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ABSTRACT

This paper describes the prototype of an on-site High Sensitivity Early Warning Monitoring System, using Photo-Induced Fluorescence, for pesticide monitoring in natural waters (HSEWPIF). To obtain a high sensitivity, the prototype was designed with four main features. Four UV LEDs are used to excite the photoproducts at different wavelengths and select the most efficient one. Two UV LEDs are used simultaneously at each wavelength to increase the excitation power and then the fluorescence emission of the photoproducts. High-pass filters are used to avoid the saturation of the spectrophotometer and to increase the signal-to-noise ratio. The HSEWPIF prototype also employs UV absorption to detect any occasional increase of suspended and dissolved organic matter, which could disrupt the fluorescence measurement. The conception of this new experimental set-up is explained and described, then analytical applications are carried out online for the determination of fipronil and monolinuron. We obtained a linear calibration range from 0 to 3 μg.mL^{-1} with limits of detection of 1.24 ng.mL^{-1} for fipronil and 0.32 ng.mL^{-1} for monolinuron. A mean recovery of 99.2% for fipronil and 100.9 % for monolinuron show that the method is accurate, moreover a standard deviation of 1.96 % for fipronil and 2.49 % for monolinuron show that the method is repeatable. Compared to other methods for the determination of pesticides by photo-induced fluorescence, the HSEWPIF prototype has good sensitivity with better limits of detection, and good analytical performances. These results show that HSEWPIF can be used for monitoring pesticide in natural waters to protect industrial facilities against accidental contamination.

1. Introduction

Pesticides are extensively used to achieve good agricultural production yields. Since they are mostly soluble, they are leached into flowing water and pollute groundwater long after application. Pesticide residues, at levels exceeding World Health Organization guidelines, i.e. 2 to 20 ng.mL^{-1} [1] are also hazardous to human health through the consumption of contaminated food and water [2,3]. Some industrial facilities, such as drinking water production, breweries or canneries, are very exposed to an accidental rise in the concentration of pesticides in the water supply. They must

therefore be protected at all times against accidental pollution by pesticides. In this objective the present work introduces a new High Sensitivity Early Warning water quality monitoring system using Photo Induced Fluorescence (HSEWPIF) for pesticide monitoring.

Many pesticides are not fluorescent, but UV irradiation could transform them into fluorescents photoproducts, it corresponds to the photo-induced fluorescence method (PIF). This method has been often used for the analysis in natural waters of herbicides [4-7] and insecticides [8-11]. For example Gil-García et al. [12] and Mbaye et al. [13] reach limits of detection in the range 1 to 22 ng.mL^{-1} for the determination of flufenoxuron, lufenuron, hexaflumuron, triflumuron and propanyl. Bakhoum *et al.* [14] and Diaw et al. [15], have proposed a PIF method using laser irradiation to determine fenuron and diflubenzuron in natural waters while reaching detections limits of respectively 1.5, and 4.8 ng.mL^{-1}. Thiare et al. [16] have determinate fenvalerate, by classical-PIF method with satisfactory mean recovery values in the range 95-105%.

To obtain a higher sensitivity the HSEWPIF is designed with four main new features.
- Firstly, it uses new UV-C fluorescent lamp, with a higher proportion of 254 nm line than classical mercury discharge lamps [17], to form a highest quantity of photoproducts.
- Secondly, it uses four UV LEDs, at 250, 260, 280 and 300 nm to excite the fluorescent photoproducts, chosen to correspond to the optimal excitation wavelength of a majority of PIF photoproducts. The wavelength leading the highest intensity could then be keep for analysis.
- Thirdly, it uses simultaneously two UV LEDs of the same wavelength to increase the excitation power.
- Fourthly, it uses high-pass filters to cut the Rayleigh signal generated by the LEDs and avoid saturating the spectrophotometer.

The HSEWPIF prototype also employ UV absorption to detect any important increase of suspended and dissolved organic matter, as it could occur for example after a thunderstorm, which will disrupt the fluorescence measurement and lead to miss an alert.

This HSEWPIF prototype is then used to investigate fipronil (FIP) and monolinuron (MNL), naturally non-fluorescent, as examples of pesticides widely used in Senegal. The analytical performances of the method are then evaluated on natural waters and tap water.

2. Material and methods

2.1 Reagents and samples

Fipronil and monulinuron (technical-grade purity > 99%, Supplementary materials - Table 1) and methanol (analytical reagent grade) were from Sigma Aldrich (St Quentin Fallavier, France). Ultra-pure water (Millipore Mro-MQ System) was used for working solutions. For the applications we used samples of tap water from Mboro (Niayes district near Dakar, Senegal) and groundwater drawn at two meters depth from Cean agricultural area (near Mboro).

2.2 Materials

Solid phase extraction C18 cartridge (LiChrolut RP-18E 200 mg 40–63 mm). Peristaltic pump (Minipuls, Gilson, Middleton, WI, USA). Teflon tube coil (3m long, 3mm internal diameter).

Cylindrical quartz flow cell (0.5 mL volume). UV-C fluorescent compact E27 15W 240V Germicide TUV HNS 55x230mm (Bailey Electric & Electronics bv, Oosterhout, Netherlands). 250, 260, 280 and 300 nm, 1 mW UV LEDs with typical specifications 8 V and 80 mA and LED driver 'DC2200' with a current resolution of +/- 0.1 mA (Thorlabs Inc, New Jersey). High-pass filters WG295 and WG320 (IDEX Health & Sciences, West Henrietta, NY state, USA). Diode array 200–800 nm spectrometer named 'Flame' and OceanView acquisition software (Ocean Insight, Duiven, The Netherlands). Cary Eclipse fluorimeter (Agilent, Les Ulis, France). Statgraphics 19 software (The Plains, Virginia, USA).

2.3 Methods

Stock solutions were prepared in methanol (approximately 300 mg.L^{-1}), dilutions where then done in ultrapure water. All analytical application was carried out on raw natural samples, however in case of organic contaminations found in the samples, we removed them by solid phase extraction on reverse phase C18 cartridges. The cartridges were first washed by 5 mL of methanol to remove any organic traces, then rinsed by 5 mL of ultrapure water to eliminate the methanol, afterwards 50 mL of natural sample was passed throw the cartridges to remove organic contamination and then kept for analytical applications.

