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Toxic effect of triphenyltin on *Lemna polyrhiza*

Zhihui Song[1]* and Guolan Huang[2]

[1]College of Material and Environmental Science, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China
[2]College of Environmental Science and Engineering, Nankai University, Tianjin 300071, People's Republic of China

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Little data about toxic effect of triphenyltin (TPT) on aquatic plants is available. The purpose of this paper is to study the toxic effect of TPT on duckweed, *Lemna polyrhiza*, and the bioconcentration factor of TPT by *Lemna polyrhiza*. At 5 μg/l concentration TPT treatment, a toxic effect on growth of *Lemna polyrhiza* appeared. The 8 day IC_{50} of TPT to *Lemna polyrhiza* was 19.22 μg/l. TPT stimulated peroxidase activity and nitrate reductase activity at 2 and 5 μg/l. TPT reduced chloroplast activity of *Lemna polyrhiza* at 2 and 5 μg/l. Bioconcentration factors of TPT for *Lemna polyrhiza* were 4.3 and 10.9 at 2 and 5 μg/l, respectively. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: triphenyltin; *Lemna polyrhiza*; bioconcentration factor; toxicity

INTRODUCTION

Organotin compounds are used in a variety of consumer and industrial products including marine antifouling paints, agricultural pesticides, wood preservatives and plastic stabilizers. It is widely accepted that antifouling paints are the most important contributors of organotin compounds to the marine environment, where they have been responsible for many deleterious effects on non-target aquatic life.[1] There are many sources of contamination by organotin compounds in coastal areas, with high levels of organotin compounds observed in marinas, moorings and near vessel repair facilities. Triphenyltin (TPT) was found in sediment, plankton and mussels from the port of Osaka and Otsuchi Bay.[2] Concentrations of TPT in sediments from four sites in the Göta älv Estuary, Southwest Sweden, ranged from 1.5 to 71 ng/g dry weight (d.w.).[3] Fish (blue gill, largemouth bass and channel catfish) from a pond near a pecan orchard in central Georgia (USA), which had been sprayed with commercial TPT hydroxide mixtures, contained TPT as well as diphenyltin (DPT) and monophenyltin (MPT).[4] TPT and triphenyltin (TBT) are known to be immunotoxic and cause renal and hepatic damage; the relative order of organotin toxicity in astrocyte cultures is TPT > TBT.[5] Organotins (TPT and TBT) directly inactivate cytochrome P-450 because of interaction with critical sulfhydryl groups of the hemoprotein, and TBT is metabolized more readily than TPT in rat, hamster and human.[6] The IC_{50}s of TPT to hepatic glutathione *S*-transferase activity in marine fish *Siganus canaliculatus* and *Sparus sarba* are 10 and 28 μM, respectively.[7] Three types of hybrid catfish (*Clarias gariepinus, Clarias macrocephalus*) cell culture were established to screen toxicity of TPT.[8] There is little information on the effect of TPT on fresh water aquatic plants. Few reports are available on the bioconcentration of TPT by aquatic plants. The purpose of this work was to study the toxic effect of TPT on duckweed, *Lemna polyrhiza*, and the bioconcentration factor of TPT by *Lemna polyrhiza*.

MATERIAL AND METHODS

Duckweed (*Lemna polyrhiza*) was collected from Weijin River in Tianjin city. TPT and other toxic pollutants were not detected in the river. *Lemna polyrhiza* was cultured in Hoagland medium in the laboratory for 2 weeks. The Hoagland medium consisted of 0.303 g/l KNO_3, 0.222 g/l $CaCl_2$, 0.246 g/l $MgSO_4 \cdot 7H_2O$, 0.686 g/l KH_2PO_4, 0.010 g/l $FeSO_4$, 0.200 g/l EDTA and 2 ml soil extraction solution in 1 l distilled water. One healthy *Lemna polyrhiza* plant was selected for culture in new Hoagland medium. When the amount of plant was sufficient, healthy *Lemna polyrhiza* was the tested.

The IC_{50} test was conducted in a 250 ml beaker with 200 ml test solution. Four concentrations of TPT and a control (0, 5, 10, 25, 50unsSn μg/l in Hoagland medium) were used; each

*Correspondence to: Zhihui Song, College of Material and Environmental Science, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China.
E-mail: songhuey@sina.com
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test was repeated three times. Thirteen fronds (leaf blade) of *Lemna polyrhiza* were put in to each beaker and covered with glass. The Beaker was lit at 4000 lx for the entire time at 25 °C. The test lasted for 8 days.

