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Inhibitive Effect of Organotin Compounds on the Chlorophyll Content of the Green Freshwater Alga *Scenedesmus quadricauda*

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Algae have been considered to be good indicators of the bioactivity of industrial wastes (Walsh and Merrill 1984). Unicellular algae vary in their response to a variety of toxicants (Tadros et al. 1994). Little is known, however, about the toxicity of organotin compounds (OTC) to freshwater green algae. The work reported here was done to examine the effect of selected OTC on the green alga Scenedesmus quadricauda and on its chlorophyll production. Since chlorophyll *a* comprises 1 to 2 % of the dry weight of most algal cells and since it is easily quantified spectrophotometrically, this photosynthetic pigment is widely used for the determination of phytoplankton and periphyton biomass (APHA 1985),

Many OTC are toxic to various organisms and they are used to a limited but significant extent as biocidal agents in agriculture and technology. Many studies on the toxicology of Sn have focused on OTC (McDonald and Trevors 1988). Organotins are present in four main forms, each with varying levels of toxicity. The general form is R_nSnX_{4-n} where R is methyl, ethyl, buthyl, and phenyl group(s), and X is a halide such as Cl^-. Triorganotin compounds (R_3SnX) are probably the most widely studied tin compounds. They are the most biologically active group, and are responsible for the majority of the biocidal effects. The R group in this case determines the level of toxicity towards specific organisms (Davies and Smith 1980). Variation of the inorganic radical X appears to have little effect on the biological activity (Davies and Smith 1980). Tributyltin and triphenyltin are highly toxic to algae (McDonald and Trevors 1988).

This paper reports the studies on the effects of OTC on the chlorophyll (total, a, b) content of green alga S quadricauda. Toxicity bioassays were performed under controlled conditions, using the changes of chlorophyll contents as an indication of OTC toxicity.

MATERIALS AND METHODS

Scenedesmus quadricauda (TURP.)BREB., strain Greifswald 15, was kindly supplied by the Institute of Botany AS CR, Trebon, Czech Republic. During the tests, the culture was incubated under continuous light at 25±1 °C and a light intensity produced by three 40 W white fluorescent lamps. The culture was maintained in liquid medium containing (g/L): KNO_3 0.1; $K_2HPO_4.7H_2O$ 0.01; $MgSO_4.7H_2O$ 0.001; $FeCl_3.6H_2O$ 0.001; soil extract 50 mL; pH = 7.18. During the tests, the alga grew in 100-mL Erlenmayer flasks with a 25-mL cultivation medium supplemented with OTC. Each OTC was tested in four concentrations (mg/L): 0.001; 0.01; 0.1 and 1.0. Each concentration was duplicated three times. Approximately 25,000 coenobia (four cells connected into one unit) were inoculated in test and control media. After 7 d cultivation, 1 mL of OTC, at the appropriate concentration, was added to the cultivation medium. The cultivation lasted 2 d longer under the same conditions and then the amount of chlorophyll was determined by the method published by Harris (1989). Chlorophyll was extracted into the 95 % ethanol and its amount was calculated under the following equations:

$$\text{total chlorophyll } (chl\_t) = 6.10(A_{665}) + 20.04(A_{649})$$
$$\text{chlorophyll } \_a\ (chl\_a) = 13.70(1_{665}) - 5.76(A_{649})$$
$$\text{chlorophyll } \_b\ (chl\_b) = 25.80(A_{649}) - 7.60(A_{665})$$

in µg/mL culture. The inhibitory effect of OTC on chlorophyll production by the alga S quadricauda was determined by the using equation: I = (1 - c/Ko).100 % (c - chlorophyll content for determinate concentration of tested OTC; Ko - chlorophyll content in control).

Twelve OTC synthesized at the Department of Organic Technology, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovak Republic were tested. The diorganotin compounds (type R_2SnX_2) that were tested were:

A - dibutyl-tin-bis-N,N-diethyl-dithiocarbaminate
B - dimethyl-tin-bis-N,N-diethyl-dithiocarbaminate

The other tested compounds were triorganotins (type R_3SnX):

C - triphenyl-tin-chloride
D - triphenyl-tin-acetate
E - triphenyl-tin-N,N-diethyl-dithiocarbaminate
F - tribenzyl-tin-bis-N,N-diethyl-dithiocarbaminate
G - tribenzyl-tin-chloride
H - bis-tributyl-tin-3,4,5,6-tetrachlor-phthalate
I - tributyl-tin-sulphaminate
J - tributyl-tin-N,N-diethyl-dithiocarbaminate
K - tributyl-tin-naphtenate
L - tributyl-tin-oxide (TBTO)

The results were statisticaly evaluated by Student's test at P = 0.05.

