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Impact of a pulsed light process on phytosanitary products and ecotoxicity of viticultural wastewaters

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ABSTRACT

Pulsed light (PL) treatment of three viticultural wastewaters (WWs) was performed with increasing fluence from 0 to 91 J/cm^2. The evolution of the concentrations of the pesticides was monitored by High-Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC-MS/MS). PL enabled a significant decrease in concentration of the majority of the pesticides present in the wastewaters (WW): 6 out of 12 pesticides were degraded by 62 to 92 % in WW1, 7 out of 10 by over 80 % in WW2, and the only pesticide observed, fluopicolide, was degraded by 87 % in WW3. Only four pesticides were not significantly affected by PL: ametoctradin, benalaxyl, dimethomorph and tebuconazole. PL treatment was found to be less effective on wastewater than on pesticide-spiked deionised water, likely due to the wastewater matrix components absorbing the radiation. Fifteen of the 36 targeted photoproducts were observed following PL treatment of wastewater. The main potential reactions of the halogenated compounds were dehalogenation or substitution of halogen by hydroxyl groups. A photoproduct and possibly natural metabolite of fenbuconazole (*m/z* 303) was observed in an untreated sample. Acute toxicity tests were performed on the bacteria *A. fischeri*. A significant reduction in toxicity was observed in the three tested wastewaters: by a factor of 4.1, 6.5 and 4.3 in WWs 1, 2 and 3 respectively. However, the wastewaters remained highly toxic even after the highest fluence applied, probably due to very high copper concentrations in the three wastewaters, and to the absorbance of the samples reducing the degradation efficiency of the PL. Further studies should focus on adapting PL treatment to wastewater; the first step should be a pilot study performed on a semi-industrial scale. Toxicity analyses should be carried out on various freshwater trophic levels to ensure the harmlessness of the process.

KEYWORDS: HPLC-MS/MS, acute toxicity, pesticides, transformation products, photodegradation, advanced oxidation process

INTRODUCTION

Wine production depends on the quantity and quality of the harvested grapes. However, pests and pathogens are responsible for serious damages, which have an impact on the quality and quantity of the grapes. Diseases are caused by various pathogens, such as fungi and viruses. The most common vine diseases are downy mildew, powdery mildew, grey mould, black rot and grape vine trunk disease, which can affect all parts of the plant and lead to significant yield losses. Pesticides are used to protect crops against such pests and pathogens, with numerous pesticide active substances being authorised in viticulture. After phytosanitary products have been sprayed on a vineyard plot, the sprayer is rinsed in a dedicated area and the resulting viticultural wastewater is collected for treatment. Given that, according to the International Organisation of Vine and Wine (OIV), the total area of vineyards in the world in 2022 was approximately 7.2 million hectares, viticulture is responsible for a significant volume of wastewater each year. Viticultural wastewaters are complex matrices composed of, for example, organic matter, mud, soil particles, engine oil, phytosanitary products, fertilisers and heavy metals. Concentrations of relevant compounds that are of public health and environmental concern (fertilisers, pesticides and heavy metals) range from ng/L to µg/L in non-treated viticultural wastewater (Kyzas *et al.,* 2016; Massot *et al.,* 2012).

Viticultural wastewater treatment comprises three processes: separation, biodegradation and advanced oxidation. Separation processes, such as reverse osmosis, have been shown to be highly effective for purifying and reducing the ecotoxicity of winery wastewaters (Ioannou *et al.,* 2013), but to our knowledge no study has focused on reducing the pesticide content and ecotoxicity of viticultural wastewaters. Moreover, separation processes are responsible for the production of specific concentrated industrial waste. In the case of biological treatments, while some studies have explored and shown their ability to reduce the toxicity of viticultural wastewaters (Massot *et al.,* 2012), their ability to degrade bio-recalcitrant compounds, such as pesticides and polyphenols, has been refuted in the literature (Davididou & Frontistis, 2021). On the other hand, advanced oxidation processes are receiving growing interest, in particular photochemical techniques for degrading phytosanitary products, such as photocatalysis (Deshmukh & Deosarkar, 2022) and UV-photodegradation processes (Celeiro *et al.,* 2017). These techniques are cost-effective and do not require the addition of filtration adjuvants.

Recent studies have shown that photodegradation processes are effective in breaking down pesticides and pharmaceutical compounds in aqueous solutions (Gao *et al.,* 2023; Gómez-Morte *et al.,* 2021). Photodegradation works by absorbing photons, which increases the energy level of the atomic bonds of molecules until it reaches such a high level that the molecular bonds change (photoisomerisation) or break; other groups, such as hydroxyl groups, may also be added. (Coly & Aaron, 1994; Patria *et al.,* 1995).

Among these techniques, pulsed light has demonstrated efficiency in degrading various phytosanitary products (Baranda *et al.,* 2012; Baranda *et al.,* 2014; Baranda *et al.,* 2017). Pulsed light (PL) is a non-thermal technology that relies on a capacitor to store high discharge voltages. This energy is then released as intermittent short pulses through a light source filled with xenon gas, resulting in a wide wavelength range from 200 to 1000 nm.

Recently, we reported (Clavero *et al.,* 2025) the degradation of twenty pesticides in aqueous solution using PL. By modulating the PL parameters, namely fluence and voltage, it was possible to optimise the degradation of pesticides. The gradual increase in fluence showed the gradual formation of 74 photodegradation products. Part of the photodegradation products was subsequently degraded at the highest fluence. Moreover, a high reduction in toxicity to the bacteria *Aliivibrio fischeri* occurred after PL treatment of water samples spiked with pesticides. Therefore, PL is a promising process for degrading pesticides in contaminated wastewater with a concomitant toxicity reduction.

The current study aimed at investigating the potential of using PL technology as a novel process for viticultural wastewater treatment. In such a complex matrix, interfering reactions may occur and the efficiency of the process and harmlessness of the treated samples must be checked. First, the PL degradation of pesticides in three different viticultural wastewater samples was studied using a targeted approach comprising High-Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC-MS/MS). Second, the formation and evolution of photoproducts in all three wastewaters was investigated. Finally, toxicity bioassays on the bacteria *A. fischeri* were conducted on each sample to evaluate the remediation potential of PL when used as viticultural wastewater treatment.