3. Results and discussion

3.1 Experimental setup development

The HSEWPIF prototype is describes in the Figure 1. It uses a peristaltic pump for sampling and sending it through the system. The photoreactor is composed of a Teflon tube coil (3m long, 3mm internal diameter). The speed of the pump allows adjusting the irradiation time. Then the sample passes through the detection cell (cell 1, cylindrical, 0.5 mL).

For fluorescence measurements, to obtain a high sensitivity, the prototype was setup with the following new characteristics:

- Three UV-C lamps are used as they emitted a higher proportion of 254 nm line than the classical mercury discharge lamps [17], allowing to increase the formation of PIF photoproducts. The lamps are disposed in the photoreactor centre (3 cm distance of the tube at the closest point).
- Four UV LEDs (250, 260, 280 and 300 nm, Figure 2) are disposed along the cell-1 to excite the fluorescent photoproducts and are sequentially switched on to record the four spectra. An electronic controller is used to precisely maintain the current intensity (80 +/- 0.1 mA) in order to avoid the variation of the power of the LEDs. For each LED, a check of the Rayleigh intensity is regularly performed on ultrapure water.
- At each wavelength, two UV LEDs are used simultaneously to increase the excitation power to 2 mW and enhance the fluorescence emission of the photoproducts. The two LEDs are disposed face to face on both sides of the cell-1. A total of eight LEDs are therefore used by the HSEWPIF.

- High-pass filters are disposed at the output of the optic fibre to cut the Rayleigh signal of the LEDs and avoid the saturation of the spectrophotometer. The WG295 filter (transmittance of 1% at 277nm, 50% at 299nm and 90% at 333nm), is used to cut the signal of the 250 and 260nm LEDs. The WG320 filter (transmittance of 1% at 308nm, 50% at 325nm and 90% at 360mn), is used to cut the signal of the 280nm and 300 nm LEDs (Figure 2).

- Among the four recorded spectra, the most intense is kept in order to obtain the best sensitivity.

For absorbance measurement, the three-way valves allow to bypass the photoreactor and send the sample directly through a second quartz flow cell (cell-2). The emission of UV-C lamps is collected by an optic fibre to the cell-2. Another optic fibre collects the transmitted light to the detection system. It allows to record absorbance at 254 nm without additional cost.

Detection of fluorescence and absorbance are done on the 'Flame' spectrometer, chosen for its moderate price and its sensitivity. The spectra are acquired with the following settings: Data update rate = 5 ms; Integration time = 10 s; Scans to average = 3; Electric dark correction = On. Therefore, the acquisition of one spectrum needs 30 s and the average of 3 spectra is kept as the experimental measure, consequently one experimental measurement requires about 2 mn. The spectra are then smoothed by a Savitzky-Golay procedure (+/- 3) nm. A one-minute cleaning of the flow system is subsequently performed by ultra-pure water between each sampling. The total time for a measurement can therefore be considered to be approximately 5 minutes. An irradiation time of 2 mn per sample, considering the volume of the irradiated part of the tube coil of 13 mL, need a flow rate of 6.6 mL.mn^{-1}. Thus, a triplicate measurement on each sample requires a total sample volume of 25 mL.

The prototype was designed to be integrated into a stand-alone cabinet, controlled by a touchscreen interface, and then installed near a river or water supply for automatic monitoring. The sampling frequency will be programmable by the user. An hourly or bi-hourly measurement may be sufficient for good monitoring while reducing the frequency of system maintenance.
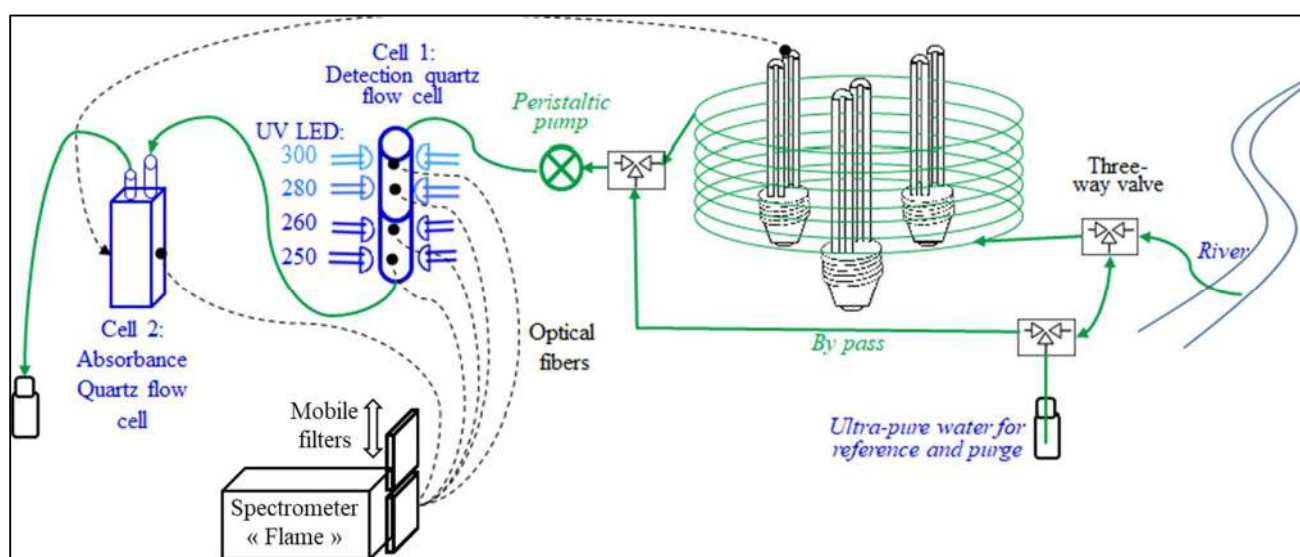

