Cah. Biol. Mar. (2006) 47 : 39-45

Inhibition of microphytobenthic photosynthesis by the herbicides atrazine and diuron

Hélène LEGRAND[1], Olivier HERLORY[2], Jean-Marc GUARINI[1], Gérard F. BLANCHARD[2] and Pierre RICHARD[1]*

(1) *Centre de Recherche sur les Ecosystèmes Marins et Aquacoles de L'Houmeau (UMR 10 CNRS-IFREMER), B.P. 5, 17137 L'Houmeau, France*

(2) *Laboratoire de Biologie et Environnement Marins (LBEM, FRE 2727), CNRS-Université de La Rochelle, Bâtiment Marie Curie, Avenue Michel Crépeau, 17042 La Rochelle, France*

** Author for correspondence: Fax 05 46 50 06 60. E-mail: prichard@ifremer.fr*

Abstract: Atrazine and diuron are two herbicides present in sediments of the intertidal mudflats in the Pertuis Charentais (Atlantic coast, France). They may negatively affect photosynthesis processes in microphytobenthos, the most productive primary producer in these environments. The impact of these herbicides on benthic diatoms was investigated using PAM fluorimetry on natural microalgal communities, once extracted from sediment, by following the variations of the effective quantum yield of PSII (Φ_{PSII}) during exposure to atrazine and diuron concentrations in a range 0.1 µg L^{-1} - 10 mg L^{-1}. No significant effect was noticed for 0.1 µg L^{-1} concentrations, but progressive reduction in photosynthetic efficiency, always occurring within 15 min, was associated with increasing herbicide concentration. Diuron appeared to be more toxic than atrazine as shown by the complete inhibition of the photosynthetic processes at concentrations of 100 µg L^{-1} of diuron, and 10 mg L^{-1} of atrazine. A synergistic effect between these two herbicides was noticed only for the lowest concentrations tested.

Résumé : *Inhibition de la photosynthèse du microphytobenthos par les herbicides atrazine et diuron.* Les herbicides atrazine et diuron sont retrouvés en concentrations non négligeables dans les sédiments des vasières littorales des Pertuis Charentais, sur la côte atlantique. Ils ont donc potentiellement un effet toxique sur le microphytobenthos, le compartiment primaire le plus productif de ces milieux intertidaux, par l'action directe qu'ils ont sur les processus même de la photosynthèse. Cet effet a été étudié par fluorimétrie PAM sur des communautés naturelles, extraites du sédiment et soumises à des concentrations d'herbicides de 0,1 µg L^{-1} à 10 mg L^{-1}. Les variations du rendement effectif Φ_{PSII} du photosystème II en fonction du temps d'exposition aux concentrations indiquées permettent de quantifier l'effet relatif des herbicides sur l'activité photosynthétique. Cet effet est très rapide puisqu'il s'exprime en moins de 15 minutes. L'exposition à des concentrations de 0,1 µg L^{-1} d'atrazine ou de diuron n'indique aucune réduction significative du rendement photosynthétique. Au-delà de cette concentration, Φ_{PSII} diminue progressivement jusqu'à une inhibition totale du processus photosynthétique pour des valeurs de 100 µg L^{-1} pour le diuron, et 10 mg L^{-1} pour l'atrazine. Le diuron apparaît donc comme un inhibiteur plus fort que l'atrazine. Seuls les mélanges des plus faibles concentrations d'herbicides montrent un effet synergique.

Keywords: Microphytobenthos; Chlorophyll fluorescence; PAM; Atrazine; Diuron

Reçu le 11 juillet 2005 ; accepté après révision le 24 janvier 2006.
Received 11 July 2005; accepted in revised form 24 January 2006.

