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# Unicellular algae used as biosensors for chemical detection in Mediterranean lagoon and coastal waters

Claude Durrieu*, Houssemeddine Guedri, Franck Fremion, Laurence Volatier

*ENTPE, Université de Lyon, rue Maurice Audin, 69518 Vaulx-en-Velin cédex, France*

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**Abstract**

Lagoons and coastal waters are contaminated by a large number of chemicals discharged directly or carried by rivers and runoff water that drain catchment areas in which agricultural activities take place. The inflow of these exogenous compounds constitutes a genuine risk for the health of ecosystems. It is therefore important to detect their presence in the natural environment before they cause irreversible damage.

Here we present a study aimed at developing a tool for rapid detection of pesticides and other chemicals in environments liable to be contaminated, in order to propose an early warning system for decision-makers. The study carried out focuses on two herbicides commonly encountered in the environment, i.e. diuron and glyphosate, as well as several of their photodegradation products (DCPU, DCPMU, AMPA). The results presented contribute toward developing a biosensor based on measuring the metabolic activities of immobilized unicellular marine algae. The sensor's operation is based on measuring the esterase localized on the external membrane of the algae cells and chlorophyll fluorescence. The tests carried out show that the signal emitted by the sensor is disturbed by the presence of the two herbicides studied. The system proposed appears useful as a tool for controlling environments requiring monitoring.


*Keywords:* Marine algae; Herbicides; Esterase activity; Chlorophyll fluorescence; Biosensors

## 1. Introduction

Mediterranean lagoon and coastal waters are exposed to different types of chemical contaminants carried by continental waters, runoff resulting from rainfall episodes and direct discharges. Pesticides make up a large amount of this pollution (Hüskes and Levsen, 1997). Due to their strong affinity for organic matter, they accumulate in sediments and can return to the water column due to disturbance by strong winds and heavy swells (Grémare et al., 2003). They are also often present in the surface microlayer where they reach high concentrations (García-Flor et al., 2005). The compounds maintained on the surface are subject to intense photodegradation processes capable of generating compounds whose toxicity can sometimes be higher than that of the initial product (Barcelo and Hennion, 1997; Landry et al., 2005). Therefore, all these exogenous compounds can jeopardize the health of ecosystems (Louchart et al., 2000).

Here we present a study performed in the framework of a research program focusing on the distribution, degradation and ecotoxocological impact of pesticides on microorganisms in Mediterranean lagoon and coastal waters. The study concerns the site of the lagoons of Bage and Thau and the bay of Banyuls (Languedoc Roussillon Region, southern France). A number of herbicidal compounds were identified in the surface waters of the study area, including diuron, terbuthylazine, simazine, glyphosate and two degradation products (AMPA and desethyl-terbuthylazine). Our study focuses on the two products most widely represented at the site examined, diuron and glyphosate, as well as on several of their photodegradation products (DCPU and DCPMU for diuron and AMPA for glyphosate).

* Corresponding author.
*E-mail addresses:* Claude.durrieu@entpe.fr (C. Durrieu), Houssemeddine.guedri@entpe.fr (H. Guedri), Franck.fremion@entpe.fr (F. Fremion), Laurence.volatier@entpe.fr (L. Volatier).

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The objective of this work was to develop a biosensor capable of detecting pesticides in contaminated areas. The tool developed is based on studying metabolic disturbances of immobilized unicellular marine algae. The advantage of such a system is that it permits continuous observation of the environments to be monitored. Besides the fact that it forms the basis of trophic chains, algae can accumulate large amounts of pollutants (Mc Cormick and Cairns, 1994). Therefore, they act as pertinent indicators of toxicity and for evaluating damage done to ecosystems exposed to pollutant emissions; biosensors developed using entire and non-modified unicellular algae are thus of great ecological interest (Keddy et al., 1995; Moacir et al., 2008). This is the advantage of this tool in comparison to biosensors using modified cells which can be used in the field and biosensors based on pure enzyme whose length of life is very short in a natural environment. The metabolic functions investigated hereafter are the enzymatic activity of membrane esterase, which has been highlighted in previous studies on freshwater algae with specific sensitivity to certain herbicidal compounds (Chouteau et al., 2005), and chlorophyll fluorescence, which is known to be modified by the presence of herbicides (Eullaffroy and Vernet, 2003; Vedrine et al., 2003).

