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# *IN SITU* EVALUATION OF HERBICIDE EFFECTS ON THE COMPOSITION OF RIVER PERIPHYTIC DIATOM COMMUNITIES IN A REGION OF INTENSIVE AGRICULTURE

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BIOINDICATION
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DIATOMS
EUTROPHICATION
HERBICIDES
PERIPHYTON
POCIS
RIVERS

ABSTRACT. – A field study in a region of intensive agriculture was carried out in order to assess the *in situ* effects of herbicides on natural periphytic diatom communities. Artificial substrates were placed in rivers at ten sites and for five 3-week periods, in spring and in autumn 2008. The composition of diatom communities at the end of each period was related to water chemical conditions. River contamination by herbicides was estimated with passive samplers (POCIS) which can integrate variations in concentrations over a few weeks. Multivariate analysis resulted in the identification of two distinct groups of samples differing by their diatom community compositions and by nutrient and herbicide concentrations. It was difficult to discuss separately the effect of nutrients and herbicides because of the high correlation of these two factors. Diatom communities from the most polluted sites had lower species diversity. Some species of the genera *Achnanthidium*, *Cocconeis* and *Rhoicosphenia* appeared like potential bioindicators of perturbation by agriculture. The observed variations in diatom community composition probably resulted from eutrophication and herbicide toxicity. However in some cases with such investigation methods, the herbicides found in those rivers did not seem to have a prominent effect on diatom communities.

## INTRODUCTION

The use of fertilizers in agriculture has a well-known effect on river, lake and coastal sea eutrophication (Schindler 2006). The effect of agricultural pesticides on aquatic ecosystems has been less investigated (DeLorenzo *et al*. 2001). Most studies are ecotoxicological tests of one molecule on a simplified biological compartment and in experimental conditions. Only a few focus on the real impact of pesticides in a natural environment (Schulz 2004, Pesce *et al*. 2008, Morin *et al*. 2009).

One major difficulty of field studies on the effects of pesticide contamination is an appropriate in situ exposure characterization. Pesticides are transferred to the rivers via soil water runoff and spray drift. The conjunction of pesticide spraying and rain events results in peak concentrations with a low time extent especially in small streams. Thus the mean pesticide concentrations in rivers are difficult to estimate with sporadic point samples. Passive samplers such as POCIS (Alvarez *et al*. 2004, Mazzella *et al*. 2007) integrate variations in concentrations over a few weeks and provide accurate estimations of the mean exposure level of benthic organisms to toxicants.

The toxicity of pesticides can affect non-target aquatic organisms and thus perturbate ecosystems. Herbicides may be toxic especially for aquatic photosynthetic organisms including algae. Periphytic diatoms are a major component of the biofilm growing at the light exposed surface of immerged substrates. As primary producers they are at the basis of the lotic trophic web. Diatoms have been extensively used as bioindicators of trophic and organic pollution (Prygiel *et al*. 1999). However, more knowledge is needed on the response of their communities to toxic pollutions in order to use them as indicators of river contaminations (Guasch *et al*. 1999, Morin *et al*. 2010). It has been experimentally demonstrated that herbicides alter diatom communities even at low concentrations (Kosinski 1984, Goldsborough & Robinson 1986, Schmitt-Jansen & Altenburger 2005, Ricart *et al*. 2009). In the natural environment, the effect of herbicides might vary according to their concentrations, their mode of action (Debenest *et al*. 2009) and their interactions between each others (Relyea 2009) and with other environmental factors (Lozano & Pratt 1994, Guasch & Sabater 1998).

In this study, the effect of herbicides on diatom communities was evaluated directly in rivers draining a region of intensive agriculture. In order to do this, the composition of diatom communities colonizing artificial substrates was related to the mean herbicide concentrations determined by passive samplers during the period of colonization.

## MATERIAL AND METHODS

*Study area*: The field study took place in the “Coteaux de Gascogne” in South-Western France (Fig. 1). It is a rural region whose surface is mostly covered by intensive annual crops. The main cultures are maize, wheat, sunflower and rape. The slope of the fields, the common use of irrigation, the low soil permeability and the heavy rain fall during storms in spring result in high surface water run-off and make the rivers particularly vulnerable to contamination by pesticides.