Introduction

Herbicides are widely used to protect crops against adventitious plants in agricultural areas but also in urban areas for example to prevent weed development along roads and railways. Nevertheless, a large fraction of these products finally enters aquatic ecosystems through soil leaching and agricultural runoff. It is estimated that close to 90% of the french surface waters are contaminated nowadays, sometimes much above European safety standards (IFEN, 2001). The two herbicides the most used in France were for long atrazine and diuron, the first one is now totally forbidden, the second only allowed during some months a year, but they are still found in consequent quantities in the environment. Their most direct potent impact concerns their effect on photosynthesis of primary producers (DeNoyelles et al., 1982; Bester et al., 1995; Dorigo & Leboulanger, 2001), that may lead to decreased primary productivity at the ecosystem level and thus potentially affect food web functioning (Pratt et al., 1997).

As in many estuaries, the strong productivity of the littoral ecosystems of the "Pertuis Charentais", a suite of shallow-water bays on the Atlantic coast of France, is largely due to the high level of microphytobenthic production at the surface of the intertidal mudflats (Guarini, 1998). Microphytobenthos is the main food resource for most of benthic invertebrates (Riera, 1995; Riera *et al*., 1996) and also for cultivated filter-feeding molluscs (Riera & Richard, 1996). This production is potentially impeded by herbicides brought from agricultural catchments by rivers and also, in the Pertuis Charentais, from adjacent saltmarshes drained and turned to diverse cultivations such as corn or cereals. Nevertheless, the effect of herbicides on marine benthic diatoms has never been analyzed and this preliminary study was aimed at investigating short-term response of natural microphytobenthic communities to atrazine and diuron exposure.

Materials and Methods

Microphytobenthos sampling

Benthic diatoms were originating from the intertidal mudflats of the southern part of Aiguillon Bay, North of La Rochelle city, on the Atlantic coast of France. On these very fine sediments, microphytobenthos is quite exclusively composed of epipelic diatoms that migrate to the mud surface during diurnal emersions and form dense biofilms. During the study period (March to June), the communities were dominated by *Navicula* sp. and *Pleurosigma angulatum*.

Surficial sediment was taken at low tide, sieved on a 500 µm mesh sieve to remove any small macrofauna and spread into plates to a 2 cm depth. Three 100 µm nylon nets were layed over the mud and the plates were left in the laboratory behind a window under natural daylight to initiate upwards diatom migration. After a few hours diatoms accumulated on the upper net were rinsed out. Chlorophyll *a* concentration was estimated using the PAM fluorometer. The diatom suspension was diluted with filtered seawater as to get always the same fluorescence signal (as a proxy of the Chl. *a* concentration) at the beginning of the experimentations.

Experimental design

Four 100 ml stock solutions (0.5, 50, 500 µg L^{-1} and 50 mg L^{-1}) of analytical grade atrazine (99.2 %, Riedel-de-Haën, Germany) and diuron (98%, Sigma, USA) were prepared in filtered seawater after previous dissolution in 1 ml dimethylsulfoxide. Experiments were done using a 50 ml total (including herbicide) volume of algal solution, in an Erlenmeyer flask under continuous magnetic stirring, and light (120 to 180 µmol.photons.m^{-2}.s^{-1}) provided both by fluorescent tubes and attenuated natural daylight coming from a window of the laboratory. These culture aliquots were spiked with stock solutions as to get in experiments relative to the effect of each herbicide, final concentrations of 0, 0.1, 10, 100 µg L^{-1} and 10 mg L^{-1}, in a range of values found in the Bay of Marennes-Oléron or in the effluents of channels draining cultivated adjacent fields (Munaron, 2004). The possible synergistic effect between atrazine and diuron was tested for 3 mixture concentrations (0, 0.1, 10 µg L^{-1}), the null concentrations being used as a comparative check with the single herbicide experiments.

