HERBICIDE MONITORING IN SURFACE WATER SAMPLES WITH A
PHOTOSYSTEM-II BASED BIOSENSOR

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Abstract: Photosystem II is the enzymatic complex supporting photosynthetic electron transport, the splitting of water and the production of ATP and NADPH in photosynthetic organisms. Recent work has demonstrated the possibility to isolate stable PSII particles to be immobilised on a Clark electrode for obtaining a biosensor with a very high sensitivity to some herbicides [1] In the present work we tried to test the practical application of the biosensor to monitor under real operational conditions. We analyzed the modification of PSII activities in various water samples. We observed that PS II activity is stimulated by 14-21% in normal water and the nature of the stimulation was attributed to the presence of divalent ions which cause a different aggregation in the membrane with consequent changes in electron transfer capacity.

Abbreviations:

\textbf{atrazine}: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; \textbf{diuron}: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; \textbf{DQ}: tetramethyl-p-benzoquinone; \textbf{ioxynil}: 4-hydroxy-3,5-diodobenzonitrile; \textbf{simazine}: 6-chloro-N,N-diethyl-1,3,5-triazine-2,4-diamine.

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INTRODUCTION

Photosystem II (PSII) is the enzymatic complex supporting photosynthetic electron transport, the splitting of water and the production of ATP and NADPH in photosynthetic organisms. For its inherent features, PSII is extremely suitable for the realization of biosensors. Upon illumination it develops oxygen, runs electrons to an acceptor and fluoresces. The simplicity of the biological transduction is, among other advantages, since the activity can be monitored directly in presence of an electron acceptor without requiring other markers or transducer molecules. Another advantage is that PSII is extremely susceptible and selective to some classes of agents which are widely used in agriculture as herbicides. Phenylurea, triazine, diazine and phenolic derivatives represent economically very important compounds since they are widely used in the chemical, pharmaceutical and agricultural industries. They represent about 50% of all herbicides used at the present time in agriculture, with a worldwide usage of many thousands of tons.

The maximum values allowed for herbicides in surface water is 0.500 ppb, which corresponds, for herbicides in the molecular weight range 200-300, to about $10^{-9}$ M. The seasonal concentrations of herbicides vary dramatically: maximum values are reached in the spring and at the beginning of summer, while winter concentrations are considerably lower than the limit imposed by law. The European Drinking Water Act (1980) does not allow concentrations of herbicides in drinking water to exceed 0.1 $\mu$g/L of any individual herbicide and 0.5 $\mu$g/L of total herbicides.

Herbicide analysis in surface, ground- and drinking-waters are generally performed by GC, HPLC, recently by Capillary Electrophoresis (CE) and, in particular, by Micellar Electrokinetic
Capillary chromatography. HPLC and CE are more useful when polar or acidic compounds have to be analyzed. Prior to analysis, water samples are pre-concentrated (typically 100-1000 folds) by Solid-Phase extraction or Liquid-Liquid extraction. The limit of detection (LOD) depends on the molecular structure of the herbicide and on the analytical and extraction technique utilized.

In HPLC studies, LODs for Diuron and Ioxynil range from $4 \times 10^{-10}$ - $4 \times 10^{-11}$ M in surface waters [4-6] to $10^{-10}$-$10^{-11}$ M in ground and drinking water [4,7,8]; LODs for Atrazine and Simazine are $5 \times 10^{-10}$-$10^{-11}$ M in surface waters [4,9] and $2 \times 10^{-11}$ - $5 \times 10^{-12}$ M in ground and drinking waters [4,7].

CE analysis has a lower sensitivity than HPLC due to the very small injection volumes (nL) although it is more efficient: Atrazine and Simazine detection limits are about $2 \times 10^{-9}$ - $5 \times 10^{-10}$ in surface waters [10, 11] and $10^{-10}$ in drinking or ground waters [12]. A LOD of $5 \times 10^{-10}$ M has been reported for Diuron [12].

About one half of the herbicides used at present in agriculture inhibit the light reactions in photosynthesis, mostly by targeting the Photosystem II (PS II) complex.

The biosensor system can reveal herbicides at the following LODs: Diuron, $5 \times 10^{-10}$; Atrazine, $2 \times 10^{-9}$; Simazine, $1 \times 10^{-8}$; Ioxynil, $9 \times 10^{-9}$ M [1], without a preconcentration step.

In a recent work we have demonstrated the possibility of realizing a quite stable PSII-based biosensor [1].

In the present work the problems met during the application of the biosensor under real operational conditions were analyzed and the biosensor application was compared with HPLC and electrophoretic capillary techniques. Successful application should lead to a versatile, easy-to-use apparatus, low cost, specific technology with a presumably lower environmental impact than many other traditional techniques for
herbicide detection, able to reveal specific herbicides and their degradation products in water.