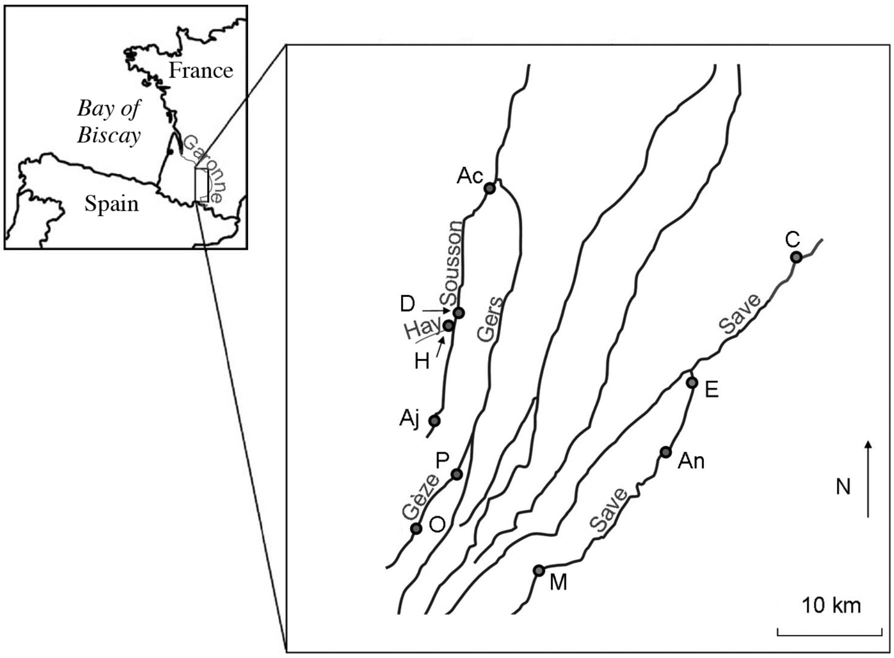


Fig. 1. – Map of the 10 sites sampled in this study (Ac: Auch, D: Daréous, H: Clermont, Aj: Aujan, P: Peyret, O: Organ, C: Cazaux, E: Espaon, An: Anan, M: Montmaurin).

A total of 10 sites presenting a wide range of water quality were selected on 4 rivers:

- 4 on the Save river (Montmaurin, Anan, Espaon, Cazaux) a tributary of the Garonne river which flows toward the Atlantic Ocean;
- 3 on the Sousson (Anan, Daréous, Auch) and 2 on the Gèze River (Organ, Peyret), two tributaries of the Gers which also feeds the Garonne River;
- 1 on the Hay stream (Clermont), a small tributary of the Sousson.

Mean depths of the streams were about 10-20 cm at the shallow stations of Clermont, Aujan and Organ, 20-50 cm at stations Daréous, Auch, Peyret and Montmaurin and over 50 cm at the other stations of the Save river. The bottom of the rivers was made of rock and stones.

*Diatom collection and identification:* Artificial substrates (glass slides 320 $cm^2$) were placed in the rivers at each site for 5 colonization periods of 3 weeks: 3 consecutive periods in spring from end of April to July and 2 consecutive periods in autumn from October to November 2008. The substrates were contained in perforated plastic racks floating at the surface of the deep rivers. This helped to reduce variations of current and light conditions among sites for substrate colonization by diatoms (Morin *et al*. 2007). At the end of each period, periphytic diatoms were sampled by scraping the glass slides with a razor blade. Diatom samples were preserved in 5 % formaldehyde solution. There could not be a sample for each site × period couple because of accidental loss of material in the rivers especially during the second period when high flood occurred.

In the laboratory, a two step digestion of the samples was carried out in boiling hydrogen peroxide (30 %) first and then in hydrochloric acid. The remaining cleaned diatom frustules were put on a glass microscope slide in a high refractive medium (Naphrax©, Brunel Microscopes Ltd UK). Diatom species were identified at ×1000 magnification from taxonomic literature of Central Europe (Krammer & Lange-Bertalot 1986-1991) and recent nomenclature updates. The relative abundance of each species was calculated from a minimum total count of 400 diatom valves. Diatom diversity on the slides was assessed using the Shannon index.

*Chemical point measurements:* At the first and last day of each colonization period, basic water physico-chemical parameters were determined at each site (temperature, pH, conductivity, dissolved oxygen). Also 2 litres of stream water were collected for nutrient analysis (phosphate-$PO_4$, nitrate-$NO_3$, nitrite-$NO_2$, ammonium-$NH_4$) and the determination of suspended particulate matter (SPM), its organic fraction (OM), total phosphorus (Ptot) and Kjeldahl nitrogen (Ntot). Analytical measurements followed French and international standards (NF T90-023, NF EN ISO 11732 and NF EN ISO 13395, NF EN 872 and NF EN 25663).

*Herbicide time-weighed average concentrations:* Polar organic chemical integrative samplers (POCIS) (Alvarez *et al.* 2004) spiked with a performance reference compound (Mazzella *et al.* 2007) were deployed and used for determining herbicide time-weighed average concentrations. After the exposure in water, each POCIS was opened and the sequestration medium (i.e. Oasis HLB) was transferred in a 50 mL glass beaker with 2 × 20 mL washes of HPLC grade water. The sorbent was transferred into a 1 mL empty solid-phase extraction (SPE) tube with a polyethylene frit and packed under vacuum by using a Visiprep SPE Manifold. Afterwards, another polyethylene frit was added to the top of the SPE cartridge. All the cartridges were washed with 20 mL of HPLC grade water and dried with a stream of nitrogen for 30 minutes. Elutions were achieved with 5 mL of