MATERIALS AND METHODS

1. Chemicals

Stock solutions of 88 pesticides at concentrations of between 300 mg/L and 896 mg/L (Table S1) were prepared in acetonitrile (HPLC grade, VWR International SAS, Rosny-sous-Bois, France) for each single pesticide.

Acetonitrile (ACN Optima® LC-MS grade, 100 %) used for chromatographic separation (was supplied by Fisher chemical (Illkirch, France). Ultrapure water was obtained with a Milli-Q Reference system (Merck Millipore, Fontenay-sous-Bois, France). The mobile phases used for HPLC separation were acidified with 0.1 % of formic acid (CAS RN: [64-18-6], high purity grade, 100%, from Amresco Inc., Solon, Ohio, USA).

2. Samples

Three viticultural wastewater samples were obtained from three different wine estates in the Bordeaux region: Wastewaters 1 and 2 (WW1 and WW2) were from wine estates that have a conventional vine treatment itinerary

in which synthetic pesticides are used, and Wastewater 3 (WW3) was from a vineyard that applies organic practices.

3. Pulsed light treatment

3.1 Instrumentation

An LP Box (Sanodev, Limoges, France) was utilised for the pulsed light treatments (Figure 1). Light pulses are generated by storing electrical energy in a capacitor, which is then rapidly discharged into a xenon lamp, producing the light pulses. The LP Box was operated at an optimal 4 kV and 3 Hz, as established in previous work (Clavero *et al.,* 2025). Pulsed light technology relies on the emission of polychromatic light spanning a broad emission spectrum, with the LP Box covering a range of 200 to 1000 nm. The spectral output of the LP Box at 4 kV comprised 3.2 % UV-C, 6.2 % UV-B and 19 % UV-A. These spectra were measured within a defined area at a distance of 32 cm from the lamp using an Ardop Qwave spectrometer (Pessac, France). The fluence per flash of the LP Box at 4 kV in the defined area was 0.05723 J/cm². This fluence was measured with a sensor and monitor from Thorlabs Inc. (Newton, USA). The total fluence, H, of each sample was calculated by multiplying number of flashes by fluence per flash. The treatment area comprised a rectangle measuring 5 cm by 20 cm.

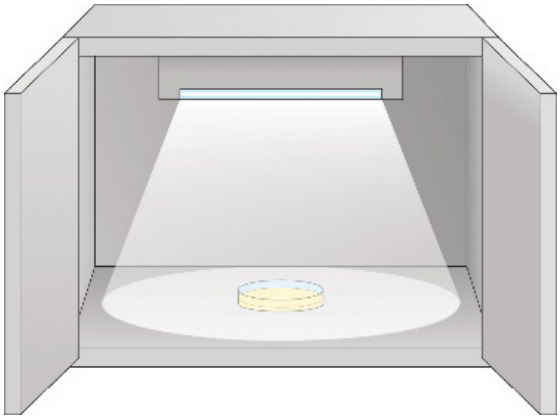


FIGURE 1. Pulsed light system (LPBox) showing lamp (top), petri dish (bottom) and delimited area (in white).

3.2 PL treatment of samples

PL treatments were performed on the three different viticultural wastewater samples. For each treatment, 10 mL of the wastewater was placed in a glass Petri dish in the centre of the delimited area. Any evaporation caused by the highest fluence treatment was compensated for by adding Milli-Q water with a maximum value of 20 % of the initial volume for the highest treatment. The maximum temperature value observed was 42 °C for the highest treatment. In the various experiments, four different fluences were applied to the three types of viticultural wastewater: 11, 22, 45 and 91 J cm². Each experiment was carried out in triplicate.

4 HPLC-MS/MS Analysis

4.1 Instrumentation

A high-performance liquid chromatograph coupled with a tandem mass spectrometer (HPLC-MS/MS) was employed to evaluate the effect of PL on non-volatile pesticides in MRM mode (Multiple Reaction Monitoring). The HPLC-MS/MS system consisted of an Agilent Technologies 1260 Infinity HPLC coupled to a 6430 triple quadrupole mass spectrometer (Massy, France).

The HPLC system comprised a 1260 binary pump, a 1260 high performance degasser, a 1260 autosampler and a 1290 thermostatted column compartment, which was maintained at 40 °C. The mass spectrometer was equipped with an electrospray ionisation (ESI) source that utilised nitrogen as a drying, nebulising and collision gas, which was generated by a Gengaz NiGen LCMS 40-1 generator (Wasquehal, France). The ESI interface operated in both positive and negative modes with a capillary voltage of 3000 V, nebuliser pressure of 40 psi, drying gas flow of 11 L/min and gas temperature of 350 °C. The system was operated using the MassHunter Workstation software version B.05.00.

Reverse-phase chromatographic separation was achieved using a mobile phase system consisting of water (A) and acetonitrile (B), each containing 0.1 % formic acid. The elution followed a gradient programme: 0-15 min, 95-0 % A; 15-25 min, 0-0 % A; 25-28 min, 0-95 % A, followed by a 6 min column equilibration period before the next injection, resulting in a total run time of 34 min. The separation was carried out on a Poroshell 120 CS-C18 column (2.1 × 150 mm, I.D. × length; 2.7 µm particle size). Electrospray ionisation was performed in positive mode (ESI^+) for all the pesticides, except for fludioxonil, which was analyzed in negative mode (ESI^-).

Quantification was achieved in MRM (Multiple Reaction Monitoring) mode as described in Clavero *et al*. (2025). The MRM transitions are reported in Table S1. The quantification of the photoproducts is only relative as no standard was available.

For the quantification, matrix-matched calibrations were performed on each highest fluence PL-treated wastewater sample spiked with a mix of 88 pesticides at 4 concentration levels (Table S1). Two ranges without dilution and two with a 10-fold dilution were performed, in order to quantify samples with high pesticide concentration; *i.e.*, out of the calibration range.

The limits of detection (LOD) and quantification (LOQ) (Table S2) were evaluated using linear regressions with compound response on the Y axis and expected concentration on the X axis based on the following formula:

$$LOQ=\frac{10 * \sigma b}{a} \qquad LOD=\frac{3 * \sigma b}{a}$$

with α = slope of the calibration line σb = standard deviation of the intercept.

