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Effect of Triazine Compounds on Freshwater Algae

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The continued input of pesticides into the aquatic environment possess a potential threat to the aquatic ecosystem by their direct action on aquatic flora. The killing of the algae in the aquatic habitat polluted with pesticides leads to disturbances in the primary food chain and ultimately to the ecosystem (Palmer 1980). Pollution of water with several classes of pesticides and PCBs will upset the natural balance among algal species as well as other microorganisms found in water bodies (Canter and Lund 1968; Brooker and Edwards 1973). Such effects will lead to subsequent changes in water quality and its possible uses.

Although the effect of some pesticides on certain microorganisms has been reported, still this aspect needs much more attention. Algae are commonly used for assessing the water quality of diverse habitats (Cain et al. 1979). Therefore, the main objective of this study was to assess the effect of triazine compounds on the Nile water algae as well as on changes in diversity and redundancy of existing species.

MATERIAL AND METHODS

The common and chemical names of the studied triazines are given in Table 1. The compounds were selected to represent various chemical structures within a chemically related group of organic herbicides. Gardoprim and gesapax (99.9% purity) were kindly supplied by Ciba-Geigy, Switzerland; standard solutions were prepared in methanol. Extraction of the triazines from the aqueous media was run by 15% methylene chloride in hexane. Recovery of gardoprim and gesapax from the aqueous media amounted to 95%. Residues of triazines were identified and determined using GC equipped with electron capture detector (Ni_{63}), glass column with an internal diameter 1/8 in and 6 ft length packed with 1.5% OV 17 + 1.95 OV 210 coated on 80/100 chromosorb W. Temperatures of column, injector and detector were 190°C, 220°C and 250°C respectively. Nitrogen was the carrier gas (30 mL/min), (EPA, 1974).

Samples were collected from the Nile River water using a grab

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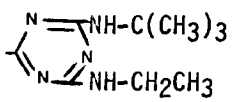
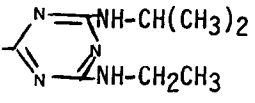
Table 1. Common and chemical names of studied triazines.

Common name	Chemical name	Chemical structure
Gardoprim	2,4-diamine, N-(1,1-dimethyl-ethyl)-N'-ethyl, 6-chloro-S-triazine	
Gesapax	2,4-diamine, N-(1-methyl-ethyl)-N'-ethyl-6(methylthio)-S-triazine.	

sampler. The algae were concentrated via the Sedgwick-Rafter method (APHA 1965) to form the material to be used as inoculum for bioassay tests. The bulk represented green algae, blue-green algae and diatoms. The Algal Assay Procedure Bottle Test (APHA 1989) was used and performed under controlled conditions using changes in chlorophyll "a" content, algal counts, diversity, and redundancy of algal species as indication of triazine herbicides effect. Bioassay flasks were incubated at 24 $\pm$ 2°C under continuous white fluorescent light 2500 ft C. (foot candle). Flasks were shaken once per day to prevent clumping of the cells. Different concentrations of the tested herbicides were added to each algal culture. From each flasks a known quantity of medium was filtered through a 0.45-µm membrane filter to determine chlorophyll "a". Chlorophyll "a" (Chl."a") was extracted with 90% acetone and measured spectrophotometrically at 665 nm, 645 nm and 630 nm. Chl."a", growth rate and algal counts were calculated according to APHA (1989). Experiments were run in triplicate for 10 d to follow the changes induced by the applied herbicides.

Two community composition parameters namely, diversity (H') and redundancy index (R) were estimated according to Shannon's equations, (1948). Diversity was estimated using Shannon's equation:

$$H' = - \sum_{i=1}^{S} (n_i / N) \log_e (n_i / N)$$

where:
S = number of taxa samples
n_i = number of individuals of i-th taxon.
N = sample size.

In addition, redundancy (or dominancy) index (R) was estimated for each group using the following equation:

$$R = \frac{H' (max/S) - H'}{H' (max/S) - H' (min/S)}$$

where:
H' (max/S) and (min/S) are maximum and minimum values of H' at given S.

