Supporting information of

A reliable procedure to obtain environmentally relevant nanoplastics proxies

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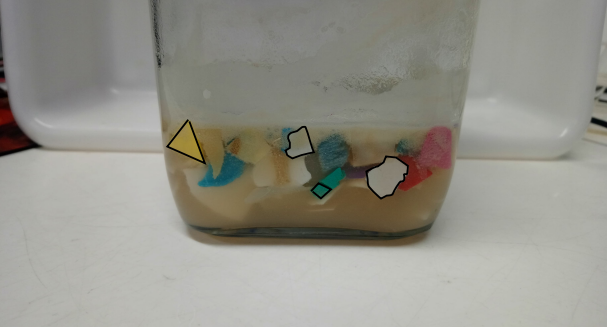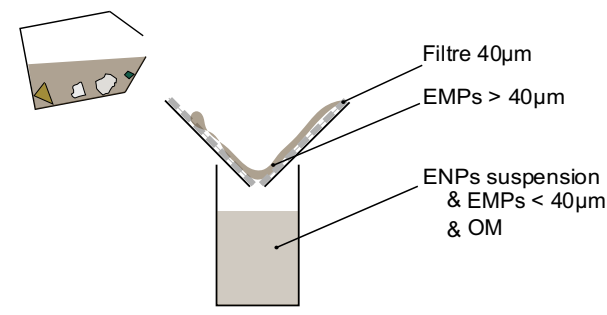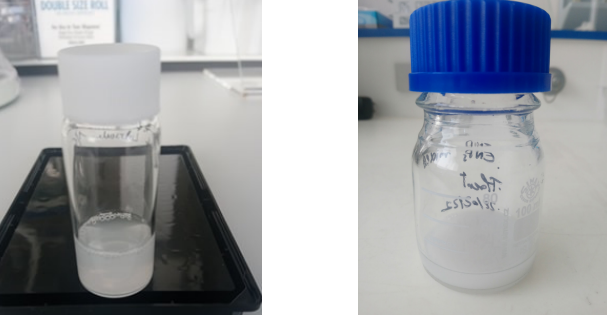
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S1. Workflow presenting the ENPs preparation protocol

1. Plastics Selection

1.a. Select plastics between 1cm < Plastics < 10 cm with non negligible weathered layer.

1.b. Add dionized water (D.I.) at a 1:2 ratio in square bottle.

2. ENPs extraction

2.a. Let agitate the suspension for 2 days at 250 rpm.

2.b. Then apply 1 hour of sonication.

3. Microplastics separation

3.a. Take away microplastics with 40 μm cut-off filter.

Filtre 40μm

EMPs > 40μm

ENPs suspension & EMPs < 40μm & OM

4. OM removing & ENPs separation

4.a. Apply UVC during 5 h prior adding H_2O_2 (1% v/v).

4.b. Separate microplastics (<40μm) from ENPs with a 1.2 μm cut-off.

4.c. Remove H_2O_2 by concentrating/diluting* with D.I.the suspension 5 times.

5. ENPs concentration

5.a. Quantify the total organic carbon

5.b. Concentrate the suspension as wanted with an ultrafiltration cell

* this operation is operated with the ultrafiltration cell

Figure S1. Workflow of the environmental nanoplastics preparation protocol.

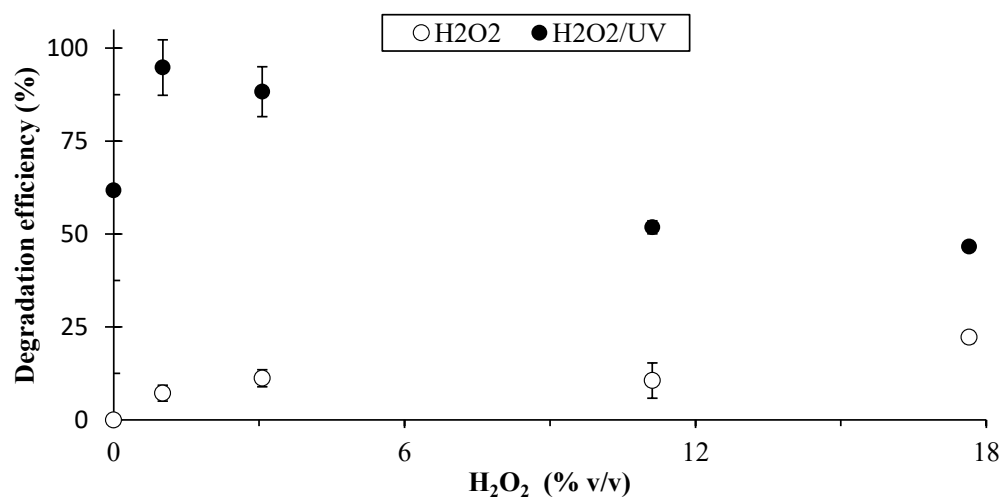## S2. Preliminary experiment for OM oxidation with H_2O_2