In the specific case of deltamethrine, and because of the low sensitivity of the HPLC-MS/MS detection for this molecule, LOQ and LOD were determined visually from the concentrations with a peak area of 5000 and 1500 a.u. (arbitrary unit) respectively.

4.2 Sample preparation for HPLC-MS/MS quantification

Quantification of the pesticides in the different wastewater samples was performed after QuEChERS extraction (Quick, Easy, Cheap, Effective, Rugged and Safe) using the EN 15662 method. Extractions were carried out on 10 mL of wastewater sample, treated or untreated by PL, in a 50 mL Falcon® tube. First, samples were shaken with 10 mL of ACN (HPLC grade) on an IKA HS 501 digital reciprocating shaker (VWR International S.A.S, Fontenay-sous-Bois, France) at 320 rpm for 15 min. Then, the content of a pouch of citrate buffer salts (roQ QuEChERS kits AH0-9041, Phenomenex, Le Pecq, France) consisting of 4 g magnesium sulphate, 1 g sodium chloride, 0.5 g disodium hydrogencitrate sesquihydrate and 1 g trisodium citrate dihydrate was added, and the Falcon® tube was shaken again horizontally for 5 min. After centrifugation (Thermo Scientific™ Sorvall™ ST 8 Small Benchtop Centrifuge equipped with a HIGHConic III fixed angle rotor, Fisher Scientific SAS, Illkirch, France) at 3000 g for 5 min, 1 mL of the supernatant was filtered through a 0.2 µm PTFE syringe filter.

5. Toxicity bioassay

The marine bacterium *A. fischeri*, known for its bioluminescent properties, was used as the reference organism in the Microtox® assay. The Microtox test kits were obtained from Modern Water Inc. (New Castle, Delaware, the United States of America). The assays were conducted in accordance with the NF EF ISO 11348-3 guidelines (ISO, 2007). The Microtox reagents were kept at -24 °C until use, and the analyses were performed after rehydrating the lyophilised bacteria using 1 mL of Milli-Q water in test cuvettes at 5 °C.

A negative control solution was prepared with 2 % NaCl. Positive control solutions were prepared with an aqueous solution of $K_2Cr_2O_7$ (0.2 g/L) at concentrations of 25, 50, 100 and 200 mg/L in 2 % NaCl. Samples were also buffered with 2 % NaCl and the pH of each sample was checked and adjusted when necessary (with HCl or NaOH aqueous solutions) to between 6 and 8.5. The bacteria were exposed to the various test solutions for 30 min at 15 °C. The bioluminescence emitted by the bacterial culture was quantified using Modern Water's M500 Toxicity Analyzer Luminometer. Sample dilution was between 10 and 0.1 % of the initial effluent for WW 1 and WW 3 and between 50 and 0.4 % for WW 2. These ranges were used to determine the EC50s for each of the effluents. To ensure the robustness of the results, each point of each range was carried out in triplicate within a maximum of 2 hr following bacteria rehydration, in accordance with the protocol outlined by (Gómez *et al.,* 2023).

6. Spectrophotometric analysis

A Shimadzu UV 2600i spectrophotometer was used to measure the absorbance spectrum of the three WW samples. The WW samples were diluted to one tenth with Milli-Q water and then placed in a 10 mm quartz cuvette. The absorption spectrum of the samples were measured as being between 200 and 1000 nm.

7. Quantification of copper

Copper was quantified by the EPOC laboratory. The samples were first acidified with 10 % nitric acid before being analysed by ICP OES (Inductively Coupled Plasma Optical Emission Spectrometer (Spectrometer ICP-OES 700®, Agilent Technologies).

8. Statistical analysis

Data from all bioassays and pesticide quantifications were statistically analysed with a Kruskal Wallis test (p-value < 0.05) followed by a post hoc test: Fisher test with a correction of the p-value with the method Bonferroni using Rstudio 2022.02.2+485.

RESULTS AND DISCUSSION

1. Pesticide evolution with PL treatment

Three viticultural wastewaters (WW) were treated by PL at 11, 22, 45 and 91 J/cm². Treated and untreated WW samples underwent extraction using the QuEChERS EN 15662 method and quantified in LC-MS/MS by standard addition of 88 pesticides.

The quantification of each untreated sample showed major differences between the wastewaters: out of the 88 pesticides, WW1 contained 12 pesticides (Figure 2a, Table S3a), WW2 10 pesticides (Figure 2b, Table S3b) and WW3 only 1 pesticide (Figure 2c, Table S3c). It was not expected to detect any pesticides in WW3, as it was from an organic wine estate. In addition to containing the highest number of pesticides, WW1 also showed the highest concentrations of pesticides, four being over 1 mg/L, three over 500 µg/L, and five being between 20 and 100 µg/L. WW2 had only two pesticides at concentrations higher than 1 mg/L, and the other pesticides were at concentrations of between 20 and 200 µg/L. The only pesticide present in WW3 was at a low concentration (142.1 µg/L), which is probably due to its organic itinerary.