PAM fluorescence measurements

The herbicide effect on photosynthetic processes has been estimated using a PAM fluorometer (Diving-PAM, Walz, Germany), that measures variations of the effective quantum yield of PSII (Φ_{PSII}), reflecting the level of the blockage of electron transport (Genty et al., 1989). The effective quantum yield of PSII (Φ_{PSII}) is calculated from the steady-state fluorescence (F_t) at an actinic light (200 µmol.photons.m^{-2}.s^{-1}, see below), and the maximal fluorescence (F_m') after a saturating light pulse (5000 µmol.photons.m^{-2}.s^{-1} during 0.8 s) on light-adapted algae :

$$\Phi_{PSII} = (F_m' - F_t) / F_m' = \Delta F / F_m' \quad (1)$$

The optimal quantum yield (results not shown) was first evaluated for each replicate as to normalize possible differences between samples collected on different days, and as a check for the physiological state of microalgae. It was calculated from the minimal fluorescence (F_0) at the end of a period of dark adaptation and the maximal fluorescence (F_m) after a saturating light pulse on dark-adapted algae. It was always very close to 0.7, that can be considered as a good physiological state when compared to values obtained by Kromkamp et al. (1998) and Parkhill et al. (2001).

Following preliminary trials, the duration of dark adaptation was established at 10 min, the light for F_t measurements was set to 200 µmol.photons.m^{-2}.s^{-1} during 1.5 min, combination that is not likely to induce photoinhibition in benthic diatoms since Blanchard et al. (2004) showed that photoinhibition was induced after 90 min under 2000 µmol. photons.m^{-2}.s^{-1}. This light was provided by a circular fluorescent tube Imperia 6500°K encircling the measurement tube, and measured using a LiCor quantum sensor LI-193.

Fluorescence measurements were done every 15 min up to 90 min of herbicide exposure and each experiment was made in triplicate. Three ml of the tested cell suspensions were placed in a transparent plastic tube (50 x 15 mm) and allowed to settle during the dark adaptation period. The tip of the PAM fluorometer optical fiber was positioned 10 mm above the diatom biofilm in the tube.

Data analysis

A one-way analysis of variance (ANOVA) was performed to analyse the effects of herbicides levels on the effective quantum yield of PSII. Significant main effects were further examined with Tukey a posteriori comparisons. Control stability was tested through F test on linear regression analysis.

Interactive effects, either additive, synergistic or antagonistic, between atrazine and diuron in mixtures were estimated from the calculation of the interaction ratio (IR) obtained by dividing the observed percent inhibition by the expected percent inhibition in the effective quantum yield of PSII due to interactive effects of atrazine and diuron in mixture (Gisi, 1996). The expected percent inhibition I_{exp} was obtained by applying Abbott formula:

$$I_{exp} = A + D - AD/100 \qquad (2)$$

where A and D are the percentages of inhibition given by atrazine and diuron separately. All inhibition percentages were estimated relative to controls for each herbicide exposure duration. IR values < 0.5, in the range 0.5–1.5 and > 1.5 indicate antagonistic, additive and synergistic effects, respectively (Gisi, 1996).

Results

Atrazine effect

Variations of the effective quantum yield of PSII during the time of exposure (Fig. 1) showed that herbicide effect was completed within the first 15 min for all tested atrazine concentrations, the effective quantum yield of PSII stayed very stable thereafter.

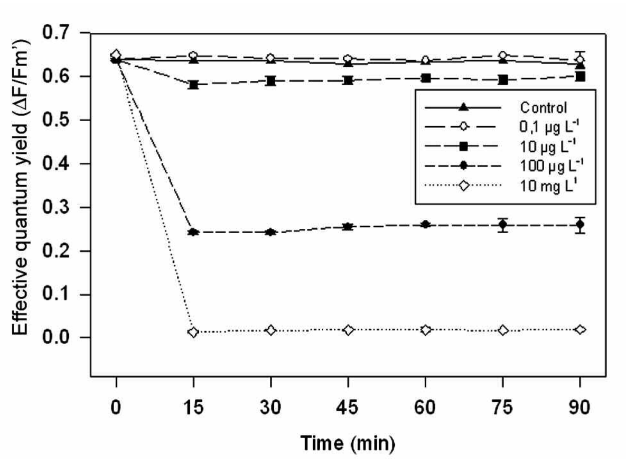

Figure 1. Variations of effective quantum yield of PSII of microphytobenthic communities during exposure to different atrazine concentrations (mean ± SD, n = 3).

