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Growth Response of Freshwater Algae to Continuous Flow of Terbutryn

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The single species approach, although advantageous in comparability of results, has its limitations. The most important one is that the standard test species may not be the dominant species in natural water environment, so the algal assay test result may be irrelevant to that particular in the environment. Furthermore, phytoplankton in the aquatic environment is a mixed and balanced community. Algal species composition and interactions in the community during algal assay are important parameters for observing the algal response to the environmental stress (Del Giorgio et al 1991).

Toxicant effects on aquatic microorganisms have been emphasized. Much of this research has dealt with phototrophic algae and cyanobacteria, which are the main primary producers in the hydrosphere (El-Dib et al 1989). A common contaminant of surface water of agricultural regions are triazine herbicides (Morris and Ron 1981). The volume of herbicide used and introduced into the aquatic environment as a final reservoir has grown markedly. Although research concerning the effects of herbicides on algae has been extensive, few studies have examined the impact on algal productivity. Long-term bioassay effects, using continuous system, is a relatively new approach in the screening of complex environmental compounds.

The purpose of this study was to determine the changes in both growth and population of fresh water algae under stress of continuous flow of terbutryn concentrations and the rate of accumulated terbutryn algal cells.

MATERIALS AND METHODS

A laboratory-scale unit (continuous flow system) was designed to study the effect of triazine herbicide terbutryn (2,4-diamine-N-dimethylethyl) N-ethyl-6(methylthio)-5 triazine) (99.9% purity) obtained from CIBA Geigy (Swizerland) on the algal growth under continuous flow rate.

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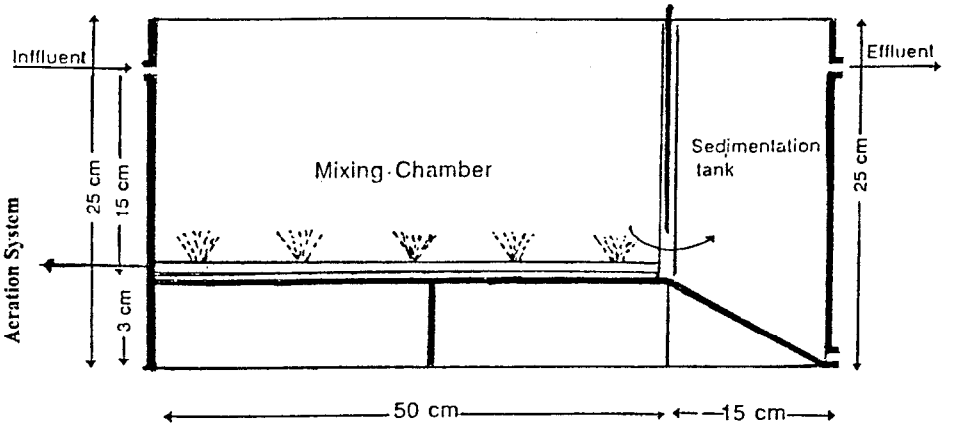

Figure 1. Schematic Diagram of the Test System

A schematic diagram is given in figure (1). The system is equipped with diffused aeration system to provide complete mixing and prevent clumbing the algal cells. The system was subjected to continuous fluorescent of light (≅ 1750 Lux) and operated at the room temperature 24 ± 2 °C. The process was started by seeding the pond with phytoplankton collected from the River Nile water after concentrated via phytoplankton net. Concentrated Nile water algae consisted mainly green algae, blue-green algae and diatoms. The system was fed continuously with media at constant hydraulic load of 0.67 $m^3.m^3.d^{-1}$ to give a total hydraulic detention time of two days. After reaching the steady state terbutryn is fed to the system.

Biomass production was monitored daily by determining chlorophylls (a, b and c) content using spectrophotometric method according to American Public Health Association (1992) and the changes in the community structure according to the key of the fresh water algae (Streble and Krauter 1978). Total protein content was measured according to Chapman and Pratt (1978). Total carbohydrate content was estimated as glucose using the spectrophotometric method described by Dubois et al (1956).

Accumulation of terbutryn in produced biomass was determined by using GLC. The algal samples were washed with distilled water (purified with hexane) three time to remove attached particles. The weight was determined after being dried at 60°C (24 h) and at 105°C (24 h). About 5 mg of dried phytoplankton was kinfe-homogenized in 10 ml acetone and 10 ml of hexane was added. The mixture was treated in an ultrasonic bath for 10 min. The hexane was separated with 50 ml NaCl solution (2%) and the remaining aliquote rinsed with 2x2 ml hexane. The hexane portions were combined, evaporated to 500-1000 µl and c1eaned up with sulphuric acid (Larsson 1987). Extracts were analyzed by GLC using a Perkin Elmer GC 8320 fitted with an electron capture detector (Ni 63), and stainless steel column was packed with 1.5 % ov - 17 + 1.95 ov - 210 coated on 80/100.