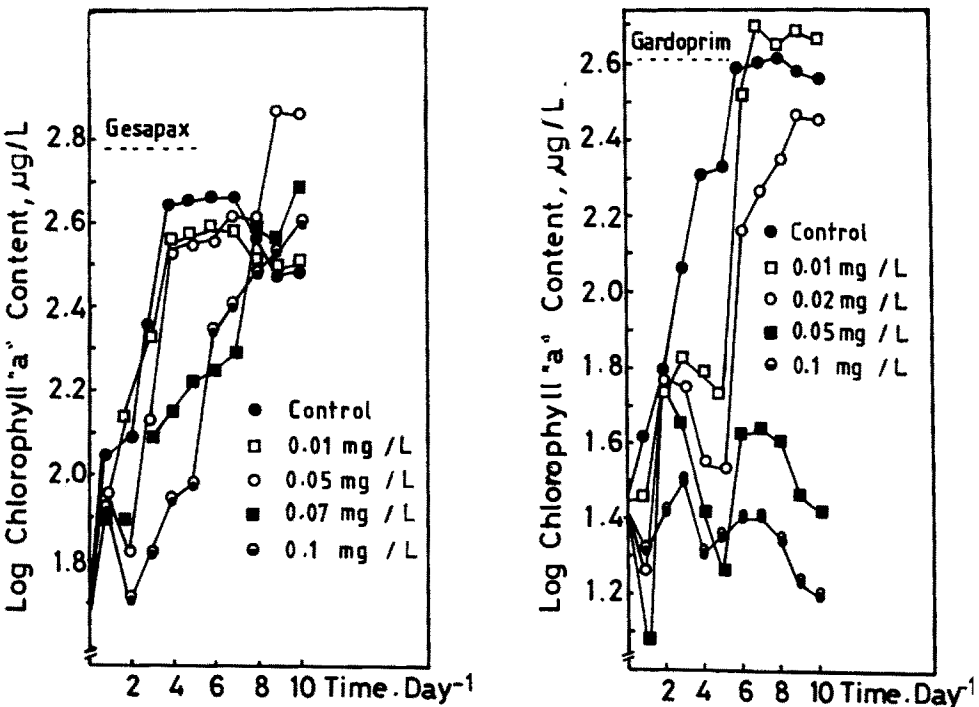


Figure 1. Effect of triazen compounds on algal growth

RESULTS AND DISCUSSION

The response of freshwater algae to different concentrations of gardoprim, namely, 0.01, 0.02, 0.05 and 0.1 mg/L are presented in Figure 1. Irregular pattern in Chl."a" content was detected in case of 0.01 and 0.02 mg/L treatments up to the 5th d of incubation. Thereafter the resistant species were able to exhibit visible growth (Table 2). Increasing gardoprim concentration to 0.05 and 1.0 mg/L in the media increased the toxicity effect levels to different algal species. Gesapax concentrations (0.01, 0.05, 0.07 and 0.1 mg/L) had no harmful effect on Nile water algae. Only some inconsistency in algal growth took place at the beginning of the run followed by active growth (Figure 1). This is due to changes in algal community structure.

Values of specific growth rate (Table 2) showed positive relation with gardoprim concentration. The maximum specific growth rate decreased at 0.05 and 0.1 mg/L gardoprim, indicating the inhibitory effect of those two doses. However, the subcultures from both two concentrations secured high biomass and the maximum algal growth was 1.5 mg/L and 1.4 mg/L Chl."a" for 0.05 and 0.1 mg/L gardoprim respectivley. In addition, the recovered cells increased in numbers with high growth rate which reached 0.72 and 0.63 d^{-1} for 0.05 and 0.1 mg/L, respectively. In case of gesapax, no clear variation was detected in algal growth rate between nontreated and treated culture with different gesapax concentration (Table 2). In general, the inhibitory effect of the studied herbicides was in the following order: gardoprim > gesapax.

Table 2. Growth parameters of Nile River water algae at different doses of herbicides.

Growth parameters / Treatment mg/L	Maximum growth rate "μ" d^{-1}	Chl."a" at maximum growth	% Chl."a" of control
	Gardoprim		
0.00	0.805	0.43	100.0
0.01	1.817	0.59	118.0
0.02	1.450	0.29	68.6
0.05	-	0.05	12.7
0.10	0.253	0.03	7.5
	Gesapax		
0.00	0.943	0.46	100.00
0.01	0.713	0.38	82.60
0.05	0.920	0.74	160.87
0.07	0.620	0.49	106.52
0.10	0.828	0.40	86.96

Virmani et al. (1975) and DeNoyelles et al. (1982) found that 0.5 mg/L atrazine often caused an almost complete inhibition of algal growth and photosynthesis of freshwater algae. El-Dib et al. (1989) recorded that 0.2 mg/L gardoprim inhibited the growth of freshwater algae Scenedesmus, while the inhibitory of gesapax was 0.05 mg/L.