Table I. – Mean herbicide concentrations at the 10 sites as measured by POCIS. The 5 values correspond to the 3-week periods of substrate colonization (nd: not determined).

| herbicide ($\mu$g.L$^{-1}$) | metolachlor | | | | | acetochlor | | | | | alachlor | | | | | atrazine | | | | | DEA[a] | | | | | DIA[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| period | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| SITES: Auch | 1.78 | nd | 0.75 | 0.23 | nd | 0.03 | nd | 0.12 | 0 | nd | 0 | nd | 0.03 | 0 | nd | 0.05 | nd | 0.39 | 0.68 | nd | 0.06 | nd | 0.15 | 0.2 | nd | 0.39 | nd | 0.03 | 0 | nd |
| Daréous | nd | nd | 1.5 | 0.95 | 0.71 | nd | nd | 0.3 | 0 | 0 | nd | nd | 0.09 | 0 | 0 | nd | nd | 1.16 | 32.3 | 2.2 | nd | nd | 0.41 | 0.52 | 0.22 | nd | nd | 0.12 | 0.19 | 0 |
| Clermont | 0.18 | nd | 0.44 | 0.24 | 0.32 | 0.1 | nd | 0 | 0 | 0 | 0 | nd | 0 | 0 | 0 | 0.03 | nd | 0.05 | 0.21 | 0.07 | 0.11 | nd | 0.14 | 0.57 | 0 | 0.6 | nd | 0 | 0 | 0 |
| Aujan | 0.27 | 0.39 | 0.38 | 0.03 | 0.11 | 0.18 | 0.04 | 0.02 | 0 | 0 | 0 | 0.18 | 0.09 | 0 | 0.04 | 0 | 0.08 | 0.21 | 0.17 | 0 | 0.03 | 0.06 | 0.16 | 0.18 | 0.09 | 0.1 | 0 | 0.07 | 0 | 0 |
| Peyret | 0.14 | nd | 0.05 | 0.11 | 0.06 | 0.41 | nd | 0.06 | 0.05 | 0 | 0.73 | nd | 0 | 0 | 0 | 0 | nd | 0 | 0 | 0 | 0.05 | nd | 0.01 | 0 | 0 | 0.17 | nd | 0 | 0 | 0 |
| Organ | 0.08 | 0.11 | 0.04 | 0 | 0 | 0.02 | 0.09 | 0.1 | 0 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0.03 | 0.01 | 0 | 0 | 0 | 0.01 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cazaux | 1.43 | nd | nd | 0 | 0.16 | 0.79 | nd | nd | 0 | 0 | 0.05 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0.04 | nd | nd | 0 | 0 | 0.17 | nd | nd | 0 | 0 |
| Espaon | nd | nd | 0.03 | 0 | 0.03 | nd | nd | 0.01 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0.01 | 0 | 0 | nd | nd | 0 | 0 | 0 |
| Anan | 1.52 | 0.31 | 0.06 | 0 | 0 | 1.24 | 0.14 | 0.02 | 0 | 0 | 0.04 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0 | 0 | 0 | 0.03 | 0.02 | 0.02 | 0 | 0 | 0.12 | 0 | 0 | 0 | 0 |
| Montmaurin | nd | nd | 0.01 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0 | nd | nd | 0 | 0 | 0 |

a: deethylatrazine, b: deisopropylatrazine

methanol. 10 $\mu$L of a stock solution (acetonitrile) containing 100 ng $\mu$L$^{-1}$ of atrazine D5 was added before the evaporation of the methanol with a gentle stream of nitrogen. The final extract was dissolved within 1 mL of ethyl acetate prior to the GC-MS analyses. Further details regarding the analyses can be found elsewhere (Mazzella *et al*. 2008).

*Data analysis*: Multivariate analyses were conducted using the R software (http://www.r-project.org/, Ihaka & Gentleman 1996): principal component analysis (PCA) on the physico-chemical variables (means between beginning and end of period), correspondence analysis (CA) on diatom community compositions and linear discriminant analysis (LDA) on an identified group of diatom samples. Only the taxa representing at least in one community more than 1 % of diatom cells were considered in CA and LDA (76 taxa). A hierarchical ascendant classification was performed on diatom communities using Chi square distance and the Ward aggregation method in order to identify distinct groups. The IndVal descriptor (Dufrêne & Legendre 1997) was used to extract potential indicator species from a group of communities. The comparison of chemical and biological parameters between groups was done with Welch's t-test.