Cromosorb W. The column injector and detector temperature were 190,220 and 270 °C, respectively. Nitrogen as a carrier gas, was used at a flow rate of 30 ml/min. Samples were run in triplicate with blank. Calibration program was verified by measurement of one or more concentrations of each standard. Quality control of synthetic sample was analyzed with the samples. The relative standard deviation ranged between 10% to 10% of the mean.

RESULTS AND DISCUSSION.

Control, the system was operated without terbutryn addition. The system reached steady state after 46 days with an average Chl(a) content 18.68 mg/l. At this stage, a continuous feeding if 0.5 mg/l terbutryn took place. During the first 10 days of 0.5 mg/l terbutryn addition no clear variation in the biomass production was observed from that obtained in the control. Then slight decrease (Fig 2) in Chl(a) content was observed, representing 92% of control culture.

The increasing of terbutryn concentration to 1.0 mg/l led to a reduction in algal growth with an average Chl(a) content reached 10.5 mg/l which representing 56.2% of control. Similarly, at 2.0 mg/l terbutryn, another decrease in Chl(a) content was detected with an average value 7.34 mg/l. At 4.0 mg/l, the average Chl(a) content was 4.27 mg/l which represent 77% reduction compared with control.

Shehata et al (1995) found that, algal Chl(a) content in continuous flow system of Nile water algae, was reduced from 70% to 90% when the system was treated with 0.02 and 0.05 mg/l of gardoprim (triazine compound) than those obtained from control run. Respectively, Virmani et al (1975) and DeNoyelles et al (1982) found that, 0.5 mg/l a triazine often caused an almost complete inhibition of algal growth and photosynthesis of fresh water algae. El-Dib et al (1989) recorded that, 0.2 mg/l ardoprim inhibited the growth of fresh water alga *scenedesmus quadrcaudia*. Shehata et al (1993) found that, increasing of gardoprim concentration to 0.5 and 1.0 mg/l increased the toxicity effect to different algal species. Osama et al (1984) concluded that, bladex in the concentration of 0.04 mg/l accelerated the inhibitory effect of *Scenedesmus quadrcaudia* cells which reached a maximum within three days exposure in continuous flow system.

The treated algal cells were allowed to recovery. The community of algal cells restored their vitality after one day of inoculation of treated cell in terbutryn herbicide free media. Cell recovery indicated by the gradual increase in Chl(a) content, and maximum growth was attained after 10 days. Thereafter, a gradual decrease in Chl(a) content was recovered by the end of the experiment. The average growth of the recovered cells was 8.9 mg/l which was 52%, still lower than that obtained in the control. El-Dib et al (1989) found that in cultures treated with gardoprim, gesapex and terbutryn, Chl(a) content of the recovered cells of *Scenedesmus* either approached that of the control or exceeded it but the maximum growth rate was always less than that of control even after 10 days of inoculation of treated cell in herbicide free media. Hence the response of algal cell in streams

