast activity. The effect of TPT on chloroplast activity is serious. Chlorophyll is an important pigment in chloroplast and its content can indicate the chloroplast

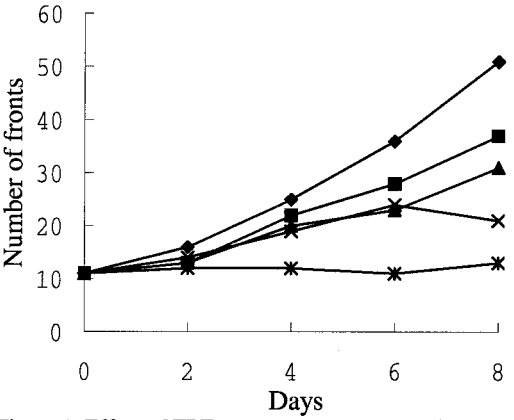
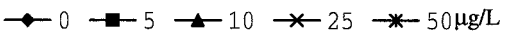


Figure 1. Effect of TPT on *Lemna minor* growth rate



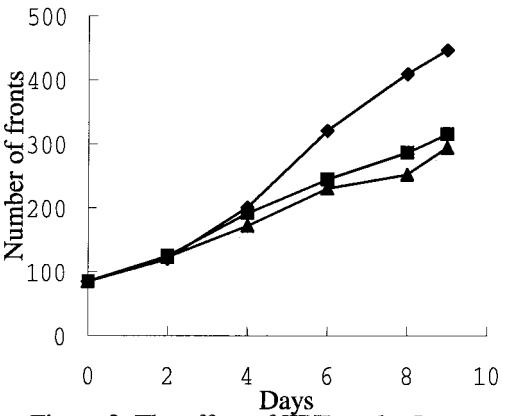
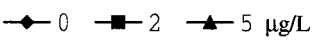


Figure 2. The effect of TPT on the *Lemna minor* growth rate at low concentration



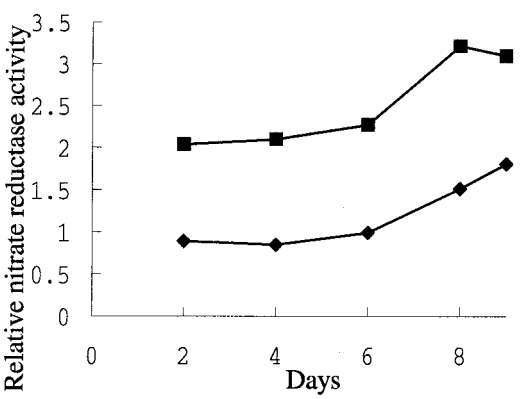
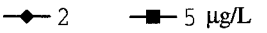


Figure 3. Effect of TPT on *Leman minor* nitrate reductase activity



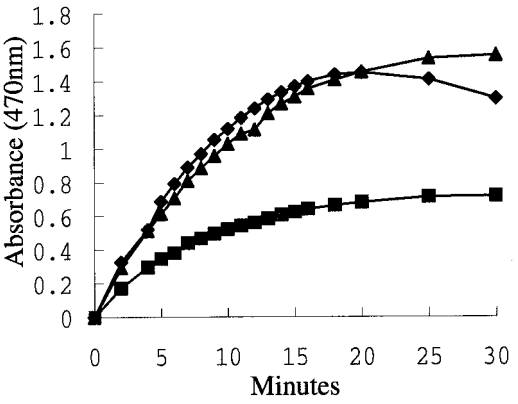
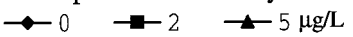


Figure 4. Effect of TPT on *Lemna minor* peroxidase activity



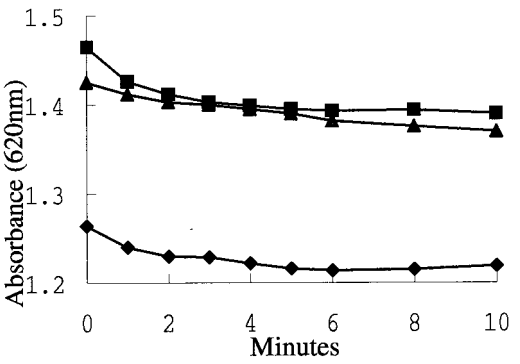
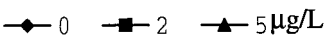


