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# Recovery of *Nostoc muscorum* Previously Exposed to Some Triazine and Phenylurea Herbicides

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Organic herbicides are heavily used to control aquatic weeds. As such compounds reach surface water, they lead to significant changes in the aquatic ecosystem. Herbicides are liable to exert algistatic, inhibitory or toxic effects on algae and/or induce morphological changes (Austin 1991; Ordog and Kuivasniemi 1989). Changes in chlorophyll (a) [Chl (a)] content, growth rate and algal count are the most sensitive parameters for the evaluation of the impact of herbicides on aquatic algae (Nyholm 1985).

Few reports are concerned with the long term effect induced on algae after exposure to herbicides for few days. This effect simulate the changes in natural surface water body during the process of self-purification by either inhibition or promotion of algal flora. Therefore, this study aimed to assess the growth characteristics and the growth pattern of the cyanobacterium Nostoc muscorum previously exposed to herbicides, when transferred to nutrient media free from such compounds. Two triazine herbicides namely gardoprim and terbutryn, and two phenylureas, neburon and linuron and their mixtures were selected for this study.

## MATERIALS AND METHODS

The cyanobacterium N. muscorum was isolated from Nile River water and used as test organism in this study. Cultures were cultivated in sterile modified Watanabe media (El-Nawawy et al. 1958) for 7 d intervals where cells were in the logarithmic-phase. Cultures were exposed for 7 d to series of herbicide concentrations to give sufficient contact period. Gardoprim (2,3-diamine, N-(1,1-dimethylethyl) -N ethyl, 6-chloro-S-triazine), terbutryn (2,4-diamine, N-(1,1-dimethylethyl)-N ethyl - 6(methyl thio)-S-triazine), neburon (1,N-butyl-3- (3,4 dichlorophenyl)1- methylurea) and linuron (3-(3,4-dichlorophenyl)-1- methoxy 1-methylurea) (98% purity) were obtained from CIBA-Geigy (Swizerland). Solution of each individual herbicide was prepared in methanol. The applied concentrations of the studied herbicides singly or in mixtures are shown in Table 1. Three replicates from each treatment and control

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Table 1. Applied measured concentrations of the individual herbicides and their mixtures.

| Group Treatment | Triazines mg/L | | | Phenylureas. mg/L | | |
|---|---|---|---|---|---|---|
| Gardoprim (G) | 0.05,0.13,0.45,1.01,2.27 | | | | | |
| Terbutryn (T) | 0.018,0.088,0.19,0.7,0.937 | | | | | |
| Neburon (N) | | | | 0.09,0.187,0.375,0.75,4.5 | | |
| Linuron (L) | | | | 0.045,0.375,0.75,3.0,4.5 | | |
| Mixture | G | | T | N | | L |
| I | 0.05 | + | 0.018 | 0.09 | + | 0.045 |
| II | 1.01 | + | 0.937 | 4.5 | + | 4.5 |
| III | 0.13 | + | 0.088 | 0.375 | + | 0.375 |
| IV | 0.05 | + | 0.937 | 0.09 | + | 4.5 |
| V | 1.01 | + | 0.018 | 4.5 | + | 0.045 |

were used. The bioassay flasks were incubated at 25°C ± 2 under continuous white fluorescent light (3000 lux), and were shaken once a day to prevent clumping of algal cells. A known volume (50 mL) of each algal suspension was withdrawn and centrifuged at the end of incubation period (7 d). The algal cells were washed with distilled water several times and resuspended in 500 mL Erlenmeyer flasks containing a herbicide-free sterile media. Chl (a) content was extracted using hot 100% methanol (Sartory and Grobbelaar, 1984). Chl (a) was spectrophotometrically measured at three wavelengths. Chl (a) concentration was calculated as: Chl (a) = 11.85 (OD 664)−1.54 (OD 647) − 0.08 (OD 630), where: Chl (a)= concentrations of Chl (a) mg/l and OD= Corrected optical density (with 1 cm light path) at respective wavelength. Chl(a) content, growth rate (U), and maximum growth rate (Umax) were calculated according to Amer. Publ. Health Assoc. (1985, p. 1069). The growth rate was calculated as:
growth rate = (ln Chl (a) at T2 - ln Chl (a) at T1) / (T2-T1)
Where T1 and T2 are the starting and ending time in days.

