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Effect of Organic Four Solvents on Natural Phytoplankton Assemblages: Consequences for Ecotoxicological Experiments on Herbicides

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Herbicides play an important role in agricultural practices, particularly for cereals (Bérard 1994). The increase in use of herbicides has elicited extensive research into herbicide effects on non target organisms such as algae. For example the s-triazine herbicides and urea-herbicides inhibit the photosynthesis electron transport system (Ducruet 1991). Thus, their potential effect on the aquatic primary producers is particularly important, and has to be studied in ecotoxicological experiments.
Triazines and urea-herbicides have a low water-solubility (Table l), therefore organic solvents are often used in ecotoxicological aquatic experiments with these herbicides (Table 2). When used in high concentrations, for example in chlorophyll extraction, these solvents damage algal cells. The risk of using solvents in ecotoxicological experiments (even in lower concentrations) could therefore interfere with and even mask the herbicide effect on algae. Some papers have been published about the combination effect of organic solvents and pesticides (e.g., on fungi, Stratton et al. 1982). The purpose of this study is to study the potential effect of these solvents on natural phytoplankton assemblages that contain both particularly sensitive and tolerant species and interspecific interactions. Therefore, this study complements other studies done in our laboratory, which address solvent effects on single species of algae (*El Jay personal communication*).

MATERIAL AND METHODS

Two experiments (September and October 1994) were performed. Samples of natural phytoplankton assemblages collected from the light-saturated layer of the Lake Geneva (France-Switzerland) with a manual pump, were distributed in 500mL glass bottles. Large zooplankton were removed by a 200-µm mesh size screen. Samples were enriched with soluble P, N and Si to get a nutrient medium close in composition to the natural spring water of the light-saturated layer of the lake (Orthophosphates: 0.02 mgP/L; mineral Nitrogen: 0.60 mgN/L; SiO_2: 1.3mgSiO_2/L). Phytoplankton were grown at 20°C +/- 1°C under constant illumination; the light intensity at the surface of the culture vessel was $1.2x10^{16}$ quanta $cm^{-2}s^{-1}$. In order to minimize wall growth, clumping, sedimentation, and insure that each bottle receved the same light intensity, the bottles were placed in a holder which was continually rotated during the experiment. The space between each bottle was 20cm.

After the initial measurements of chl a and community composition., each bottle received either 0.05% or 2% final concentration of one of the followmg solvents: ethanol (95-96% purity, PROLABO, EEC), dimethyl sulfoxid (DMSO, 99.9% purity, SIGMA ALDRICH, Steinheim, Germany), NNdimethyl formamid (DMF, 99.9% purity, SIGMA ALDRICH, Steinheim, Germany) and methanol (95-96% purity, PROLABO, EEC). There were four replicates for each concentration and each solvant, and six replicates for the control. Experiments lasted 4 days because during this period the species composition was roughly parallel to the phytoplankton succession in the lake (Sommer 1985).

Table 1. Water solubility of some herbicides

Herbicide	Herbicide family	Solubility (mg/L) 20°C	Reference
Ametryne	Triazine	185	W.Q.W.S. 1988
Atrazine	Triazine	33	W.Q.W.S. 1988
Dcmu	Urea	42	W.Q.W.S. 1988
Pendimethalin	Triazine	0.5	W.Q.W.S. 1988
Propanil	Amide	500	W.Q.W.S. 1988
Simazine	Triazine	3.5	W.Q.W.S. 1988
Terbutryne	Triazine	58	ACTA 1991