Figure S2. OM degradation yield on algae solution (i.e. TOC(t)/TOC(t0)*100) relative to the H2O2% and with (Black point) and without (White point) UV exposition.

S3. DLS autocorrelation function treatment information

The particle motion is characterized by a correlation of the scattered light intensity according to time and thereby represented by an autocorrelation function (ACF). Originally colloids in solution have an ACF forms by a decreasing exponential function from coherent to incoherent movements. To better illustrated the ACF, this function was normalized and presented on a y-axis logarithm scale. Through this transformation, the ACF of monodisperse standard are composed of a straight line with a negative slope and a curvilinear line including noises. The first part is related to the hydrodynamic radius of the particles and the latest part assured that particles are submitted to Brownian motion as expected for colloids. Note that the ACF of spherical standards were recorded in order to compare it to ACF from sample's particles. These spherical standards (100, 200, 500 and 900 nm) were purchased from ThermoFisher, and analysed at a concentration of 100 mg L^{-1} without ionic strength. Size distribution were determined by fitting the autocorrelation function with Sparse Baysian learning algorithms (SBL, Tipping, 2001). SBL algorithm deconvolutes the signal in a way which allow to identify the different population size associated with their distribution. Note that each DLS analyses were carried in 6 replicates with an analyses period of 120s. Only results for replicates with reliable residue (r<0.01) compared to the modelling were accepted

S4. Potentiometric information

Prior analysis, the pH probe was calibrated during the titration of an external acidic solution (1mM HNO_3, Honeywell Fluka). The ionic strength of all solution was set to 10 mM with $NaNO_3$ (Fisher Scientific) and all titration started below pH 4. The operational determination of the acid/base functional group was done as presented by (Spadini et al. 2018). Briefly, the proton released from the polymer surface, H_{surf}, was related to the pH of the solution (eq 1). Then, the total surface reactivity, Hs_{tot}, was defined as the H_{surf} released between pH 4 to 8, which implicitly do no take in account deprotonated function group at pH ≤ 4. Note that, the CO_2 diffusion during the experiment was taken in account by calculating the Hs_{tot} for a blank solution (i.e. deionized water).

$$[H^+] = [H_{init}] + [H_{base}] + [H_{surf}] + [OH^-] \qquad (1)$$

The meaning of the symbols used in eq. 1 are:

$[H^+]$, the free proton concentration, known at every time with the pH probe.

$[H_{init}]$, the concentration of the strong acid added to fix the pH to 3 prior the titration.

$[H_{surf}]$, is the concentration of the proton released by the surface titrated, the only unknown.

$[OH^-]$, the concentration proton released from the water auto-dissociation, at pH 7 $[H^+] = [OH^-]$.

