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EFFECT OF GLYPHOSAT ON ALGAL GROWTH AND PHYTOPLANKTONIC DIVERSITY

Hoda F. Abou-waly

water pollution Control Department. National Research

Center Dokki. Cairo, Egypt

ABSTRACT

The effect of glyphosate on the growth of fresh water algae namely, Anabaena flos-aquae and Seleastum capricornutum was investigated. The impact of this herbicide on the diversity of Nile water phytoplankton was also assessed. The herbicide was applied at various concentrations and the impact of treatments was measured in terms Chl (a) content. Glyphostae at a concentration exceeding 1.0 mg/L resulted in decrease in Chl (a) content. the tolerance of algae to glyphosate was in the order: A. flos- aquae > S. Capriconutum > Nile water algae. Glyphosate exhibited a drastic effect on the diversity and natural balance between phytoplankton population of Nile river water where diatomes represented the most tolerant group.

INTODUCTION

Glyphosate is used for its broad effectiveness as a nonselective , post - emergent herbicide recognised for use in the control of grasses , sedges and broad - leaved weeds (WSSA , 1983) . The extensive use of glyphosate and the subsequent exposure of aquatic communities may pose a serious thread to algal growth and population diversity . The significance of phytoplankton as primary producers as well as their ability to alter the balance of aquatic ecosystem has warranted greater concern for the toxic effects of this widely accepted herbicide (Sullivan et al 1981, Holtby and Bailie 1989).

Several studies refferred to the toxicological effects of glyphosate on aquatic plants grown in laboratory culture but few studies involving natural in situ plant communities (Hartman and Martin 1985). Glyphosate concentration at 89 and 890 mg/L suppresed photosynthetic rates of short term lentic preiphyton (Goldsborangh and Brown, 1988).

The objectives of this study were to determine the effects of glyphosate on the growth of two widely distributed species of freshwater algae and to ascertain the effects of this herbicide on the diversity of Nile water phytoplankton assemblage.

MATERIAL AND METHODS

Cultures

A septic culture of Anabaena flos-aquae (blue-green alga) and Selenastrum capricornutum (green alga) were obtained from University of Texas at Austin Culture Collection and grown in an aqueous medium (American Public Health Association, APHA 1985). Aliquots of cultures were transferred weekly to maintain algal growth in the log phase.

Chemical

A glyphosate (99.9% purity) were obtained from Chem. Service, Inc. (West Chester, Pennsylvania 19381), and dissolved in distilled water. The range of concentrations was determind with preliminary range-finding bioassays.

Algal Bioassay

Erlenmeyer 1-L bioassay flasks were filled with 500ml algal suspension media, and grown for a period of 10 days. All cultures were exposed to a continuous light source from fluorescent tubes with an intensity of ~ 1800 Lux for A. flos - aquae and ~ 2800 Lux for S. capricornutum. The flasks were covered with aluminum foil with small holes for gas exchange and were incubated at 24+2°C. Flasks were shaken daily to prevent clumping of the cells. The cencentration levels were 0.1, 0.5, 1.0, 1.6, 2.4, 3.2 and 3.7 in case of S. capricornutum while in case of A. flos-aquae were 0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0 mg/L. Algal growth as chorophyll (a) concentration was measured spectrophotometrically at different growth stages. For chl (a) measurement, a known volume of algal samples was filtered through a 0.45 u membrane filter and extracted with hot methanol (Sartory and Grobbelaar 1984). The chl (a) cncentration and growth rate (u) of the algae grown in each concentration, as well as the controls, were determined according to (APHA 1985). Each test alga was maintained in triplicate.