Clear changes in the distribution pattern of the three algal groups took place in the presence of triazine compounds (Figures 2 and 3).

In reviewing the distribution of the three algal groups of control and in the different gardoprim concentrations (Figure 3), a considerable increase in green algal counts was observed in the control. In presence of 0.01 mg/L gardoprim, a slight decline in green algal count was recorded. Further increase of gardoprim concentration, led to decline in total green algal counts especially at 0.05 and 0.1 mg/L gardoprim. The most dominant genera were Scenedesmus sp. and Eudorina sp. With regard to the blue-green algae, the results indicated that with increase of gardoprim concentration, the total blue-green algae counts decreased with reference to control. Thus, it may be concluded that different doses of gardoprim suppresses the growth of blue-green algae. However, the most tolerant species to different doses of gardoprim was Oscillatoria. Considerable increase in diatoms group was detected in presence of 0.01 mg/L gardoprim compared with the control. Marked decline in diatoms numbers was observed as the concentration of gardoprim increased. However, the number of diatoms in the presence of 0.1 mg/L gardoprim were approaching that of control. This indicated that low concentration of gardoprim promoted the diatoms growth. The most dominant genera were

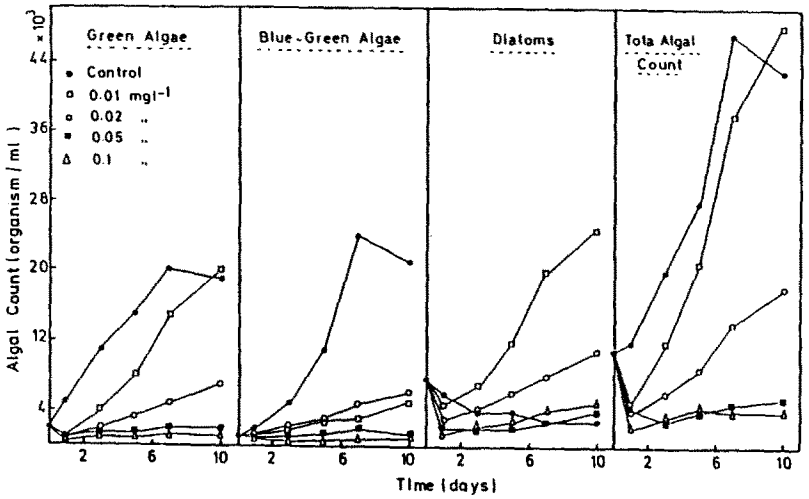


Figure 2. Effect of gardoprim treatments on algal count

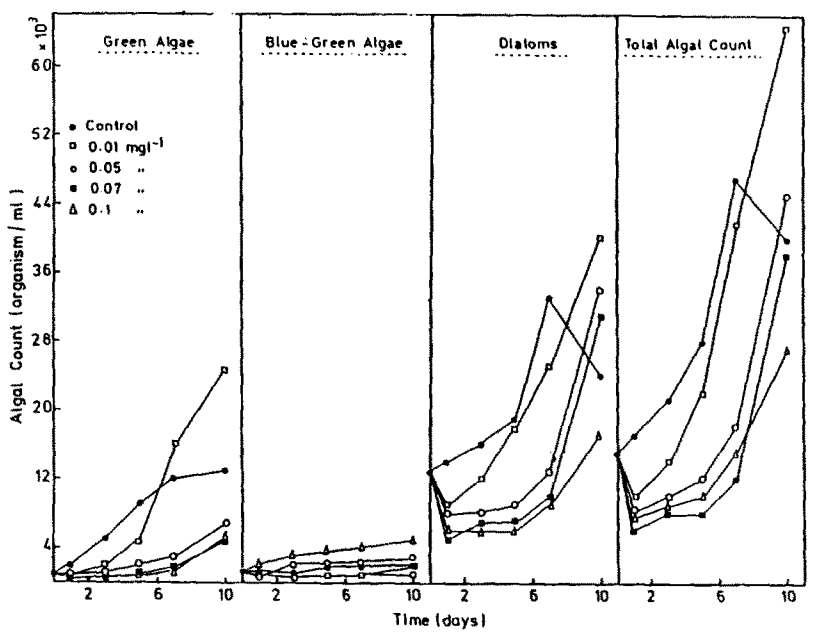


Figure 3. Effect of gesapax treatments on algal count

Melosira, Cyclotella, Navicula and Nitzschia. Generally, the total algal counts declined as the gardoprim concentration increased in media (Figure 2).