The statistically significant effect of time and herbicide concentration on growth rates were determined using repeated measurements analysis of variance (Duncan 1955).

## RESULTS AND DISCUSSION

Recovery of N muscorum after 7 d exposure to various concentrations of gardoprim, terbutryn and mixture of them was measured in terms of Chl (a) content and results are shown in Fig.1. Available data revealed that algal cells exposed to low concentration of gardoprim (0.05 and 0.13 mg/L) showed an inhibition of growth which amounted to 30% and 50% compared with the control, respectively. However, the algae recovered after 3 d of incubation in herbicide-free media and the growth rate exceeded that of the control. Algal cells previously exposed to the higher concetration levels of gardoprim (1.01 and 2.27), showed low levels

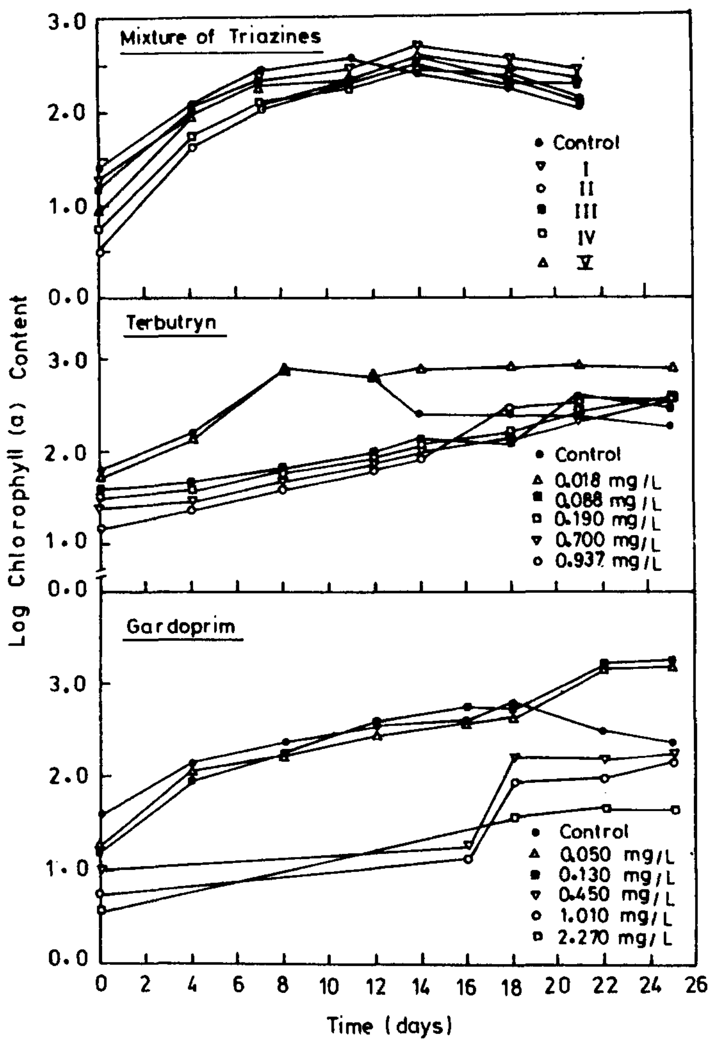


Figure 1. Growth pattern of Nostoc muscorum previously exposed to triazines in a herbicide-free media.

of Chl (a) content (Table 2) and low growth rates which reflected the after effects of the herbicide. Under such conditions, recovery of Nostoc cells was delayed up to 3 wks (Fig 1). Terbutryn, at a concentration of 0.018 mg/L had no significant effect on N. muscorum recovery and maximum Chl (a) content attained a not significant value compared to control (Table 2). However, other concentrations tested supressed the growth of Nostoc cells up to 25 d.