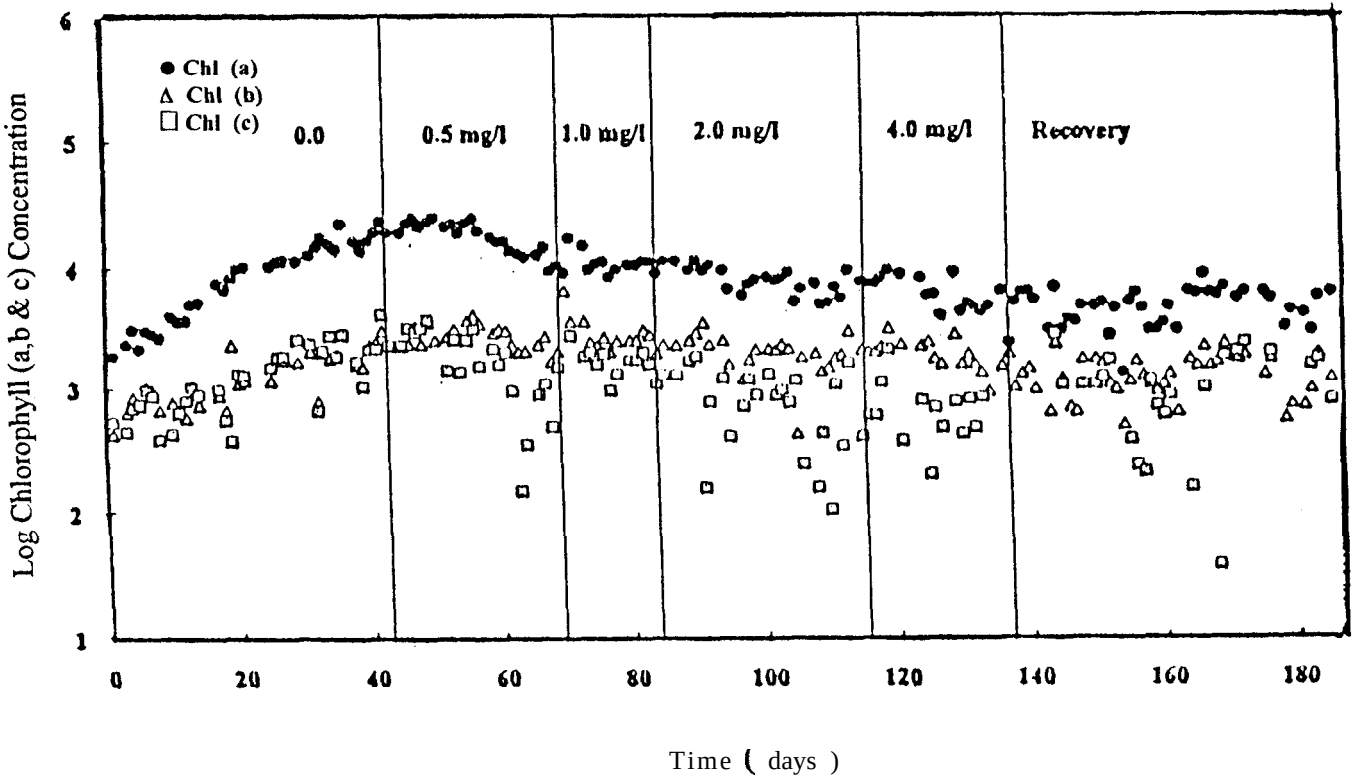

Figure 2. Effect of continuous flow o terbutryn on algal growth.

receiving triazines will depend on the effective concentration and the cells are able to recover as time and dilution factor proceed.

Terbutryn was found to affect the ratio of Chl(a)/Chl(b)/Chl(c) of Nile water algae. In general, Chl(a) dominate over chl(b) and &chlC). The ratio of chlorophylls (a, b and c) was changed to 6.03 : 0.97 : 1, 8.86 : 1.63 : 1, 9.96 : 2.74 : 1, 10.3 : 2.96 : 1 and 7.7 : 2.05 : 1 when the system was exposed to 0.5, 1.0,2.0 and 4.0 mg/l terbutryn herbicide respectively (Fig. 3). El-Dib et al (1989) state that at high concentration levels of 0.5,1.0 and 0.2 mg/l gardoprim, the ratio (a/b) of *Scenedesmus* cell decline to 1.77, 1.44 and 1.12 respectively. This trend is in agreement with that reported by Tonecki (1975) for the effect of herbicides on higher plants and by Saroja and Bose (1983) for algae.

Table 1. Effect of continuous flow terbutryn on Chlorophyll(a), protein and carbohydrate content.

	Average		
Conc. of terbutryn mg/l	Chlorophyll (a) mg/l	*Protein mg/l	*Carbohydrate mg/l
0.0	18.68	247.2	2.8
0.5	17.23	250.9	2.8
1.0	10.52	247.8	2.63
2.0	7.43	232.7	2.61
4.0	4.72	231.8	2.02
Recovery	8.9	248.7	2.42

* Average of 5 value for each run.

Good Relationship between Chl(a), carbohydrate and protein content were observed at different terbutryn levels. In lower terbutryn concentration, no clear variation in total carbohydrate synthesis in algal cell with respect to control run (Table 1). The value of carbohydrate synthesis which matched with depression in Chl(a) synthesis at higher concentration of terbutryn may be attributed to a respective inhibition of photosynthsis. These observation are in harmony with the findings of Shabana and Abou-waly (1995) and Jones and Winchell (1984). According to hypothesis by Devlin et al (1983) an trazine blocked photosynthesis electron transport might explain the significant reduction in total carbohydrate accumulation with any further increase in herbicide concentration.

Protein nitrogen content attained the same pattern of observation as carbohydrate content (Table 1). So one may concluded that terbutryn affected the physiology and biochemical events which favor more utilization of carbohydrate for nitrate reduction and synthesis of amino acids and protein.

