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Toxicity of Triphenyltin to *Spirulina subsalsa*

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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 to nontarget aquatic life (Alzieu 1991; Ruiz et al. 1995). Triphenyltin (TPT) is one of the most toxic compounds in organotin compounds to aquatic organisms ever introduced deliberately to water.

According to the anti-radiation, anti-cancer, anti-caducity and anti-mutation (Chao 1994), the *Spirulina* spp. lead the researchers to investigate all over the world. However, there are a few toxicity tests using *Spirulina* spp. (Chen 1998; Zhou 1997) as the test organism.

The toxicity of TPT to algae has seldom been reported. Freshwater algae *Senedesmus quadricauda* has been used to study the effect of TPT on organisms (Fargasova 1997, 1998). TPT is used as antifouling agents in paints applied to boat huss, which has caused the pollution in the estuarine and oceanic environment. Because estuarine and oceanic water is different from fresh water, it is necessary to study the effect of TPT on estuarine and oceanic algae. *Spirulina subsalsa* can grow in salt water, which suggests that the *Spirulina subsalsa* can be used to study the toxicity of toxicant to estuarine and oceanic environment.

The results of this paper show that *Spirulina subsalsa* is easy to culture and sensitive to the toxicant (TPT). The *Spirulina subsalsa* can be used to evaluate the contamination on the estuarine and oceanic environmental pollution.

METHODS AND MATERIALS

The *Spirulina subsalsa* was obtained from the Institute of Aquatic Organism of China. The algae were cultured with the Zorrouk solution containing 0.15 mol/L KNO_3 as the nitrate reductase substrate.

Toxicity tests were conducted in 250 mL Erlenmeyer flasks within 60 mL of test

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solution and cultures were incubated under 16 hr light (2200 ± 100 Lux) / 8hr dark photoperiod at 25 ± 1°C . Each test was replicated three times. Every two days, algal density, chlorophyll content, phycocyanin content and nitrate reductase activity were determined. The tests lasted 8 days.

There is linear relationship between value of absorbance at 560 nm and dry weight of *Spirulina*. When the value of absorbance at 560 nm is 1.00, the dry weight of *Spirulina* in the solution is 0.522 mg/mL (Zhu 1990). Thus, the absorbance of test solution at 560 nm was determined every two days for the algal growth curve.

Every two days, 5 mL of each test solution was put into the centrifuge tube for the determination of the chlorophyll a content. The solution was centrifuged at 3000 Xg for 15 minutes, and the deposit was extracted with alcohol for chlorophyll content. The extraction solution was kept in the refrigerator at 4 °C for 24 hour. Then the extraction solution was centrifuged at 3000 X g for 15 minutes. The absorbance of the clear solution was determined at 665 nm.

Every two days, 5 mL solution of each test was moved into the centrifuge tube for the determination of phycocyanin content. The solution was centrifuged at 3000 X g for 15 minutes, and the deposit was extracted with the 10 mmol/L phosphate buffer solution (pH 7.0) containing 1 mmol/L NaN_3 and 1 mmol/L sulfhydrylethanol. The extraction solution was kept in the refrigerator under 0 °C until wholly frozen. Then it was kept at 4 °C until wholly molten. The procedure of freezing and melting was carried out three times. Then the' solution was centrifuged at 3000 X g for 15 minutes. The absorbance of the clear solution was determined at 618 nm.

Nitrate reductase catalyzes nitrate to convert into nitrite, and the nitrite is released from cells to the test solution. The concentration of nitrite shows the nitrate reductase activity. The method of Zhu (1990) was used to determine the nitrite concentration in the test solution. Each test 1 mL solution was added to 2 mL 15% HCl containing 1% sulfanilamide and was added to 2 mL 15% HCl containing 0.02% N-1-naphtylethylendiamine dehydrochloride. Absorbance of the above solution was measured after 30 min at 520 nm. The standard curve was made with sodium nitrite solution.