After describing techniques for immobilizing algae used in sensor design, we first present results obtained from bioassays on free algae before testing the response of algae immobilized on biosensors. Tests on free algae, which are very easy to implement, are carried out on microplates and permit carrying out large series of tests that show the general trend of the effects caused by the different compounds investigated. The comparison of results obtained from free algae and biosensors enables evaluating the viability of algae following immobilization. This leads to a discussion on the advantages of pursuing the development of these tools in view to monitoring natural environments in situ.

## 2. Materials and methods

### 2.1. Algal cultures

Two species of unicellular marine algae were used: *Dunaliella tertiolecta* as green algae (chlorophyceae) and *Phaeodactylum tricornutum* as diatom, which are common in marine waters They were kept under standard culture conditions (20 °C; 16 h light, 8 h dark) by bi-weekly transplanting in reconstituted saline medium (Instan Ocean at 33 g/l) enriched with silica by Guillard f/2 (Sigma) marine water enrichment solution at a concentration of 90 mg/l.

### 2.2. Herbicide solutions

Diuron, glyphosate, DCPU (N-(3,4 dichlorophenyl)-urea), DCPMU(N-(3,4 dichlorophenyl)-N-methyl urea) and AMPA (aminomethylphosphonic acid) were obtained from Sigma Aldrich. Solutions of 40 mg/l of water were prepared for diuron, DCPU and DCPMU and solutions of 10 mg/l of water for glyphosate and AMPA. The different concentrations tested were prepared using these solutions and the culture medium concentrated twice was added to the herbicide solutions at 1:1 V/V to conserve the ionic strength required to keep the algae alive.

### 2.3. Bioassays on free algae

Tests on free algae involve measurements of esterase activity. They were performed after 48 h culture, after which the algae were introduced in herbicide solutions to obtain a final mixture with a cellular concentration of 3 million cells per ml. The measurements were performed after 2 h, 24 h and 48 h of algae/herbicide contact in 96-well microplates. 100 µl of the algae/herbicide mixture was placed in each well, as were variable concentrations (0–20 µM) of fluorescein diacetate (FDA; Sigma) used as substrate by algal esterase and degraded into fluorescein, a fluorescent product (excitation 480 nm, emission 538 nm) detected with a fluorimeter (Fluostar BMG). Esterase activity was proportional to the fluorescence produced.

### 2.4. Producing the biosensors

A biosensor is an analytical device designed to convert a biochemical phenomenon into a measurable signal. It combines biological components called bioreceptors and transducers that provide means of detection (Tran-Minh, 1991, Fig. 1). Here we propose two types of biosensor based on monitoring membrane esterase activity and the photosynthetic activity of immobilized algal cells. In both cases, the bioreceptor is a suspension of algal cells. The two sensors can be distinguished by their transduction system: the first biosensor is conductometric while the second is optical.

#### 2.4.1. Conductimetric biosensor

The transducer is composed of two identical pairs of interdigitated gold electrodes sold by the Kiev Institute of Semiconductor Physics (Ukraine). The two pairs of interdigitated gold electrodes 15 nm thick were deposited on a ceramic

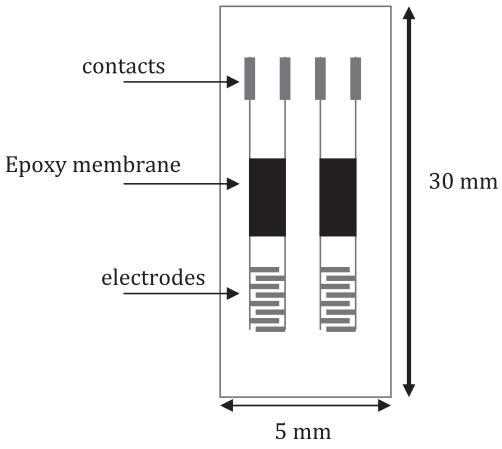


Fig. 1. Schematic representation of a conductometric algal biosensor.