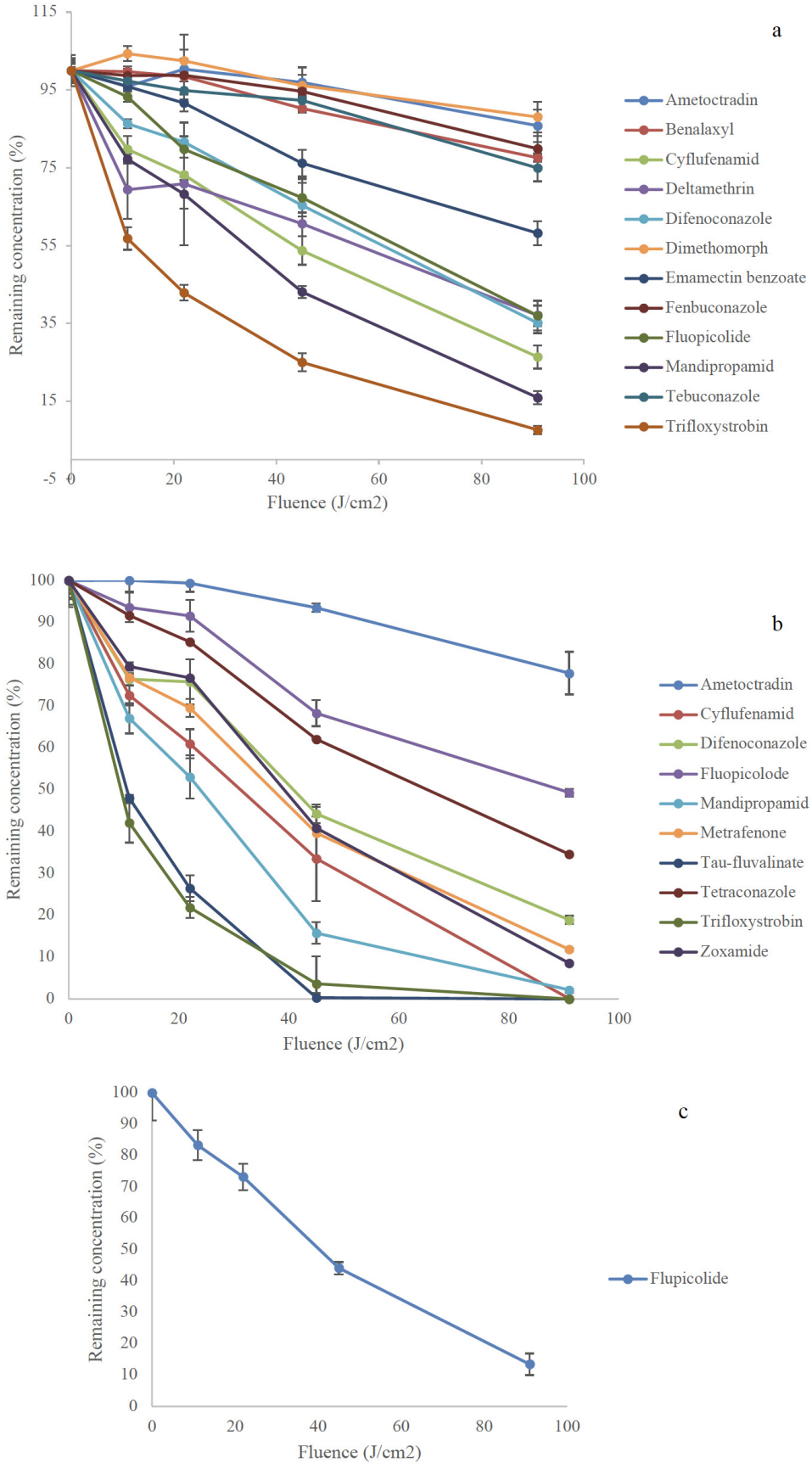


FIGURE 2. Pesticide degradation rate in (a) WW1, (b) WW2, and (c) WW3 following PL treatment at different fluences. Represented as average concentration of a triplicate ± SD.

The presence of this compound might be due to the fact that the winegrowing estate converted only recently to organic farming and to the ineffective rinsing of the retention tank or adsorption of the product on the tank walls. In the three WW samples, 16 different pesticide active substances were detected, six of which were the same in at least two WW samples. Fluopicolide was present in all three WW samples. Ametoctradin, cyflufenamid, difenoconazole, mandipropamid and trifloxystrobin were found in both the WW samples obtained from the conventional wine estates.

In all three tested wastewaters, the PL treatment led to a significant decrease in the majority of the pesticides (Kruskal Wallis followed by a post hoc test: Fisher test, p-value < 0.05), suggesting that active substances had been degraded. The gradual increase in fluence resulted in a gradual decrease in pesticide concentrations, and, as expected, the highest fluence induced the highest decrease. In WW1 (Figure 2a), 7 out of 12 compounds were degraded by over 42% (Table S3a) at the highest fluence, six of which were even degraded by between 63 and 92 %, of which two (trifloxystrobin, mandipropamid) by over 80 %. The five other compounds were degraded by between 12 % and 25 %, which is still a significant concentration reduction, particularly in the case of fenbuconazole, whose concentrations in the most heavily treated sample fell by more than 200 µg/L relative to the control sample, which is not reflected in the degradation percentage. The reduction percentage was noted to be independent of the initial concentration. WW2 (Figure 2b) showed a higher degradation ratio than WW1, with 9 out of 10 compounds being reduced by over 50 % (Table S3b), and 7 compounds by over 80 %. A significant reduction in the concentrations of fluopicolide was even observed in WW 3 (Figure 2c); *i.e.*, 87 % reduction in the most treated sample relative to the control sample (Table S3c).

Only four pesticides were not significantly affected by the PL treatment: ametoctradin, benalaxyl, dimethomorph and tebuconazole. Conversely, these compounds showed significant degradation in a previous study on Milli-Q water samples (Clavero *et al.,* 2025). This may be due to the absorbance of the wavelength responsible for the degradation of these compounds, because all the studied wastewater samples were turbid and loaded with colouring matter.

This hypothesis is corroborated by the results of Clavero *et al*. (2025) obtained from deionised water samples: the 5 aforementioned compounds had much higher degradation constants (k) and lower half fluences ($H_{1/2}$) than in the present work (Table 1). For first-order kinetics, the degradation rate constant (k) was determined by plotting the logarithm of the ratio initial concentration (C_0) to concentration at a given fluence (C_H) against total fluence (H), according to the equation $Ln(C_0/C_H) = kH$. Subsequently, the half fluence ($H_{1/2}$; representing the total fluence required for a 50 % reduction of pesticide concentration) could be calculated using the equation $H_{1/2} = Ln2/k$. At the least, the $H_{1/2}$ values for dimethomorph in wastewater were 6.5-fold those in Milli-Q water, and at the most they were 40.4-fold for fenbuconazole. This can be explained by the high absorbance of radiation, despite the experiment having been carried out on thin layers of effluent to prevent such interferences occurring as much as possible.