Microscopic examination showed that during the control run, no clear variation in the algal community structure than that obtained in the initial algal community. High diversity of green algae was observed and the most dominant species were

Pediastrum simplex, Coelastrum microporum, Scenedesmus quadrcaudia and *Mougeotia scalans.*

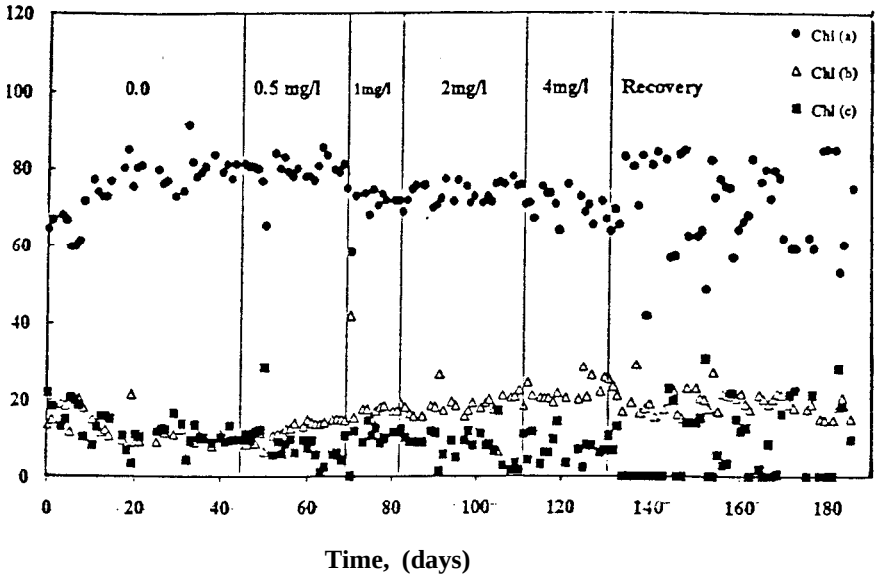

Figure 3. Percentage of chlorophyll a, b & c of total chlorophylls content.

Of blue-green algae, *Microcystis aeruginosa, Merismopedia glauca, Oscillatoria formosa* and *Oscillatoria mougeotii* were the most dominant species. A good diversity of diatoms was observed and the dominant species were *Diatoma elongatum, synedra ulna* and *Melosira granulata* (Table 2).

After adding terbutryn, changes in the diversity and redundancy of algal population took place (Table 2). The more sensitive algal species disappeared completely, while the tolerant form resisted the condition and increased its numbers. At different concentration of terbutryn *Mougeotia scalans* (Green algae), *Oscillatoria formosa, Oscillaton'a mougeotii* and Lyngbya *limnetica* (blue-green algae) were the most dominant species. However, gross pollution causes a great reduction in number and kinds of algae (Hooper 1969 and Mitchell and Buzzell 1971).
When the tolerant algal species were subjected to recovery from terbutryn stress, no substantial changes in the algal taxa was observed. Changes in growth rate and/or algal counts tend to be more sensitive parameters for the evaluation of the inhibitory effect of pesticides on algae (Nyholm 1985).

Terbutryn accumulated in settled algae collected at the end of each run were 1.0042,1.953, 2.004 and 5.514 mg /gm dry weight for concentrations 0.5, 1.0,2.0 and 4.0 mg/l terbutryn, respectively, The results showed that there was a relation between the length of exposure time, terbutryn concentration and its accumulation in algal cells.

Table 2. Change in algal community structure with terbutryn concentration.

Algae Taxa	Initial	Control	Concentration (mg/l)				Recovery
			0.5	1.0	2.0	4.0	
Green Algae							
Coelastrum microporum.	+	3+	+	+	±	±	+
Pediastrum simplex.	2+	2+	+	+	±	±	+
Pediastrum clathratum	+	+	±	±	±	±	+
Pediastrum duplex	±	+	±				
Ankistrodesmus falcatus.	+	+	±				
Dictyosphaerium ehrenberglanum.	+	+	±				
Oocystis pusilla.	+	+	±	±	±	±	2+
Scenedesmus qu adricauda.	+	3+	2+	±	±	±	+
Staurastrum paradoxum.	+	+	±				
Mougeotia scalaris.	+	4+	4+	4+	2+	+	3+
Spirogyra communis.	±	+	±				
Blue-green Algae.							
Oscillatoria formosa.	+	2+	4+	4+	4+	4+	4+
Oscillatoria chlorina	+	+	±				
Oscillatoria mougeotii	±	±	+	+	+	+	+
Merismopedia glauca.	+	2+	±				
Microcystis aeruginosa.	2+	2+	±	+	+	+	+
Anabaena constricta.	±	+	+	±	±	±	±
Lyngbya limnetica.	±	+	+	2+	2+	2+	2+
Diatoms.							
Diatoma elongatum.	4+	4+	+	±	±	±	±
Synedra ulna.	4+	4+	+	±	±	±	±
Melosira granulata	2+	3+	+	±	±	±	±

4+ Dominant 3+ Plenty 2+ Many + Apreciable no. ± Little

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