Figure 5. Effect of TPT on *Lemna minor* chloroplast activity



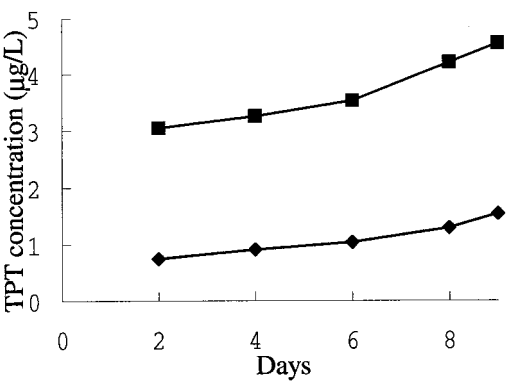


Figure 6. Concentration of TPT in the test

Table 1. The effect of TPT on *Lemna minor*

TPT concentration (μg/L)	Chlorophyll content (mg/g*)	Sugar content (mg/g*)	TPT content in fronds (μg/g*)	Relative nitrate reductase activity at the end of tests	Peroxidase activity (ΔA_{470}/g*)	Chloroplast activity (ΔA_{620} / mg chlorophyll)
0	1.310 ±0.007	4.667 ±0.012	-	1	1.34 ±0.05	0.042 ±0.002
2	1.334 ±0.010	3.958 ±0.026	0.0109 ±0.0005	1.81 ±0.13	1.39 ±0.07	0.081 ±0.014
5	0.900 ±0.008	2.604 ±0.037	0.0138 ±0.0015	3.10 ±0.45	1.41 ±0.03	0.037 ±0.006

* fresh weight

activity. At 2 μg/L TPT, the chlorophyll content was 102% of that in control tests, while the chloroplast activity is 193% of that in control tests. This would suggest that chloroplast activity may change before the chlorophyll content changes when exposed to pollutants. Thus, chloroplast activity is a more useful parameter than chlorophyll content and can be used to evaluate the toxicity of pollutants to plants.

Figure 6 shows that at the beginning of test, duckweed absorbed TPT quickly. When the duckweed concentration of TPT was near the limited range, the absorption rate of TPT slowed and the concentration of TPT in test water became higher. In these tests, the absorbing TPT approached a stable status. Bioconcentration factors (BCF)s of TPT in duckweed were 7.03 and 3.02 at 2 and 5 μg/L at 8th day, respectively. There could be two processes when determining duckweed absorptional TPT: one was the diffusion process depending on the difference concentration between test water and duckweed, and the other one was the organism active absorbing process. At 2 μg/L TPT, the organism active absorbing process might play a main role in the TPT absorption in duckweed, while at 5 μg/L TPT, the diffusion process of TPT could be an important part in TPT absorption in duckweed. At 5 μg/L TPT, the harmful effect on *Lemna minor* was more serious than at 2 μg/L TPT, which would decrease the ability of the organism's activity absorbing process.

Radiolabeled TPT was slowly metabolized by the fathead minnow. Only 2% of the TPT was metabolized to DPT after 6 days of exposure via water (Kannan and Lee 1996). The BCFs results of TPT indicate that the concentration of TPT can enlarge following the food chain, which is dangerous because of the high toxicity of TPT and low biodegradation. TPT has a degenerative effect on the fish microsomal monooxygenase system (Fent et al. 1998). TPT are potent inhibitors of glutathione S-transferase (GST)-mediated detoxication of xenobiotics in the liver and kidney of tropical marine fish (Al-Ghais and Ali 1999). These results, together with this study, suggest that TPT have negative effects on various

enzyme systems, not only in animals but also in plants. The results of this study show the TPT effects on various physiological functions of plant.

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