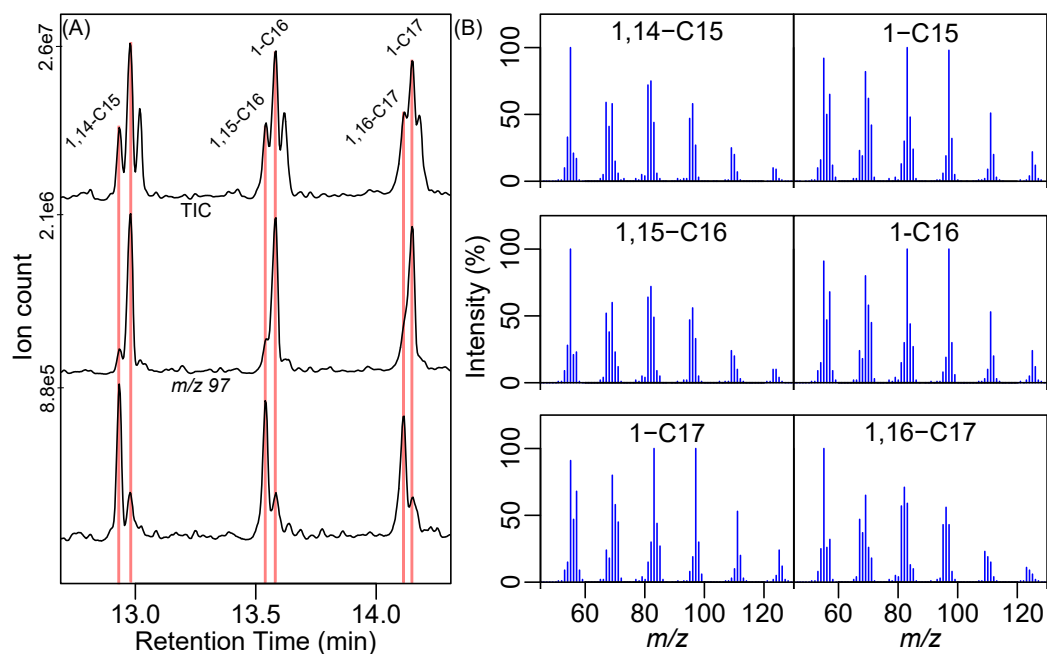## S5. PE and PP library used for polymer identification

Figure S3: (A) PE micropellet pyrograms for TIC, *m/z* 97 and 81. (B) Mass spectra of pyrolyzates recorded at the PP marker retention times used to build the library. 1,14-C15 for 1,14-pentadecadiene; 1-C15 for pentadecene; 1,15-C16 for 1,15-hexadecadiene; 1-C16 for hexadecene; 1,16-C17 for 1,16-heptadacadiene; 1-C17 for heptadecene.

The Figure S3 present the mass spectrum from the pyrolyzates recorded to allow the PE identification in unkown sample. Table S1 and S2 present the Kovats retention indices calculated for the pyrolyzates used to identify PE and PP polymer, respectively.

Table S1: PE pyrolyzates used for its identification. * Indicates pyrolyzates with no interference from OM distinctives pyrolyzates and the occurrence in the. ** Non-isothermal Kovats retention indices. 1,14-C15 for 1,14-pentadecadiene; 1-C15 for pentadecene; 1,15-C16 for 1,15-hexadecadiene; 1-C16 for hexadecene; 1,16-C17 for 1,16-heptadacadiene; 1-C17 for heptadecene.

Pyrolyzates	PE pellet sigma	
	RI**	Ref.
1,14-C15*	1487	This study
1-C15	1493	
1,15-C16*	1586	
1-C16	1593	
1,16-C17*	1687	
1-C17	1693	

Table S2: Pyrolyzates used for PP identification. (*) pyrolyzates with no interference from OM distinctives pyrolyzates and the occurrence in the. (**) Non-isotherma Kovats retention indices. C9 for 2,4-dimehtyl-1-heptene, C12 for 2, 4, 6-trimethyl-1-nonene (meso form), C15 Isotactic for 2, 4, 6, 8-tetramethyl-1-undecene, C15 Syndiotactic for 2, 4, 6, 8- tetramethyl-1-undecene.

Pyrolyzates	PP pellet sigma	
	RI*	Ref.
C9*	855	(Blancho et al. 2021)
C12*	1084	
C15i*	1312	
C15s*	1330	

S6. Pyrolyzates from OM

Table S3: Pyrolyzates markers for typical OM and their occurrence in major plastic polymers. The table summarize the precursors of these markers found in literature. * Indicated Pyr-GC/MS analysis realized at the laboratory beside literature data.

| Specific selected OM marker	Class	Origin	References	Presence in							
				Algae*	Marine DOC	Riverine DOC	SOM*	PS*	PP*	PVC*	PE
2C-Phenol	Phenol	Unspecific (Peptides, proteins, lignin, tannins, polysaccharides)	(Gillam et Wilson 1985; Dignac et al. 2005; Nierop et al. 2005; Kaal et al. 2016)	X	X	X	X	-	-	-	-
2C-2-Cyclopentenone	Cyclopentone	Carbohydrates	(Nierop et al. 2005; Kaal et al. 2016; Rouches et al. 2017)	X	-	X	X	-	-	-	-
Indole	N-containing	Proteins	(Kaal et al. 2016; Biller et Ross 2014)	X	X	X	-	-	-	-	-
			References	(Gillam et Wilson 1985; Ross et al. 2009; Van Heemst et al. 1996; Maddi et al. 2011)	(Ishiwatari, Yamamoto, et Handa 1995)	(Christy et al. 1998; Dignac et al. 2005)	(Huang et al. 1998; Kaal et al. 2016; Nierop et al. 2005)			(Tsuge, Ohtani, et Watanabe 2011)	