Fig. 1. Diagram of the High Sensitivity Early Warning PIF prototype.
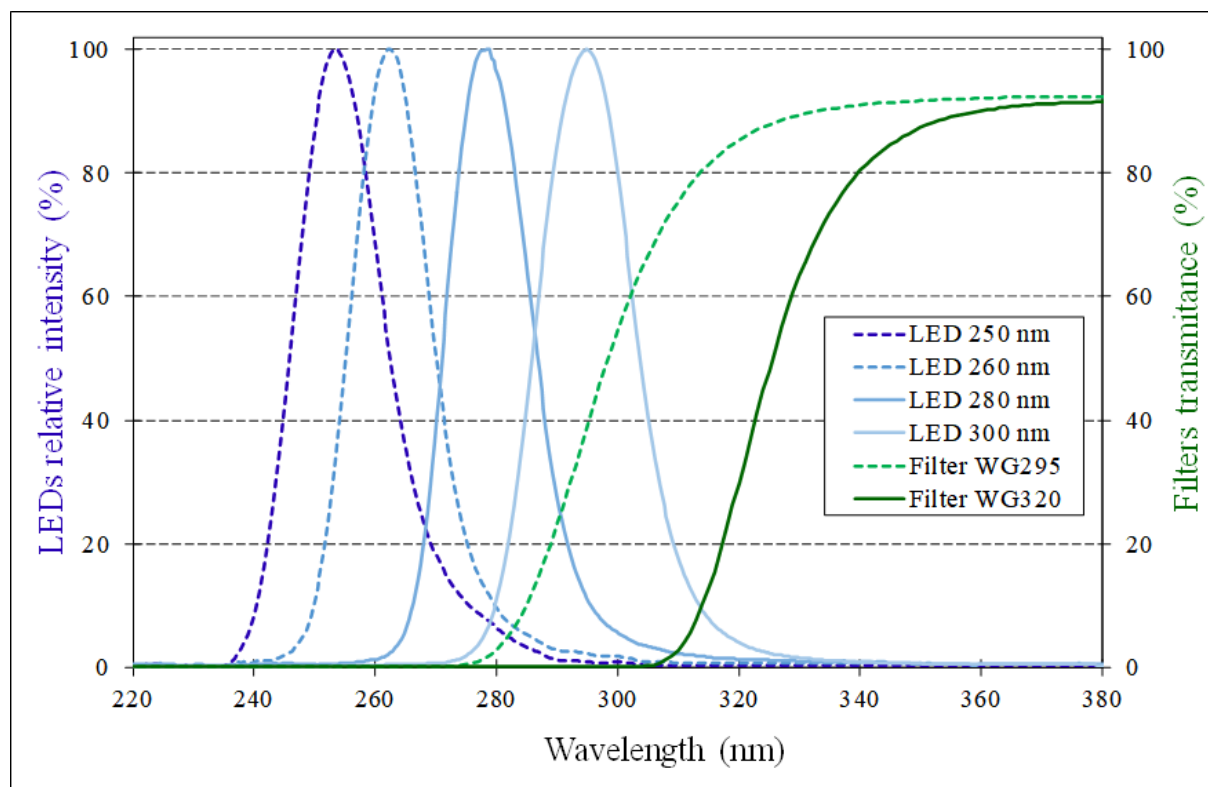
Fig. 2. LEDs emission spectrum (left Y-axis) and filters transmittance (right Y-axis)

3.2 Photo-induced fluorescence properties

The UV-C lamp are used to irradiate the pesticide in aqueous solutions (20 mL, 2 µg.mL^{-1}). The excitation spectrum (Figure 3 and Table 1) shows one band for MNL at 280 nm, and two bands for FIP at 215 and 265 nm. One PIF compound appears for both pesticides with an emission at 360 nm for MNL and and 460 nm for FIP. The amount of photoproducts becomes maximal after an irradiation of 1.5 mn for MNL and 9 min for FIP (Figure 4 and Table 1).

Fig. 3. PIF excitation and emission spectra of fipronil and monolinuron (2 µg.mL^{-1}) in water after respectively 9 and 1.5 mn of irradiation time.
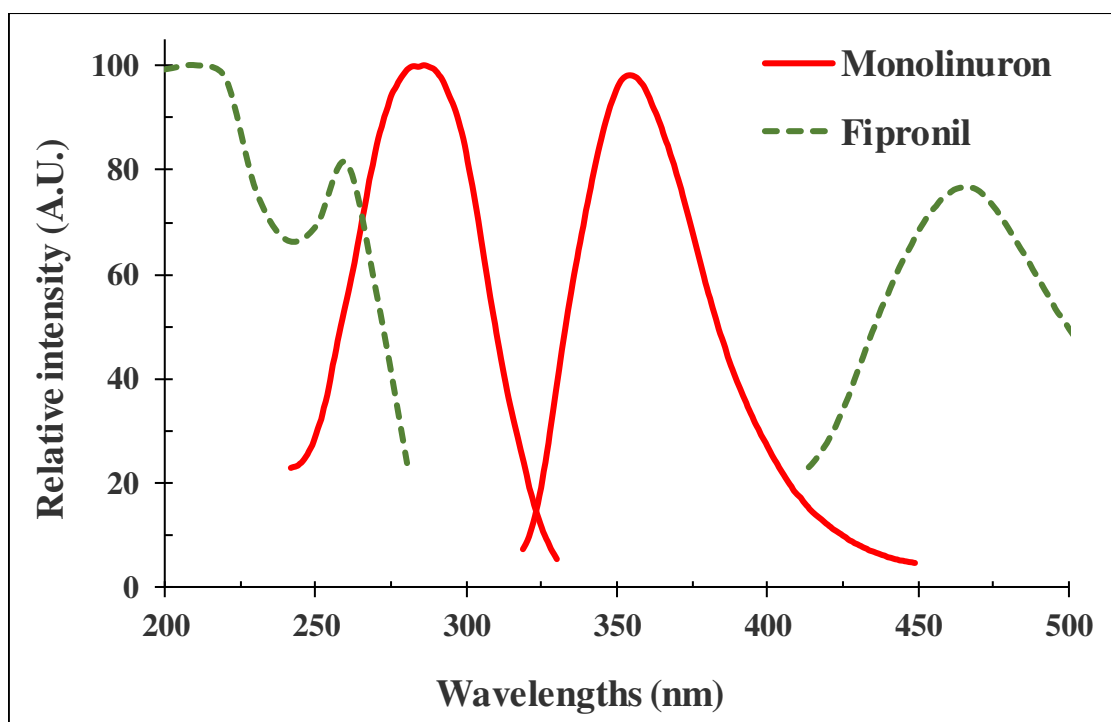

