Journal of Applied Phycology **14:** 41–48, 2002.

Use of microbenthic algal communities in ecotoxicological tests for the assessment of water quality: the Ter river case study

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Received 15 September 2000; revised 7 March 2001; accepted 7 March 2001

Key words: Atrazine, Copper, Cyanobacteria, Diatoms, Ecotoxicity, Environmental assessment, Fluorescence, Green algae, Mediterranean, River, Photosynthesis

Abstract

The tolerance of microbenthic algal communities to two model toxicants, atrazine and copper, was studied in the Ter river during spring and summer. Artificial substrata were colonised at 9 sites and used to perform short-term (1–4 h) toxicity tests in the laboratory and to obtain photon yield as the ecotoxicological endpoint. The tolerance was lower in spring than in summer for both toxic substances and varied according to the site studied. Copper toxicity was associated with physico-chemical conditions (total suspended solids and water pH) and, especially, with several biomass-related parameters, whereas atrazine toxicity was related to algal abundance and species composition. Temporal and spatial changes in nutrient concentration may alter the biomass and species composition of the communities and thus affect their tolerance to toxic substances. It has to be therefore considered that the environmental characteristics of the river system may determine relevant direct and indirect effects on the algal communities, then affecting their specific ecotoxicological responses. Once this is assumed, the empirical expressions obtained on calculating EC_{50} and EC_{10} can be used to predict the community-level transient effects of toxic exposures.

Introduction

Most ecotoxicological tests are performed in the laboratory, on small populations of certain species and, although they provide useful information on the effect of these toxicants, they are not fully reliable to forecast effects in natural systems (Cairns and Niederlehner 1995), and require complementary studies on natural communities (e.g., Ivorra et al. 2000). The high variance of tests involving multi-species assemblages can be reduced with appropriate knowledge of the ecological function of the most relevant components of the community (Round 1991). Therefore, the use of natural communities from many river systems does not imply loss of reliability. The community-scale approach is ecologically sound, since it integrates the specific tolerances of all the taxa present in a given community (Blanck and Wängberg 1988), as well as their own interactions and environment-relationships.

Physiological tests are used to ascertain the immediate response of algal communities to toxicants (Blaylock et al. 1985; Tubbing et al. 1996). These tests provide a short-term complement to the long-term response of communities, when the exposure to a toxicant can affect their structure (Paulsson et al. 2000).

This study was carried out in the Ter, a chemically and biologically well characterised (Caixach et al. 1990; Espadaler et al. 1997; Sabater et al. 1991, 1995) Mediterranean river with various human influences. We selected two common model toxicants, atrazine and copper, which result from agricultural, farming and industrial inputs. The first is a usual herbicide whose concentrations in river systems range from 0.017 to 0.19 μg L^{-1} (Readman et al. 1993; Solomon et al. 1996). The latter is a heavy metal with concentrations between 30 and 60 μg L^{-1} in moderately polluted sites (Armengol et al. 1993). Both toxicants

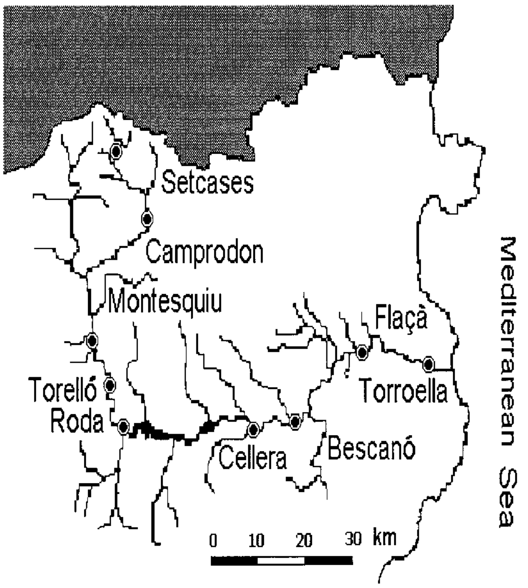

Figure 1. The Ter, showing the sites where the ecotoxicological response of microbenthic communities was analysed.

can reach peak values of several orders of magnitude higher than the above-described (Huber 1993; Van Beelen and Doelman 1997).

The aim of this study was first to show the reliability of an approach based on community tests in the framework of a well-described ecological context; and second, to predict easily the ecological consequences of a given input of toxicant in a given location.