## RESULTS

### *Chemical conditions*

The most commonly measured herbicides with the POCIS belonged to the group of the chloroacetamids (metolachlor, acetochlor and alachlor) and the triazines (atrazine, DEA: deethylatrazine, DIA: deisopropylatrazine) (Table I). Among chloroacetamids, metolachlor was the most frequent herbicide and had the highest time-weighed concentrations (up to 2 $\mu$g.L$^{-1}$). Then came in decreasing order of concentrations acetochlor (max 1.24 $\mu$g.L$^{-1}$) and finally alachlor whose use has been forbidden in France since June 2008. Triazines were less frequent and most often under the form of breakdown products (DEA and DIA) which is consistent with the prohibition in France of atrazine and simazine since 2003. However extremely high integrated concentrations of atrazine (> 30 $\mu$g.L$^{-1}$) were recorded in the Sousson River in October (period 4) when the use of herbicides for agriculture is normally reduced. This suggests an illegal agricultural use of the product or an accidental point contamination of the river by another activity.

Principal component analysis shows relatively high positive correlations of herbicides (metolachlor, DIA, DEA and atrazine) and nutrients ($NO_3$, $PO_4$, $NO_2$ and $NH_4$) and conductivity which also reflects the trophic level (Fig. 2a). Among herbicides, DIA appears mostly correlated with metolachlor which occurs quite independently from atrazine logically correlated with its breakdown product DEA. Acetochlor and alachlor are poorly represented in the plane of the PCA. The plot of the

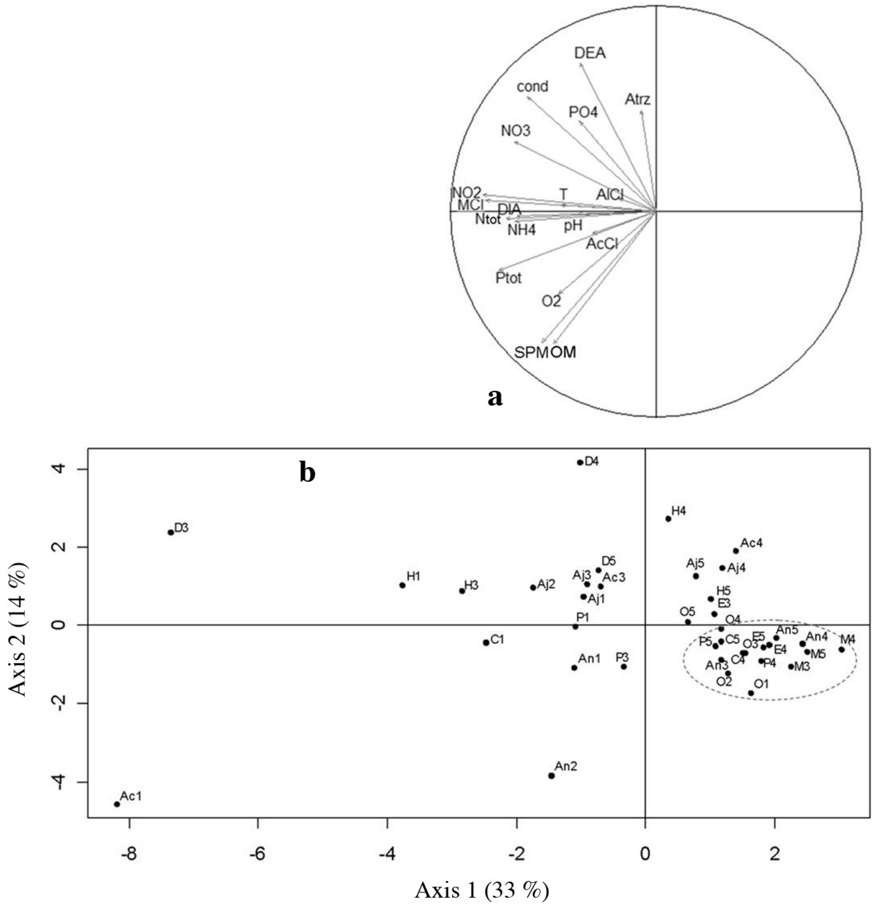


Fig. 2. – Principal component analysis made on the physico-chemical conditions: (a) correlation circle and (b) plot of the samples (site×period) on the 2 first axes. The dashed ellipse shows the low disturbance group (Atrz: atrazin, MCl: metolachlor, AcCl: acetochlor, AlCl: alachlor).

samples (site × period, Fig. 2b) shows a concentration of points in the bottom right hand corner and a high scattering in the left hand side reflecting higher nutrient or herbicide concentrations and in some cases higher turbidity (Ac1 and An2, flood period).

The group at the bottom right hand corner is characterized by low trophic and contamination levels and corresponds to good water quality ('low disturbance' group, Table II). As to the rest of the plot, the correlations between trophic and toxic factors do not allow to identify conditions of dominance of one or the other stressor.