Table 2. Ecotoxicological experiments performed with some pesticides in solvents

Solvent	Pesticide	Organism	Reference
methanol (0.01%) controls with methanol	atrazine	*Selenastrum capricornutum* *Anabaena flos-aquae*	Abou-Waly et al. (1991)
methanol (0.1%) controls with methanol	atrazine, simazine terbutryne	*Chlamydomonas geitleri*	Francois & Robinson (1990)
DMSO (0.05%) controls with DMSO	simethryne	various freshwater algal taxa	Kasai et al. (1993)
methanol	42 herbicides (*e.g.*, ametryne, atrazine, simazine)	*Chlorella pyrenoidosa*	Kratky & Warren (1971)
ethanol (0.1%) controls with ethanol	dcmu, propanil atrazine	various green algae and cyanobacteria taxa	Maule & Wright (1984)
DMSO (0.5%) controls with DMSO	simazine	various freshwater algal taxa	O'Neal & Lembi (1983)
methanol	gardoprim gesapax	natural phytoplankton	Shehata et al. (1993)
ethanol or DMSO (≤0.05%)	14 pesticides (*e.g.*, dcmu)	*Dunaliella salina*	Yarden et al. (1993)

Chl (a) concentration estimations were measured daily by *in vivo* fluorescence (Fluorimeter Backscat-1101 LP, Turner, USA). Calibration between fluorescence intensity and chl (a) was made using the chlorophyll extraction procedure of Strickland and Parsons (1968): at the end of the experiments the chl a concentration was determined on mixturs of each replicate set.

Phytoplankton cells were counted at the beginning and end of each experiment. Samples were immediately fixed in Lugol's solution, sedimented in Utermöhl's chambers and counted with an inverted microscope (Axiovert 35 Zeiss, Germany).

Two of the four replicates were counted for each solvant at 0.05% concentration, and at least 500 cells were counted by duplication, which yields a precision of +/- 10% within 95% confidence limits if algae are randomly distributed (Lund et al. 1958).

A community composition diversity parameter (H') was estimated using Shannon-Weaver equation (Shannon and Weaver 1963):

$$H' = \sum_{i=1}^{s} \frac{N_i}{N} * \log 2 * \frac{N_i}{N}$$

H' = diversity index (specific composition)
S = number of taxa samples
N_i = number of individuals of i-th taxon
N = sample size

RESULTS AND DISCUSSION

No significant difference in chl (a) was detected among any of the solvent treatments and control. Except for ethanol treatment. At 0,05% ethanol curve growth was inhibited in both experiments, while at the 2% curve growth was inhibited in the fast experiment but stimulated in experiment 2.(Figure 1).

Total growth as measured by chl a curves gives only a simplified image of the phytoplankton complexity. The specific composition or diversity of the phytoplankton communities among the samples treated with 0.05% of the different solvents was calculated using the Dhannon-Weaver index. Phytoplankton taxa encountered in the study are listed in Table 3.

Table 3. Phytoplankton taxa encountered in the study, (taxa in which the number of individual exceeded 2% of the total number are underlined)

Cyanophyceae	Desmidiaceae
Aphanizomenon flos aquae	*Closterium sp*
Oscillatoria limnetica	*Mougeotia gracillima*
Oscillatoria rubescens	*Staurastrum cingulum*
Pseudanabaena sp	
Cryptophyceae	Chrysophyceae
Rhodomonas minuta	*Desmarella brachycalyx*
Rhodomonas minuta var. *nannoplanctica*	*Dynobryon divergens*
	Ochromonas sp
	Salpingoeca sp
Bacillariophyceae	Chlorophyceae
Asterionella formosa	*Chlamydomonas sp*
Cyclotella sp	*Chlorella vulgaris*
Diatoma elongatum	*Elakatotrix gelatinosa*
Fragilaria crotonensis	*Flagelate diameter 10µm*
Navicula sp	*Flagelate diameter 5µm*
Nitzschia sp	*Micractinium pusillum*
Synedra acus v angustissima	*Paulschulzia pseudovolvox*
Synedra acus v radians	*Tetraselmis cordiformis*

In the experiments the diversity index (Table 4) varied from 2.40 bits/cell to 3.87 bits/cell, within the range common values to ecosystems (Frontier and Pichod-Viale 1991). Diversity is lower in the first experiment (2.49 bits/cell in the control) than in the second experiment (3.85 bits/cell in the control), indicating a difference in the assemblages collected at different times of the year. Variations among treatments and control are lower in the first experiment.