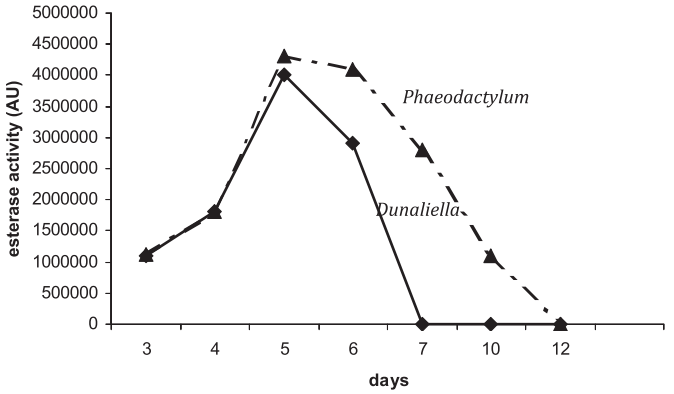


Fig. 2. Esterase activity during the different stages of the culture.

substrate. A layer Ti 50 nm thick was used to improve adhesion of gold on the substrate. The width of the fingers of the interdigitated electrodes was 10 µm, which was also the space between them, while their length was about 1 mm, providing a sensitive area on each of the two electrodes of about 1 $mm^2$. The central part was covered in epoxy resin to define the sensitive part of the sensor (Fig. 2). Measurements were based on detection of variations in conductivity of the sensitive areas linked to the movement of ions between the anode and cathode. The activity of the esterase located on the algal membrane led to catalytic reactions producing ionic species resulting in measurable changes of conductivity (Nishida, 1979).

For this type of sensor, algae were immobilized using the self assembly monolayer (SAM) technique as described in our previous work (Guedri and Durrieu, 2008). To achieve this, interdigitated electrodes were first cleaned by ultrasound for 10 min in water, then subjected to chemical reduction by immersion in piranha solution ($H_2O_2/H_2SO_4$, 1:3 V:V). The electrodes were then rinsed with absolute ethanol, dried under a nitrogen flow and immersed in a solution of 3-mercaptopropionic acid (MPA) at 2 mM for 10 h at ambient temperature to permit formation of SAMs. MPA adsorbed on the surface of the electrode was eliminated by rinsing with water before depositing 0.5 µl of algal suspension (preconcentrated by centrifugation at 3 million cells per ml) on the electrode. The deposit was maintained for 12 h, after which the algal cells not held by the SAMs were eliminated by rinsing with distilled water. After this rigorous washing phase, analysis with a microscope showed the algae immobilized on the surface of the gold. The formation of the monolayer was controlled by cyclic voltammetry.

Measurements of activity were performed by immersing the electrode in the culture medium in which increasing concentrations of substrate (FDA) were added.

#### 2.4.2. Optical biosensor

The transducer was an optical fiber bundle (Varian) at the end of which algal cells were immobilized in a quartz fiber membrane (Millipore) by simple physical adsorption. In this case, the optical biosensor was used here to measure the chlorophyll fluorescence of the algal cells. The optical fiber bundle in contact with the algae was linked to a spectrofluorimeter and conveyed the exciting light (wavelength 640 nm), provoking emission of photons (wavelength 680 nm) redirected by collector bundles to the fluorimeter for amplification and signal processing. The sensor was operated in batch mode: the optical fiber was fixed to the membrane holding the algae immobilized by a polypropylene cell into which was introduced a solution formulated from the culture medium that also contained the compounds to be tested.

## 3. Results and discussion

### 3.1. Measurement of esterase activity at different stages of culture

Esterase activity was monitored at different stages of culture (between 3 and 12 days) for the two species of algae studied. Fig. 3 shows that the evolution of esterase activity is the same through time for both species. Maximum activity is reached after 5 days in both cases, after which a rapid decrease was observed as the culture aged. All esterase activity measurements described below were performed on cultures between 3- and 4-days old.

### 3.2. Measurement of esterase activity on free and immobilized algae

Measurement of esterase activity performed on free and immobilized algae (using the SAMs technique) for different concentrations of FDA substrate allowed us to plot the kinetics

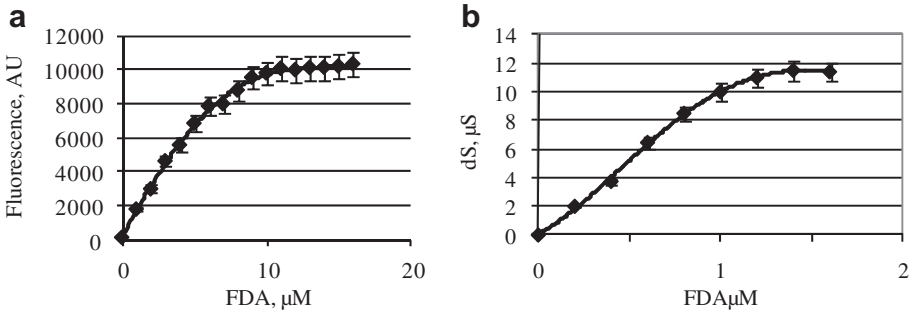


Fig. 3. Esterase activity measurement on free algae (a) and immobilized algae (b). Bars represent SD obtained with 3 replicates.