S7. TOC evolution during the OM oxidation
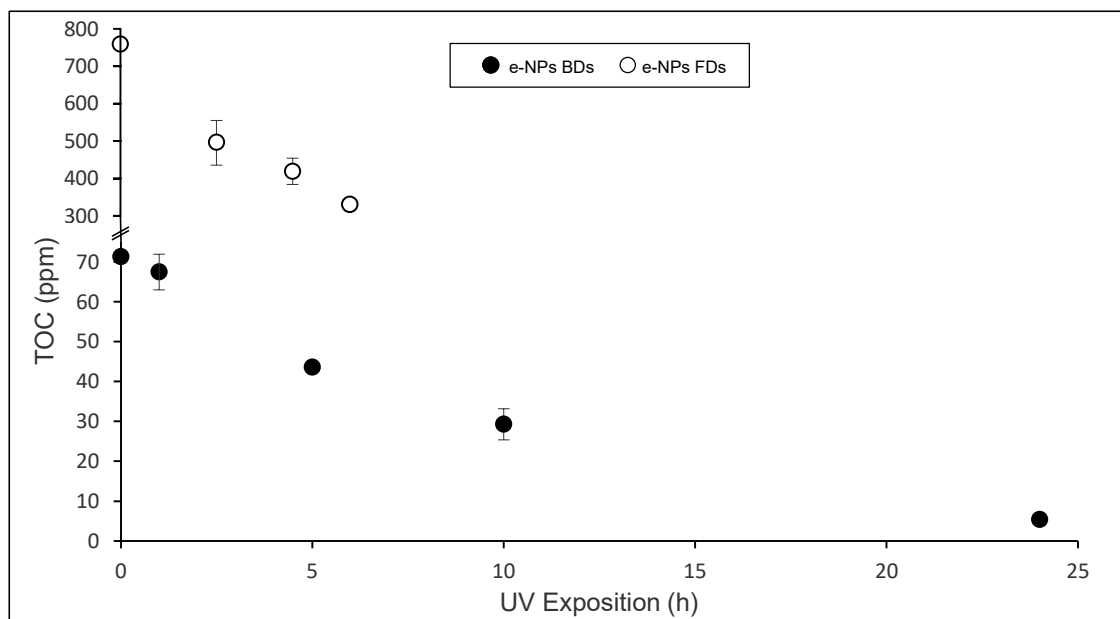
Figure S4. Evolution of TOC concentration during the application of OM degradation protocol applied on the ENPs suspension from PDs and BDs extraction, filled dot and unfilled dot, respectively.
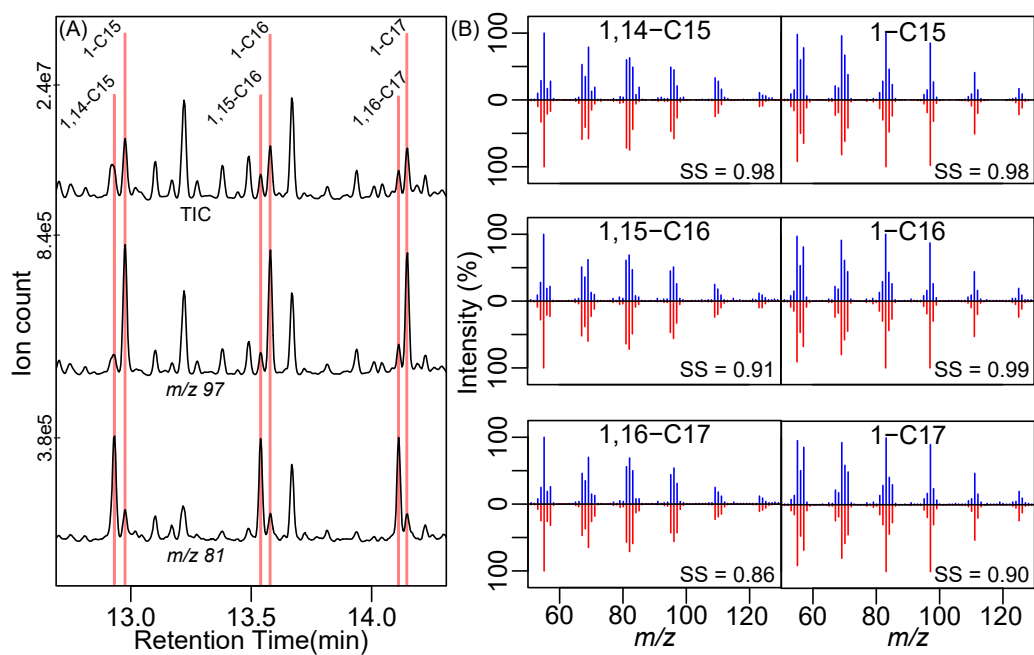
S8. Polymer identification on ENPs