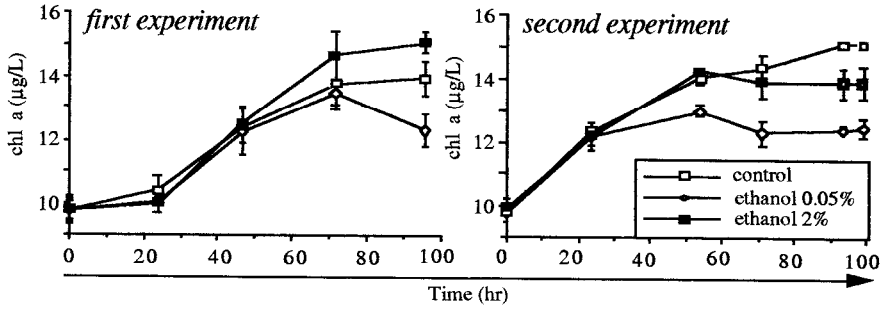

Figure 1. Effect of ethanol on total growth phytoplankton community expressed by the chl (a) estimation. Treatment means are plotted with vertical bars that indicate confidence interval ($P<0.05$, using Student test)

Table 4. Changes in the *Shannon-Weaver* index after treatment with different solvents (0.05% concentration)

	H' (bits/cell)				
Solvent	control	ethanol	DMSO	DMF	methanol
First experiment:	2.49	2.40	2.49	2.51	2.40
Second experiment:	3.85	3.22	3.86	3.87	3.78

Phytoplankton diversity of ethanol treatment is low compared to the control in both experiments (2.40 and 3.22 bits/cell compared to 2.49 and 3.85 bits/cell respectivly). This is due both to the presence of dominant species in high number (eg., *Ochromonas sp* in both experiments), and to the absence of some rare species (eg., *Oscillatoria limnetica* in second experiment). Diversity in methanol treatments is close to ethanol treatment (especialy in the first experiment), and diversity in DMSO and DMF treatments is close to controls' one.

Figure 2 shows the important changes of algal communities grown in the presence of the different solvents.

With the ethanol treatment, only 46% and 33% of taxa (compared to the total number of taxa) remained numerically close (between -2 and 2) to those of the control for experiments 12 and 2 respectivly (Figures 2A and 2B). The decrease of *Rhodomonas minuta* var. *nannoplanctica* is particulary important (-60 in Figure 2B) and has to be emphasized since *Rhodomonas* is reported to be tolerent to atrazine (*Rhodomonas pusilla:* De Noyelles et *al.* 1982, *Rhodomonas minuta:* Hamilton et al. 1988). Methanol treatment showed intermediate percentages of taxa similar to the control (54% and 60%), then DMF and DMSO treatments showed higher percentages of taxa similar to the control (77% and 87% for DMSO, and 77% and 73% for DMF, in experiments 1 and 2 respectivly). However, in the DMF treatment *Rhodomonas minuta* were very numerous (+60.3 times the control) and in case of DMSO treatment *Asterionella formosa* showed low number (- 10.6 times less than the control) in the second experiment (Figure 2B).

The impact of each treatment on the phytoplankton (assemblages) was different between first and second experiment. Solvents treatments tended to stimulate phytoplankton in the first experiment whereas they tended to inhibite phytoplankton in the second experiment (Figures 1: ethanol 2% and 2). For example *Desmarella brachycalyx* is stimulated by DMSO during the first experiment (+6.1) and is inhibited during the second one (-10.6). Since the experiments were performed on phytoplankton assemblages collected at two different times (September and October 1994), innoculum species composition should vary from experiment to experiment