In case of mixtures of both triazines, recovery progressively increased within 4 d from incubation as indicated by the value of U max. Meanwhile Chl (a) values

Table 2. Growth characteristics of N. muscorum previously exposed to triazines.

| Conc.mg/L | %Chl(a)* at zero time | U Max. | Max.Chl(a) values ug/L | %Max. Chl(a)* content |
|---|---|---|---|---|
| Gardoprim | | | | |
| Control | 100 | 0.335 (4)** | 654 | 100 |
| 0.05 | 70 | 0.483 (") | 1600 | 245 |
| 0.12 | 49 | 0.442 (") | 1880 | 288 |
| 0.45 | 32 | 0.155 (18) | 179 | 27 |
| 1.01 | 16 | 0.154 (") | 161 | 25 |
| 2.27 | 7 | 0.130 (") | 48 | 7 |
| Terbutryn | | | | |
| Control | 100 | 0.326 (8) | 870 | 100 |
| 0.018 | 87 | 0.340 (") | 882 | 101 |
| 0.08 | 64 | 0.113 (21) | 494 | 57 |
| 0.19 | 51 | 0.106 (") | 420 | 48 |
| 0.7 | 43 | 0.107 (25) | 403 | 46 |
| 0.9 | 23 | 0.163 (18) | 424 | 49 |
| Mixtures | | | | |
| Control | 100 | 0.41 (4) | 396 | 100 |
| I | 124 | 0.31 (") | 468 | 118 |
| II | 12 | 0.68 (") | 325 | 82 |
| III | 60 | 0.48 (") | 312 | 79 |
| IV | 21 | 0.58 (") | 339 | 86 |
| V | 33 | 0.58 (") | 386 | 97 |

* Compared with control ** Days after incubation.

ranged between 312 and 468 ug/L after two wks (Table 2). Hence, the two triazines showed an extended algistatic effect on algae even after the algae were transferred to herbicide-free media. In contrast El Dib et al (1989) reported the ability of Scenedesmus sp to recover in herbicide free media, after 10 d exposure to gardoprim, terbutryn and gesapax. In addition the Chl (a) content of the recovered Scenedesmus cells either approached that of control or exceeded it, but the maximum growth rates were always less than that of control. However, Abou Waly et al (1991) showed that the growth rate of recultivated Anabaena flos aquae previously exposed to atrazine exceeded that of control after 5 d in a pesticide - free media. Yee et al (1985) found that full recovery of Chlamydomonas regins previously exposed to prometryne was observed on pesticide-free nutrient agar. Similar recovery was not obtained in washed Anabaena sp. Goldsbororough and Robinson (1983) showed that recovery of algal communities following decreased simazine and terbutryn concentrations began within 1 wk,