Calculation:

The chlorophyll content was calculated as follows:

$$C_{chl\text{-}t} = A_{665\text{-}t} \times 14.3$$

The phycocyanin content was calculated as follows:

$$C_{cyanin\text{-}t} = A_{618\text{-}t} \times 13.4$$

The nitrate reductase activity was calculated as follows:

First to make a standard curve of the nitrite concentration vs. absorbance at 520 nm:

$$C_{nitrite\text{-}t} = (A_{520\text{-}t} - 0.015) \times 12.048$$

Then the nitrate reductase activity (U_t) was calculated as follows:

$$U_t = C_{nitrite\ t} / A_{560\text{-}t}$$

The relative nitrate reductase activity (U_{rt}) was calculated as follows:

$$U_{rt} = U_t / U_{0t}$$

The growth rate was calculated as follows:

$$V_t = \ln (N_t / N_0) / t$$

Where, t was the test time, $A_{665\text{-}t}$, $A_{618\text{-}t}$, $A_{560\text{-}t}$, and $A_{520\text{-}t}$ was the absorbance at 665, 618, 560, 520 nm at t time, respectively, $C_{chl\text{-}t}$ was chlorophyll content of each test solution at t time, $C_{cyanin\text{-}t}$ was phycocyanin content of each test solution at t time, $C_{nitrite\text{-}t}$ was the nitrite concentrate in test solution at t time, U_{rt} is the relative nitrate reductase at t time, U_{0t} is the nitrate reductase activity of control test at t time. V_t was the growth rate at t time, N_t represented the $C_{chl\text{-}t}$, $C_{cyanin\text{-}t}$ and $A_{560\text{-}t}$, N_0 was the value of the chlorophyll content, phycocyanin content and solution aborbance at 560 nm at initial time.

The growth rate inhibitory effect was calculated as follows:

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

Where, V_0 was the growth rate or the N_{rt} at 8th day of control tests, V, was the growth rate or the N_{rt} at 8th day of each contamination test. I was regressed with the value of logarithm of TPT concentration so as to calculate the value. of IC_{50} (median inhibitory concentration).

RESULTS AND DISCUSSION

The effects of TPT on the growth rate, chlorophyll content, phycocyanin content and nitrate reductase activity of *Spirulina subsalsa* are showed in figure 1, 2, 3 and 4, respectively. The IC_{50}s of TPT on *Spirulina subsalsa* are showed in Table 1.

Table 1. The effect of TPT on the *Spirulina subsalsa* (8 days IC_{50}, μg/L)

Parameters	Growth rate	Chlorophyll content	Phycocyanin content	Nitrate reductase activity
TPT	15.63	9.38	31.45	6.05 (EC_{50})

It can be seen in Fig.1 - Fig.3 that the toxicity of TPT on the growth rate, chlorophyll a content and phycocyanin content of *Spirulina subsalsa* increased with the concentration of TPT increase. Compared with the control test, there appeared 30%, 27%, 25% and 93% in growth rate, chlorophyll content, phycocyanin content and nitrate reductase activity in treatment test, respectively when the concentration of TPT was 10 μg/L. The toxicity of TPT was the highest to nitrate reductase activity among the above four parameters, which demonstrated that physiological parameters could represent the toxicity of TPT at low concentration.

Fig. 4 shows that when the concentrations of TPT were 5, 10 and 20 μg/L, the relative nitrate reductase activity decreased with test time going on. At the beginning of tests, the nitrate reductase activity in treatment tests was higher than