RESULTS AND DISCUSSION

In a concentration of 1.0 mg/L, compounds A,C,D and L reduced the content of chl _t_ about 95 %. In these cases the contents of chl _a_ and chl _b_ also decreased very rapidly (Tab. 1.). In all cases chlorophyll contents were inhibited significantly or highly significantly in comparison to the control, with the exception of compound E when the chl _t_ and chl _b_ contents were determined.

Table 1. The chlorophyll contents in the alga _Scenedesmus quadricauda_ after application of organotin compounds in concentration 1.0 mg/L

Comp.	Pigment content (µg/mL) ± Sx		
	chl t	chl a	chl b
A	0.063±0.002** (-91.1)	0.039±0.001** (-83.6)	0.024±0.001** (-94.8)
B	0.353±0.010** (-62.7)	0.119±0.006** (-79.5)	0.208±0.015* (-36.9)
C	0.046±0.002** (-93.4)	0.014±0.001** (-99.1)	0.035±0.002** (-90.4)
D	0.033±0.002** (-95.3)	0.006±0.001** (-97.5)	0.027±0.001** (-94.1)
E	0.811±0.013ns (-14.8)	0.333±0.004* (-42.7)	0.173±0.005ns (-24.3)
F	0.049±0.002** (-86.3)	0.027±0.001** (-78.2)	0.022±0.001** (-90.5)
G	0.628±0.016** (-55.8)	0.242±0.003** (-64.9)	0.386±0.014* (-47.3)
H	0.291±0.003** (-79.5)	0.128±0.001** (-81.4)	0.163±0.002** (-77.8)
I	0.628±0.021** (-67.0)	0.360±0.010** (-66.0)	0.268±0.010** (-68.4)
J	0.936±0.027* (-50.8)	0.494±0.017* (-53.4)	0.442±0.013* (-47.6)
K	0.626±0.024** (-67.1)	0.356±0.019** (-66.4)	0.270±0.013** (-68.0)
L	0.004±0.0001** (-98.9)	0.003±0.0002** (-94.3)	-----

The value in bracket indicate percent inhibition (-) or promotion (+) in the presence of OTC, as compared to the corresponding control.
Sx - standard deviation; ns - not significant; * - significant differences P<O.05; ** - high significant differences P<O.01

In a concentration of 0.1 mg/L, the inhibitive effect of the chlorophyll content was also very strong (Tab. 2.). Again, the strongest effects in this concentration

Table 2. The chlorophyll contents in the alga *Scenedesmus quadricauda* after application of organotin compounds in concentration 0.1 mg/L

Comp.	Pigment content (μg/mL) ± Sx		
	chl t	chl a	chl b
A	0.157±0.008** (-77.4)	0.049±0.001** (-80.3)	0.108±0.003** (-76.4)
B	0.418±0.012* (-56.1)	0.210±0.010** (-63.9)	0.234±0.005ns (-36.9)
C	0.067±0.002** (-90.4)	0.032±0.002** (-86.6)	0.035±0.001** (-92.3)
D	0.078±0.003** (-88.8)	0.024±0.001** (-80.9)	0.054±0.002** (-88.2)
E	0.228±0.009** (-76.2)	0.105±0.002** (-81.9)	0.123±0.006* (-66.8)
F	0.168±0.008* (-52.9)	0.055±0.002* (-55.6)	0.113±0.003* (-51.3)
G	0.428±0.016* (-69.9)	0.165±0.005** (-88.6)	0.263±0.008* (-64.3)
H	0.376±0.017** (-73.6)	0.134±0.004** (-80.6)	0.242±0.003* (-67.0)
I	1.025±0.048* (-46.2)	0.458±0.014* (-56.9)	0.567±0.016ns (-32.8)
J	0.634±0.017* (-66.7)	0.240±0.007** (-77.4)	0.394±0.012* (-53.3)
K	1.202±0.021ns (-36.9)	0.669±0.017ns (-36.9)	0.533±0.018ns (-30.9)
L	0.048±0.002** (-86.8)	0.023±0.001** (-81.5)	0.025±0.001** (-89.2)

Details as in Table 1.

were for compounds A,C,D and L, but the overall effect of this concentration was lower than the effect of 1.0 mg/L. The strongest effects in this concentration were for compounds A,C,D,E,G,H,J and L. An inhibitive effect higher than for the concentration of 1.0 mg/L was identified only for compounds E,G and J. A significant difference was found between these two concentrations (0.1 and 1.0 mg/L) only for compound E. For two other cases (compounds G,J), no significant differences were determined when compared with higher concentration (1.0 mg/L). When compared with the control, all OTC, except compound K and compounds B and I when chl *b* was determined, had a significant or highly significant inhibitive effect on chlorophyll content.