***Diatom communities***

The first axis of the correspondence analysis (Fig. 3) clearly separates a group of diatom communities on the left-hand side of the plot. On the right-hand side, the second axis opposes communities from oligotrophic upstream sites at the bottom and autumnal communities from more downstream sites on the top. The low disturbance samples identified in the PCA (Fig. 2b) are all on the right side of the CA plot. Reciprocally, the isolated group in the left-hand side of the CA plot appears among the scattered points in the PCA and seems thus to correspond to perturbed communities developed in higher concentrations of nutrients and herbicides ('high disturbance' group). The hierarchical ascendant classification (Fig. 4) based on diatom community compositions confirms and better delimitates the clusters defined from the CA. At the highest level of consideration, two clusters can be distinguished: (1) high disturbance communities (left branch) encompassing upstream sites of Clermont and Aujan at all studied periods and downstream sites in spring, and (2) the rest of the communities (right branch) with the same sub-division as on the CA plot. Mean total herbicide and phosphorus concentrations in the high disturbance group are consistent with high agricultural pressure (Table II).

The discriminant analysis was performed on these 2 clusters and resulted in a satisfying separation of them (Fig 5a). Correlations of the various physico-chemical parameters with the discriminant factor reveal the preponderance of trophic factors in the determination of the high disturbance cluster: nitrate (corr. = 0.8), conductivity and organic nitrogen. Among herbicides metolachlor contributes the most with a 0.5 correlation.

Figure 3 indicates some taxa which are particularly associated with some communities of the high disturbance group. However, some species like *Pinnularia microstauron* or *Gomphoneis minuta* are mostly present at one site and are not representative of the whole group. The taxa which have the higest IndVal scores and can thus be considered as potential indicators of perturbation are:

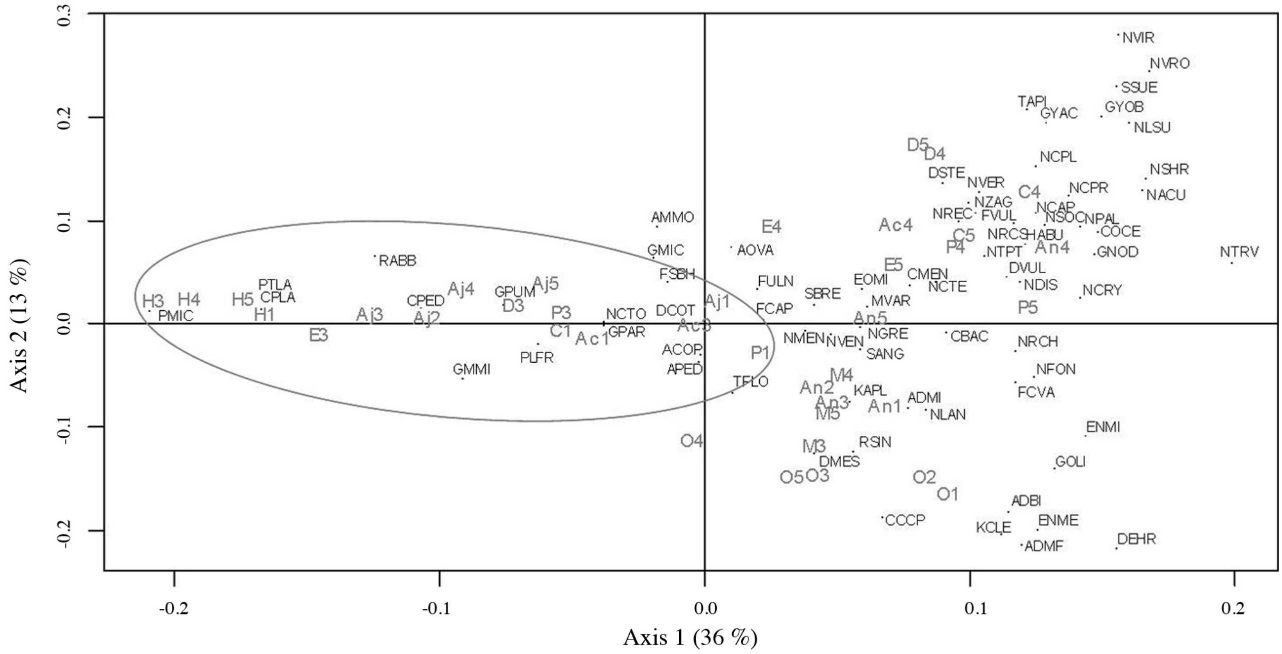


Fig. 3. – Correspondence analysis. Plot of the communities (site × period) and the diatom species on the 2 first axes. The ellipse delimits the high disturbance group. ACOP: *Amphora copulata*, ADBI: *Achnanthidium biasolettianum*, ADMI: *Achnanthidium minutissimum*, AMMO: *Amphora montana*, AOVA: *Amphora ovalis*, APED: *Amphora pediculus*, CBAC: *Caloneis bacillum*, CCCP: *Cyclotella cyclopuncta*, CMEN: *Cyclotella meneghiniana*, COCE: *Cyclotella ocellata*, CPED: *Cocconeis pediculus*, CPLA: *Cocconeis placentula*, DCOT: *Diadesmis contenta*, DEHR: *Diatoma ehrenbergii*, DMES: *Diatoma mesodon*, DSTE: *Discostella stelligera*, DVUL: *Diatoma vulgaris*, ENME: *Encyonema mesianum*, ENMI: *Encyonema minutum*, EOMI: *Eolimna minima*, FCAP: *Fragilaria capucina*, FCVA: *Fragilaria capucina* var. *vaucheriae*, FSBH: *Fallacia subhamulata*, FULN: *Fragilaria ulna*, GMIC: *Gomphonema micropus*, GMMI: *Gomphoneis minuta*, GNOD: *Gyrosigma nodiferum*, GOLI: *Gomphonema olivaceum*, GPAR: *Gomphonema parvulum*, GPUM: *Gomphonema pumilum*, GYAC: *Gyrosigma acuminatum*, GYOB: *Gyrosigma obtusatum*, HABU: *Hantzschia abundans*, KPLO: *Karayevia ploenensis*, KCLE: *Karayevia clevei*, MVAR: *Melosira varians*, NACU: *Nitzschia acula*, NCAP: *Navicula capitata*, NCPL: *Nitzschia capitellata*, NCPR: *Navicula capitatoradiata*, NCRY: *Navicula cryptocephala*, NCTE: *Navicula cryptotenella*, NCTO: *Navicula cryptotenelloides*, NDIS: *Nitzschia dissipata*, NFON: *Nitzschia fonticola*, NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, NLIN: *Nitzschia linearis*, NMEN: *Navicula menisculus*, NPAL: *Nitzschia palea*, NRCH: *Navicula reichardtiana*, NRCS: *Navicula recens*, NREC: *Nitzschia recta*, NSHR: *Navicula schroeteri*, NSOC: *Nitzschia sociabilis*, NTPT: *Navicula tripunctata*, NTRV: *Navicula trivialis*, NVEN: *Navicula veneta*, NVER: *Nitzschia vermicularis*, NVIR: *Navicula viridula*, NVRO: *Navicula rostellata*, NZAG: *Nitzschia angustatula*, PLFR: *Planothidium frequentissimum*, PMIC: *Pinnularia microstauron*, PTLA: *Planothidium lanceolatum*, RABB: *Rhoicosphenia abbreviata*, RSIN: *Reimeria sinuata*, SANG: *Surirella angusta*, SBRE: *Surirella brebissonii*, SSUE: *Surirella suecica*, TAPI: *Tryblionella apiculata*, TFLO: *Tabellaria flocculosa*.

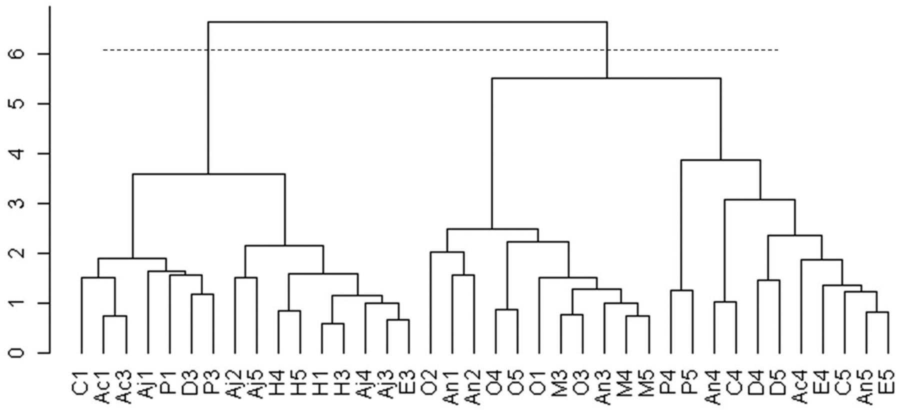


Fig. 4. – Classification tree resulting from a cluster analysis made on diatom communities. The left branch corresponds to the high disturbance group.

*Planothidium lanceolatum* (0.80), *Cocconeis placentula* (0.79), *Rhoicosphenia abbreviata* (0.66), *Planothidium frequentissimum* (0.66), *Cocconeis pediculus* (0.63), *Amphora pediculus* (0.57), *Gomphonema pumilum* (0.54) and *Gomphonema parvulum* (0.45).

It seems also on Figure 3 that the high disturbance communities have fewer characteristic taxa than the others. Indeed the comparison of the Shannon indices of the two clusters (Fig. 4) highlights the lower diversity of the high disturbance communities (Fig. 6).