Figure S5: (A) ENPs BDs pyrograms for all TIC, m/z 97 and m/z 81. **(B)** Mass spectra comparison between the MSs of pyrolyzates recorded at the PE marker retention times (blue) and their library MSs (red).

Table S4. **PE pyrolyzates used for its identification. * Indicates pyrolyzates with no interference from OM distinctives pyrolyzates and the occurrence in the. ** Non-isothermal Kovats retention indices. 1,14-C15 for 1,14-pentadecadiene; 1-C15 for pentadecene; 1,15-C16 for 1,15-hexadecadiene; 1-C16 for hexadecene; 1,16-C17 for 1,16-heptadacadiene; 1-C17 for heptadecane.**

Pyrolyzates	PP pellet sigma		ENPs PDs		ENPs BDs	
	RI**	Ref.	RI**	SS	RI**	SS
1,14-C15*	1487	This study	-	-	1485	0.98
1-C15	1493		-	-	1493	0.98
1,15-C16*	1586		-	-	1587	0.91
1-C16	1593		-	-	1594	0.99
1,16-C17*	1687		-	-	1687	0.86
1-C17	1693		-	-	1693	0.90

Table S5. Pyrolyzates used for PP identification. (*) pyrolyzates with no interference from OM distinctives pyrolyzates and the occurrence in the. (**) Non-isotherma Kovats retention indices. C9 for 2,4-dimehtyl-1-heptene, C12 for 2, 4, 6-trimethyl-1-nonene (meso form), C15 Isotactic for 2, 4, 6, 8-tetramethyl-1-undecene, C15 Syndiotactic for 2, 4, 6, 8- tetramethyl-1-undecene.

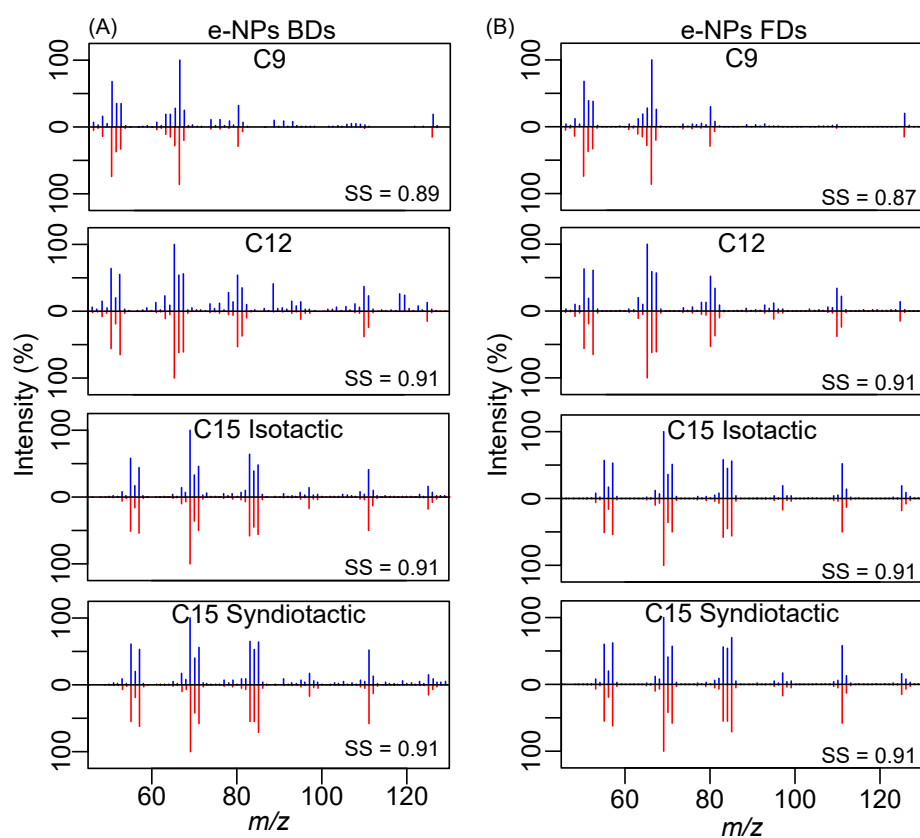| Pyrolyzates	PP pellet sigma		ENPs PDs		ENPs BDs	
	RI*	Ref.	RI*	SS	RI*	SS
C9*	855	(Blancho et al. 2021)	852	0.89	853	0.87
C12*	1084		1086	0.91	1085	0.91
C15i*	1312		1313	0.91	1312	0.91
C15s*	1330		1331	0.91	1330	0.91