MATERIALS AND METHODS

Biosensor assembly

PSII-biosensor assembly has been previously reported [1]. The activity of the PSII particles was revealed in a flow cell provided with a Clark's electrode. In the present study we used PSII particles from the thermophilic cyanobacterium *Synechococcus elongatus* obtained using the non-ionic detergent heptylthioglucoside.

The analyses were performed in a measuring buffer containing 15mM MES pH6.5, 5mM MgCl₂, 100mM NaCl, 0.5M mannitol, in the presence of $2 \times 10^{-4}$ M DQ (electron acceptor) and $5 \times 10^{-5}$ M CAP.

Herbicide analyses by HPLC with solid-phase extraction.

A Beckman 110B double pumping system with a 730 controller and a 340 organizer provided with an Altex injector was utilized connected with a Shimadzu SPD-M6A diode array UV-Vis detector. Elution was performed on a C18 Altima column (250x4.6 mm) (Alltech). A 250 mg Carbograph 1 cartridge (Superchrom) was utilized for SPE. All solvents were HPLC grade.

Real Water Samples
To check presence of interfering compounds in surface waters we tested the PSII-biosensor using three natural waters from Lazio region. We measured the biosensor activity, in samples taken in winter to exclude the presence of herbicides: Tiber river in Monterotondo, Aqua Marcia and Valle del Sorbo brook in Rome (Italy). Nevertheless, Tiber river water was tested for Atrazine, Simazine and Diuron herbicides content by HPLC [4]. Simazine and Diuron were not found and Atrazine was present at the level of ppt.

RESULTS AND DISCUSSION

We tested the PSII-biosensor in three river samples. To determine the possibility of interfering compounds in water samples.

![Graph](image)

**Figure 1.** Photosystem II biomediator activity during the biosensor utilization: series 1, Tiber water-buffer; series 2, deionized water-buffer

Although the PSII inactivation was exponential, the decrease of its activity during the measurement was considered linear, since the detection was carried out in a quite short time respect to the PS II lifetime.
Figure 2. Photosystem II biomediator activity during the biosensor utilization: the activity was measured during successive fluxes of buffers prepared in deionized water, Tiber river, Valle del Sorbo river, Rome Aqua Marcia. The graph indicates different activities of PSII in deionized and natural waters. The standard deviation of data obtained from replicates on the same sample was less than 3%.

Surprisingly, the use of real samples instead of deionized water to prepare the buffer, caused a PSII activation. In all tested samples, an activation in the range of 14?21% was found (see Figures 1-2).

Since it is known that PSII activity depends on the presence of charge ionic species (mainly divalent cations) and consequent membrane aggregation [2-3], we studied the possibility that the activation was due the presence of divalent or univalent cations.

We determined that the activation was in part correlable to the presence of high concentrations of divalent cations in the water (Table 1). It should be noted that the PSII-biosensor was also tested with a salinity (due to NaCl 0.2M) similar to sea water with a negligible effect on its activity (see Table 1).
Table 1. Comparison between PSII activation and water conductibility

<table>
<thead>
<tr>
<th>Water</th>
<th>Salt conc. mM</th>
<th>Conduc. mS</th>
<th>PSII Activity</th>
</tr>
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<tbody>
<tr>
<td>Deionized water</td>
<td>MgCl(_2)</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.6</td>
<td>109</td>
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<td></td>
<td>15</td>
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<td>6.4</td>
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<td></td>
<td>0.15</td>
<td>9.3</td>
<td>105</td>
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<tr>
<td></td>
<td>0.2</td>
<td>9.6</td>
<td>105</td>
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<tr>
<td></td>
<td>? 0.2</td>
<td>?9.6</td>
<td>88</td>
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Comparing the present non-traditional system for measuring herbicide in water with the current and new analytical methods above reported, this device provides some advantages, among which: a low cost, a high sensitivity to some herbicides without a preconcentration step and the possibility to use the same biological mediator for several assays after washing out the inhibitor. We found that there is no apparent inhibition in our water samples due to humic acids or the interference of unknown compounds.

Among the disadvantages, we have to consider that the system only provides the inhibitor herbicide class (photosynthetic inhibitors) but not the inhibitor chemical structure. We determined that the biosensor can be useful for herbicide analysis in sea water but attention should be paid to salt concentrations.

In conclusion, the PSII-based biosensor responds to a range of compounds and is suggested for use in the rapid screening of surface
water samples, essentially focusing laboratory analysis to determine only suspected positive samples.

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REFERENCES