Study area

Several sites scattered throughout the main stretch of the River Ter (Figure 1) were analysed during spring and summer. The sites ranged from the headwaters to the mouth, and had very distinct influences (Table 1): nivo-pluvial in the upper stretch, strongly Mediterranean in the lower stretch (Sabater et al. 1995). Three reservoirs located in the middle part interrupt the river and alter its physical and chemical characteristics downstream (Puig et al. 1987). In the periods studied, benthic algae grow at the maximal rates and show well-differentiated physical and chemical characteristics (Sabater and Sabater 1992).

Materials and methods

Artificial substrata (etched glass 1.4 cm^{-2} of surface area) on perspex supports were placed at the sites, and allowed to colonise for four weeks. The physical (temperature, light transmittance, water velocity) and chemical (pH, dissolved oxygen) parameters of the sites were measured at the beginning and at the end of the experiment. Chemical data (chemical water parameters, atrazine and copper concentrations) of the sites were obtained from the Catalan Agency for Water, which monitors them routinely. The artificial substrata were placed avoiding littoral, slow-moving waters; the current velocity ranged from 4 cm s^{-1} (Roda de Ter, Flaçà) to 80–100 cm s^{-1} (Ter headwaters: Setcases, Camprodon). Transmitted light at the substrata surface was measured with an underwater light sensor, and recorded as the difference between the data at the river surface and the location of the glass.

After colonisation, the glass were transported to the laboratory (maximum travel time of 2h) and separated for community analysis (2 glass), chlorophyll *a* analysis (5 glass) and ecotoxicological tests. Community analysis included the identification of the taxa, enumeration (following Utermöhl's technique after sonication, Sabater et al. 1998) and estimate of cell biovolume (Muñoz et al. 2000). Cell counts were used to calculate the Shannon-Wiener diversity index (Shannon and Weaver 1963). Chlorophyll-a was estimated after extraction with 90% acetone and spectrophotometric measurements (Jeffrey and Humphrey 1975). For the ecotoxicological tests, two protocols were followed. Since copper requires long-term incubation, the glass were placed on artificial channels (Navarro et al. 2000). Since the water for incubation was from the site, the channels were used in a recirculation mode (4 h). Water temperature and pH were adjusted to those of the sites at the beginning of incubation (± 1 °C for temperature, ± 0.01 for pH). pH was carefully controlled in order to ensure the chemical speciation of this heavy metal. Eight channels were used, including one control and 7 copper concentrations, with five glass replicates each. For atrazine, incubation (1h) was performed in buckets containing the glass with the herbicide concentrations (7) and the control. In both tests, light was provided by halogen lamps (110 μmol photon m^{-2} s^{-1} at the glass surface).

Fluorescence was measured using intact periphyton. The photon yield (Fm'-F)/Fm' quotient (Falkowski and Raven 1997) was measured after incubation

Table 1. Physical and chemical characteristics of the sites on the Ter studied in spring and summer 1999. Standard deviations for mean values are given in italics.