In order to study the effect of glyphosate on Nile water phytoplankton assemblage, water samples were collected from the Nile river and concentrated via the Sedgwick - Rafter method to form the material used as inoculum for study. The concentrate represented green algae, blue - green algae and diatoms. The Algal Assay procedure Bottle test (APHA,1989) was used and performed under controlled conditions. Triplicate concentrations of 0.05, 0.1, 0.15, 0.2, 0.5, 1.0, 1.6 and 2.4 mg/L of glyphosate as well as three control flasks with no herbicide were maintained under the previously stated conditions for a period of 10 days. At maximum growth, phytoplankton were counted. The cells were identified according to a key of fresh water algae in Palmer (1980). The phytoplankton community composition parameters of the control and treated cultures were measured using Shannon's equation (1948)

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

where, S is number of taxa samples and ni is the number of individuals of ith taxon

Results

The response with time of S. capricornutum, A. flos - aquae and Nile water algae to ranges of glyphosate, measured in terms of Chl (a) concentration, is represented in Figure (1). On the faith of the preliminary experiment the concentration of glyphosate less than 0.05 mg/L is excluded from subsequent studies.

It seems reasonable to infer that glyphosate concentrations less than 0.1 had no effect on short term algal growth. After one day of exposure, a sharp decrease in Chl (a) content was recorded as a result of increase of glyphosate concentrations. In all treated cultures, algal cells started to grow with high growth rates after the 3rd day of experiment and recorded its highest values by the end of the run

In case of A. flos - aquae, 1.0 mg/L glyphosate caused 10% reduction in algal growth compared to control.. The same reduction value was attained by applying

Table 1 - Changes in algal properties as a result of glyphosate treatments

Conc mg/L	Maximum growth rate u/day	Max Chl (a) content ug/L
	A. flos - aquae	
0.00	0.64	1098.3
0.5	0.56	1032.4
1.0	0.61	988.5
2.0	0.43	823.7
2.5	0.32	549.1
3.0	0.31	439.3
4.0	0.24	362.4
5.0	0.07	107.8
	S. capricornutum	
0.00	0.72	1436.4
0.1	0.62	1378.56
0.5	0.55	1292.8
1.0	0.5	1149.1
1.6	0.36	718.2
2.4	0.32	646.4
3.2	0.22	474.0
3.7	0.11	186.73
	Nile water Algae	
0.00	0.79	480.4
0.05	0.69	446.8
0.1	0.60	432.36
0.15	0.50	389.1
0.2	0.46	336.28
0.5	0.38	278.6
1.0	0.34	244.6
1.6	0.32	182.6

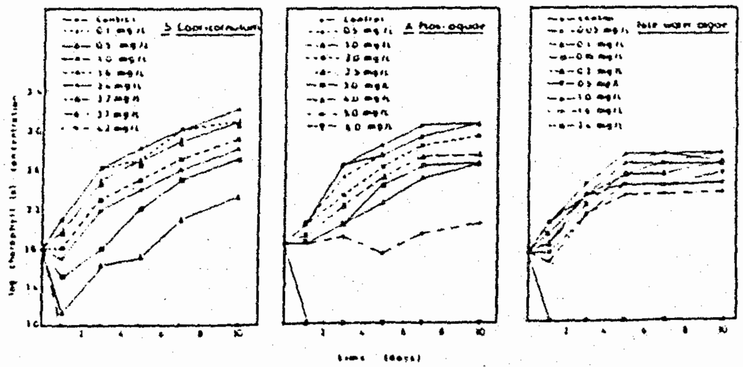

Fig 1 Response of algal growth to glyphosate.

glyphosate at 0.5 and 0.1 mg/L in case of S. capricornutum and Nile water phytoplankton respectively (Table 1)

In presence of 5.0 mg/L glyphosate, 90% reduction in Chl (a) content of A. flos-aquae culture was observed while in case of S. capricornutum, glyphosate at 3.7 mg/L caused 87% reduction in algal Chl(a) content. Complete inhibition in Nile water algae was observed at 2.4 mg/L up to the end of experiment. Generally, the tolerance of algae to glyphosate was in the following order:

A. flos-aquae > S. capricornutum > Nile water algae .

Table 2. EC_{50} values of Glyphosate measured at various time intervals

Algal species	Calculated EC_{50} (mg/L) after different days of incubation			
	3 d	5 d	7 d	10 d
S. capricornutum	1.89	1.92	2.04	2.15
A. flos - aquae	2.3	2.75	2.9	2.9
Nile water algae	1.3	1.14	1.08	1.53