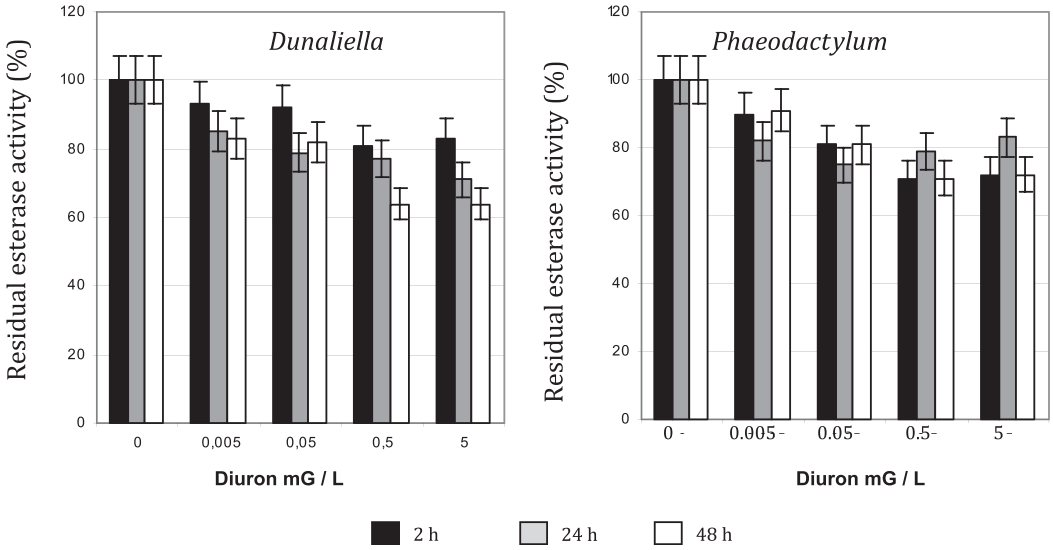


Fig. 4. *D. tertiolecta* and *P. tricornutum* esterase activity after 2, 24 or 48 h exposure at different diuron concentrations. Results are expressed as percent activity of the control. Bars represent SD obtained with 3 replicates.

linking the fluorescence produced with the concentration of substrate. Fig. 4 provides a comparison of the curve of this kinetics which in both cases can be assimilated with the Michaëlis–Menten model. Algae immobilization does not appear to affect membrane esterase activity. It should be noted, as already demonstrated by us in previous studies performed with freshwater algae (Chouteau et al., 2004), that the quantities of substrate required to obtain an optimal signal are 10 times higher for free algae than for those on biosensors. Immobilization of algae by SAMs makes it possible to obtain a monolayer of algae and work with much lower cell concentrations on biosensors. This therefore improves the sensitivity of the test. By using the SAM technique, each algal cell is exposed to the substrate in the same way whereas, when using microplate bioassays, algae at the bottom of the wells probably come into contact with lower quantities of substrate (Fig. 5).

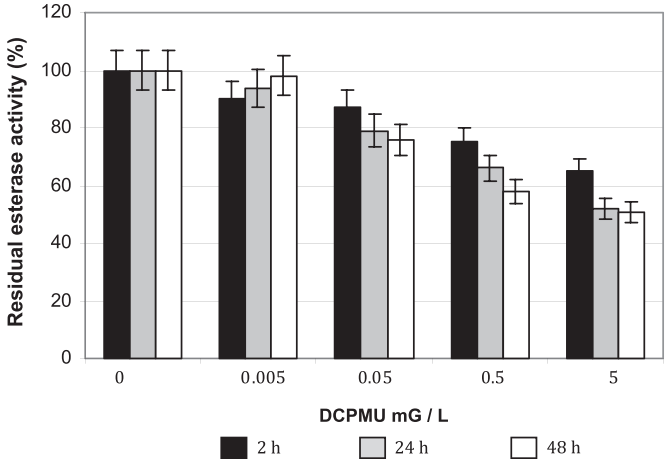


Fig. 5. *P. tricornutum* esterase activity after 2, 24 or 48 h exposure at different DCPMU concentrations. Results are expressed as percent activity of the control. Bars represent SD obtained with 3 replicates.