Every 2 days, the test solution was renewed and the number of fronds in each beaker was counted. At the end of the test, whole fronds in each beaker were homogenized with 10 ml acetone to extract chlorophyll. The extraction solution was put into a refrigerator for 96 h at 4 °C. Then the solution was centrifuged at 3000 g for 15 min. The absorbance of the clear solution was determined at 663 and 64.5 nm.

The chlorophyll content was calculated as follows:

$$C_{chl} = 20.2A_{645} + 8.02A_{663} \quad (1)$$

Growth inhibition was calculated as follows:

$$V = \ln(N_t/N_0)/t \quad (2)$$

$$I = (V_0 - V_t)/V_0 \times 100\% \quad (3)$$

where C_{chl} is the chlorophyll content, A_{645} and A_{663} values of absorbance of clear solution at 645 and 663 nm, respectively, V the growth rate, N the number of fronds of *Lemna polyrhiza*, or chlorophyll content (C_{chl}), and N_t and N_0 are N at time t and initial time. V_0 is the growth rate of control, V_t the growth rate of treatment test at time t and I is the growth inhibition. I was regressed with values of the logarithms of TPT concentrations so as to calculate the IC_{50} value.

The bioconcentration factor (BCF) test was conducted using a 500 ml beaker containing 400 ml test solution. Two concentrations of TPT and a control (0, 2, 5 Sn μg/l in Hoagland medium) were used; each test was repeated three times. Beakers were lighted at 4000Lx for the entire time at 25 C. The test lasted 8 days.

The test solution was renewed every 2 days and the number of fronds in each beaker counted. An of 2 mL test solution in each beaker was used to determine nitrate reductase activity every 2 days. At the end of the test, five, 10 or more fronds of *Lemna polyrhiza* were used to determined chlorophyll content; five, 10 or more fronds were used to determine sugar content, 10, 20 or more fronds were used to determine peroxidase activity; and 20, 30 or more fronds were used to determine chloroplast activity. The remaining fronds in each beaker were used to determined TPT content.

The methods of determining and calculating nitrate reductase activity, peroxidase activity, chloroplast activity and TPT content in duckweed have been published.[9]

RESULTS AND DISCUSSIONS

The effects of TPT on growth, chlorophyll content, nitrate reductase activity, peroxidase activity, chloroplast activity and TPT content in the test solution are shown in Figs 1–6 and Table 1.

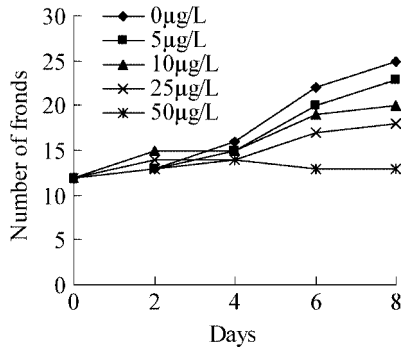

Figure 1. Effect of TPT on the growth of *Lemna polyrhiza*.

Table 1. Chlorophyll content in *Lemna polyrhiza* with TPT treatment at the end of the test

Concentration of TPT (μg/l)	0	5	10	25	50
Chlorophyll content in fronds of *Lemna polyrhiza* (mg/g)	0.902	0.815	0.582	0.534	0.378

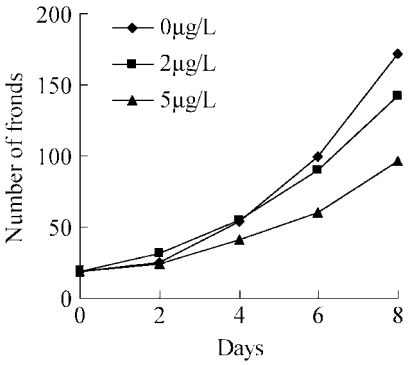

Figure 2. Effect of TPT on the growth of *Lemna polyrhiza* at low concentration.