Figure S6:Mass spectra comparison between the MSs of pyrolyzates recorded at the PP marker retention times (blue) and their library MSs (red) for PDs (A) and (B) BDs, respectively. SS, spectrum similarity calculated with MSD Chemstation algorithm.
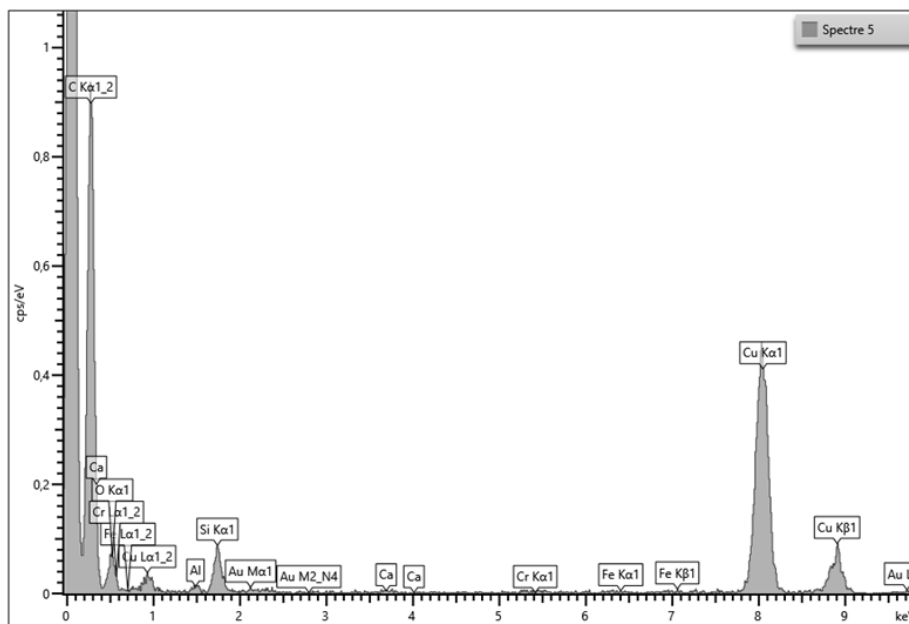
S9. EDX Analysis

Figure S7: EDX spectra of the copper grid having an amorphous carbon deposit on its surface. The spectra were acquired with a 5nm probe.
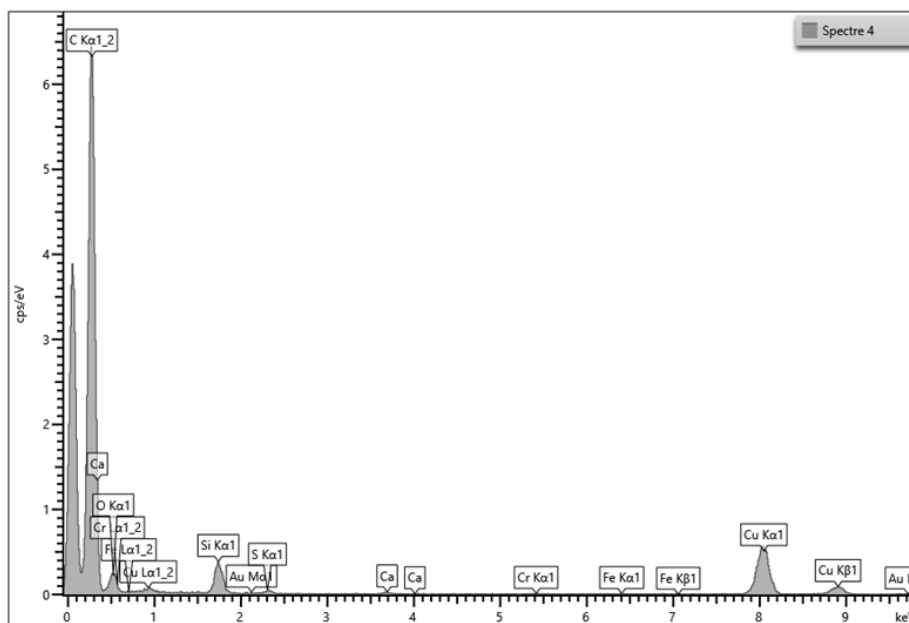