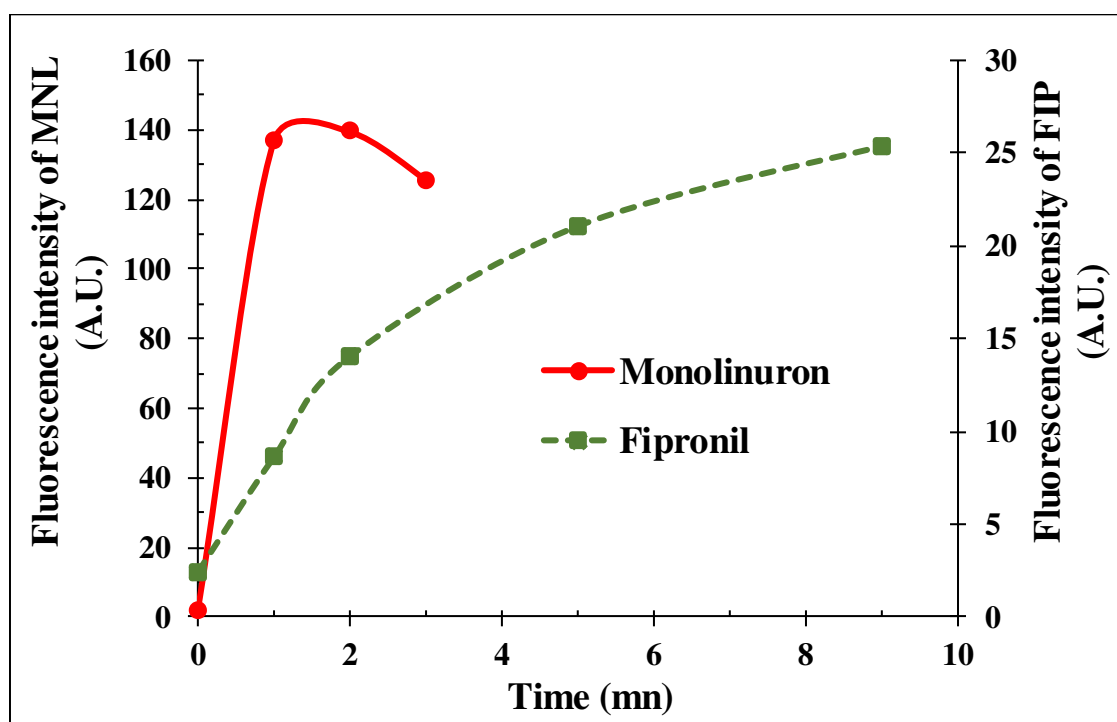**Fig. 4**. Formation of the PIF photoproducts of fipronil and monolinuron (2 µg.mL^{-1}) in water as a function of the UV irradiation time.

Table 1.

UV absorption and fluorescence properties of fipronil, and monolinuron.

Pesticides	Native pesticide absorption (nm)	Optimal irradiation time (mn)	Photoinduced compound excitation (nm)	Photoinduced compound emission (nm)
Fipronil	223; 285	9.0	215; 265	460
Monolinuron	243	1.5	280	360

3.3. Method validation and applications for pesticide determination

Experimental evaluation of new setup.

To evaluate the pertinence of the new added features we have use a full factorial experimental design 2^2 for qualitative factors [18], and we have measured the signal to noise ratio in the different experimental configurations (Supplementary material - Table 2) i.e.: with one or two LEDs (-1; +1 in coded levels); without and with high-pass filter (-1; +1 in coded levels). We have use a concentration of 3 µg.mL^{-1} for the two pesticide and the same irradiation time of 2 mn as a compromise between the optimal irradiation time of each pesticides and the acceptable duration of a measurement to perform a sufficient number of analyses per hour. The excitation wavelength selected was 260 nm for FIP and 280 nm for MNL.

The models obtained (equations 1 and 2) shows firstly that each feature uses separately improves significantly the signal to noise ratio (p-value < 5%). With one LED, adding a filter increase the signal to noise ratio from 1 135 to 2 313 for fipronil (Supplementary material - Figure 1A) and from 4 495 to 8 086 for monolinuron (Supplementary material - Figure 1C). Without filter, adding a second LED increase the signal to noise ratio from 1 135 to 2 539 for fipronil (Supplementary material - Figure 1A) and from 4 495 to 13 148 for monolinuron (Supplementary material - Figure 1C). It is worthwhile to note that, contrary to what one might think, adding the filter has an important effect on the signal-to-noise ratio, almost as great than adding the second LED.

Secondly, a positive interaction is also significant (p-value < 5%) between the two features. Adding a filter and a second LED increase further the signal to noise ratio from 1 135 to 6 375 for fipronil (Supplementary material - Figure 1B) and from 4 495to 22 875 for monolinuron (Supplementary material - Figure 1D).

These results demonstrate the pertinence to use simultaneously a high-pass filter and a second LED to increase both the sensitivity and the signal to noise ratio of the method.