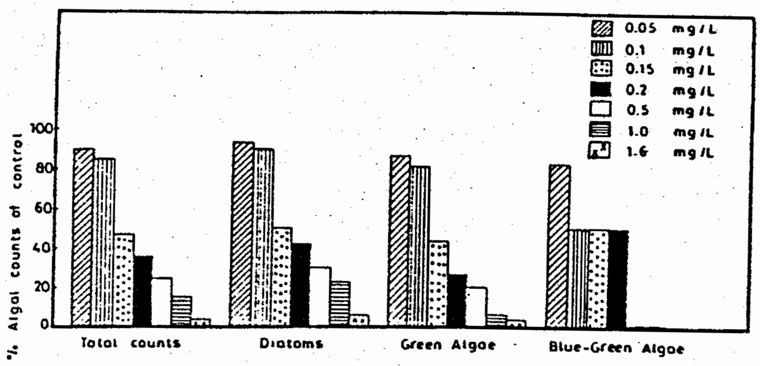

Fig. 2. Changes in algal counts as a result of glyphosate treatment.

The expected concentration of glyphosate that reduce algal growth by 50% (EC_{50}) compared to the untreated culture are presented in Table (2). A part of some minor fluctuation the value of EC_{50} increases as the contact time of glyphosate with algae increased.

All glyphosate coencentrations reduced the total algal numbers. The reduction ranged between 10 & 98 % of control. In case of diatoms the reduction ranged between 7 & 92 % of control. High reduction in green algal counts took place especially at high concentrations. In addition, a pronounced decline in the blue-green algal count was detected even disappeared completly at high glyphosate concentrations (Fig.2).

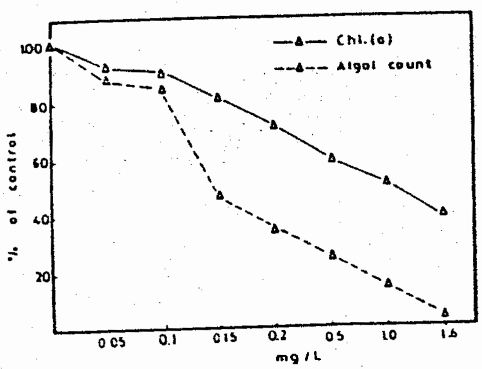

Fig. 3. Relation between total algal count and chlorophyll (a) concentration.

Table 3 Phytoplanktonic Diversity of Culture Exposed to Glyphosate at Maximum Growth

Genus	Glyphosate Conentraion (mg/L)							
	0.0	0.05	0.1	0.15	0.2	0.5	1.0	1.6
Green Algae								
Scenedesmus	40*	39	37	20	13	10	3	1
Eudorina	33	29	26	14	8	5	3	2
Oocystis	10	8	8	2	2	1	0	0
Ankistrodesmus	7	4	4	2	1	1	0	0
Selenastrum	2	1	1	0	0	0	0	0
Pediastrum	2	1	1	1	1	1	0	0
Microactinum	0	0	0	0	1	1	0	0
Closterium	1	0	0	1	0	0	0	0
Botryococcus	1	1	1	1	0	0	0	0
Diversity	1.4	1.3	1.3	1.3	1.3	1.3	0.7	0.6
Blue-green algae								
Microcystis	2	2	1	1	1	0	0	0
Merismopedia	1	1	1	0	0	0	0	0
Coelosphaerium	1	0	0	0	0	0	0	0
Oscillatoria	1	1	1	1	1	0	0	0
Anabaena	1	1	0	1	1	0	0	0
Diversity	1.6	1.3	1.1	1.1	1.0	0.0	0.0	0.0
Diatoms								
Melosira	65	60	60	40	35	24	19	1
Diatoma	54	50	47	19	15	11	8	6
Fragilaria	1	1	1	0	0	0	0	0
Cyclotella	2	2	1	1	1	2	2	0
Synedra	1	1	1	1	1	1	0	0
Asterionella	0	1	1	0	0	0	0	0
Cymbella	1	1	1	1	1	0	0	0
Surirella	1	0	0	0	0	0	0	0
Navicula	1	1	1	1	0	0	0	0
Diversity	0.96	0.98	0.95	0.91	0.86	0.89	0.82	0.41
Total Genera	21	19	18	16	14	10	5	4

* Each value Represents organism Number x 104 and Indicates a Mean of three Replicates.