Figure S8. EDX spectra of the e-NPs 1. The spectra were acquired with a 5nm probe.

Table S6. Relative atomic composition obtained from EDX analysis on e-NPs 1 and its embedded black cubic particle.

% Atomic	ENPs 1	Ti oxide
C	96.60	65.70
O	21.58	21.58
Na	-	-
Al	0.07	0.25
Si	0.63	0.50
S	0.06	0.06
K	-	-
Ca	0.21	0.10
Ti	-	11.80

S10. UV lamp characteristic

Table S7. UV radiation characteristic from the RPR 2537A Lamp (Rayonet).

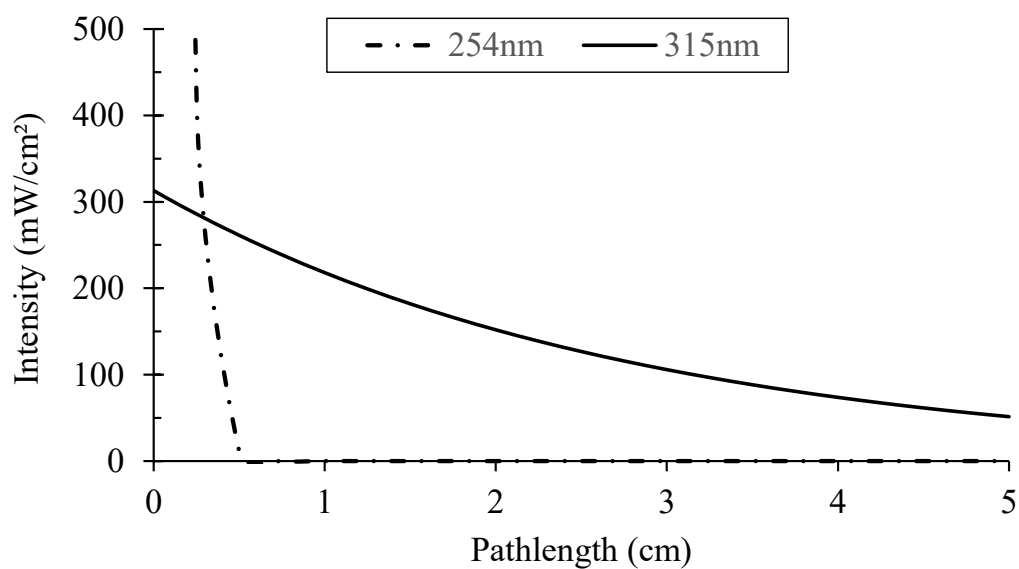| Wavelength (Å)	Intensity (mW/cm²)
2537	12800
2652	388
2804	14
2894	18
2967	66
3022	32
3129	250
3654	213
4047	250
4359	768
5461	428
5780	91