Distribution of the three algal groups in the control culture and different treated cultures with gesapax are illustrated in Figure 3. Great increase in green algal count was observed in culture media treated with 0.01 mg/L gesapax. Treatment with 0.05, 0.07 and 0.1 mg/L led to a marked decline in total green algal counts. Scenedesmus, Eudorina, Pediastrum and Ankistrodesmus were the most dominant genera. Blue-green algal counts reached its maximum growth at 0.1 mg/L gesapax (Figure 3). The most

dominant species were Oscillatoria, Cylindrospermum and Agmenellum. Also, maximum diatom counts were recorded in culture medium treated with 0.01 mg/L gesapax, followed by 0.05 mg/L and 0.07 mg/L treatment. Melosira, Cyclotella, Bacillaria and Synedra were the most dominant genera. In general, culture treated with 0.01 mg/L recorded the maximum total algal counts. Other gesapax concentrations decrease the total algal counts if compared with control.

Atrazine at a level of 0.1 mg/L usually caused > 90% inhibition of the green algal growth (Virmani 1975). Blue-green algae are more resistant to this herbicide (Rohwer and Fluckiger 1979). Atrazine is significantly more toxic than its degradation products when tested towards algae and cyanobacteria (Stratton 1984).

As shown in Table 3 diversity and redundancy values reflected the changes of algal community based on genera numbers in the presence of different herbicides. Green algae exhibited a high diversity at all investigated herbicide concentrations and ranged from 1.03 to 1.97. Also, a high diversity of diatoms was recorded in case of gardoprim and ranged from 0.77 to 1.36. In contrast diatoms exhibited a high redundancy in case of gesapax due to the presence of Melosira with high number. Also, blue-green algae

Table 3. Changes in algal community properties at various levels of Herbicides.

	Gardoprim concentrations									
Algal	0.00 mg/L		0.01 mg/L		0.02 mg/L		0.05 mg/L		0.1 mg/L	
group	H	R	H	R	H	R	H	R	H	R
Green	1.2	0.0	1.6	0.0	1.5	0.0	1.5	0.0	1.0	0.0
Blue-green	0.3	1.0	0.8	0.9	0.4	1.0	0.0	1.0	0.3	1.0
Diatoms	1.1	0.0	0.8	1.0	1.4	0.1	1.1	0.0	0.9	0.2
	Gesapax concentrations									
Algal	0.00 mg/L		0.01 mg/L		0.05 mg/L		0.07 mg/L		0.1 mg/L	
group	H	R	H	R	H	R	H	R	H	R
Green	2.0	0.0	1.8	0.0	1.5	0.0	1.5	0.0	1.7	0.0
Blue-green	1.2	0.6	1.0	0.6	0.8	1.0	0.9	1.0	1.5	0.2
Diatoms	0.6	1.0	0.5	1.0	0.8	1.0	1.0	0.8	0.8	1.0

H = Diversity within the group. R = Redundancy (dominancy).

exhibited high redundancy in case of gardoprim, due to the dominance of Oscillatoria, while high diversity was recorded in case of gesapax and ranged between 0.82-1.46 (Table 3).

Mitchell and Buzzel (1971) has pointed out that oligotrophic waters are characterized by a diversity range from 0.7 to 1.0,

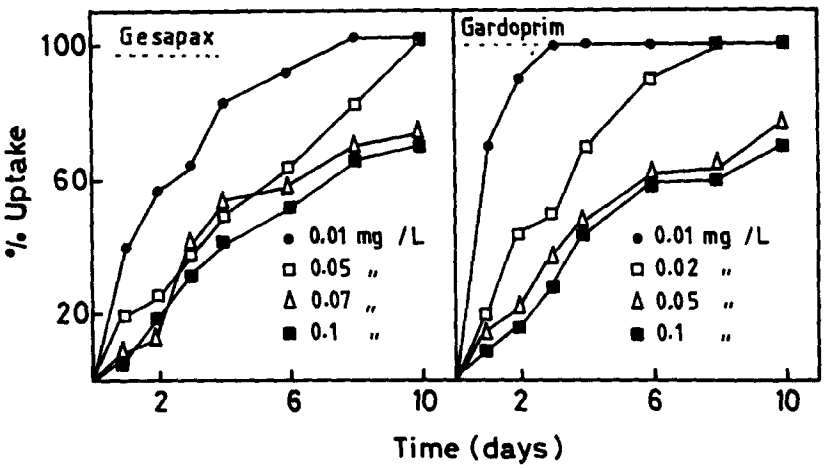


Figure 4. Percentage uptake of triazine by Nile water algae

while 0.3 or less is found in eutrophic water. Nelson et al. (1976) stated that a high diversity of algal population indicated high water quality, or in other words, higher diversity was interpreted to imply lower toxicity.