### *3.3. The action of herbicides on the esterase activity of free algae*

The microplate tests on free algae were initially carried out on two species of algae exposed beforehand to different concentrations of herbicides for periods of 2 h, 24 h and 48 h. Three replicates were carried out for each concentration and each time exposure. Fig. 6 shows test results obtained for diuron. They are expressed in terms of residual activity, i.e. the percentage of activity compared to a control treated under the same conditions of the test, but with the diuron replaced by the culture medium. Diuron was observed to inhibit the esterase activity of *D. tertiolecta* with an increasing dose–effect

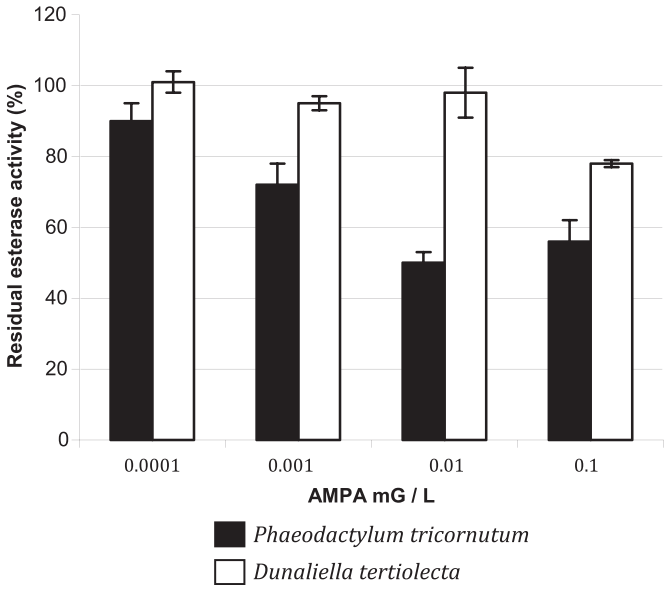


Fig. 6. *D. tertiolecta* and *P. tricornutum* esterase activity after 24 h exposure at different AMPA concentrations. Results are expressed as percent activity of the control. Bars represent SD obtained with 3 replicates.

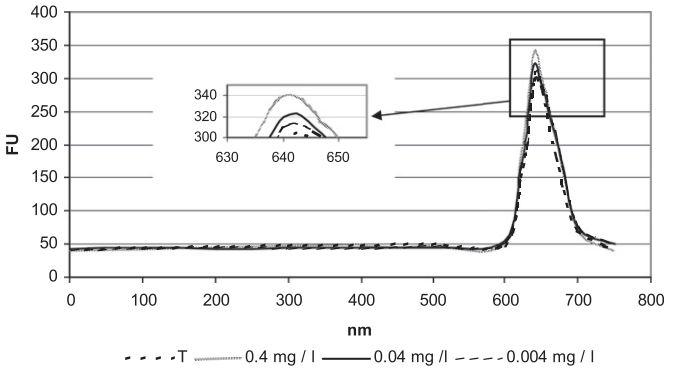


Fig. 7. Emission fluorescence spectrum, with excitation at 460 nm, of *Dunaliella* cells immobilized on an optical biosensor after exposure to different concentrations of diuron.

relationship up to a concentration of 0.5 mg/l, which led to a residual activity of 62% of the basic activity for 48 h exposure. A concentration of 5 mg/l also gives the same order of residual activity for 48 h of exposure. The rate of activity inhibition increases with time exposure as shown in Fig. 6. Thus, it seems that maximum inhibition is obtained with a concentration of 0.5 mg/l for an exposure time of 48 h. It was not possible to test for longer exposure times, since the esterase activity of *Dunaliella* decreased rapidly after 6 days of culture. The results obtained were very different for *P. tricornutum*: the concentrations of diuron tested did not reveal a dose–effect relation. The effect obtained appeared to be independent of exposure time. According to these results, *D. tertiolecta* appeared more advantageous for detecting the presence of diuron than *P. tricornutum*.