Figure 1. Evolution du rendement quantique du PSII ($\Delta F/F_m$') au cours du temps en fonction de différentes concentrations d'atrazine (moyenne ± écart-type, n = 3).

In the controls, effective quantum yield of PSII was stable all along the experiment (mean value ± SD = 0.635 ± 0.005), it has not been considered useful to normalize the data relative to the controls. Significant differences of the effective quantum yield of PSII existed between the 5 tested atrazine concentrations (ANOVA: F = 1.4 10^4, P < 0.001). There was no significant effective quantum yield of PSII decrease for the 0.1 µg L^{-1} atrazine concentration (ANOVA: F = 4.16, P > 0.05), while $\Delta F/F_m$' decreased by 7% at 10 µg L^{-1}, by 60% at 100 µg L^{-1}, and by 97% at 10 mg L^{-1}.

Diuron effect

Effective quantum yield of PSII variations during exposure to diverse diuron concentrations are shown on Fig. 2. The mean control value (0.606 ± 0.003) was also very stable along the experiment but slightly below the control value for the atrazine experiment. As for atrazine, the variability between replicate was extremely low, except for the 10 µg L^{-1} concentration, probably because the diatom community sampled for one replicate of this experiment was taken after a strong rainfall that may have affected the physiological state of diatoms.

Like atrazine, diuron had a quick effect on photosynthesis, as it was completed within the first 15 min, and differences between concentrations were significant (ANOVA: F = 4083, P < 0.001). There was no significant decrease for the 0.1 µg L^{-1} atrazine concentration (ANOVA: F = 6.65, P > 0.05), while $\Delta F/F_m$' decreased by 64% at 10 µg L^{-1}, and by close to 98% at 100 µg L^{-1} and 10 mg L^{-1}.

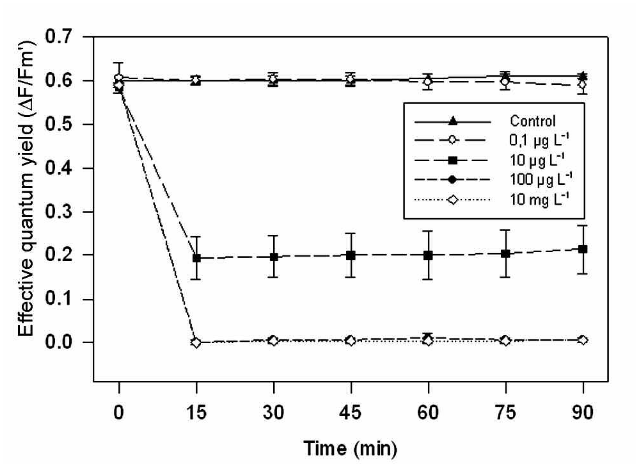

Figure 2. Variations of effective quantum yield of PSII of microphytobenthic communities during exposure to different diuron concentrations (mean ± SD, n = 3).

Figure 2. Evolution du rendement quantique du PSII ($\Delta F/F_m$') au cours du temps en fonction de différentes concentrations de diuron (moyenne ± écart-type, n = 3)

Effect of mixtures of atrazine and diuron

Nine concentration combinations (0, 0.1, 10 µg L^{-1}) were tested and the effective quantum yield of PSII variations during exposure to these mixtures are shown on Fig. 3.

The mean control value (0.612 ± 0.002) was very stable along the experiment. No decrease occurred for the mixtures 0.1 µg L^{-1} atrazine + 0 µg L^{-1} diuron and 0 µg L^{-1} atrazine + 0.1 µg L^{-1} diuron. Mixtures consisting of 10 µg L^{-1} atrazine + 0 µg L^{-1} diuron, 0.1 µg L^{-1} atrazine + 0.1 µg L^{-1} diuron and 10 µg L^{-1} atrazine + 0.1 µg L^{-1} diuron led to a small (around 7%) decrease of $\Delta F/F_m$'.