From Figs 1 and 2, the toxic effect of TPT on *Lemna polyrhiza* appeared at 2 μg/l concentration. At 5 μg/l TPT the growth rate of *Lemna polyrhiza* was 88% of the control. At 50 μg/l TPT, the growth rate of *Lemna polyrhiza* was 10% of the control, and the fronds of *Lemna polyrhiza* became yellow. At the end of the test, chlorophyll content in fronds of *Lemna polyrhiza* exposed to 50 μg/l TPT was 42% of the control. The 8 day IC_{50} of TPT on *Lemna polyrhiza* frond number was 19.22 μg/l. For chlorophyll content, the 8 day IC_{50} of TPT on *Lemna polyrhiza* was 5.76 μg/l. This showed that chlorophyll content was more sensitive to TPT than frond number as an endpoint parameter. Values of 8 day IC_{50} of

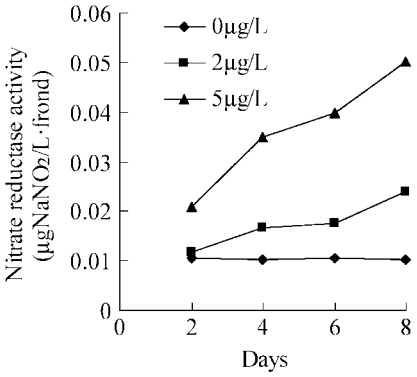

Figure 3. Effect of PCP on the nitrate reductase activity of *Lemna polyrhiza*.

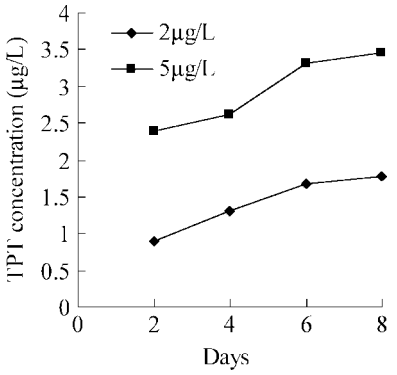

Figure 6. TPT concentration in the test solution.

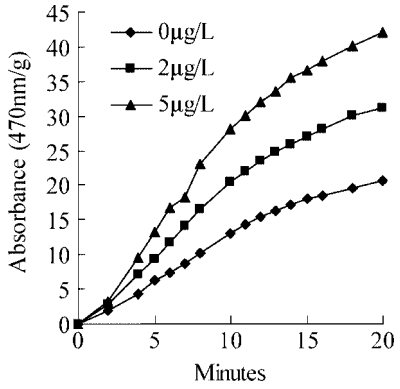

Figure 4. Effect of TPT on the peroxidase activity of *Lemna polyrhiza*.

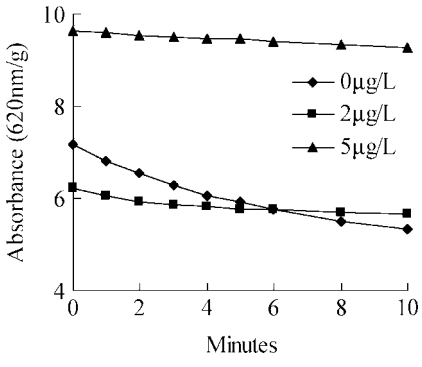

Figure 5. Effect of TPT on chloroplast activity of *Lemna polyrhiza*.

TPT for *Spirulina subsalsa* were 15.63 and 9.38 μg/l for growth rate and chlorophyll content, respectively.[10] The chlorophyll content of the freshwater alga *Scenedesmus quadricauda* with 10 μg/l TPT treatment was 28% of the control.[11] These data suggest that *Lemna polyrhiza* was the same sensitivity as blue-green algae and is less sensitive than green alga to TPT. The results suggest that TPT can decrease the chlorophyll content of plants, which further damages photosynthesis the 48 h EC_{50} value for TPT in *Daphnia magna* was 10.2 μg/l,[12] which suggests that *Lemna polyrhiza* is less sensitive to TPT than *Daphnia magna*.

From Fig. 3, nitrate reductase activity increased at low TPT concentration. Values of nitrate reductase activity were 2.3 and 4.8 times the control at 2 and 5 μg/l TPT, respectively. This showed that, at low concentration, TPT stimulates nitrate reductase activity. Nitrogen is an important nutrition element for plant survival. The abnormal nitrate reductase activity stimulated by TPT will cause a nitrogen metabolism disorder, leading to plant death.

Figure 4 shows that the peroxidase activity of *Lemna polyrhiza* increased at low TPT concentration. The values of peroxidase activity were 1.6 and 2.1 times the control, respectively. Peroxidase is one type of detoxification enzyme system. Higher peroxidase activity indicated a detoxification enzyme system working to reduce the damage from pollutants. Arochlor 1254 (a commercial mixture of polychlorinated biphenyl congeners) causes the *Lingulodinium polyedrum* (dinoflagellate) cells to exhibit increased ascorbate peroxidase activity (50%).[13] Human glutathione peroxidase activity increased with chlorpyrifos-ethl (an organophosphate insecticide) at low concentrations.[14] However, peroxidase activity at too high or low average levels will induce the detoxification of plants. The peroxidase activity of *Lemna minor*, another species of duckweed, at 2 and 5 μg/l TPT is slightly higher than the control.[9] These data show that TPT, like other pollutants, can stimulate plants peroxidase activity.