Tests were also carried out on the photodegradation products of diuron, DCPU and DCPMU. Only DCPMU appeared to have a concentration-dependent inhibiting action on *P. tricornutum* (Fig. 7), but had no marked effect on *D. tertiolecta*. Likewise, DCPU had no marked action on the two species.

The tests carried out with glyphosate highlighted considerable inhibition of esterase activity at higher concentrations tested for 48 h exposure (residual activity $< 40\%$) for the two species of algae (Table 1). The glyphosate used here was not in its Roundup® formulation linking it to the surfactant, polyoxyethylene amine (POEA), used to facilitate penetration into cells, as showed by Hedberg and Wallin (2010). The glyphosate used here was in salt form, which according to the literature, is of lower toxicity (Tsui and Chu, 2003), which might explain the minimum time of 48 h required to obtain an effect. Conversely, we observed an induction of esterase activity (residual activity $> 120\%$) for the lowest concentration of glyphosate tested. In previous studies, we highlighted induction phenomena for low concentrations of pollutants, in particular for heavy metals (Chouteau et al., 2005). This decrease in activity could correspond to detoxification mechanisms that occur for very low concentrations of toxic substance as described by Hodgson and Levi (1996).

AMPA, a photodegradation product of glyphosate, was also tested on the two species studied. After 24 h exposure, it causes considerable inhibition of activity at the highest concentrations for the two species of algae. No activity induction phenomenon was observed with this product. Therefore, AMPA appears to be more toxic than its original product, glyphosate.

These different tests show that the membrane esterase activity of the algae studied is disturbed in varying degrees depending on the algal species and the compound studied. Given the variability in results, it is estimated that inhibition of esterase activity is significant when residual activity after exposure changes by 10% versus initial activity. Fig. 6 shows that in *Dunaliella*, residual activity obtained after 24 h exposure to 5 µg/l of diuron is lower than 90% of initial activity; thus, the inhibition obtained can be considered as significant. In *Phaeodactylum*, we observed residual activity following 48 h exposure to 0.1 µg/l of glyphosate higher than 110% of initial activity. This increase in activity can also be considered as significant. In brief, it appears that the esterase activity of *Dunaliella* is an interesting marker for detecting the presence of diuron, while that of *Phaeodactylum* is more suitable for detecting DCPMU. The esterase activity of both species provides a useful marker for detecting glyphosate and its photodegradation product, AMPA.

*D. tertiolecta* therefore appears adapted to the design of a biosensor capable of detecting diuron and glyphosate.

### *3.4. Detecting herbicides with conductimetric biosensors*

The tests were carried out on sensors using *Dunaliella* cells immobilized in monolayers (SAMs) according to the protocol used in a previous study to measure alkaline phosphatase activity on freshwater algae (Guedri and Durrieu, 2008). To do this, we selected several sensors for which the variability of the signal obtained by the different electrodes does not exceed 10%. The tests were performed after 2 h and 24 h immersion

Table 1
*D. tertiolecta* and *P. tricornutum* esterase activity after 2, 24 or 48 h exposure at different glyphosate concentrations. Results are expressed as percent activity of the control.

| Glyphosate mg/l | *D. tertiolecta* | | | *P. tricornutum* | | |
|---|---|---|---|---|---|---|
| | 2H00 | 24H00 | 48H00 | 2H00 | 24H00 | 48H00 |
| 0.0001 | 102 ± 3 | 100 ± 5 | 125 ± 6 | 98 ± 4 | 104 ± 7 | 135 ± 8 |
| 0.001 | 109 ± 2 | 90 ± 4 | 65 ± 4 | 98 ± 5 | 100 ± 2 | 70 ± 4 |
| 0.01 | 98 ± 3 | 102 ± 5 | 55 ± 5 | 104 ± 4 | 99 ± 8 | 40 ± 6 |
| 0.1 | 90 ± 10 | 101 ± 8 | 46 ± 5 | 99 ± 7 | 95 ± 8 | 42 ± 3 |
| 1 | 95 ± 9 | 104 ± 6 | 38 ± 10 | 92 ± 5 | 96 ± 7 | 34 ± 4 |

Table 2
*D. tertiolecta* esterase activity detected with conductometric biosensor after 2 and 24 h exposure at different herbicide concentrations. Results are expressed as percent activity of a control biosensor. Duplicates were realized for diuron and AMPA.