Equation 1

Signal to noise ratio fipronil = -991 – 758 . FilterWG295 + 2724 . LED260
+ 1338 . FilterWG295 . LED260

Equation 2

Signal to noise ratio monolinuron = -5430 – 1275 . FilterWG320 + 11721 . LED280
+ 3068 . FilterWG320 . LED280

Photo-induced fluorescence determination and calibration

Calibration curves were done in triplicate (concentration ranges of 0.005–3 µg mL^{-1}), at an irradiation time of 2 minutes (Figure 5). The MNL lead to the most sensitive result (slope = 1098 AU.mL.mg^{-1}), while FIP was less sensitive (459 AU.mL.mg^{-1}). The calibration linearity was tested by variance analysis (Table 2). For both pesticides the regression is explicative (regression variance, V_{REG}, significantly higher than residual variance, V_{RES}, p -value ≤ 5%); and the model is descriptive (lack of fit variance V_{LOF}, not significantly higher than pure error variance V_{PE}, p-value > 5%). Moreover, the intercepts are not significantly different from zero (Table 3, Student t test, p-value > 5%). In these experimental conditions, we have obtained detection limits of 1.24 ng.mL^{-1} for FIP and 0.32 ng.mL^{-1} for MNL. However, better detection limits could be reach at the optimal irradiation time for each pesticide. With an optimal irradiation time of 9 mn, one can see on Figure 5 that the fluorescence intensity of FIP is almost the double (130 instead of 70 A.U.) and logically we obtain a LOD almost two times lower of 0.68 ng.mL^{-1}. For MNL as the irradiation time of 2 mn is close to its optimal time of 1.5 mn, consequently the improvement of the LOD is very small with 0.30 ng.mL^{-1} instead of 0.32 ng.mL^{-1}.

Fipronil **Monolinuron**
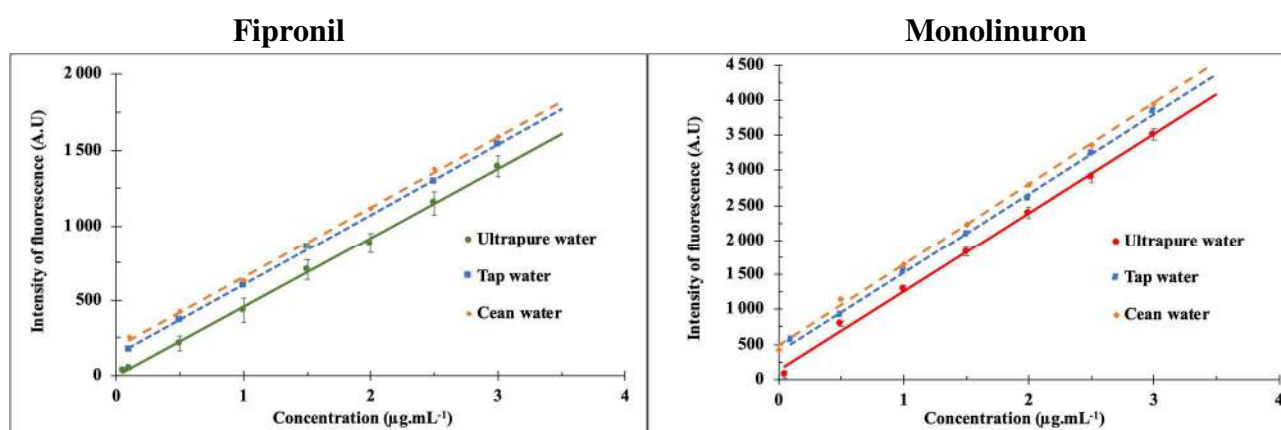
Fig. 5. Calibration and standard addition curves of fiprolil and monolinuron.

Table 2

Evaluation parameters of the linear functions by variance analysis at a confidence level of 5%.

		Monolinuron	Fiprolil
ANOVA 1	V_{REG}	8.33 10^6	2.99 10^6
	V_{RES}	4.76 10^3	2.16 10^3
	p value	0.00	0.00
ANOVA 2	V_{LOF}	6495.46	798.36
	V_{PE}	3380.04	3339.78
	p value	0.24	0.94

Analytical applications in natural matrix

We performed standard addition method on raw natural samples from Mboro city tap water (Niayes district, Senegal) and Cean agricultural district groundwater (near Mboro). The samples were fortified at 0.2 µg.mL^{-1} for FIP and MNL, then increasing concentrations of FIP and MNL were

added (Figure 5). The standard addition curves are linear (variance analysis) and parallel to the calibration curve (student t test showing no significant difference between the slopes at 5% confidence level), showing the absence of matrix effect (Table 3).

A recovery study was then conducted and the results plotted as a function of the concentration (Figure 6). This chart is a substitute of the recovery tables [19,20], the dotted lines correspond to the maximum permissible deviation (MPD, +/- 5% except +/- 10% for the two lowest calibration point), and the centre corresponds to a 100% recovery. One can see that the results obtained are within the MPD limits showing the good repeatability of the method. Moreover, for the two pesticides studded the mean recovery are close to 100%, showing the accuracy of the method (99.95 ± 3.18 and 98.44 ± 0.74 for FIP in tap and Cean waters respectively; and 99.97 ± 1.87, 101.84 ± 3.12 for MLN in tap and Cean waters respectively). These good results show that the method is well adapted for the determination of pesticides in river and tap water samples.

Table 3

Comparison of the standard addition curve and calibration curve slopes by Student t tests.

	Monolinuron			Fipronil		
	Ultrapure water	Tap water	Cean water	Ultrapure water	Tap water	Cean water
Slope	1098.46	1133.78	1153.95	459.06	467.8	465.28
Intercept	108.66	393.3	490.76	-4.82	133.57	186.47
Intercept STD	41.51	32.00	34.60	19.25	12.40	9.90
P value	0.057			0.941		
Different to 0	No			No		
LOD (ng.mL^{-1})	0.32			1.24		
LOQ (ng.mL^{-1})	0.96			3.72		
t_{DIFF}		0.211	0.269		0.159	0.081
t_{TAB} (5%)		2.145	2.145		2.145	2.145
d.o.f		11	11		11	11
Different to calibration		No	No		No	No