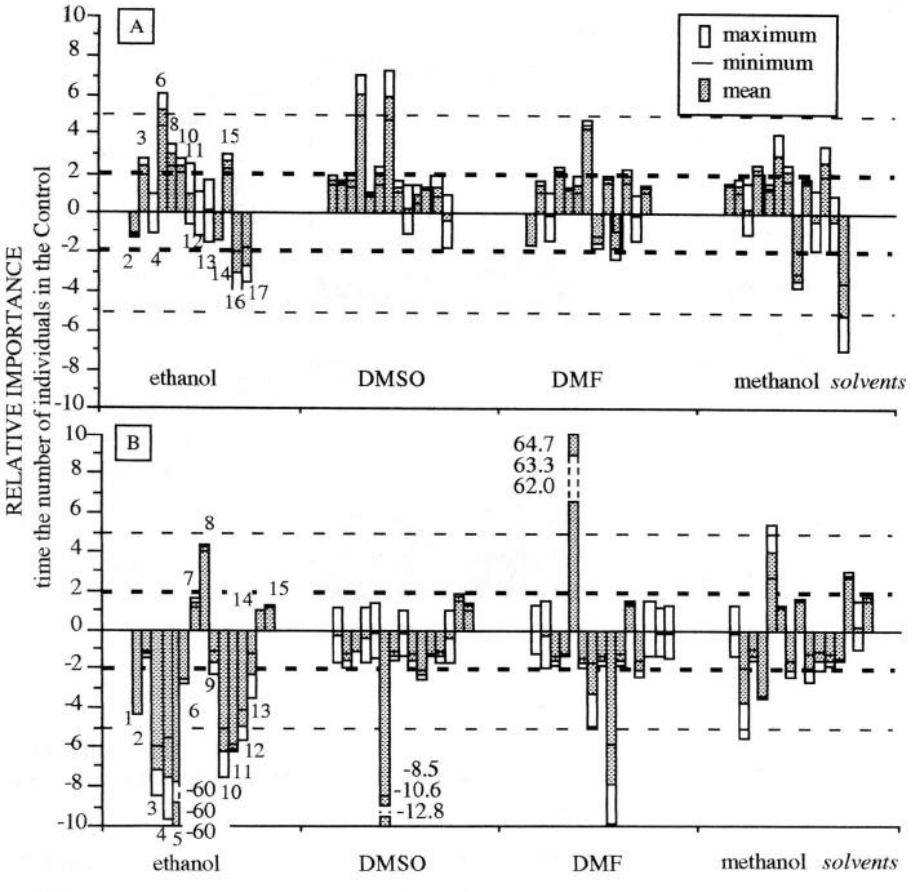

Figure 2. Relative importance of the dominant taxa in each treatment (0.05%) compared to the control, This relative importance varies from >10 times to <10 times the number of individuals in the control. Identity with the control number is defined as y=0. We consider a species is numerically close to the control when the relative importance is between -2 and 2. A: first experiment; B: second experiment. *(1)Aphanizomenon flos aquae, (2)Oscillatoria limnetica, (3)Oscillatoria rubescens, (4)Pseudanabaena sp, (5)Rhodomonas minuta var. nannoplanctica, (6)Desmarella brachycalyx, (7)Salpingoeca sp, (8)Ochromonas sp, (9)Asterionella formosa, (10)Cyclotella sp, (11)Diatoma elongatum, (12)Frugilaria crotonensis, (13)Chlamydomonas sp, (14)Chlorella vulgaris, (15)Flagelate diameter l0 µm, (16)Flagelate diiameter 5µm, (17)Mougeotia gracillima*

(Figure 3). This difference was also noticeable in the controls at zero time and remained over the course of the experiment (Figure 3): the first experiment was characterized by an adaptation phase, a lower growth, a stable presence of *Chlorella vulgaris*, a large decrease of *Cyclotella sp* and an increase of *Fragilaria crotonensis.* The second experiment was characterized by higher growth and no adaptation phase, a decrease of *Chlorella vulgaris* and an increase of various taxa which were rare in the beginning of the experiment. The impact of a pertubation on algae communities is dependent on the composition of the algal community at the time of