SPRING	pH	T (°C)	Cond (μS cm^{-1})	TSS (mg L^{-1})	O_2 (mg L^{-1})	BOD_5 (mg L^{-1})	PO_4-P (mg L^{-1})	NH_4-N (mg L^{-1})	NO_3-N (mg L^{-1})	TOC (mg L^{-1})
Setcases	8.14	6.2	78.0	8.0	10.3	–	0.033	0.110	0.112	1.0
Camprodon	7.9	10.0	211.0	13.5	10.4	1.9	0.051	0.375	0.758	1.7
		1.2	*42.4*	*4.8*	*0.4*	*0.2*	*0.021*	*0.291*	*0.329*	*0.2*
Montesquiu	8.4	10.3	219.8	7.9	11.2	2.2	0.045	0.108	0.747	1.1
		0.6	*38.4*	*0.7*	*0.1*	*0.1*	*0.008*	*0.038*	*0.383*	*0.0*
Torelló	8.0	12.3	334.7	24.5	10.7	4.4	0.103	0.183	0.727	2.1
		0.9	*15.6*	*2.5*	*0.3*	*1.9*	*0.016*	*0.094*	*0.115*	*0.2*
Roda de Ter	7.9	13.2	633.2	24.2	9.6	6.6	0.091	2.042	0.645	4.5
		0.5	*46.9*	*6.0*	*0.4*	*2.2*	*0.026*	*0.455*	*0.548*	*1.3*
Cellera de Ter	7.9	8.7	514.8	3.4	9.9	2.0	0.112	0.443	2.001	2.9
		0.8	*52.6*	*0.1*	*0.6*	*0*	*0.098*	*0.513*	*1.281*	*0.4*
Bescanó	7.6	12.3	535.3	14.8	10.5	2.1	0.048	0.107	1.693	2.3
		2.5	*79.7*	*11.8*	*0.1*	*0.7*	*0.013*	*0.05*	*0.397*	*0.1*
Flaçà	7.6	18	729.3	36.0	10.7	4.4	0.174	0.344	2.581	2.9
		0.5	*116*	*35.3*	*0.5*	*0.9*	*0.059*	*0.024*	*0.546*	*0.5*
Torroella	7.7	18.6	737.2	68.6	10.0	3.9	0.137	0.303	2.481	3.5
		0.7	*134.1*	*44.8*	*1.4*	*1.9*	*0.008*	*0.033*	*0.631*	*0.4*
SUMMER										
Setcases	8.1	10.0	80.0	8.0	10.3	–	0.035	0.311	0.067	1.0
Camprodon	8.1	15.7	273.7	12.1	8.5	2.2	0.083	0.504	0.469	1.3
		1.4	–	*0.4*	*1.5*	*0.7*	*0.353*	*0.259*	*0.101*	*0.5*
Montesquiu	8.4	15.8	264.7	13.0	9.2	2.8	0.059	0.190	0.429	0.9
		1.1	–	*1.1*	*1.3*	*1.2*	*0.025*	*0.129*	*0.243*	*0.2*
Torelló	8.2	19.7	334.7	59.2	7.9	4.8	0.086	0.173	0.580	1.9
		1.9	–	*1.6*	*2.0*	*1.8*	*0.015*	*0.087*	*0.352*	*0.5*
Cellera de Ter	7.6	14.6	544.7	3.7	9.3	3.0	0.390	0.150	2.642	2.3
		5.6	–	*2.4*	*1.8*	*1.4*	*0.127*	*0.102*	*0.541*	*0.8*
Flaçà	7.7	22.7	796.3	12.3	8.7	3.6	0.127	0.317	2.059	2.6
		1.0	–	*0.3*	*0.04*	*0.5*	*0.042*	*0.052*	*0.036*	*0.4*

by means of a Pulse Amplitude Modulation (PAM) fluorometer, which is a clean, non-destructive technique. In steady light and temperature conditions, it is proportional to the photosynthetic rate (Hofstraat et al. 1994). The measurements were used to estimate the effective concentration that reduced the photon yield by 50% (EC_{50}) and by 10% (EC_{10}). These parameters were quantified by log-linear interpolation, which provides the photon yield in samples exposed to toxic substances as a percentage of the average activity of the controls (which is set to 100%).

The relationships between the ecotoxicological, biological and environmental data were analysed by Pearson Correlation tests.

Results

Physical and chemical characteristics

In the River Ter conductivity increased downstream by several orders of magnitude (Table 1). However, the reservoirs caused major discontinuity in this pattern (Table 1), and also affected the total suspended solids (TSS). The highest TSS concentrations were found at the sites immediately upstream of the reservoirs and at the vicinity of the river mouth, and the lowest at the sites downstream of the reservoirs. The highest concentrations of nutrients (422–540 μg L^{-1} soluble reactive P and 8–12 mg L^{-1} nitrate-N) were detected at the lowermost sites and upstream of the reservoirs.

Table 2. Diversity, biomass (chlorophyll-*a*), biovolume and percent growth forms for each algal community from the sites on the Ter.