Table II. – Chemical parameters of the low and high disturbance groups defined from multivariate analysis (means ± standard error).

| | pH | Conductivity | Suspended particulate matter | Nitrates | Nitrites | Ammonium | Kjeldahl Nitrogen | Phosphates | Total phosphorus | Total herbicides |
|---|---|---|---|---|---|---|---|---|---|---|
| Code | pH | cond | SPM | NO3 | NO2 | NH4 | Ntot | PO4 | Ptot | |
| Unit | | $\mu S.cm^{-1}$ | $mg.L^{-1}$ | $mg\ N.L^{-1}$ | $mg\ N.L^{-1}$ | $mg\ N.L^{-1}$ | $mg\ N.L^{-1}$ | $mg\ P.L^{-1}$ | $mg\ P.L^{-1}$ | $\mu g.L^{-1}$ |
| Low disturbance (n = 16) | 7.99 ± 0.06 | 218 ± 14 | 27 ± 11 | 1.55 ± 0.25 | 0.012 ± 0.002 | 0.036 ± 0.005 | 0.62 ± 0.05 | 0.025 ± 0.008 | 0.08 ± 0.01 | 64 ± 21 |
| High disturbance (n = 16) | 8.10 ± 0.04 | 497 ± 32*** | 138 ± 112 | 7.22 ± 0.91*** | 0.047 ± 0.014* | 0.073 ± 0.017* | 2.15 ± 0.49** | 0.057 ± 0.012* | 0.68 ± 0.27* | 1273 ± 280*** |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

## DISCUSSION

### *Combined effect of herbicides and nutrients*

The specific effect of herbicides on diatom periphytic communities was difficult to assess because of the positive correlation of herbicide concentrations and the trophic level whose high influence on diatom assemblages is well known (Kelly & Whitton 1995). It is a recurrent problem in field investigations since intensive agriculture by the concomitant use of fertilizers and pesticides generates river gradients of both trophic and toxic factors. Variations observed in field communities are thus difficult to attribute to one or the other factor. Morin *et al*. (2009), on a larger set of spring data, managed to delineate the effect of the two stressors using artificial neural networks. However, the identified groups of samples remained only slightly contrasted as regards trophic conditions and their characterization was dependent on the nutrients considered (nitrate or phosphate). In this study, the collection of samples in autumn when herbicide input to rivers is usually lower than in spring made possible the comparison of diatom communities from the same site at various exposure levels. Diatom communities from downstream sites like Auch, Cazaux or Espaon showed seasonal variations (Fig. 3) which might be due to a higher agricultural pressure in spring. Nevertheless small streams running between agricultural fields like in Clermont or Aujan exhibited a more constant composition of their diatom communities along the year. Maybe a longer time is needed for diatom communities to recover after perturbation in these upstream sites with a low water discharge.

### *Bioindicators of agricultural perturbation*

It is not possible from this study to identify diatom species strictly indicating a contamination by herbicides. However, potential bioindicators of perturbation by agriculture (herbicides + nutrients) can be derived from multivariate analysis and species screening using the IndVal descriptor. Most of the selected species in this study were proposed as indicators of pesticide contamination in another field study (Morin *et al*. 2009). Particularly the cited species of the genus *Planothidium* are known to be tolerant to pollution (Germain 1979, Lange-Bertalot 1979) and were experimentally found at high herbicide concentrations (Kosinski 1984, Pérès *et al*. 1996) as well as the species *Cocconeis placentula* (Goldsborough & Robinson 1986, Debenest *et al*. 2009).

Species of the genera *Achnanthidium* and *Cocconeis* have a prostrate growth form (Hudon & Legendre 1987). Their deep position in the biofilm, close to the substrate, may confer them higher protection than other forms against stream water contamination (Rimet *et al*. 2009), unless their higher abundance results from a change in the grazing pressure at the high disturbance sites.