For mixtures of 0 µg L^{-1} atrazine + 10 µg L^{-1} diuron and 0.1 µg L^{-1} atrazine + 10 µg L^{-1} diuron a larger decrease (around 59%) was observed while the largest reduction (67%) in the effective quantum yield of PSII was noticed for the mixture 10 µg L^{-1} atrazine + 10 µg L^{-1} diuron.

Values of IR, the interaction ratio, that indicates the level of interaction between herbicides, are given in Table 1.

IR was very close to 1 for the mixture with the highest concentrations (10 µg L^{-1} Atrazine + 10 µg L^{-1} Diuron), only slightly < 1 for the mixture containing 10 µg L^{-1} and high for the low herbicide concentrations in mixture.

Table 1. Interaction ratio* calculated for the different tested mixtures.

Tableau 1. Rapport d'interaction* estimé pour les différents mélanges d'herbicides.

Diuron (µg L^{-1})	Atrazine (µg L^{-1})	
	0.1	**10**
0.1	4.31	0.81
10	0.92	1.01

*defined in text / défini dans le texte

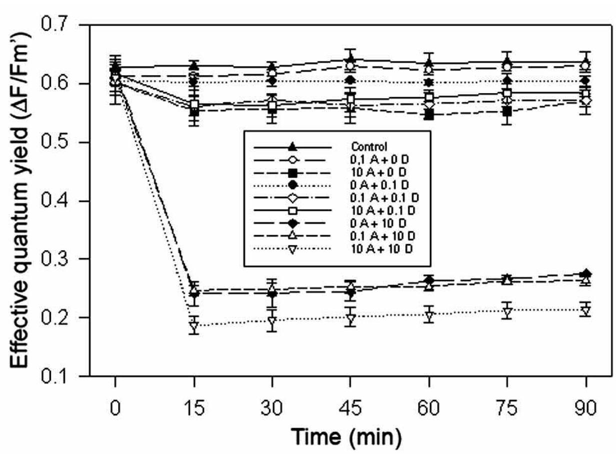

Figure 3. Variations of effective quantum yield of PSII of microphytobenthic communities during exposure to different combinations of atrazine and diuron (mean ± SD, n = 3). Concentrations of each group are given in Table 1.

Figure 3. Evolution du rendement quantique du PSII ($\Delta F/F_m$') au cours du temps en fonction de différents mélanges d'atrazine et de diuron (moyenne ± écart-type, n = 3).

Discussion

Results of our experiments showed that atrazine and diuron effects were completed within a very short time of exposure of benthic diatoms (c.a. 15 min). Atrazine is well known for its fast uptake (Nikkilä et al., 2001; Guasch et al., 2003), although other studies in seagrasses and corals showed that the reduction of the photosynthetic capacity can be completed only after a few days, even if most of the effect was observed within 1h (Ralph, 2000; Jones et al., 2003). The range of inhibiting atrazine concentrations found in this study ranged within those reported in other experiments on microalgae or aquatic plants. There was no visible short-term effect for the 0.1 µg L^{-1} concentration and a small effect for 10 µg L^{-1} to be compared to the detection limit of the atrazine effect in a range 3-16 µg L^{-1} reported by El Jay et al. (1997) and Hartgers et al. (1998).

Diuron appeared to be more toxic than atrazine for similar concentrations as often described in the literature (El Jay et al., 1997; Hartgers et al., 1998; Ralph, 2000; Jones et al., 2003). The 0.1 µg L^{-1} diuron treatment had no significant short-term effect on microphytobenthos photosynthesis, but the effect was consequent for higher concentrations. The smallest detectable effect has been previously reported to be in a range 1-4.5 µg L^{-1} (El Jay et al., 1997; Hartgers et al., 1998; Jones et al., 2003).