| [C] (mg/l) | Diuron | | Glyphosate | | AMPA | |
|---|---|---|---|---|---|---|
| | 2H00 | 24H00 | 2H00 | 24H00 | 2H00 | 24H00 |
| 1 | 42/50 | 30/57 | 98 | 102 | 65/72 | 40/20 |
| 0.1 | 75/82 | 66/60 | 95 | 90 | 75/56 | 78/62 |
| 0.01 | 80/95 | 72/83 | – | – | 82/68 | 72/61 |

of the sensors in solutions of diuron, glyphosate and AMPA. The control sensors were immerged for 2 h and 24 h in the culture medium. The results are expressed in the same way as for the tests performed on free algae, by comparing the residual activity obtained with the control sensors treated under test conditions.

Table 2 summarizes all results obtained for the compounds tested. They are consistent with the results obtained for free algae. Inhibition caused by diuron is greater than that caused to free algae. We obtained a residual activity of barely 30% for one of the sensors exposed to a concentration of diuron of 1 mg/l. This result could be explained by the low number of algae immobilized (monolayer) on the sensor. In this case, all the algae were exposed to the same concentration of diuron, whereas for bioassays, algae at the bottom of the microplate wells may not have been in contact with the toxic compound (Fig. 4). We have already mentioned this point by comparing the concentrations of substrate required to obtain a detectable signal between the bioassays and the biosensors. No notable effect was observed for glyphosate, contrary to AMPA, which led to significant inhibition after only 24 h exposure.

Given the variability of the results obtained, it will be necessary to test a larger number of sensors with lower concentrations of toxic compounds in order to determine the detection thresholds for each product. It is necessary to develop sensors using *P. tricornutum* to confirm the interest of this tool and identify the possible specific responses of each sensor.

### 3.5. Detecting diuron with an optical biosensor

The optical biosensor was also produced using *Dunaliella* cells, since the esterase activity of this species had been shown to be sensitive to diuron. Many studies have been performed on the action of herbicides on photosynthesis and its associated mechanism, chlorophyll fluorescence. In previous works, we demonstrated the interest of this type of sensor for detecting atrazine in freshwater (Vedrine et al., 2003).

It shows a fluorescence emission spectrum generated by excitation at 460 nm. Maximum emission was observed at 640 nm. The spectrum was produced after exposing the sensor to different concentrations of diuron (0.4, 0.04 and 0.004 mg/l). A positive linear relationship between fluorescence and the concentration of diuron was observed. Thus the optical sensor based on chlorophyll fluorescence measurement appears interesting for detecting diuron in aquatic environments. Measurements of other herbicide compounds will permit comparing detection thresholds and possible specific characteristics of these different tools.

In conclusion, this study highlights the action of two herbicides commonly found in the environment (diuron and glyphosate) on two specific metabolic activities of unicellular algae (esterase activity localized on the external membrane of the cells and chlorophyll fluorescence). The tests on free algae, performed on two species of algae, showed that the metabolic activities tested can be used as pertinent markers for detecting herbicide compounds studied in this work. The two species respond differently from each other. Therefore, it would be interesting to test other species in a future study in order to identify other possible specific characteristics.

The biosensor tests were only performed on one of the two species used for the tests on free algae. They showed that the activity of the algae did not appear to be affected by immobilization and the tests performed following exposure of the sensors to herbicide compounds gave results in agreement with results obtained with free algae. It could also be presumed that the response of the sensors accurately reflects the metabolism of the algae in the natural environment. The strongest concentrations of herbicides tested were high and do not reflect the values found in the environment, contrary to the lowest concentrations tested which are regularly found and which produce here an effect on enzyme activities. These initial tests enabled us to assess the response potential of the sensors. The lowest values tested corresponded to the limit of values authorized for drinking water. The results obtained show that detection of these concentrations is possible. However, the lowest threshold has to be identified for each pollutant; further works will be necessary to do so. This first study using marine algae sensors shows the advantage of using this type of tool for monitoring the quality of lagoon and coastal waters. Furthermore, measurements were performed on full living cells and thus the results obtained are of ecological interest, providing information on potential disorders at the first level of the trophic chain, the algal compartment.