Figure 5 shows the absorbance change curves at 620 nm in the chloroplast activity determining test. The control curve decreases more rapidly than that with 2 and 5 μg/l TPT treatment. The values of chloroplast activity of *Lemna polyrhiza* at 2 and 5 μg/l TPT treatment are 33.8 and 13.6% of control, respectively. However, at the end of the test, chlorophyll contents of *Lemna polyrhiza* at 2 and 5 μg/l TPT treatment were nearly the same as the control. This implies that chlorophyll content is a perfect indicator of photosynthesis

activity. The toxic effect of pollutants on photosynthesis may appear before chlorophyll content changes. TPT affects intersystem electron transport in uncoupled chloroplasts, and uncoupled whole chain electron transport in the presence of methyl viologen under saturating illumination is inhibited by 75% at TPT concentrations >80 μM, with a half-maximal effect at about 20 μM.[15] The pollutant can destroy the chloroplast construct without reducing the chlorophyll content. Photosynthesis cannot work without the whole physiological structure, although chlorophyll content is not changed by the pollutant.

From Table 1, the sugar contents at 2 and 5 μg/l TPT treatments are 49 and 13% of the control, respectively. Sugar indicates the assimilation status of plant. The chloroplast is damaged and the assimilation at sugar is broken down. This result is like the effect of Cd on wheat.[16]

From Fig. 6, TPT concentration in the test solution was nearly stable at the end of test. This suggests that the uptake TPT from the test solution by *Lemna polyrhiza* is balanced. The BCFs of TPT for *Lemna polyrhiza* were 4.3 and 10.9 at 2 and 5 μg/l TPT, respectively. TPT can be bioconcentrated by benthic organisms, and at higher trophic levels in the food chain biodegradation products of TPT were not found.[17] Microbial degradation of radio labeled TPT in soil or sediment samples was slow, with only 5% degradation during a 14-day incubation period.[4] The concentrations of TPT in fish muscle from rivers and sea areas in Osaka, Japan were in the range 0.001–0.130 mg/kg wet weight. The concentrations of TPT were highest in fish liver, and relatively high concentrations of TPT were found in heart and brain.[18] TPT BCFs at pH 8 were 2200, 680 and 190 for *Thymallus thymallus*, *Chironomus riparius* and *Daphnia magna*, respectively.[19] TPT was detected in *Crassostrea. gigas* with concentrations up to 678 ng/g from the Chinhae Bay System, Korea.[20] In the foodchain of a shallow freshwater lake in the Netherlands, zebra mussels, eel, roach, bream, pike, perch, pike perch and cormorant showed high levels of organotin compounds. At the lower trophic levels, phenyltin concentrations were high in benthic species, and at the higher trophic levels, high net bioaccumulation resulted in high TPT concentrations.[21] Concentration of TPT in milkfish flesh in a brackish water pond was about 230 ng TPT/g wet tissue.[22] These means that TPT can be bioconcentrated by animals and plants, and biodegradation is difficult. TPT could be transported to higher trophic level organisms through the food chain, including duckweed, which could lead to greater toxicity of TPT to more organisms.

Dunaliella tertiolecta was exposed to TPT; swollen mitochondria were observed and disruption of the thylakoid membranes of the chloroplast was also observed. It is suggested that inhibition of respiration and photosynthesis metabolic processes could take place before structural damage to the responsible organelles is observed.[23] The external pectin theca, the limiting membrane and inter-photosynthetically active lamellae in the *Spirulina subsalsa* cell were the targets that were easily damaged by TPT.[24] Those data suggest that TPT reduces photosynthetic activity by damaging the physiological structure.

CONCLUSIONS

At 5 μg/l TPT concentration treatment, a toxic effect on the growth of *Lemna polyrhiza* appeared. The growth rate of *Lemna polyrhiza* decreased with increasing TPT concentration. The nitrate activity and peroxidase activity of *Lemna polyrhiza* increased at 2 and 5 μg/l TPT concentration. The chloroplast activity *Lemna polyrhiza* was significantly decreased by TPT treatment. BCFs of TPT by *Lemna polyrhiza* were 4.3 and 10.9 at 2 and 5 μg/l concentrations.

Acknowledgments

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