Our results on mixture effects showed different levels of interaction depending on herbicides concentrations. According to Gisi (1996), IR values < 0.5, in the range 0.5-1.5 and > 1.5 indicate antagonistic, additive and synergistic effects, respectively. It thus appeared that the mixed lowest concentrations has a clear synergistic effect (IR = 4.31), while the other have an additive effect according to that classification. However, considering the combinations where one herbicide is at the higher concentration tested in mixture (10 µg L^{-1}), no additional effect, comparatively to the effect of this single herbicide in the same concentration (Figs. 1 & 2), could be detected (Fig. 3). This could be due to the similar way of inhibition of the two herbicides: atrazine and diuron behave the same way on photosynthetic processes, by blocking the electron transport chain of Photosystem II (PSII) which produces oxygen from the photochemical oxidation of water (El Jay et al., 1997). Excitation energy generated by PSII is thus lost as heat or fluorescent light, and it is not used for CO_2 assimilation within the Calvin cycle. There are few studies on the combined effect of different herbicides or herbicides and other contaminants such as metals on marine aquatic plants and microalgae. Most of them concerned diuron and showed a so-called “diuron effect” that seems to decrease the toxicity of some metals like copper (Teisseire et al., 1999) or some herbicides like chlorophtalim (Okhi et al., 1997) or oxyfluorfen (Geoffroy et al., 2002). This interaction is likely due to different modes of action of the involved contaminants, that is not the case for atrazine and diuron and explains why in this study only the mixtures with the lowest concentrations of atrazine and diuron present a synergistic effect, and the weak additive effect for the others.

Due to excessive herbicides loads applied in agricultural practices, high levels of herbicides, up to 200 µg L^{-1} can be found in some surface waters (Solomon et al., 1996). In rivers, herbicides concentrations are usually much lower but can still reach values that have a detectable effect on microalgae photosynthesis (Battaglin & Goolsby, 1999). In our study area, the very few data available concern mainly atrazine and its degradation products brought by the Charente river in Marennes-Oléron Bay. Their concentrations along the Charente estuary is only controlled by dilution processes (Munaron, 2004) and estimated at a level in a range 0.4 to 1 µg L^{-1}. However, all along the shore of Marennes-Oléron Bay and Aiguillon Bay extend large surfaces of saltmarshes that are progressively drained and used for pastry and now for corn, cereals or sunflower cultivation. In Marennes-Oléron Bay, water draining these fields have higher levels (2-7 µg L^{-1}), that may be extremely high (up to 140 µg L^{-1}) in a few situations, and lead to 1-1.5 µg L^{-1} values in the upper part and 0.5-0.7 µg L^{-1} values in the lower part of the main effluent channel (Anras, 1997). Some of these values are in the range of detectable short-term effects on microphytobenthic photosynthesis but on the mudflats benthic diatoms encounter much lower concentrations. At high tide, atrazine concentration in the seawater above intertidal mudflats is ten-fold lower. Benthic diatoms are more directly concerned by herbicides present in the sediment interstitial waters or adsorbed on clay particles, but the few measurements presently available show that herbicides levels are even lower, around 0.05 µg L^{-1} for atrazine, 0.1 µg L^{-1} for its degradation products and 0.02 µg L^{-1} for diuron (Munaron, pers. com.).

From our results, these concentrations should not have any short-term effect on microphytobenthic photosynthesis. However, we cannot exclude that these levels can induce other effects on microphytobenthos at a larger scale. These algal communities include species in a very large size range, that could have different responses to herbicides as shown for phytoplankton and periphyton species (Seguin et al., 2001; Bérard et al., 2003; Weiner et al., 2004). This could lead to changes in community structure by selecting herbicide-resistant species (Hartgers et al., 1998; Leboulanger et al., 2001; Seguin et al., 2001; Seguin et al., 2002) with diverse production capabilities (Bester et al., 1995). This may have consequences on primary productivity that could propagate to the trophic levels of microphytobenthos consumers. Other contaminants present in the environment, such as metals like copper or zinc which are found in quantity in the studied mudflats, could also interact with herbicides (Teisseire et al., 1999; Guasch et al., 2003).