STD: standard deviation; d.o.f.: degrees of freedom; t_{DIFF}: calculated Student value of the difference between the two slopes; t_{TAB}: tabulated Student t value.
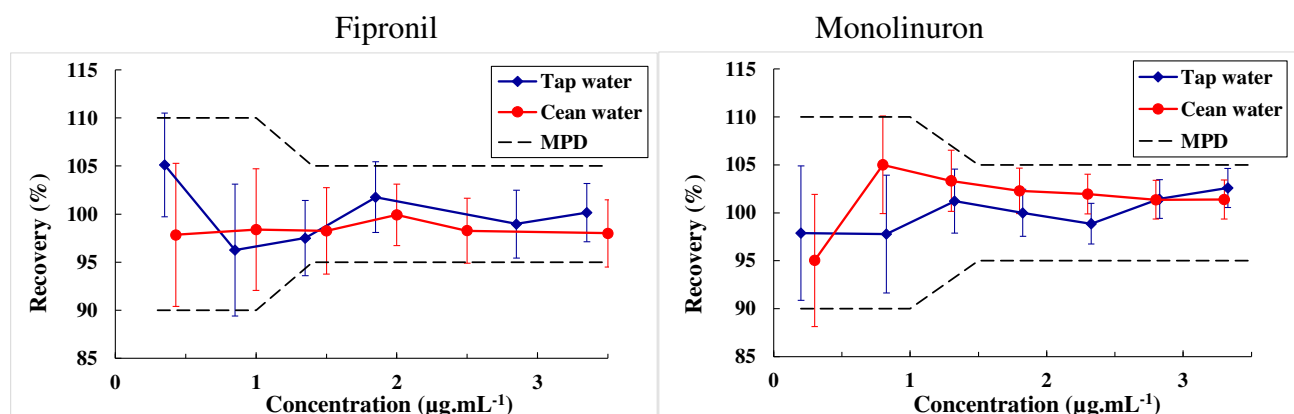
Fig. 6. Recovery plots for fipronil and monolinuron in tap water and river water. The maximum permissible deviation (MPD), corresponds to the dotted lines (+/- 5% and +/- 10% for the lowest spiked point).

Blind tests

We have also performed blind tests in triplicate at different concentrations than those uses presciently (Table 4). The concentration found are not significantly different to the added one by a Student t test. This shows that the method is accurate.

Table 4

Comparisons of test point experimental results to added values by a Student *t* test

Pesticides	Tap water					Cean water				
	Added (μg. mL^{-1})	Found (μg. mL^{-1})	t_D	$t_S(5\%)$	SD	Added (μg. mL^{-1})	Found (μg. mL^{-1})	t_D	$t_S(5\%)$	SD
FIP	0.84	0.90±0.09	1.20	2.36	NO	0.82	0.75±0.07	1.55	2.36	NO
	2.35	2.42±0.08	1.36	2.36	NO	1.80	1.79±0.08	0.14	2.36	NO
MNL	1.30	1.23±0.13	1.44	2.57	NO	1.30	1.40±0.13	1.42	2.57	NO
	2.20	2.10±0.016	1.05	2.57	NO	2.20	2.27±0.17	0.80	2.57	NO

t_D: calculated Student value of the difference between the two slopes; *t*: tabulated Student *t* value; SD significant difference

Interference study of other species

To assess the selectivity of our method, we investigated the potential influence of other species (pesticides and inorganic ions) that may be present in Senegal natural waters on the photoinduced fluorescence measurements. The tolerance limit of the interfering species is defined as the concentration for which the percentage change of the PIF signal does not exceed ± 5 % by using the following formula, where F0 and F corresponds respectively to the PIF signal recorded without and with interfering species:

$$\Delta F(\%) = \frac{F-F0}{F0}.100$$

We have a fixed concentration of, respectively, 0.043 μg mL^{-1} for fipronil, and 0.036 μg mL^{-1} for monolinuron.

To evaluate interferences from pesticides at surrounding concentrations of fipronil and monolinuron, we add increasing concentration of chlorosulfuron (0.030 à 1.80 μg mL-1), metsulfuron méthyl (0.039 à 1.90 μg mL-1), and oxadiazon (0,037 à 1.50 μg mL-1). We found no changes in the spectra shape and no shifts in the emission wavelengths of fipronil and monolinuron

in these conditions (Table 5). However, some variations in PIF intensity were observed with increasing concentration of the interfering pesticides, with tolerance limits ranging from 0.15 to 0.51 μg mL-1 in the case of fipronil and from 0.17 to 0.31 μg mL-1 in the case of monolinuron. It can be seen that a 5% variation in the signal is caused by concentrations of contaminating pesticides of the same order as those of fipronil and monolinuron, which is proportionally a significant co-contamination. It can therefore be concluded that the method remains reliable and tolerates high interference.

To evaluate interferences from inorganic ions, we add increasing concentrations of KNO_3, $CaCl_2$ and $NaNO_3$ (0,0035 à 39,00 μg mL^{-1}). The PIF fluorescence intensities of both pesticides show significant variations, with tolerance limits ranging, depending on the type of ion, from 0.15 to 1.51 μg mL-1 in the case of fipronil and from 0.21 to 5.2 μg mL-1 in the case of monolinuron (Table 5). This shows a quenching that can be significant depending on the type of ions present in the matrix, which is a classical phenomenon in fluorescence. Our method has been tested and validated on aqueous matrices from some regions of Senegal, it is obvious that it will have to be recalibrated and reevaluated for each specific aqueous matrix at its place of use, and reference spectrum has to be recorded a in absence of any organic contamination.

Table 5

Interferences stydy of other pesticides and inorganic ions, for fixed concentration of 0.33 μg mL^{-1} of fipronil, and 0.35 μg mL^{-1} of monolinuron.

Pesticides	Interference with fipronil		Interference with monolinuron	
	Concentration ranges (μg mL^{-1})	Tolerance limits (μg mL^{-1})	Concentration ranges (μg mL^{-1})	Tolerance limits (μg mL^{-1})
Fipronil	-----	-----	0.040 à 1.90	0.14
Monolinuron	0.040 à 1.80	0.33	-----	-----
Chlorosulfuron	0.040 à 1.80	0.51	0.040 à 1.80	0.31
Metsulfuron méthyl	0.039 à 1.90	0.38	0.038 à 1.90	ndi
Oxadiazon	0.037 à 1.50	0.15	0.037 à 1.50	0.17
Inorganic ions				
$K^+NO_3^-$	0.0050 à 35.00	1.51	0.0050 à 35.00	0.23
$Ca^{2+} 2Cl^-$	0.0045 à 36.00	0.15	0.0045 à 36.00	0.21
$Na^+ NO_3^-$	0.0035 à 39.00	0.25	0.0035 à 39.00	5.20

ndi : no detected interference. *Tolerance limit*: concentration of the interfering for which the percentage of variation of the PIF signal does not exceed the ± 5 %.