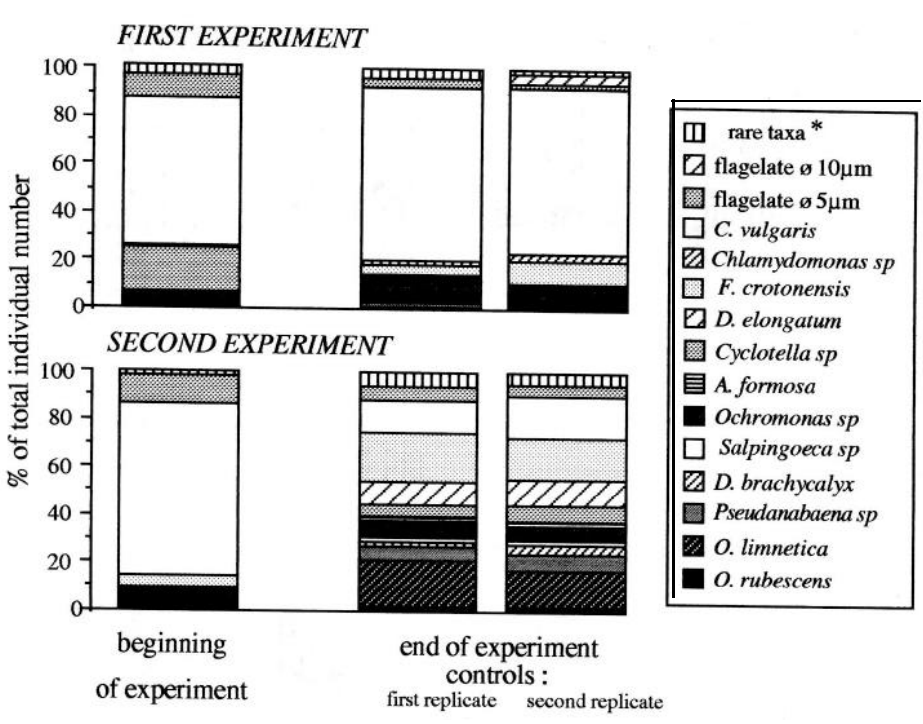

Figure 3. Difference in the species composition of the inoculum and the controls in the both experiments. * rare taxa: taxa in which the number of individual was less than 2% of the total sample population.

exposure, as shown by Herman et al. (1986) with herbicide. This could explain the observed differences between first and second experiment monitored in the same conditions (light, temperature ...). but with differences in species composition of the inoculum.

Each solvent showed an effect on the phytoplankton communities. In general the impacts of the studied solvents on lacustrine phytoplankton growth and specific composition (H') are (in order from the greatest to the lovest impact) ethanol>methanol>DMF ≥DMSO. In a preliminary experiment (*Bérard and Druart, unpublished data*) to determine the impact of atrazine (in 0.05 % ethanol) on natural phytoplankton assemblages, Cryptophycae *Rhodomonas* showed a decrease. This species, known for its tolerence to atrazine, was probably inhibited by the organic solvent and not by the triazine.

Overall DMF and DMSO seemed to have the least impact on phytoplankton assemblages. Studies on *the* algae *C. vulgaris* and *Selenastrum capricornutum (El Jay personal communication)* showed no effect of DMSO at 0.05 %. However, this work on natural phytoplankton assemblages, has shown that DMSO at 0.05% does have an impact on other algae (Figure 2). The natural composition of algal community, with interspcific interactions, increases the complexity of the system studied. It is possible that this complexity increases the sensitivity of our phytoplankton system to a stress like DMSO solvent (Lampert et al. 1989).

These results confirm the potential effect of solvents on natural assemblages of phytoplankton and the necessity to perform preliminary experiments with DMSO when the use of organic solvent in ecotoxicological phytoplankton experiments is

required. These results show also the variability of the impact of pertubations on phytoplankton assemblages probably dependent on initial composition of algal community.

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