Considering atrazine and diuron effects only on effective quantum yield of PSII in short-term experiments could underestimate their overall effect on microphytobenthic production. In particular, it has been shown that microphytobenthos production may be thermoinhibited on intertidal mudflats where temperature may be as high as 34°C in the surficial layers in summer (Guarini, 1998). Such inhibition could be enhanced by even low concentrations of contaminants. In a same way, intertidal benthic diatoms keep their full photosynthetic capability even at the high irradiance levels encountered at the mud surface at low tide (> 2000 µmol.photons.$m^{-2}.s^{-1}$), but only during a limited period of time, that is estimated to 90 min (Blanchard et al., 2004), but that could be reduced by the presence of herbicides. Some environmental parameters such light and heavy metals have been shown to interact with herbicide effects

(Guasch & Sabater, 1998; Guasch et al., 2003) and in case microphytobenthic responses to extreme light and temperature are altered, even by small levels of herbicides, drastic changes in primary production could occur.

Acknowledgements

This work was partly funded by the CNRS Programme "Environnement Vie et Sociétés" 02N55/0004. We want to thank 2 anonymous referees for valuable comments on the manuscript.

References

Anras L. 1997. Influence du réseau hydraulique sur la qualité des eaux de surface dans un marais littoral agricole: rôle des processus biogéochimiques à l'interface eau-sédiment. Thèse de Doctorat, Université de Poitiers, 179 pp.

Battaglin W.A. & Goolsby D.A. 1999. Are shifts in herbicide use reflected in concentration changes in Midwestern rivers? *Environmental Science and Technology*, **33**: 2917-2925.

Bérard A., Dorigo U., Mercier I., Becker-van Slooten K., Grandjean D. & Leboulanger C. 2003. Comparison of the ecotoxicological impact of the triazines Irgarol 1051 and atrazine on microalgal cultures and natural microalgal communities in Lake Geneva. *Chemosphere*, **53**: 935-944.

Bester K., Hühnerfuss H., Brockmann U. & Rick H.J. 1995. Biological effects of triazine herbicide contamination on marine phytoplancton. *Archives of Environmental Contamination and Toxicology,* **29**: 277-283.

Blanchard G.F., Guarini J.-M., Dang C. & Richard P. 2004. Characterizing and quantifying photoinhibition in intertidal microphytobenthos. *Journal of Phycology,* **40**: 692-696.

DeNoyelles F., Kettle D.W. & Sinn D.E. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. *Ecology*, **63**: 1285-1293.

Dorigo U. & Leboulanger C. 2001. A pulse-amplitude modulated fluorescence-based method for assessing the effects of photosystem II herbicides on freshwater periphyton. *Journal of Applied Phycology,* **13**: 509-515.

El Jay A., Ducruet J.-M., Duval J.-C. & Pelletier J.P. 1997. A high-sensitivity chlorophyll fluorescence assay for monitoring herbicide inhibition of photosystem II in the chlorophyte *Selenastrum capricornutum*: Comparison with effect on cell growth. *Archives für Hydrobiologie,* **140**: 273-286.

Genty B., Briantais J.-M & Baker N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica Biophysica Acta,* **990**: 87-92.

Geoffroy L., Teisseire H., Couderchet M. & Vernet G. 2002. Effect of oxyfluorfen and diuron alone and in mixture on antioxidative enzymes of *Scenedesmus obliquus*. *Pesticide Biochemistry and Physiology*, **72**: 178-185.

Gisi U. 1996. Synergistic interaction of fungicides in mixtures. *Phytopathology*, **86**: 1273-1279.

Guarini J-M. 1998. Modélisation de la dynamique du microphytobenthos des vasières intertidales du basin de Marennes-Oléron. Effets des synchroniseurs physiques sur la régulation de la production. Thèse de Doctorat, Université Pierre et Marie Curie (Paris 6), 177 pp.

Guasch H. & Sabater S. 1998. Light history influences the sensitivity to atrazine in periphytic algae. *Journal of Phycology*, **34**: 233-241.

Guasch H., Admiraal W. & Sabater S. 2003. Contrasting effects of organic and inorganic toxicants on freshwater periphyton. *Aquatic Toxicology*, **64**: 165-175.