Absorbance control

Dissolved organic matter in river water could increase suddenly after heavy rain or thunderstorm inducing an important rise of the absorbance. In this case, it will disturb the fluorescence measurement by reducing the emission intensity. Therefore, an absorbance control is required to ensure the quality of the fluorescence measurement.

The absorbance is measure at 254 nm (the more intense emission of the UV-C lamp). A linear calibration has been obtained on dissolved organic matter (Supplementary material - Figure 2). One can see that a concentration of only 0.65 µg.mL^{-1} leads to an absorbance equal to 1; meaning that only 10% of the light pass through the sample. Therefore, in this case, the fluorescence signal of any pesticides present in the water will be reduced by 90% and the detection alert will be missed. Continuous monitoring of absorbance therefore ensures the accuracy of pesticide measurement and alerts to abnormally high levels of dissolved organic matter.

Discussion

We compared our results to literature ones by direct analysis using spectroscopic detection (UV detection, PIF detection) or after chromatographic separation coupled with spectroscopic detection or mass spectrometry (Table 6).

By focussing first to on-site technics. Using flow analysis and PIF detection our results are about ten times better than other works, for example Bakhoum et al. [11] have obtained a LOD of 5.25 ng.mL^{-1} for fluometuron and 17.45 ng.mL^{-1} for diflubenzuron; on the other hand our results are in the same range than works using flow analysis with micellar enhance PIF detection (LOD of 2.15 ng.mL^{-1} for isoproturon and 0.49 ng.mL^{-1} for flufenoxuron [23]). Other on-site technics propose to use a preconcentration step (Aquapod SPE50 [21], LOD atrazine = 0.9 ng.mL^{-1}), or an HPLC separation (SAMOS system [22], LOD simazine = 0.6 ng.mL^{-1}) and UV detection. In this case, our results are in the same limits of detection range but with a less complex system to operate.

By focussing secondly to laboratory techniques, after manual sampling and subsequent transport to the laboratory. Using direct PIF detection Bakhoum et al. [14] obtained for example LODs about ten times higher than our results (1.98 to 17.47 ng.mL^{-1} for benzoylurea pesticides); and Gil-Garcia et al. [12] obtained a LOD of 12 ng.mL^{-1} for lufenuron in river water. Other technics use chromatographic separation and different detection method. With HPLC and PIF detection, the LOD published are in the same range than our results: 0.5 ng.mL^{-1} for propanil to 3.2 ng.mL^{-1} for carboxin [25]. With preconcentration followed by HPLC and UV detection, the results publish have often very high detection limits in the range of 10 ng.mL^{-1} for diflubenzuron [26] to 80 ng.mL^{-1} for isoproturon [28] or 1000 ng.mL^{-1} For chlorofenapyr [30]. With QuEChERS extractions followed by HPLC separation and MS detection the results obtained are comparable to ours with LOD in the range of 1.4 ng.mL^{-1} for difenoconazole [32], and with ionic microextractions followed by HPLC and UV detection the results are upper to us with a LOD of 30 ng.mL^{-1} for fenvalerae [31]. The best results are obtained by QuEChERS extractions followed by GC-MS/MS, with very low limits of detections in the range of 0.015 ng.mL^{-1} (bifenthrin and permethrin [36]). However, these methods using QuEChERS extractions, chromatographic separation and mass detection are much more expensive and too complex to be use on-site for an automatic permanent monitoring.

Some recent results point to the development of very small fluorescent molecular chemosensors for the detection of pesticides. However, these chemosensors currently allow too high

limits of detection with for example 4 10^3 ng.mL^{-1} for glyphosate [37] and 30 10^3 ng.mL^{-1} for 2,4-D [38], they can also develop a fluorescent emission with other species than pesticides and their sensitivity decrease with time of use.

4. Conclusion

We have successfully developed a prototype of a high sensitivity early warning monitoring system, using photo induced fluorescence, for pesticide monitoring in natural waters. We have demonstrated that the joint use of two LEDs at each wavelength to double the excitation power and high-pass filters to cut the excitation Rayleigh, significantly improves the signal to noise ratio and decreases the detection limits. Two other features have contributed to these results: the new UV-C lamps, with a high proportion of 254 nm wavelength compared to conventional mercury vapour discharge lamps, which increase the amount of photoproducts formed; and the multi-wavelength excitation, which allows each pesticide to be excited at a wavelength close to its optimum. Moreover, the same UV-C lamps are used to perform absorbance measurements at 254 nm. The monitoring of the absorbance ensures the reliability of the fluorescence determination in case of an unusually high content in dissolved organic matter without any additional cost.

We have succeeded to obtain good performances with a moderate cost. The prototype reached LOD of 1.24 ng.mL^{-1} for fipronil and 0.32 ng.mL^{-1} for monolinuron which are lower than the WHO guideline limits (2 to 20 ng.mL^{-1}). Analytical applications and recovery study have also shown that the method is reproducible, accurate and without significant matrix effects.

We can therefore conclude that the developed prototype has good analytical performance compared to other published techniques and that we have achieved the objectives of developing a warning system, which can be applied to monitor pesticide residues in natural waters.

Table 6.
Comparison of the analytical performances of some on-site systems and laboratory systems. The table is sort by the method i.e.: on-site; laboratory after sampling. Then for each method, the table is sort by the detection technic used i.e.: PIF; laser-PIF; micellar enhanced PIF; UV; UV-DAD, MS; MS-MS; NPD and fluorescent chemosensor. At least for each detection technic the table is sort by the analysis mode i.e.: Direct analysis, flow analysis, chromatographic separation.