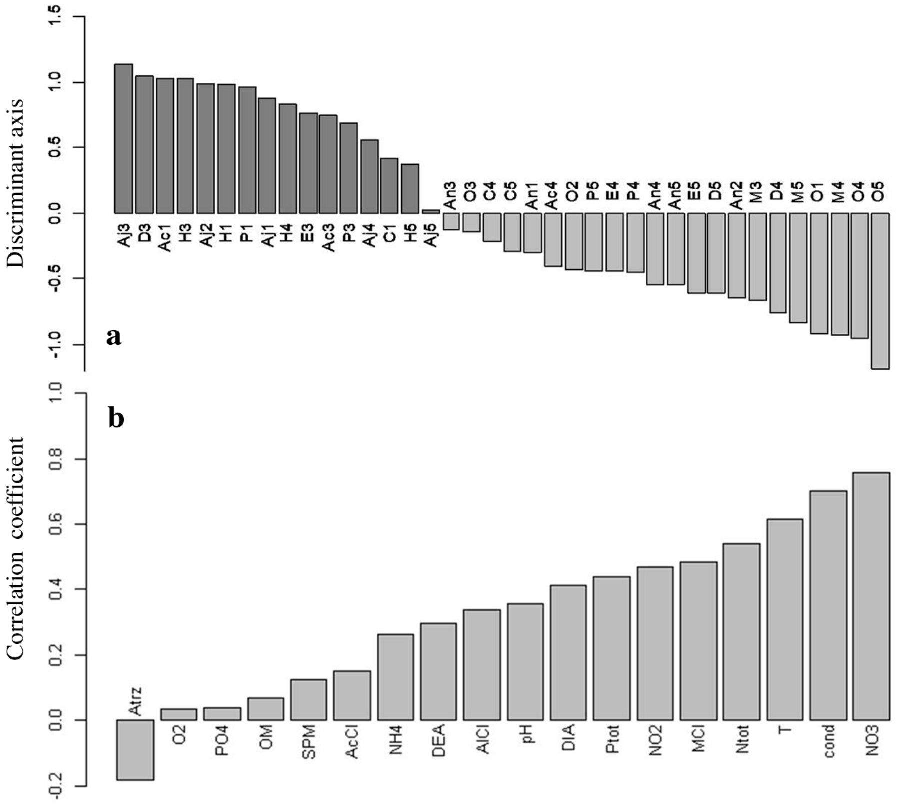


Fig. 5. – Discriminant analysis for the chemical characterization of the high disturbance group: (a) distribution of the samples on the discriminant axis and (b) correlations between the environmental factors and the discriminant factor (Atrz: atrazin, MCl: metolachlor, AcCl: acetochlor, AlCl: alachlor).

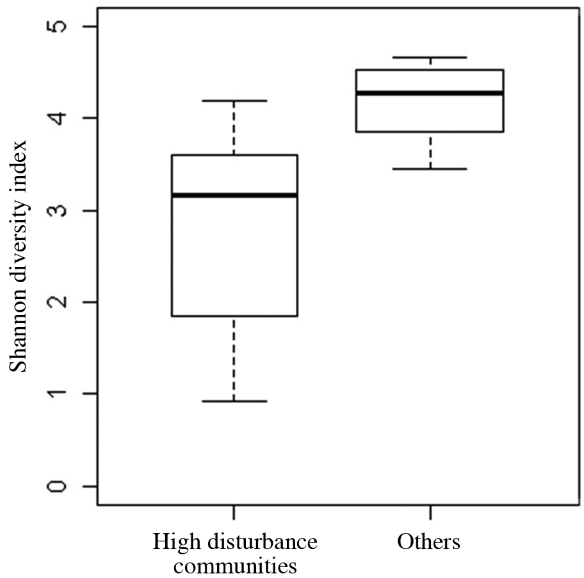


Fig. 6. – Boxplot of the Shannon diversity indices of the communities: difference between the high disturbance group and the other communities ($p < 0.001$).

If herbicides lead to the selection of tolerant species replacing more sensitive ones in a community, there should be a lower species diversity under toxic pressure. This was observed in the high disturbance communities of this study, in another field study (Morin *et al*. 2009) and experimentally (Hamala & Kollig 1985, Abdel-Hamid *et al*. 1996). Thus the increased relative abundance of tolerant species together with a decline in species diversity may be used as complementary indicators of pollution by herbicides.

***Herbicide effect assessment***

The high disturbance group is mostly defined by trophic factors and to a lesser extent by metolachlor (Fig. 5). This suggests a higher effect of eutrophication than of herbicides. The examination of diatom communities at some particular sites which fell outside the two defined groups (low and high disturbance) may confirm a relatively low effect of herbicides. At Dareous, the two communities D4 and D5 appear very similar on the CA plot (Fig. 3) whereas the recorded atrazine concentration was 15-fold higher during period 4 than period 5. It is possible that diatom communities had no time to recover from the 4th to the 5th studied period. However, these communities are close to the autumnal downstream communities developed in low herbicide concentrations (Fig. 3). It is more likely that the peak of atrazine was too short in time to affect diatom communities. If passive samplers have the great advantage over point sampling to account for every herbicide peak during a period of three weeks, they do not provide information on the amplitude of a peak. However the effect of a contaminant on a diatom community may differ whether the exposure is acute or chronic (Tlili *et al*. 2008).

Most surprising are the results from the well sampled site of Anan. The three spring communities appear quite similar (Fig. 3) in spite of very different herbicide concentrations (Table I). Moreover these communities are also similar to those from the site of Montmaurin which was never really contaminated by herbicides. This supports a low toxicity of chloroacetamids which were the dominant herbicides at the site of Anan.

## CONCLUSION

The variations observed in diatom communities are probably due to the combination of trophic and toxic pollution from agriculture. These two types of pollution are correlated because they both increase with the intensification of agricultural practices. That is why their separate effects are difficult to isolate with a general descriptor such as diatom community composition. It seems that herbicides have only a moderate effect at the concentrations measured in those rivers. Experiments in controlled nutrient concentrations may help (1) to better evaluate the individual effect of the different herbicides in acute or chronic exposure, (2) to validate the potential bioindicators identified in this study and (3) to find other sensitive descriptors of pollution by pesticides. Some integrative ecotoxicological descriptors like the frequency of teratological forms or the proportion of empty frustules may be more appropriate to address the toxic effect of herbicides. However, because of the high counting effort that they require, these descriptors should be experimentally tested before using them for the analysis of field samples at large scale.

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