Figure S9. Calculated intensity at the 254 and 310 nm wavelength in the reactor for H_2O_2 1%.
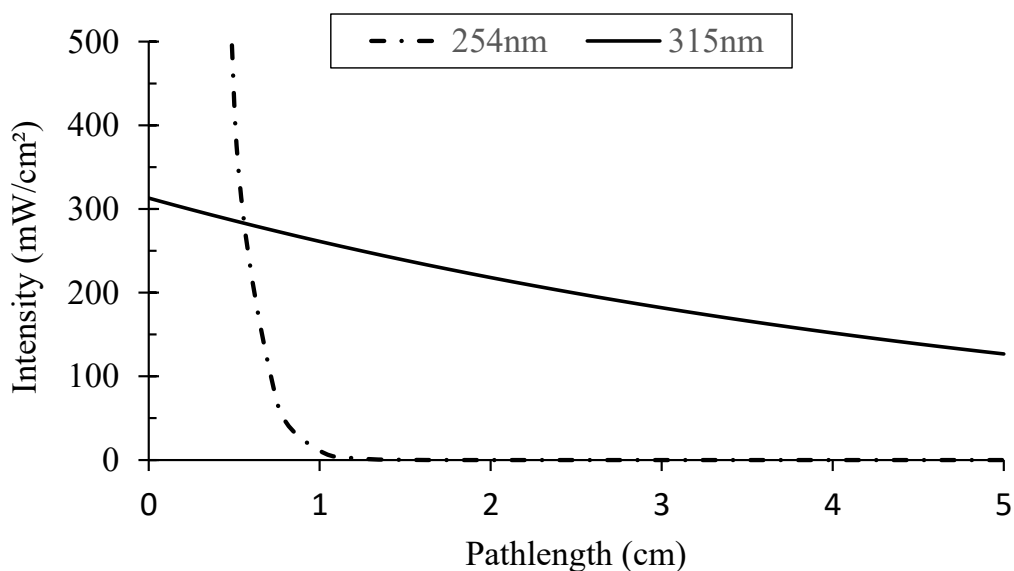
Figure S10. Calculated intensity at the 254 and 310 nm wavelength in the reactor for H_2O_2 0.5%.

S11. H_2O_2 concentration after 6 hours of UVC on ENPs suspension

The quantity of H_2O_2 was determined after the application of the OM oxidation. Through the use of the calibration curve presented on Figure S11. The H_2O_2 was quantified at 0.64% after 6 hours of UVC (Table S8).
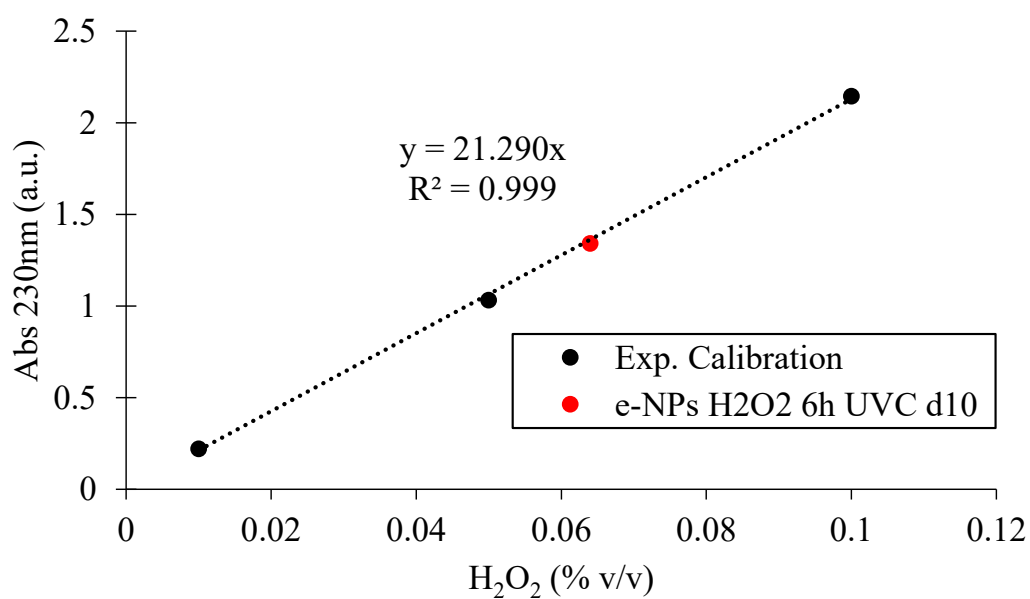

Figure S11. H_2O_2 calibration curve and the H_2O_2 determined on the ENPs sample after 6 hours of UVC exposition. Note that the sample was diluted by a factor 10 prior the q the quantification.

Table S8. H$_2$O$_2$ quantification after the UVC exposition of the ENPs suspension

UV exposition (hours)	ENPs presence	H$_2$O$_2$ (% v/v)
0	X	1
6	X	0.64