	Chlorophyll-*a* (μg cm^{-2})	Shannon-Wiener index	Density (cells cm^{-2})	Biovolume (μm^3 cm^{-2})	% Biovolume filamentous	% Biovolume encrusting	% Biovolume prostrate	% Biovolume planktonic	% Biovolume stalked
SUMMER									
Setcases	0.47	3.19	8.51×10^5	5.41×10^8	6.5	63.9	23.9	0.0	5.8
Camprodon	4.42	1.58	4.84×10^6	6.00×10^8	65.4	27.4	6.1	0.0	1.0
Montesquiu	14.15	2.99	7.91×10^6	1.65×10^9	43.3	14.2	27.5	0.0	15.0
Torelló	1.23	2.03	1.76×10^6	6.21×10^8	63.8	1.3	25.0	0.0	10.0
Roda de Ter	1.06	3.29	4.50×10^5	4.66×10^8	24.4	9.8	58.5	2.4	4.9
Cellera de Ter	2.82	2.70	2.70×10^6	3.01×10^9	13.0	0.0	82.9	0.0	4.1
Bescanó	0.64	2.38	2.90×10^6	2.18×10^{10}	15.2	0.0	77.3	0.0	7.6
Flaçà	1.84	2.95	5.27×10^5	5.70×10^9	16.7	0.0	66.7	12.5	4.2
Torroella	1.34	2.68	5.93×10^5	8.76×10^8	0.0	0.0	77.8	14.8	7.4
SPRING									
Setcases	1.02	3.28	8.79×10^5	2.43×10^8	15.0	15.0	60.6	0.0	9.4
Camprodon	4.59	3.25	2.29×10^6	7.72×10^8	31.0	16.9	48.6	0.0	3.5
Montesquiu	7.99	1.55	7.87×10^6	6.73×10^8	78.4	9.8	10.9	0.0	1.0
Torelló	0.84	3.49	3.41×10^6	8.47×10^8	30.0	29.2	26.2	7.3	7.3
Cellera de Ter	6.42	3.19	2.34×10^6	6.91×10^9	12.5	0.0	84.4	0.0	3.1
Flaçà	2.81	2.29	1.03×10^6	2.78×10^8	3.2	26.6	67.0	3.2	0.0

Monthly averages of basal copper concentrations (when detectable) during the study period ranged between 0.5 μg L^{-1} (close to detection limit, at Bescanó) and 4.5 μg L^{-1} (Roda de Ter, upstream of the reservoirs). Analogous measurements of atrazine ranged from 0.004 μg L^{-1} (at Montesquiu) to 0.03–0.08 μg L^{-1} (Roda de Ter, and river mouth).

Microbenthic community parameters

During spring, the chlorophyll-*a* concentration in the colonised glass substrata was higher (Table 2). Minimal and maximal values were detected in the headwaters and slightly upstream of the reservoirs, respectively. In summer, the differences between sites were more marked (Table 2). Diatoms were the dominant algal group in all the sites studied. They accounted for 87 to 95% of the total algal biovolume. The dominant growth forms varied according to sites and periods (Table 2). Encrusting taxa (e.g., *Chamaesiphon polonicus*) were dominant in the headwaters during spring. Filamentous taxa (e.g., *Phormidium autumnale*) accounted for about half the total taxa from the middle sites, upstream of the reservoirs. Prostrate forms (*Navicula* spp., *Cymbella* spp.) were abundant in communities at sites downstream of the reservoirs, especially during summer. Finally, planktonic taxa (like *Cyclotella* sp. and *Stephanodiscus hantzschii*) were mainly found in the lowermost sites.

The mean Shannon-Wiener' index of diversity was similar for the two periods. However, the variation between sites was much higher during summer. Moreover, the differences between sites were higher upstream than downstream of the reservoirs (Table 2).

Ecotoxicological tests

The EC_{50} for the two toxicants at all sites and during the two periods are shown in Table 3. In general, tolerance to copper was lower in spring than in summer. The EC_{50} for copper in spring and in summer ranged from 20 to 50 μg Cu L^{-1} and from 100 to 350 μg Cu L^{-1}, respectively. The headwaters (Camprodon) were the most sensitive (16 μg Cu L^{-1}) and Montesquiu (115 μg Cu L^{-1}) the most tolerant in spring. The latter was also the most tolerant in summer, but with a much higher EC_{50} (1557 μg Cu L^{-1}).

The tolerance to atrazine toxicity was higher in summer than in spring. Most EC_{50} in the latter period ranged between 6 –9 μg atrazine L^{-1}, while in summer the usual range of values went up to 30–70 μg atrazine L^{-1}. The most tolerant sites were in the Ter

Table 3. EC_{50} for copper and atrazine for photon yield (photosynthetic rate) in the sites studied.

Site	EC_{50} atr	EC_{50} Cu
SPRING		
Setcases	47.5	–
Camprodon	4.4	16.7
Montesquiu	9.9	115.0
Torelló	8.9	22.4
Roda de Ter	7.6	18.3
Cellera	9.5	26.2
Bescanó	7.9	27.4
Flaçà	7.7	29.3
Torroella	6.5	53.6
SUMMER		
Setcases	55.1	281.3
Camprodon	112.0	349.7
Montesquiu	48.9	1558.0
Torelló	36.2	95.4
Cellera	76.2	321.9
Flaçà	32.0	106.6

headwaters, with EC_{50} values ranging from 47 to 112 μg of atrazine L^{-1}.