Pesticides	Method	Targeted matrices	Protocol	Detection technic	LOD (ng mL⁻¹)	LOQ (ng mL⁻¹)	Linear range (ng mL⁻¹)	Analysis time (mn)	Ref.
Monulinuron Fipronil	On-site	River water, Tap water, Sea water	Flow analysis automatic detector.	PIF	0.32 1.24	0.96 3.72	0 – 3000‡	10	This work
Monolinuron Fluometuron Diflubenzuron	On-site	River water, Tap water, Sea water	Flow analysis automatic detector.	PIF	2.01 5.25 17.45	8.49 16.75 55.40	0 - 2000‡	10	[11]
Fipronil Acetamipride Cyprodinil	On-site	River water, Tap water	Flow analysis automatic detector.	PIF	1.12 2.81 2.99	3.37 8.43 2.99	0 - 2500‡	10	[10]
Isoproturon Flufenoxuron	On-site	River water, Tap water	Flow analysis automatic detector.	Micellar-enhanced PIF	2.15 0.49	6.45 5.88	0 - 2000‡	10	[23]
Atrazine Isoproturon Diuron	On-site, "Aquapod SPE50" system	River water	SPE pre-concentration	UV	0.9 - 1.5‡	2.7 – 4.5‡	NP	10	[21]
Simazine Chloroluron Atrazine Isoproturon	On-site, "SAMOS" system	River water	SPE pre-concentration HPLC separation	UV	0.6 – 1.0‡	1.8 – 3.0‡	NP	30	[22]
Fipronil Acetapipride	Laboratory	Ultra-pure water	Direct analysis in water.	PIF with UV-C Lamps	NP	NP	0 – 3000‡	10	[17]
Dichlorprop	Laboratory	Tomato, Fruits (strawberries, orange, plum).	50 % (v/v) methanol and pH 5 buffer solutions	PIF correlated to photoxidation.	0.8	2.4	40 - 200	10	[24]
Fenuron Diflubenzuron	Laboratory	River water, Sea water	Direct analysis in water, methanol–water 50/50	Laser induced PIF.	1.5 4.8	4.8 16.0	0 – 10.0 0 – 8.0	3	[14]
Carboxin Monalide Propanil	Laboratory	River water, Sea water	HPLC separation.	Laser induced PIF.	3.2 3.6 0.5	10.6 12.1 1.8	0 – 5.0‡	3	[25]

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Analyte	Setting	Sample	Method	Detection	LOD	LOQ	Linear range		Ref.
Diflubenzuron	Laboratory	Ground water	On-line SPE pre-concentration, HPLC separation	PIF postcolumn derivatization	10	40	2.5 - 700	20	[26]
Isoproturon, Néburon, Linuron, Diuron	Laboratory	Tap water	Flow injection analysis.	Micellar-enhanced PIF.	450 740 330 920	690 1530 410 1510	620-10310 1370-13760 50-12500 1160-11620	15	[27]
Isoproturon Propanil Diuron Linuron Neburon	Laboratory	River water	SPE pre-concentration, HPLC separation.	PIF postcolumn derivatization	80 70 23 26 35	240 210 69 78 105	400 - 20000 400 - 20000 40 - 3200 40 – 2800 60 - 4800	20	[28]
Flufenoxuron Lufenuron Hexaflumuron Triflumuron	Laboratory	River water	Direct analysis in methanol, ethanol and 2propanol.	PIF	12 22 20 16	36 66 60 48	25-1000‡	5	[12]
Fipronil	Laboratory	Commercial phytosanitary products	Flow injection analysis	PIF	167	500	500-8000	1	[29]
Fipronil Chlorfenapyr Buprofezin Hexythiazox	Laboratory	Tap water, lake water, fountain water	Ionic liquid-liquid dispersive microextraction, HPLC separation	UV-DAD	530 1050 1120 1280	1590 3150 3360 3840	200-10000‡	20	[30]
Deltamethrin Fenvalerate Permethrin Etofenprox Bifenthrin	Laboratory	River water Lake water	Ultrasound-assisted dispersive liquid-liquid microextraction, HPLC separation	UV	60 30 60 30 60	200 100 200 100 200	200 - 500‡	30	[31]
Difenoconazole Triadimenol Metalaxyl Chlorpyriphos Propiconazole And 6 others pesticides	Laboratory	Tomato	QuEChERS extraction, HPLC separation	MS/MS	1.38 1.40 2.08 4.28 16.66 0.51 – 2.29†	4.61 4.67 6.94 11.68 38.00 1.53 – 6.83†	NP	20	[32]
Propoxur Fipronil Chlorpyrifos-methyl Diazinon	Laboratory	Honey	SPE pre-concentration, GC separation	Nitrogen–phosphorous detector	45 20 10 5	150 60 30 15	10-1000‡	30	[33]
Fipronil	Laboratory	Water, soil,	SPE pre-	MS	0.08	0.24	0.3-100	20	[34]

Pesticide	Setting	Matrix	Method	Detection					Ref
Dichlorvos Diazinon Pirimicarb Chlorpyrifos-methyl Pirimiphos-methyl And 5 others pesticides	Laboratory	Cereals	QuEChERS extraction GC separation	MS	7.0 5.5 5.0 5.8 6.0 5.0 - 9.0†	20 17 15 12 12 12 - 18†	24-6250 21-5680 21-5000 23-6250 22-5200 18-6250	30	[35]
Bifenthrin Fenpropathrin Cyhalothrin Permethrin Cyfluthrin And 5 other pyrethroid	Laboratory	Mushrooms (Oyster, Shiitake, Eryngii ; Crimini, Enoki, Buneshimej)	QuEChERS extraction, GC separation	MS/MS	0.016 - 0.038* 0.034 - 0.750* 0.023 - 1.211* 0.015 - 0.059* 0.100 - 1.070* 0.072 - 1.669†	0.051 - 5.57* 0.102 - 2.25* 0.096 - 3.63* 0.045 - 0.18* 0.300 - 3.21* 0.216 - 5.01†	NP	20	[36]
2,4-D	Laboratory	Lake water, tap water	Molecular printed fluorescence chemosensor	Fluorescence	$30 \cdot 10^3$	$90 \cdot 10^3$	NP	15	[37]
Glyphosate	Laboratory	Water	Multifunctional fluorescent chemosensor	Fluorescence	$4.22 \cdot 10^3$	$12.68 \cdot 10^3$	NP	15	[38]

NP : Not Published; * Range covering the six mushrooms studded; †: Same range for all the pesticides; ‡ : Range covering several pesticides not published in detail. ‡

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