The different $H_{1/2}$ values revealed differences in the absorption of radiation between the three wastewater samples. In WW1, the $H_{1/2}$ values of ametoctradin, cyflufenamid, difenoconazole, mandipropamid and trifloxystrobin were approximately double those in WW2 (Table 1). Moreover, in WW2, trifloxystrobin, mandipropamid and cyflufenamid were degraded to concentrations lower than their LOQ (Table S2), whereas in WW1 no pesticides were degraded to such low levels (Table S3a and S3b); this indicates a difference in absorbance between the wastewater samples; WW1 appears to absorb more radiation than WW2, thus reducing degradation of the compounds. This is validated by the absorption spectra carried out between 200 and 1000 nm for each 10-fold diluted WW (Figure 3). The tendency differs for fluopicolide, however, with reductions of 63 % in WW1 and 51 % in WW2.

Similar to the results of Clavero *et al*. (2025), Table 1 shows significant differences in the sensitivity of the compounds to pulsed light, with $H_{1/2}$ values ranging from 19.4 for trifloxystrobin

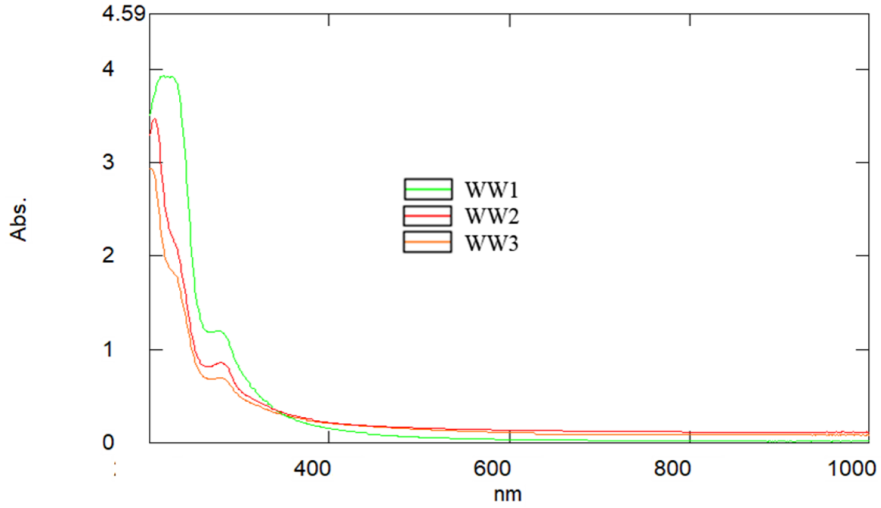


FIGURE 3. Absorbance between 200 and 1000 nm of WWs 1, 2 and 3 with 10-fold dilution.

TABLE 1. $H_{1/2}$ and k of the pesticides in the three wastewaters, WW1, WW2 and WW3.

		WW1		WW2		WW3	
Pesticide	Pesticide class	k	$H_{1/2}$	k	$H_{1/2}$	k	$H_{1/2}$
Ametoctradin	Triazolopyrimidine	0.0015	454.1	0.0027	250.4		
Dimethomorph	Cinnamic acid amide	0.0017	410.0				
Fenbuconazole	Triazoles	0.0024	287.0				
Benalaxyl	Benzylanilide	0.0029	238.8				
Tebuconazole	Triazoles	0.0030	231.3				
Emamectin benzoate	Avermectin	0.0060	113.7				
Deltamethrin	Pyrethroid	0.0102	67.5				
Fluopicolide	Benzamide	0.0104	66.6	0.0079	86.7	0.0180	36.7
Tetraconazole	Triazoles			0.0110	61.7		
Difenoconazole	Triazoles	0.0106	65.1	0.0173	40.1		
Zoxamide	Benzamide			0.0202	34.3		
Metrafenone	Benzophenone			0.0200	33.7		
Cyflufenamid	Phenylacetamide	0.0140	49.2	0.0262	26.4		
Mandipropamid	Mandelamide	0.0189	36.5	0.0350	19.8		
Tau-fluvalinate	Pyrethroid			0.0650	10.6		
Trifloxystrobin	Strobilurine	0.0356	19.4	0.0730	9.4		

to 454.1 J/cm² for ametoctradin in WW1, and from 9.4 for trifloxystrobin to 250.4 J/cm² for ametoctradin in WW2. It is interesting to note that, in WW1 and WW2, trifloxytrobin appears to be the most sensitive compound and ametoctradin the least sensitive, with fluopicolide, difenoconazole, cyflufenamid and mandipropamid (in order of increasing sensitivity) in between. In the same way, as reported in our previous study, mandipropamid was found to be slightly more sensitive than difenoconazole and much more sensitive than ametoctradin; furthermore, this sensitivity seems to be family dependent. The strobilurins were the most PL sensitive compounds in all matrices studied, followed by the mandelamides, the triazoles and the pyrethroids, and the least sensitive compounds still comprised a cinnamic acid amide and a triazolopyrimidine. This supports the hypothesis that compounds with a high number of conjugated bonds and phenyl groups are more stable than other compounds and thus less sensitive to PL treatments. Only fenbuconazole and tebuconazole showed different behaviour compared to the other triazoles; this can be explained by the fact that the matrix was able to absorb the specific wavelengths responsible for the degradation of these compounds.

Pulsed light was able to significantly degrade the majority of the compounds in three different wastewater samples even if the matrix absorbed a part of the radiation and the efficiency of pesticide concentration reduction was family dependent.

2. Evolution of photodegradation products with PL treatment

In a previous study (Clavero *et al.,* 2025), a LC-MS relative quantification method was developed to monitor the formation of 74 photodegradation products from 16 pesticides. Seven of these 16 pesticides were present in the wastewater of the present study (5 in WW1, 4 in WW2, and none in WW3), but only 15 of the 36 monitored photoproducts were detected; these were derived from benalaxyl, difenoconazole, fenbuconazole, mandipropamid, metrafenone, tebuconazole and tetraconazole. Photoproducts were only observed in WW1 and WW2, which is coherent, given the conventional treatment itinerary from which they come. At least one photoproduct was observed from each of the 7 pesticides in which degradation products were targeted using the relative quantification method, except for difenoconazole and mandipropamid in WW2, which can be explained by their low concentrations in this wastewater.