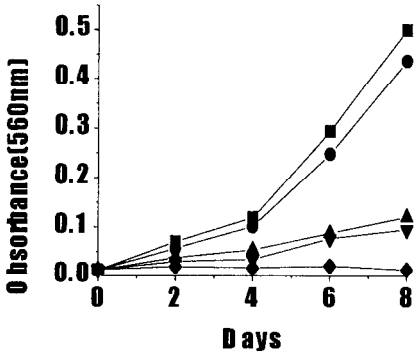

Figure 1. Effect of TPT to growth rate of *Spriulina subsalsa*

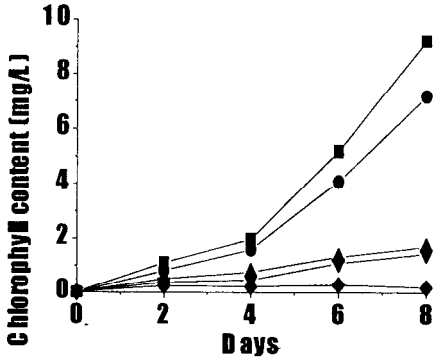

Figure 2. The effect of TPT to chlorophyll content of *Spirulina subsalsa*

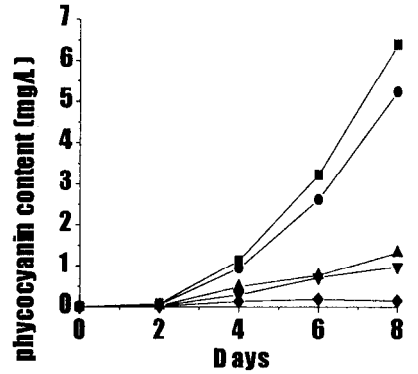

Figure 3. Effect of TPT to phycocyanin content of *Spirulina subsalsa*

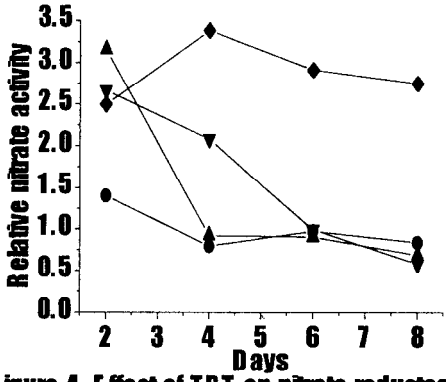

Figure 4. Effect of TPT on nitrate reductase activity of *Spirulina subsalsa*

that of control test. This means that TPT had stimulative effect on nitrate reductase activity when tests started. With the tests time going on, the inhibitory effect on nitrate reductase activity appeared and became more and more serious. At the end of tests, the nitrate reductase activity was lower than that of control test.

It can be seen in Fig. 4 that when the concentration of TPT was 40 μg/L, the nitrate reductase activity was much higher than that of control test. It is obvious in growth curve of Fig.1 that there was only small increase at the beginning of the test and descent at the end of the test at 40 μg/L of TPT. This showed that most of algal cells were dead at 40 μg/L of TPT after 8 days. This means that at 40 μg/L,

TPT caused the algal cells break, and the cellular inclusions including nitrate reductase were released into the culture solution. The nitrate reductase directly contacted the nitrate in the test solution, which resulted in that the activity of nitrate reductase outside was higher than that inside the algal cells. This result suggested that abnormal high nitrate reductase activity showed the high toxicity of TPT to *Spirulina subsalsa.*

The 8 days IC_{50}s of TBT to growth rate, chlorophyll content and phycocyanin content of *Spirulina subsalsa* are 5.09 μg/L, 5.14μg/L and 3.25μg/L, respectively (Chen 1998). It can be seen that the toxicity of TPT is lower than TBT to *Spirulina subsalsa.* The EC_{50}s of TPT to growth rate and chlorophyll a content of greenalgae *Scenedesmus quadricauda* are about 0.04 and 10 μg/L, respectively. Those results show that *Spirulina subsalsa* is less sensitive than greenalgae *Scenedesmus quadricauda* to TPT.

Nitrate reductase is very important in the nitrogen metabolism of plants and algae. Since alga acts as the energy and food supplier of the aquatic environment, the nitrogen metabolism of the algae relates the aquatic ecological stabilization. When the nitrate reductase activity changes, the nitrogen metabolism will be affected and there will appear disturbance of nitrogen circle in an aquatic ecosystem. Thus, the effect of contamination including TPT on the algal nitrate reductase is necessary to be studied in order to evaluate the influence of the pollutants on the environment.

In this paper, a convenient method for determination of algal nitrate reductase activity was established. The simple work was to centrifuge the test solution to obtain a clear test liquor, which was a modified method of Zhu (1990).

It is the first time in this research that the effect of TPT on the nitrate reductase activity of *Spirulina subsalsa* has been observed. The result indicates that the nitrate reductase activity is the most sensitive parameters of algae to TPT. Therefore, algal nitrate reductase activity is another important parameter used in the environmental assessment.

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