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Toxic Effect and Accumulation of Atrazine in Algae

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Autotrophic microorganisms are highly sensitive to some herbicides, especially to triazines. The occurrence, toxicity and accumulation of herbicides in ecosystems have been studied extensively whereas the kinetics of their sorption, which is crucial for possible decontamination of aqueous environments, have attracted far less attention.

Even very low concentrations of atrazine are known to have a toxic effect on unicellular algae. WELLS & CHAPPEL (1965) found that atrazine in concentrations in excess of 1 ppm completely inhibited the growth of the alga Chlorella pyrenoidosa. On the other hand, according to VANCE & SMITH (1969) small quantities of atrazine had no toxic effect on the growth of the alga Scenedesmus quadricauda and C. pyrenoidosa, and stimulated the growth of the alga Chlamydomonas eugametos. Intensive detoxation of atrazine was found in media inoculated by the alga Chlorosarcina sp. (KRUGLOV & PAROMENSKAYA 1970). GRAMLICH & FRANS (1964) reported a maximum inhibition of the alga C. pyrenoidosa by atrazine in a concentration of about 10^{-7} M. ASHTON et al. (1966) studied the effect of atrazine on Chlorella vulgaris. Atrazine was found to prevent normal growth and reduce the content of chlorophylls. The cells contained no starch. Different species of unicellular algae responded to the presence of atrazine in a different way (LOEPPKY & TWEEDY 1969). Chlamydomonas reinhardtii was inhibited by 0.5 ppm atrazine in the medium while C. pyrenoidosa was not inhibited even by high concentrations.

BUTLER et al. (1975) studied the growth of several freshwater algae and its inhibition by low concentrations of atrazine; most of the herbicide was present in the cultures even at the end of the 3-week cultivation.

Our work is concerned with the toxicity and kinetics of elimination of atrazine by microscopic algae under laboratory conditions. The results point to some perspectives of decontamination of aqueous environments.

MATERIALS AND METHODS

Inorganic medium described by SETLIK (1968) was used, diluted with water to a medium-to-water ratio 1:3. Before the cultivation the medium was supplemented with 0.25, 0.5, 2.5, 5.0, 10.0, 15.0 and 25.0 mg dissolved atrazine/L.

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Atrazine was used in the form of ZEAZIN containing 50% (W/W) active substance. The remaining components are not herbicidally active.

Experimental device is schematically depicted in Fig. 1. It consisted of ten 32-mm diameter glass tubes placed side by side vertically in parallel with the plane of a panel of fluorescent tubes (40W each) which illuminated the suspensions placed in the tubes. The tube height was 800 mm; each tube contained 400 mL of algal suspension. Light intensity in the plane of the tubes was 30W m^{-2}. The algal suspension, kept at 33°C, was bubbled through by a mixture of air and 3% (V/V) carbon dioxide.

Algae, C. vulgaris, were obtained from the collection of the Department of Autotrophic Microorganisms.

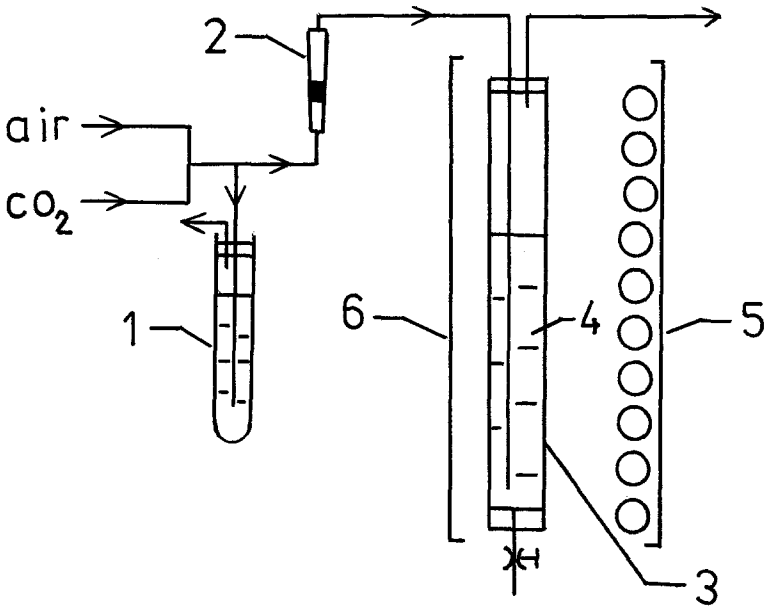

Fig. 1. Cultivation device. 1. Colorimetric control of CO_2 concentration in gas mixture. 2. Rotameter. 3. Glass tube. 4. Algal suspension. 5. Fluorescent tubes panel. 6. Reflection sheet.