In the concentration of 0.01 mg/L of OTC (Tab. 3.), compounds A,C,D,H,J and L had a highly significant inhibitive effect on all types of chlorophyll production. The inhibitive effect of other compounds was insignificant in comparison with the control except for chl *a* content when compound E was applied.

Table 3. The chlorophyll contents in the alga Scenedesmus quadricauda after application of organotin compounds in concentration 0.01 mg/L

Comp.	Pigment content (μg/mL) ± Sx		
	chl t	chl a	chl b
A	0.205±0.006** (-70.5)	0.054±0.003** (-77.3)	0.151±0.004** (-67.0)
B	0.587±0.011ns (-38.3)	0.320±0.012ns (-34.9)	0.267±0.011ns (-28.0)
C	0.193±0.005** (-72.2)	0.124±0.003** (-57.9)	0.069±0.001** (-84.9)
D	0.104±0.004** (-85.0)	0.064±0.002** (-73.1)	0.040±0.001** (-91.3)
E	0.454±0.014ns (-5.1)	0.173±0.005** (-70.2)	0.281±0.005ns (-24.3)
F	0.279±0.012ns (-21.8)	0.082±0.002ns (-31.5)	0.197±0.007ns (-16.4)
G	0.885±0.034ns (-37.8)	0.415±0.010ns (-39.8)	0.471±0.015ns (-35.7)
H	0.507±0.009** (-64.3)	0.225±0.005** (-67.3)	0.282±0.006** (-61.5)
I	1.485±0.073ns (-22.1)	0.779±0.043ns (-26.5)	0.706±0.035ns (-16.4)
J	0.269±0.010** (-85.9)	0.980±0.002** (-90.8)	0.171±0.004** (-79.6)
K	1.592±0.060ns (-16.4)	0.902±0.036ns (-14.9)	0.690±0.017ns (-18.2)
L	0.129±0.006** (-68.9)	0.069±0.002** (-68.3)	0.060±0.002** (-73.7)

Details as in Table 1.

When the lowest concentration of OTC (0.001 mg/L) was used, a significantly inhibitive effect was determined only for compounds D and J (Tab. 4.). For other compounds, the inhibition was lower than 40 %. In these tests the stimulative effect for compounds B,E,F and I was observed.

From these results chl *t*, chl *a* and chl *b* synthesis inhibition can be rank ordered as follows

chl t: 1.0 mg/L: L>D ≥ C ≥ A>F>H>K=I ≥ B>G ≥ J>E
0.1 mg/L: C ≥ D ≥ L>A=E ≥ H ≥ G ≥ J>B ≥ F>I>K
0.01 mg/L: J=D>C=A>H=L>E>B=G>I=F>K
0.001 mg/L: J>D>H>G ≥ E=A=C=L>K=B>I>F

chl a: 1.0 mg/L: C ≥ D ≥ L>A ≥ H ≥ B=F>I=K=G>J>E
0.1 mg/L: G=C>E=L=D=H=A ≥ J>B>I=F>K
0.01 mg/L: J=D>A=C>H=L>E>G=B>F=I>K
0.001 mg/L: J>D>H ≥ C>G=A=B=E>L>K>I>F

chl b: 1.0 mg/L: L>A=D ≥ C ≥ F>H>I=K>J=G>B>E
0.1 mg/L: C ≥ L=D>A>H=E ≥ G>J=F>B>I ≥ K