All the observed degradation products had peak areas that increased progressively over the course of the experiment (Figure 4). This result slightly differs from the one obtained previously in aqueous solutions, in which some compounds were formed and then degraded with the increase in fluence at the optimised voltage. This may be due to the loss of radiation resulting from absorption by the components of the matrices, as explained above, as well as to the various reactions that occur in a complex sample like wastewater, as hypothesised in the literature (Baranda *et al.,* 2014). Moreover, in the same way, three photoproducts (TPdifeno-388-2, TPbena-208 and TPfenbu-319-2) required higher fluences (45 J/cm^2) to be formed in WW1 compared to aqueous solutions. These results show that the previously developed relative quantification method is effective, and that it can be used to simultaneously monitor the removal of pesticides and the formation of their photodegradation products.

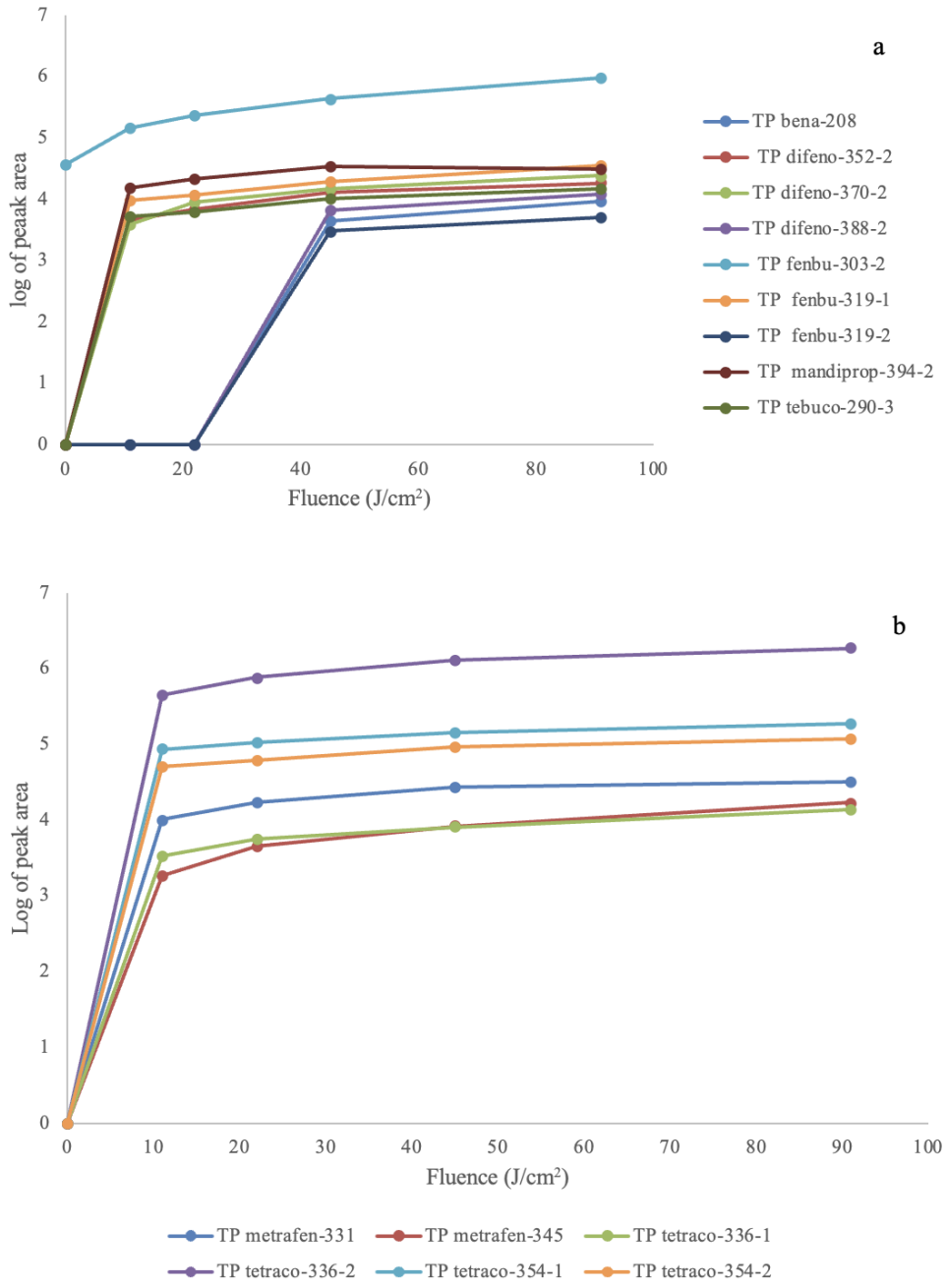


FIGURE 4. Evolution of peak area of photoproducts in (a) WW1 and (b) WW2 as a function of fluence.

The mass spectrometric analysis also shows that the mass of every studied photoproduct was generally lower than its parent compound (Clavero *et al.,* 2025). Moreover, for the most part, the observed probable reactions comprised dehalogenation or substitution of halogen by hydroxyl groups. When the compound carries more than one halogen, these reactions can reach total dehalogenation of the compound, which can lead to a reduction in toxicity, as mentioned by Baranda *et al*. (2012). Furthermore, it is highly probable that degradation products not targeted in this study were also formed.

A signal (with *m/z* = 303) observed in the untreated sample WW1 at retention time 12.34 min, which had the same fragmentation pattern as in Clavero *et al*. (2025) and which increased with fluence, indicates that a photoproduct of fenbuconazole (TP-fenbu-303-2) was already present in the untreated WW1. To our knowledge, this molecule has never previously been observed or described in the literature. It is the major compound resulting from the PL degradation of fenbuconazole in ultrapure water (Clavero *et al.,* 2025). It may therefore be possible to find this photoproduct in conditions other than by using PL treatment, given that wastewater is often stored for years before being treated, natural degradation can occur. Moreover, the high content of fenbuconazole in WW1 may have led to the formation of this compound with *m/z* = 303, even by other reactions than photodegradation. This warrants further research into the degradation of fenbuconazole *via* other processes, or even into the presence of this transformation product in food matrices treated with fenbuconazole or in water near