Correlation analysis

The interactions between ecotoxicological parameters and physical, chemical and biological variables were explored by means of correlation analysis. Among the chemical variables, pH was associated with the EC_{10} for copper, and SRP with the EC_{50} for atrazine (Table 4). However, the EC_{50} and EC_{10} were mainly correlated with algal biomass and cell densities of the growth forms (Table 4). Chlorophyll-*a* was significantly associated with the EC_{50} for copper and the EC_{10} for atrazine, with the cell densities of cyanobacteria, filamentous and stalked growth forms, and with several biovolume-related parameters (Table 4). Several cyanobacterial and algal taxa also showed significant correlations with the EC_{50} for copper and atrazine (Table 5).

Discussion

Physiological tests were useful to determine early effects on the algal communities of the Ter River. However, it was clear that strong differences occurred between the two studied periods: the algal communities were more sensitive during spring than summer to the effects of the toxicants (Table 3). This difference is attributable a variety of factors, both environmental and biological, which should be considered in the use of this type of tests in natural communities.

Among the environmental factors, flow (high during spring and low during summer) could cause the different spatial patterns in the two periods (Sabater et al. 1991). Differences between sites during the summer resulted from the lower precipitation (especially in the lower stretch), which caused water characteristics to depend on local inputs. Moreover, the reservoirs in the Ter increased the differences between the sites. Since they are in the middle part of the river (Sabater et al. 1995), they affect the dissolved and suspended solids that are transported. Although their effect on the ecotoxicological parameters of the sites upstream or downstream of the reservoirs has not been shown, TSS may adsorb and complexate heavy metals like copper (Stumm and Morgan 1981) and thus affect their bioavailability (and hence their toxicity, Table 4). The statistical analysis also revealed (Table 4) a significant correlation between the EC_{50} for copper and pH. At lower pH, the bioavailability of copper increased, since there is a higher amount of free copper (Stadorub et al. 1987).

Water chemical characteristics could also influence the ecotoxicological responses of the algal communities by affecting their respective algal biomass accumulation. Nutrients varied according to the season and the site (Table 1, Sabater et al. 1995), which probably leads to differences in algal biomass and densities. Nutrient availability strongly determines algal biomass and community composition (Biggs 1995; Guasch et al. 1995; Dodds et al. 1997). Therefore, biomass-related parameters, except TSS and pH, were the most clearly correlated with the EC_{50} and EC_{10} for copper and atrazine (Table 4). There was a positive relationship between the resistance to toxicity (higher EC_{50}) and the algal biomass. This relationship may be related to the influence that biomass accumulation has on light availability within the biofilm, as well as for that of nutrients and other dissolved substances (Hill and Knight 1988; Mulholland et al. 1995; Steinman et al. 1995). Moreover, the penetration of metals into biofilms is limited by their thickness, i.e. the accumulated biomass (e.g., Admiraal et al. 1999), since polysaccharide exudates may efficiently adsorb metals (Decho 1990; Ivorra et al. 2000), and thus reduce their penetration into algal cells.

Table 4. Significant correlations (p <0.05) between EC_{50} and EC_{10} for copper and atrazine and associated chemical and biological parameters.

	EC_{50} Cu	EC_{10} Cu	EC_{50} Atr	EC_{10} Atr
Number of stalked algae	0.91			
Chlorophyll-*a*	0.87			0.95
Number of filamentous algae	0.96			
Number of cyanobacteria	0.93			
pH		0.71		
Biovolume postrate algae		0.84		
Biovolume planktonic algae		0.55		
Algal density		0.55	0.68	
Algal biovolume		0.95		
Height above sea level			0.73	
Water flow			–0.68	
SRP			0.61	
TSS	–0.73			

Table 5. Cyanobacterial and algal taxa significantly correlated with the EC_{50} for copper and atrazine (p < 0.05)

Species	EC_{50} Atr	EC_{50}Cu
Oscillatoria limosa	0.65	
Gongrosira sp.	0.69	
Spirulina sp.	0.72	
Cocconeis placentula	0.55	
Gomphonema minutum	0.60	
Navicula radiosa		0.54

Environmental factors also determine the overall responses of algal communities: the low light availability (due to high shading) and the subsequent physiological response of the algal community probably account for the minimal atrazine toxicity observed in Setcases and other sites of the Ter headwaters (Table 3). Several laboratory and field studies indicate that atrazine toxicity is associated with photoadaptation to low light intensity, when the relative increase of carotenoids and other accompanying pigments decreases the toxicant effect (Millie et al. 1992; Guasch and Sabater 1998).