Figure (3) showed that total algal counts and Chl(a) content were positively correlated. The reduction in total algal count at maximum growth was relatively high compared to the reduction in Chl (a) content, this might be due to the changes in community structure of phytoplankton.

Results of the diversity study illustrate the potential for profound impact of gly phosate on phytoplankton population dynamics. The results specifically show an inverse relationship between glyphosate concentration and species diversity (Table 3).

Clear changes in the distribution pattern of the three major algal groups were detected at different glyphosate concentrations (Table 3). Diatoms represented the most tolerant group, Melosira and Diatoma were the most dominant genera. Scenedesmus and Eudorina were the most dominant genera of green algae. High glyphosate concentrations caused drastic effect in the blue-green algae.

Discussion

In this study, the sensitivity of algae to glyphosate is based on three parameters namely, algal counts, growth rate, maximum Chl (a) content and EC_{50}. This approach allows to ascertin that variations in susceptibilty depends on the organism type and the concentration of the compound. It also permits to study the diversity between organisms in the aquatic system.

It is evident from the present study that the effect of glyphosate is greatly differ from one species to another indicating that it depends on the concentration of the herbicide and algal species. This inagreement with that reported by Abou-waly et al (1991).

At higher concentration of glyphosate, algal growth rate attained its highest value at the end of the run. This indicates that the herbicide tested had an algistatic effect and algal cells recovered their vitality by the end of 10 days. This is inagreement with the data recorded by El-Dib et al (1989 and 1990).

Increase of EC_{50} values with time, may be attributed to the adaptation of the organisms to glyphosate. EC_{50} recorded its highest value in case of A. flos - aquae compared to S. capricornutum, consequently, blue green algae tend to be resistant to glyphosate than green ones while EC_{50} of Nile water phytoplankton was relatively low if compared with other tested ones.

Algal communities consist of a complex assemblage of organisms, differing in sensitivity to glyphosate. This explains the the difference in the EC_{50} values in treated culture of Nile water algae.

Van Rensen (1974) observed a 50% reduction in oxygen evalution by a culture of the green alga Scenedesmus sp after 60-90 min exposure to 7x10-4 M (118 mg/L) glyphosate. He concluded that glyphosate directly inhibits photosynthetic electron transport in photosystem II. Goldsbrough and Brown (1988) stated that glyphosate at concentration 89 and 890 mg/L suppressed photosynthetic rates in lentic preiphyton.

Diatoms were considered the most tolerant species in all treated concentration followed by Chlorophytes. Sullivan et al (1981) concluded that in letic systems sprayed with glyphosate, diatom community structural changes were hypothesized to have resulted from no-herbicidal environmental alterations. Austin et al (1991) noted that diatoms were numerically dominant in all treatments with glyphosate whilst bivolume was again dominated by Chloroplytes.

From the value of EC_{50} it seems that glyphosate at a concentration less than 1.0mg/L slightly affected algal growth or community. This value was relatively high if compared with the actual values that may reach aquatic environment. However, low concentrations of glyphosate stimulated algal growth and change algal diversity. The addition of glyphosate to algal community would thus appear to have little effect on subsequent successional patterns. Sullivan et al (1981) noted that post nutrient application of glyphosate increases diatom standing crop. Glyphosate could potentially act as a phosphorus source (Goldsborough and Brown, 1988, wan 1986, wan et al 1989, Goldsborough and Beck, 1989 Kreutzweiser et al 1989). Thus, glyphosate could stimulate undersirable eutrophication in water resources and not other environmental influences (Sullivent et al 1981).

It can be concluded that glyphosate up to 0.1 mg/L promotes algal growth and changes the diversity and redundancy of Nile water algae.

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