Hartgers E.M., Aalderink G.H., Van den Brink P.J., Gylstra R., Wiegman J.W.F. & Brock T.C.M. 1998. Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms. *Aquatic Ecology,* **32**: 135-152.

IFEN. 2001. Rapport 2001. Les pesticides dans l'eau. www.ifen.fr

Jones R. J., Muller J., Haynes D. & Schreiber U. 2003. Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia. *Marine Ecology Progress Series,* **251**: 153-167.

Kromkamp J., Barranguet C. & Peene J. 1998. Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. *Marine Ecology Progress Series,* **162**: 45 -55.

Leboulanger C., Rimet F., Hème de Lacotte M. & Bérard A. 2001. Effects of nicosulfuron on freshwater microalgae. *Environment International,* **26**: 131-135.

Munaron D. 2004. Etude des apports en herbicides et nutriments par la Charente : modélisation de la dispersion d'atrazine dans le bassin de Marennes-Oléron. Thèse Doctorat, Université P. & M. Curie, 344 pp.

Nikkilä A., Paulsson M., Almgren K., Blanck H. & Kukkonen J.V.K. 2001. Atrazine uptake, elimination and bioconcentration by periphyton communities and Daphnia magna: effects of dissolved organic carbon. *Environmental Toxicology and Chemistry,* **20**: 1003-1011.

Ohki A., Ohki S., Koizumi K., Sato Y., Kohno H., Böger P. & Wakabayashi K. 1997. Phytotoxicity caused by peroxidizing herbicides is alleviated by 2-substituted 4,6-bis(ethylamino)-1,3,5-triazines. *Journal of Pesticide Science*, **22**: 309-313.

Parkhill J.-P., Maillet G., Cullen J.J. 2001. Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. *Journal of Phycology*, **37**: 517-529.

Pratt J.R., Melendez A.E., Barreiro R. & Bowers N.J. 1997. Predicting the ecological effects of herbicides. *Ecological Applications,* **7**: 1117-1124.

Ralph P. J. 2000. Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll *a* fluorescence. *Aquatic Botany,* **66**: 141-152.

Riera P. 1995. Origine et devenir de la matière organique dans un écosystème estuarien à vocation conchylicole. Utilisation du traçage isotopique naturel. Thèse de Doctorat, Université Bordeaux 1, 213 pp.

Riera P. & Richard P. 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine Bay of Marennes-Oléron. *Estuarine, Coastal and Shelf Science,* **42**: 347-360.

Riera P., Richard P., Grémare A. & Blanchard G. 1996. Food source of intertidal nematodes in the Bay of Marennes-Oléron (France), as determined by dual stable isotope analysis. *Marine Ecology Progress Series*, **142:** 303-309.

Seguin F., Le Bihan F., Leboulanger C. & Bérard A. 2002. A risk assessment of pollution: induction of atrazine tolerance in phytoplankton communities in freshwater outdoor mesocosms, using chlorophyll fluorescence as an endpoint. *Water Research,* **36:** 3227-3236.

Seguin F., Leboulanger C., Rimet F., Druart J.C. & Bérard A. 2001. Effects of atrazine and nicosulfuron on phytoplankton in systems of increasing complexity. *Archives of Environmental Contamination and Toxicology,* **40:** 198-208.

Solomon K.R., Baker D.B., Richards R.P., Dixon K.R., Klaine S.J., LaPoint T.W., Kendall R.J., Weisskopf C.P., Giddings J.M., Giesy J.P., Hall L.W. & Williams W.M. 1996. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry,* **15:** 31-76.

Teisseire H., Couderchet M. & Vernet G. 1999. Phytotoxicity of diuron alone and in combination with copper or folpet on duckweed (*Lemna minor*). *Environmental Pollution*, **106:** 39-45.

Weiner J.A., DeLorenzo M.E. & Fulton M.H. 2004. Relationship between uptake capacity and differential toxicity of the herbicide atrazine in selected microalgal species. *Aquatic Toxicology*, **68:** 121-128.