Algae were grown in a nutrient medium to a concentration of about 2 g L^{-1}, separated by centrifugation and resuspended in an appropriate volume of the atrazine test medium. When the maximum concentration of biomass was achieved, the algae were separated by centrifugation and the supernatant was analysed.

Atrazine assay was conducted by gas chromatography. The residual atrazine in the supernatant was extracted for analysis according to TINDLE & GEHRKE (1968).

Optical density and pH of suspensions was controlled daily using a SPEKOL spectrophotometer and RADIOMETER pH-meter. The values of optical density shown in Table 1 were obtained by measuring of samples diluted 1:100.

RESULTS AND DISCUSSION

The time course of growth of C. vulgaris in various concentrations of atrazine in the medium and the final concentrations of algal biomass (dry weight) are shown in Table 1.

TABLE 1

Atrazine concen. mg L^{-1}	Optical density of suspension Days (1 = beginning)							Final biomass concn. g d.w. L^{-1}
	1	2	3	4	5	6	7	
0	0.06	0.10	0.11	0.13	0.14	0.13	0.14	6.88 +
0.25	0.06	0.07	0.07	0.06	0.08	0.08	0.12	4.75
0.50	0.06	0.04	0.05	0.04	0.07	0.07	0.10	3.88
2.50	0.06	0.05	0.05	0.04	0.06	0.06	0.08	3.00
5.00	0.06	0.05	0.06	0.05	0.04	0.04	0.07	2.63

+ The dry weight concentrations are not exactly proportional to the optical density.

No growth was observed above 5 mg L^{-1} atrazine. Even the lowest dose of atrazine had a marked inhibitory effect on algal growth during the first 5 days as compared with the control. Atrazine concentrations above 2.5 mg L^{-1} inhibited the growth throughout the experiment. The two lowest concentrations failed to exert inhibitory action on the last day of the experiment. The concentration of 0.5 mg L^{-1} atrazine is obviously the highest one with which the algae can still cope and restore their growth ability.

Atrazine residues in the supernatant after cultivation and separation of algae are shown in Table 2.

Efficiency and rate of atrazine removal by C. vulgaris was dependent on time. The kinetics of removal of atrazine from the aqueous environment is shown in Table 3. Initial atrazine concentration was 2.5 mg L^{-1}.

The somewhat surprising conclusion can be drawn from the table is that during a short exposure of algae to an atrazine-containing medium (less than 1 h, i.e., the time necessary for dispersion of algae and their immediate separation) more than 90% of the atrazine present was

sorbed in the algae. On a longer exposure the sorption dropped to a considerably lower level and then rose again. So, the atrazine initially sorbed by the algae is on longer exposure released back into the medium. This desorption may take place from the dead decaying cells or cell walls. Also the algae, which did not show any growth about 5 mg L^{-1} atrazine (Table 2) were able to remove atrazine from the aqueous environment. These facts suggest that the uptake of atrazine is not connected with the algal growth.

TABLE 2

Initial atrazine concentration mg L^{-1}	Final atrazine concentration mg L^{-1}	Per cent of initial concentration
0.25	0.012	4.7 +
0.50	0.012	2.3
2.5	0.064	2.6
5.0	0.20	4.0
10	0.69	6.9
15	0.83	5.6
25	4.4	18

+ The final atrazine concentration under the same conditions but without algae was always more than 85%.

TABLE 3

Cultivation time h	Final atrazine concentration ppb	Atrazine removal by algae %
0	25	90
24	84	67
48	93	63
72	89	64
96	16	94

Atrazine in an aqueous medium inhibits appreciably the growth of C. vulgaris in a batch culture in concentrations higher than 0.25 ppm. In concentrations of up to 2.5 ppm, the growth is restored after 6-7 days. Even under the conditions of growth inhibition the algae are capable of removing most atrazine present from the medium. The alga

C. vulgaris is potentially suitable for reducing residual atrazine in polluted water.

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