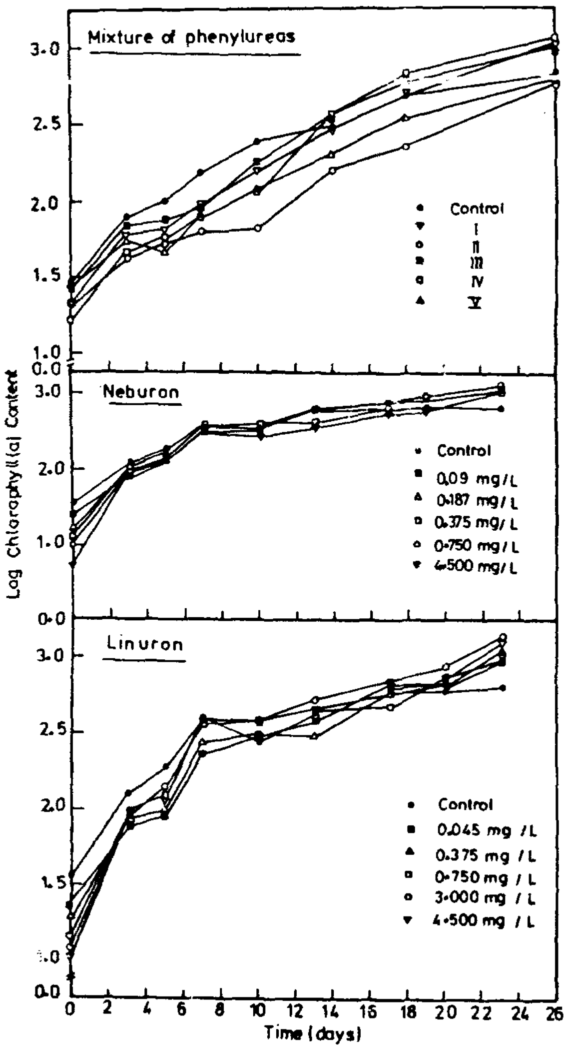


Figure 2. Growth pattern of Nostoc muscorum previously exposed to phenylureas in a herbicide-free media.

with the growth rate equal to or greater than the control. The various responses of recovery reports clearly show the impact of chemical variation of herbicide structures on the different algal species. This in turn could affect algal diversity in water resources.

In case of the individual phenylureas and their mixtures I,III and IV (Table 3), the growth rate matched that of control after 3 d. Chl (a) content of the recultured algae approached that of the control within 7 d. However, Chl (a) values exceeded the control by the end of 27 d. Algal cells previously exposed to the mixtures II and V where neburon was present at its higher level, showed a

Table 3. Growth characteristics of N. muscorum previously exposed to phenylureas.

| Conc.mg/L | %Chl(a)* at zero time | U Max. | | Max.Chl(a) values ug/L | %Max. Chl (a)* content |
|---|---|---|---|---|---|
| Linuron | | | | | |
| Control | 100 | 0.44 | (3)** | 621 | 100 |
| 0.045 | 63 | 0.41 | " | 949 | 153 |
| 0.375 | 53 | 0.50 | " | 1098 | 177 |
| 0.75 | 37 | 0.65 | " | 867 | 140 |
| 3.00 | 32 | 0.67 | " | 1420 | 229 |
| 4.50 | 25 | 0.80 | " | 1203 | 194 |
| Neburon | | | | | |
| Control | 100 | 0.41 | (3) | 659 | 100 |
| 0.09 | 71 | 0.39 | " | 1300 | 197 |
| 0.187 | 44 | 0.63 | " | 1040 | 158 |
| 0.375 | 36 | 0.71 | " | 990 | 150 |
| 0.76 | 27 | 0.79 | " | 1218 | 185 |
| 4.50 | 15 | 0.96 | " | 1063 | 161 |
| Mixtures | | | | | |
| Control | 100 | 0.34 | (3) | 669 | 100 |
| I | 74 | 0.34 | " | 992 | 148 |
| II | 73 | 0.23 | " | 568 | 85 |
| III | 91 | 0.32 | " | 941 | 141 |
| IV | 56 | 0.35 | " | 1073 | 160 |
| V | 90 | 0.26 | " | 597 | 89 |

* Compared with control ** Days after incubation.

slight decrease in the values of U max. This may be attributed to the supression in Chl (a) synthesis or related to the accumulation of herbicides within algal cells (Per Larsson, 1987). In a receiving water body, algae exposed to stress by phenylureas are liable to recover during the self- purification process leading to a mas sive growth once the dilution factor is effective. Available results are in agreement with that reported by El-Dib et al (1991) where Scenedesmus cells were capable to recover after being exposed to three phenylureas. Results presented in Table (4) reveal a statistically highly significant relationship between the applied concentrations of triazine mixtures and the growth rate during the course of the experiment especially at 16 th d ($r2 = 0.98$). However, in case of the individual herbicides, the correlation coefficient values were not significant at $P< 0.05$ as indicated in Table 4.