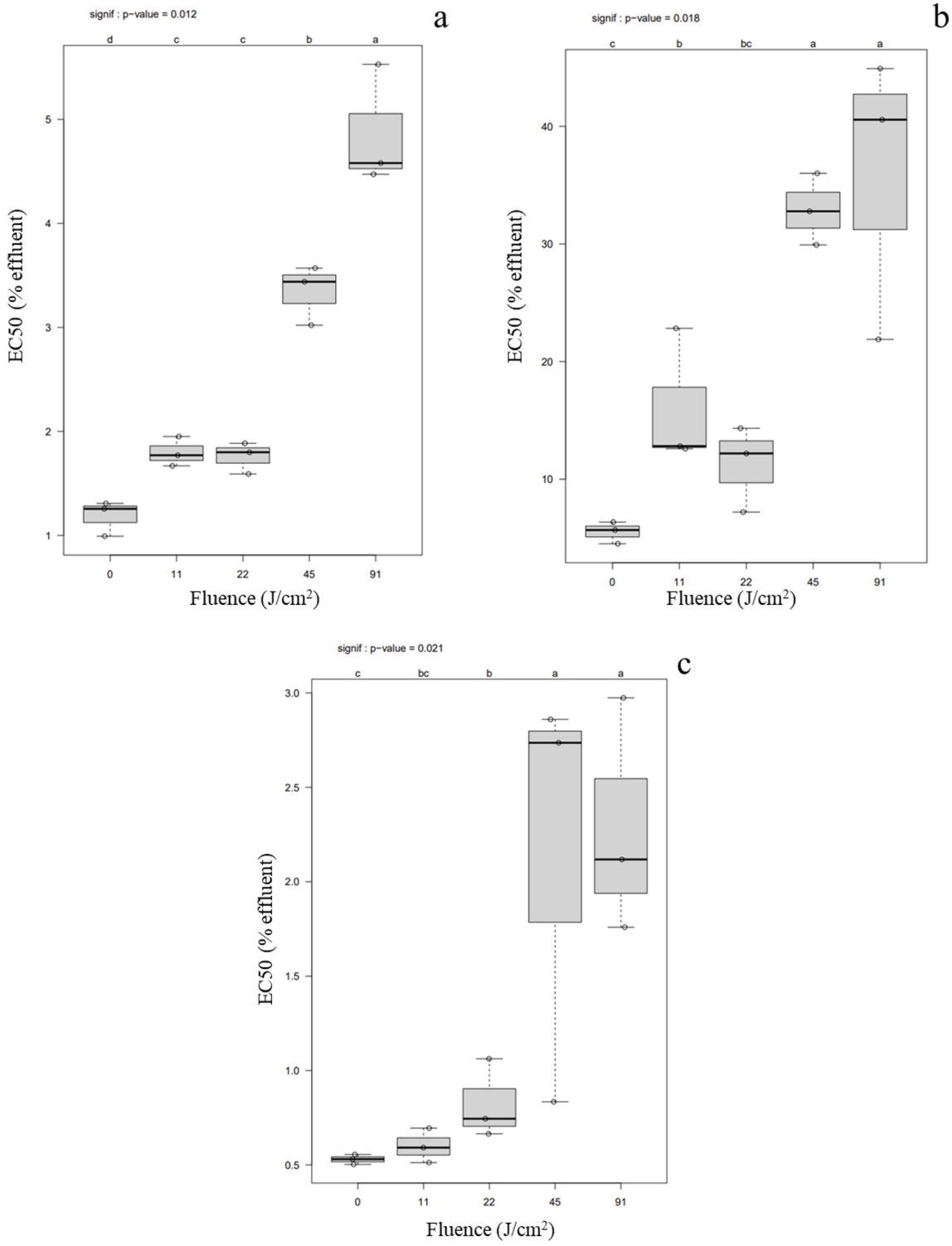


FIGURE 5. Evolution of EC50 as a function of fluence for (a) WW1, (b) WW2, (c) WW3.

TABLE 2. Copper concentrations (mg/L) in the wastewaters.

Wastewater	Copper (mg/L)
WW1	28.9 ± 3.7 (n = 2)
WW2	10.5 ± 4.1 (n = 2)
WW3	38.7 ± 7.9 (n = 3)

plots treated with fenbuconazole, as it may be an indicator of fenbuconazole degradation, which, to our knowledge, has not yet been described or monitored.

3. Toxicity assessments

The toxicity analysis reveals significant differences (Kruskal Wallis followed by the post hoc test Fisher test, p-value < 0.05) between the untreated samples and the other samples of all the tested wastewaters: the EC50 value (half maximal effective concentration) (Figure 5) indicates a progressive toxicity drop as fluence increased, even for WW3 obtained from an organic vineyard.

The EC50 of each wastewater increased gradually as the applied fluence increased, indicating that the significant reduction in toxicity to *A. fischeri* was correlated with the increase in fluence and therefore with the increase in PL treatment. Indeed, EC50 evolved inversely to toxicity level. The most highly-treated sample of each of WWs 1, 2 and 3 decreased in toxicity relative to the untreated sample by a factor of 4.1, 6.5 and 4.3 respectively. It is also interesting to note that WW2, which absorbed less radiation than WW1 according to its $H_{1/2}$ values and absorption spectrum (Figure 3), underwent a higher decrease in toxicity. This indicates that the lower the absorbance of wastewater, the higher the efficiency of the PL treatment; this could be confirmed in further studies by clarifying each sample *via* flocculation or filtration before treatment in order to reduce the absorption of light by the matrix components. The reductions in toxicity were, however, relatively similar regardless of the matrix studied, which was unexpected, as WW3 was from an organic itinerary and contained a very low quantity of pesticides compared to the other two matrices. This suggests that the drop in toxicity was not only due to a drop in pesticide concentration, but also to a decrease in concentration of other pollutants present in the matrix, wastewaters being complex matrices that can contain hydrocarbons or oils from the rinsing of sprayers. It is also possible that pulsed light had an impact on the bioavailability of copper, which is widely used in organic farming; the PL may have interacted with the organic matter in the wastewater, which may have then captured the copper, making it less available to bacteria (Kramer *et al.,* 2004).