Certain algal taxa were clearly linked with the ecotoxicological indicators. Although the density of cyanobacteria was related to the EC_{50} for copper (Table 4), the only taxa positively correlated with this ecotoxicological parameter was the diatom *Navicula radiosa* (Table 5), a common species in the middle stretch of the Ter (Sabater et al. 1991). Cyanobacteria have been described to tolerate high concentrations of heavy metals such as zinc (Say and Whitton 1982, Whitton et al. 1981), but they are not so common in copper-polluted environments (Leland and Carter 1984). In a long-term experiment Soldo and Behra (1990) determined that exposure of periphyton to high concentrations of copper (5 μM) caused the shift from the Cyanobacteria to green algae dominance. In the same experiment, diatoms remained at a similar proportion with the highest copper concentrations than in the control conditions. Takamura et al. (1989), by measuring the effect of copper on photosynthesis, also determined the high sensitivity of cyanobacteria to copper, as well as the higher resistance of green algae and of some diatom taxa. Atrazine toxicity is also associated with the higher proportion of cyanobacteria, green algae and diatoms (Table 5). Diatom communities are more tolerant than those dominated by green algae (e.g., Goldsborough and Robinson 1986; Guasch and Sabater 1998), which are 8 to 10 times more sensitive (Tang et al. 1998). Three of the algal communities most sensitive to atrazine were dominated by green algae (Bescanó, Flaçà and Torroella), whereas the least sensitive (Setcases) had a low proportion of this algal group.

Our conclusions based on natural communities are consistent with other studies carried out in more controlled conditions, in the laboratory or in mesocosms. Tests using natural communities appropriately reflect the ecological reality of a natural system (Cairns and Niederlehner 1987), and make them a valuable tool for

Table 6. Percent decrease (negative) and increase (positive) in the photosynthetic rate after the input of 10 μg L^{-1} copper for 3 h or 10 μg L^{-1} atrazine for 1 h, as deduced from the log-linear correlation between photosynthesis and the toxicant concentration (for further details see the text).

	SPRING		SUMMER	
	% effect Cu	% effect Atr	% effect Cu	% effect Atr
Setcases		0.6	–18.8	–25.3
Camprodon	–25.3	–72.0	–0.1	–15.9
Montesquiu	2.6	–50.0	2.4	–28.3
Torelló	–13.2	–55.3	–19.8	–31.5
Roda de Ter	–13.1	–59.7		
Cellera de Ter	–21.0	–52.6	1.3	–16.0
Bescanó	–0.3	–56.5		
Flaçà	–9.1	–57.7	8.2	–34.9
Torroella	22.3	–61.3		

environmental assessment. However, it is clear from our results in the Ter that the environmental characteristics of the system may determine relevant direct and indirect effects on the algal communities, then affecting their specific ecotoxicological responses. Once this is assumed, the empirical expressions obtained on calculating EC_{50} and EC_{10} can be used to estimate the effect of a concentration of toxicant. We described two scenarios that could affect the photon yield. The first is highly realistic, since it considers the concentrations detected in the Ter. It describes the 3 h-effect of 10 μg L^{-1} copper on the algal communities of the river. The second explores the response to a high concentration of atrazine (10 μg L^{-1} atrazine for 1 h) (Huber 1993) that has never been recorded in the Ter, though has been reported elsewhere (Solomon et al. 1996). Adaptation to these concentrations can occur in some sites for copper, but not for atrazine.

Each scenario shows a variety of responses. Some communities are slightly affected by the toxicant input, others show a decrease in the photosynthetic rate, and others a moderate increase (Table 6). In the case of atrazine, the effects on the photosynthetic rates are devastating for many of the algal communities of the Ter, especially during spring. Regarding copper, the moderate amount tested can cause a remarkable decrease (around 20% in some sites).

In conclusion, physiological tests provide an early prospective quantification of the transient effects of a toxic community on separate communities, predict which of these effects are not reversible and determine their intensity. This procedure could be followed to assess the effect of toxicants on algal communities, and thus extrapolate the effect to the whole river system.

Acknowledgements

This work is part of the project ‘Microbenthos’, funded by the European Community (project number ENV4-CT96-0298).

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