0.01 mg/L: D>C>J>L ≥ A ≥ H>G>B ≥ E>K=I=F
0.001 mg/L: J>D>H>G ≥ A=L ≥ C>K>I>F>E>B

From these rank orders it is evident that the chlorophyll content is influenced as much by the nature of organic groups in the side chain as by the concentration. Compound K with butyl in n-alkyl chain for concentrations 0.1 and 0.01 mg/L can be marked as the least toxic for all chlorophyll types synthesis. Compound E (phenyl in n-alkyl chain) was the least toxic for the concentration of 1.0 mg/L as was compound F (benzyl in the side chain) for the concentration of 0.001 mg/L. When the concentration 0.001 mg/L was used, the least toxic OTC for chl *b* synthesis was diorganotin compound B with methyl in the side chain. The highest inhibitive effect was found for all chlorophyll types synthesis in all concentrations of compounds C and D with phenyl group and J and L with butyl group in the n-alkyl chain. The position of diorganotin compounds (A,B) was usually in the middle of the rank orders and compound A with butyl in the side chain had a higher toxic effect than compound B with methyl. This observa-

Table 4. The chlorophyll contents in the alga *Scenedesmus quadricauda* after application of organotin compounds in concentration 0.001 mg/L

Comp.	Pigment content (μg/mL) ± Sx		
	chl t	chl a	chl b
A	0.528±0.024ns (-24.0)	0.153±0.007ns (-35.7)	0.375±0.008ns (-18.1)
B	0.885±0.029ns (-7.0)	0.379±0.015ns (-34.8)	0.506±0.020ns (+36.1)
C	0.539±0.020ns (-22.4)	0.128±0.005ns (-36.2)	0.411±0.012ns (-10.3)
D	0.246±0.004* (-64.6)	0.063±0.001** (-73.5)	0.183±0.004* (-60.0)
E	0.726±0.028ns (-23.7)	0.374±0.012ns (-35.6)	0.352±0.015ns (+28.8)
F	0.407±0.020ns (+14.0)	0.135±0.006ns (+8.9)	0.272±0.011ns (+16.8)
G	1.008±0.021ns (-29.1)	0.437±0.013ns (-36.6)	0.571±0.007ns (-22.1)
H	0.735±0.034ns (-38.3)	0.325±0.004ns (-32.8)	0.410±0.010ns (-34.1)
I	2.069±0.047ns (+8.7)	1.126±0.029ns (+6.2)	0.943±0.019ns (+11.7)
J	0.398±0.015** (-79.1)	0.184±0.006** (-82.6)	0.214±0.008** (-74.6)
K	1.757±0.065ns (-7.7)	0.901±0.032ns (-15.0)	0.856±0.028ns (+1.4)
L	0.281±0.012ns (-21.3)	0.089±0.001ns (-28.2)	0.192±0.003ns (-17.7)

Details as in Table 1.

tion completely agrees with the toxicity order of organic groups in the side chains mentioned by Thayer (1983). From these rank orders it is not possible to determine how the bounded hydrocarbon radical R (usually butyl, benzyl or phenyl) influenced the toxicity. It is only possible to say that OTC, belonging to triorganotins with butyl and phenyl radicals, inhibited the chlorophyll production in our study more than organotins with the benzyl radical. Thayer (1983) noted that the toxicity of organotins is influenced by the length of the side chain. Organotin compounds having methyl groups are considered the least toxic and toxicity increases up to the butyl group (maximum efficiency); phenyl has approximatelly the same biocidal efficiency. This state was also confirmed in our tests. Variation of the radical X (usually chloride, fluoride, oxide, hydroxide, carboxylate or thiolate) appeared to have little effect on the biological activity (Davies and Smith 1980), which completely agrees with the results we obtained. It was not possible to determine whether the chlorophyll content was more influenced by triorganotin (R_3SnX) or diorganotin (R_2SnX_2) compounds. Triorganotins are described in the literature as more toxic and biologically active than diorganotins (McDonald and Trevors 1988). This statement does not fully agree with our results. In studies on tin uptake and metabolism, bis-, and tributyltin oxide (TBTO) are often used as the representative OTC, as generally the most toxic (McDonald and Trevors 1988). In our attempts this effect was manifested fully only when higher concentrations (0.1 and 1.0 mg/L) were used. In a lower concentration (0.01 mg/L), its inhibitive effect on chlorophyll production was only about 40 %, and that is why its position in the rank orders of inhibition was in the middle (compound L).

Because of the lack of literature on the toxic effects of OTC on green algae and their chlorophyll production, it has not been possible to compare our rank orders of inhibitive effect with those of other authors. The use of tin in pesticides, antifouling paints, and in poly-Vinyl chloride stabilizers has resulted in increased levels of tin in the environment. Elevated *tin levels* are found in waters, sediments and soils in areas impacted by humans. Tin has been shown to have toxic effects both on eukaryotic and prokaryotic organisms; therefore, studies regarding the impact of tin on the environment are required for an assessment of the problem.

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