Microscopic examination of Nostoc cells, previously exposed to triazines, showed that cells exhibited a distortion of cell wall and pigments during their

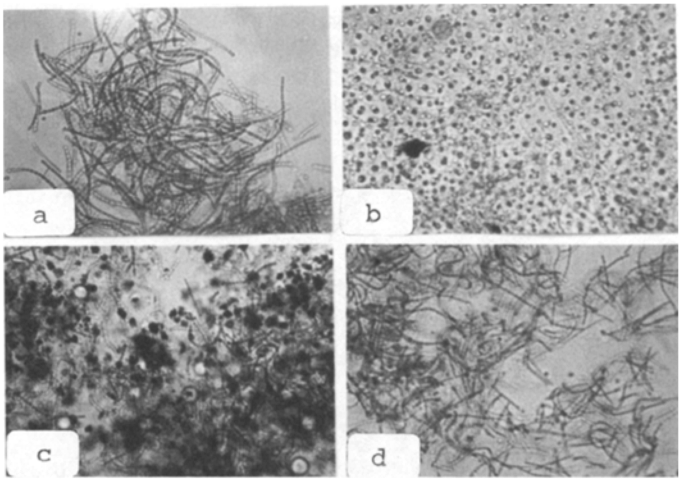


Figure 4. Morphological changes : a. Control culture b. All Gardoprim concs. c. High conc. of all other tested compd. d. Recovered culture.

Table 4. Correlation Coefficient ($r^2$) of Growth Rate of N. muscorum versus Herbicide treatment measured as Chl (a).

| | Time intervals (d) | | | | | | |
|---|---|---|---|---|---|---|---|
| Triazines | 0-4 | 0-8 | 0-12 | 0-16 | 0-18 | 0-22 | 0-25 |
| Gardoprim | 0.53 | 0.53 | 0.53 | 0.63 | 0.54 | 0.2 | 0.16 |
| Terbutryn | 0.2 | 0.29 | 0.12 | 0.002 | 0.28 | 0.3 | 0.48 |
| Mixtures | 0.87 | 0.93 | 0.91 | 0.98 | 0.97 | 0.9 | 0.9 |

| | Time intervals (d) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phenylureas | 0-3 | 0-5 | 0-7 | 0-10 | 0-13 | 0-17 | 0-19 | 0-23 |
| Neburon | 0.61 | 0.63 | 0.72 | 0.56 | 0.51 | 0.6 | 0.56 | 0.59 |
| Linuron | 0.82 | 0.7 | 0.82 | 0.59 | 0.68 | 0.69 | 0.64 | 0.77 |
| Mixtures | 0.66 | 0.16 | 0.27 | 0.18 | 0.14 | 0.12 | 0.1 | 0.09 |

recovery in a herbicide free media. In addition, all gardoprim conceutrations resulted in extreme variation in morphological characteristics of the cell where the filamentous structure of Nostoc changed to a bright unicellular-green form. (Fig 4-b)Such morphological changes

may cause problems to those concerned with algal taxonomy. In case of phenylureas, Nostoc maintained its filamentous form with bright blue-green colour. Previous studies indicated some effects of herbicides on algal morphology (Glooschenko and Glooschenko 1975; Fritz-sheriden 1982; El Dib et al. 1989 and

1991). However, the drastic change in Nostoc cells in presence of triazines may call for more detailed studies to assess the impact of such herbicides on the genetic and / or biochemical characters of Nostoc and other related algae. Also effect of mixtures of different groups of herbicides on algae needs more attention.

In general, the present study indicated that pollution with herbicides whether leading to algal inhibition or stimulation of growth could affect the quality of the receiving water body and the process of self purification.

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