While toxicity was reduced in all the studied matrices, it is worth noting that it was still high in the most highly-treated samples of WWs 1, 2 and 3, which is probably due to high concentrations of copper (Table 2) in these wastewaters. Indeed, *A. fischeri* is sensitive to copper, having an EC50 of 0.78 mg/L (Narciso *et al.,* 2023), and the EC50 of each WW appears to be related to copper content. WW3 (Figure 5c) was the most toxic wastewater, followed by WW1 (Figure 5a), with only 0.5 % and 1.2% of untreated effluent respectively needed to reach EC50, and with a copper content of 38.7 ± 7.9 mg/L and 28.9 ± 3.7 mg/L respectively. On the other hand, WW2 (Figure 5b) is the least toxic wastewater with 5.5 % of the initial effluent needed to reach EC50 and also displays the lowest copper concentration (10.5 ± 4.1 mg/L). The higher toxicity and copper content of WW3 might have been a result of its organic itinerary, in which the application of copper derivatives is the only way to treat for downy mildew, indicating the greater use of these compounds by this wine estate.

Nevertheless, despite the absorbed radiations, the PL treatment resulted in a drop in concentration of each pesticide and in toxicity of the effluent regardless of the sample studied (Figure 6). The drop in concentration of all the pesticides is clearly related to the increase in EC50. This corroborates the hypotheseses of Baranda *et al*. (2012) and Clavero *et al*. (2025) that the reduced toxicity of photoproducts is a result of halogen losses.

Finally, it can be concluded that the targeted chemical approach combined with an ecotoxicological bioassay made it possible to optimise and demonstrate the PL's ability to reduce pesticide content and toxicity, and to address the phenomena occurring in such a complex matrix like viticultural wastewater.

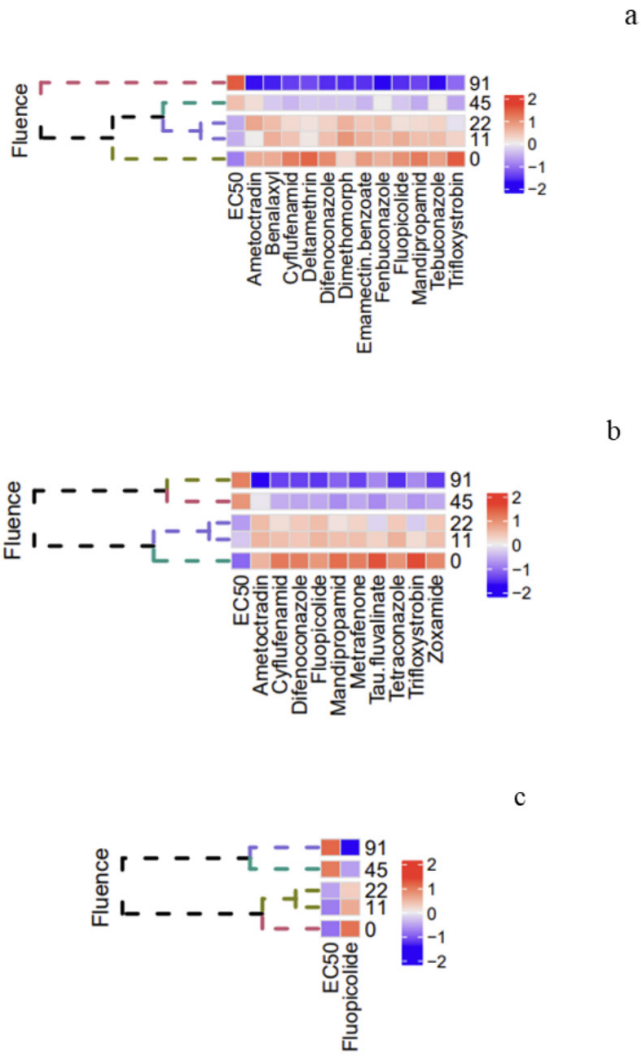


FIGURE 6. Heat map of the evolution of the monitored variables when applying fluences of between 0 and 91 J/cm² on (a) WW1, (b) WW2, and (c) WW3.

CONCLUSION

PL treatment enabled degradation of the majority of the pesticides present in the three wastewaters studied here. Although some absorption of radiation by the different matrices was observed, intense pulsed light was still highly effective, 6 out of 12 compounds being degraded by 62 to 92 % in WW1, 9 out of 10 at percentages higher than 50 % in WW2, and the only detected compound by over 90 % in WW3.

Furthermore, our results indicate that the sensitivity of the pesticides to the PL treatment may be compound-dependent and even family-dependent. The strobilurins (trifloxystrobin) were the most sensitive compounds, followed by the triazoles (difenoconazole and tetraconazole) and the pyrethroids (deltamethrin and tau-fluvalinate). The least sensitive compounds belonged to the triazolopyrimidine (ametoctradin) and cinnamic acid (dimethomorph) families. These results corroborate the findings of our previous study. However, a significant decrease in degradation efficiency was observed in this experiment because of the absorption of photons by the matrix components.

The previously developed targeted LC-MS method was used to monitor the formation and evolution of 15 of the 36 photoproducts during the experiment. It appears that the photodegradation of pesticides most often involves dehalogenation and hydroxylation reactions. In contrast to our previous work, three photoproducts showed slower formation kinetics and all the photoproducts remained stable during this experiment. This can be also imputed to the absorption of photons by the wastewater matrix components.

Moreover, the study highlighted the presence of a degradation product of fenbuconazole with *m/z* 303 that has not yet been described in the literature, and which is the main degradation product of fenbuconazole. This photoproduct may be produced under conditions other than those used in the experiment, and possibly in matrices that are in contact with fenbuconazole or even in the environment.

Finally, the toxicity assessments showed a significant decrease in toxicity of each of the studied wastewaters after PL treatment; even the organic wastewater. The toxicity decreased by a factor between 4.1 and 6.5. However, all the wastewater samples remained highly toxic, likely due to the presence of copper, which is not impacted by PL treatment.

Further studies will aim at optimising the PL process so that it can be applied at a semi-industrial scale using a continuous liquid treatment reactor in order to be able to treat thinner layers of liquid to limit the impact of radiation absorption. A pre-treatment, such as the clarification of wastewater, may be considered before applying PL to improve light penetration and avoid absorption, thus maximising degradation by PL. This type of study should also make it possible to implement more precise models in order to understand the impact of pulsed light on various pollutants (hydrocabons, pharmaceutical compounds, toxins and PFAS). Finally, another important focus of study would be the ability of PL to reduce the toxicity of viticultural wastewater taking into account different taxa and trophic levels representative of various aquatic ecosystems.

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