From these results it can be shown that the inhibition of algae, diversity of genera, and their recovery varied according to the type of herbicide, its concentration, contact time and prevailing organisms. The results of such interactions agree with the outcome of several investigations (Hewss 1972; Bakalivanov & Biochev 1979; and Gaziev 1979).

Freshwater algae showed variable ability to accumulate the studied compounds (Figure 4). In case of gardoprim, uptake level amounted to 100% after 2 d algal exposure to 0.01 mg/L gardoprim, whereas such ratio of uptake was reached after 8 d in case of the 0.02 mg/L gardoprim. By increasing the concentration of gardoprim to 0.05 and 0.1 mg/L, percentage uptake was reduced to 74% and 71% by the end of experiment which reflects the inhibitory effects of such concentrations on the growth of freshwater algae and total algal counts if compared with control.

Percentage uptake of gesapax by Nile water algae attained its maximum value (100%) in case of 0.01 and 0.05 mg/L gesapax. As the concentration of gesapax increase to 0.07 and 0.1 mg/L the percentage uptake decreased to 73% and 72%, respectively.

The results of Chl."a" content were subjected to regression coefficient analysis (Ezakel and Fox 1957) in order to detect variations in algal growth at different triazine compounds. The results obtained yielded high positive degree of correlation between Chl."a" and gardoprim concentrations ($r = -0.88$). Gesapax exhibited moderate degree of correlation ($r = -0.68$). However, a linear relationship was observed between Chl."a" content and triazine concentrations.

REFERENCES

APHA (1989) Standard Methods for the Examination of Water and Wastewater 17th ed, Washington

APHA (1965) Standard Methods for the Examination of Water, Sewage and Industrial Wastes 11th ed, NY

Bakalivanov D, Boichev A (1979) The detoxification of the herbicide dalapon in a chernozemsmalinitsa and its influence on the soil microflora. Pochvozanie Agrokhimiya 14:98-103

Brooker MP, Edwards RW (1973) Effects of the herbicide paraquat on the ecology of a reservoir. III Community metabolism. Fresh Wat Biol 3:383-389

Cain JR, Klotz RL, Trainer FR, Castello R (1979) Algal assay and chemical analysis. A comparative study of water quality assessment techniques in a polluted river. Environ Pollut 19:215-224

Canter HM, Lund JWG (1968) The importance of protozoa in controlling the abundance of planktonic algae in lakes. Proc Linn Soc Lond 179:203-219

DeNoyelles F, Kettle WD, Sinn DE (1982) The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63:1285-1293

El-Dib MA, Shehata SA, Abou-Waly HF (1989) Response of freshwater alga Scenedesmus to triazine herbicide. Water, Air & Soil Pollut 48:306-316

Environmental Protection Agency, USA (1974) Pesticide residue analysis in water. Method No. 430/1, 74-012 Washington, DC

Ezakel M, Fox K (1957) Correlation and Regression Analysis. John Wiley & Sons, New York

Gaziev MT (1979) The influence of herbicides on soil microflora in Vineyards. Khimiya Sel's Kom Khozyaistve 27:61-63

Heuss K (1972) Effects of some herbicides on limnic plankton communities. Schriftenreihe des vereins fur Wasser Boden-und Lufthygiene 37:221-229

Mitchell D, Buzzel JC (1971) Estimating eutrophic potential of pollutants. J Sanit Eng Div 97 (No SA4): 453-465

Nelson JH, Stoneburner DL, Evans ES, Pennington JR, Meisch MV (1976) Diatoms diversity as a function of insecticidal treatment with a controlled-release formulation of chlorpyrifos. Bull Environ Contam Toxicol 15:630-634

Palmer CM (1980) Algae and Water Pollution. Castle House Publications Ltd England

Rohwer F, Fluckiger W (1979) Effect of atrazine on growth, nitrogen fixation and photosynthetic rate of Anabaena cylindrica. Angew Botanik 53:59

Shannon CE (1948) A mathematical theory of communication. Bell Syst Technol J 27:379-423; 623-656

Virmani M, Evans JO, Lynn RI (1975) Preliminary studies of the effects of S-triazine, Carbonate, urea and karbutilate herbicides on growth of freshwater algae. Chemosphere 4:65

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