These tools remain purely qualitative and only allow determining the presence of pesticides in the environment, without giving their concentration. Their use needs to take into account possible interference of other elements in natural waters and does not provide an alternative to chemical dosing to measure the concentrations actually present in the environment. Hence, they can nonetheless be used for alerting decision markers. They present the advantage of giving rapid responses and being adaptable for in situ and continuous control. For this they require a miniaturization stage. They might then be proposed for continuous control in the field by

state control authorities, as rapid and cost-effective analytical tools for coastal surveillance. Before actual deployment, the system will need adequate adaptation, including robustness, sensitivity and long-term stability.

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## References

Barcelo, D., Hennion, M.C., 1997. Pesticides and their degradation products: characteristics, usage and environmental behaviour. Tech. Instrum. Anal. Chem. 19, 1–94.

Chouteau, C., Dzadevych, S., Durrieu, C., Chovelon, J.M., 2005. A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosens. Bioelectron. 21, 273–281.

Chouteau, C., Dzyadevych, S., Chovelon, J.M., Durrieu, C., 2004. Development of novel conductometric biosensors based on immobilized whole cell *Chlorella vulgaris* microalgae. Biosens. Bioelectron. 19, 1089–1096.

Eullaffroy, P., Vernet, G., 2003. The F684/F735 chlorophyll fluorescence ratio: a potential tool for rapid detection and determination of herbicide phytotoxicity in algae. Water Res. 37, 1983–1990.

García-Flor, N., Guitart, C., Abalos, M., Dachs, J., Bayona, J.M., Albaiges, J., 2005. Enrichment of organochlorine contaminants in the sea surface microlayer: an organic carbon-driven process. Mar. Chem. 96, 331–345.

Grémare, A., Amouroux, J.M., Cauwet, G., Charles, F., Courties, C., De Bovée, F., Dinet, A., Devenon, J.L., Durrieu De Madron, X., Ferre, B., Fraunie, P., Joux, F., s Lantoine, F., Lebaron, P., Naudin, J.J., Palanques, A., Pujo-Pay, M., Zudaire, L., 2003. The effects of a strong winter storm on physical and biological variables at a shelf site in the Mediterranean. Oceanol. Acta 26, 407–419.

Guedri, H., Durrieu, C., 2008. A self-assembled monolayers based conductometric algal whole cell biosensor for water monitoring. Microchim. Acta 163, 179–184.

Hedberg, D., Wallin, M., 2010. Effects of roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in *Xenopus laevis* melanophores. Toxicol. In Vitro 24, 795–802.

Hodgson, E., Levi, P.E., 1996. Pesticides: an important but underused model for the environmental health sciences. Environ. Health Persp 104, 97–106.

Hüskes, R., Levsen, K., 1997. Pesticides in rain. Chemosphere 35, 3013–3024.

Keddy, C.J., Grenne, J.C., Bonnell, M.A., 1995. Review of whole microorganisms bioassays: soil, freshwater sediment and freshwater sediment in Canada. Ecotox. Environ. Safe 30, 221–251.

Louchart, X., Voltz, M., Andrieux, P., 2000. Dynamique de la mobilisation et du transfert du diuron par ruissellement. Compte rendu de l'Académie des Sciences Paris 331, 475–481.

Landry, D., Fournier, J.C., Andreux, F., 2005. Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards. Environ. Pollut. 138, 191–200.

Mc Cormick, P.V., Cairns, J.J., 1994. Algae as indicators of environmental change. J. Appl. Phycol 6, 509–526.

Moacir, A., Torres, A.B., Marcelo, P., Barros, B., Sara, C.G., Campos, A., Ernani Pinto, C., Satish Rajamani, D., Richard, T., Sayre, D., Pio Colepicolo, A., 2008. Biochemical biomarkers in algae and marine pollution: a review. Ecotox. Environ. Safe 71, 1–15.

Nishida, Y., 1979. Immobilization of *Escherichia coli* cells having aspartase activity with carrageenan and locust bean gum. Enzym. Microb. Tech. 1, 95–99.

Tran-Minh, C., 1991. Les biocapteurs: principes, construction et application. Masson Ed, France.

Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197.

Vedrine, C., Leclerc, J.C., Durrieu, C., Tran-Minh, C., 2003. Optical whole-cell biosensor using *Chlorella vulgaris* designed for monitoring herbicides. Biosens. Bioelectron. 18, 457–463.