In Vitro Assessment Reveals the Effects of Environmentally Persistent Free Radicals on the Toxicity of Photoaged Tire Wear Particles

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Text S1. Method of thermogravimetric analysis of main constituents of TWP

Main constituents of TWP were quantified by thermogravimetric analysis (TGA, Mettler Toledo). Different components were determined by analysis the mass loss after heating the sample: (a) volatile substances (vaporize between 30- 300 °C), (b) the actual polymer (300–600 °C), (c) carbon black (600–850 °C), and the residue[1]. Temperature was increased from 30 to 850 ˚C with a constant heating rate of 20 ˚C. For purge gas, nitrogen (50 ml/min) was set from 30 to 600˚C: Nitrogen (50 ml min-1) and air (50 ml/min) was from 600 to 850 ˚C.

Text S2. Method of pyrolysis-GCMS analysis of chemical composition of TWP

Pyrolysis-GC/MS analysis was performed by using a pyrolyzer (EGA/PY3030D, Frontier, Japan), a gas chromatograph (TRACE1310, Thermo Scientific, USA) and a mass spectrometer (ISQ, Thermo Scientific, USA). The sample of TWP (approximately 1.0 mg) was pyrolyzed in the single-shot mode at 750 °C for 0.2 min. The high purity (>99.9995%) helium was used as the gas carrier with a flow rate of 1.0 mL/min, and the pyrolysis products were injected with a split ratio of 100:1. The

temperatures of the interface and transfer lines were set at 320 °C. The obtained mass spectra and the NIST MS library were used to identify the pyrolysis products and the interpretation. More parameters of pyrolysis-GC/MS analysis are presented in Table S2.

Text S3. Method of ICP-OES analysis for metals qualitative analysis of TWP

Analysis of trace metals of TWP was with a Thermo Scientific ICAP 7200 Inductive Coupled Plasma-Optical Emission Spectrometry (ICP-OES) analyzer. The machine was equipped with a Teledyne Cetac ASX-560 autosampler. The argon used to form the plasma was of high purity (Alphagaz, >99.9995%) and was supplied by Air Liquide (Belgium). The trace metals were detected by ICP-OES operated in axial mode and three characteristic spectral emission wavelengths were included for each element (as seen in Table S3). In order to avoid contamination from previous analysis, a 1% nitric acid solution (acidified bides) was used to rinse the autosampler tubing, nebulizer and spray chamber for 45 s automatically by the instrument. After every 10 samples, one of the standards and the calibration curve's blank was analyzed as

quality control. In addition, during measurement, all used glassware and recipients were first twice rinsed with a 10% nitric acid solution and subsequently with bidest water.

Text S4. EPR analysis of EPFRs and ROS on TWP

0.5 g photoaged TWP was taken for EPR analysis with using by EPR measurements using a Bruker EMXmicro-6/1/P/L spectrometer (Karlsruhe, Germany) at room temperature (~25℃) and the parameters of EPR measurement were provided in Table S2. For EPFRs detection, 50 mg partials were collected and placed in an I.D. quartz tube, then, the quartz tube was directly inserted into the cavity of the EPR instrument for EPFRs analysis. For ROS analysis, DMPO solution (100 mM), DMPO/DMSO solution (100 mM) and TEMP solution (100 mM) were used as spin-trapping agents for coupling $^{\bullet}OH$, $O_2^{\bullet-}$ and $^1O_2$, respectively. 50 mg particles were taken and mixed with 500 μL DMPO solution, 500 μL DMPO/DMSO solution and 500 μL TEMP solution for detecting $^{\bullet}OH$, $O_2^{\bullet-}$ and $^1O_2$ [2]. Analysis of non-photoaged TWP was regarded as the control.

Table S1. Information of manufacturers and use histories of the eight car tires used to produce tire tread wear particles

Nr	Brand	Tire	Type	Tire code
1	Michelin	Primacy 4	All season	235/50 R 19 99 V
2	Dunlop	SP SPORT 270	Summer	215/60 R 16 95 V
3	Bridgestone	Dueler H/P Sport	All season	215/55 R18 95V
4	Bridgestone	Dueler H/L Alenza	Summer	245/50 R 19 105 V
5	Pirelli	Cinturato P7	Summer	225/50 R 17 94W
6	Michelin	Primacy 3ZP	Summer	195/65 R15 91 V
7	Pirelli	Cinturato P7	All season	245/45 R 19 102 Y
8	Bridgestone	Turanza ER300	Summer	205/55 R 16 91V

Table S2. Overview of parameters of pyrolysis-GC/MS analysis for identifying organics of TWP

Apparatus	Parameters	Settings
Pyrolyzer (Single-shot analysis) EGA/PY-3030D	Pyrolysis temperature	750 °C
	Interface temperature	320 °C
	Pyrolysis time	0.2 min
Gas Chromatograph TRACE1310	Column, Agilent	30 m, i.d. 0.25 mm, 5% diphenyl 95% polysiloxane HP-5 ms capillary column,
	Injector port temperature	320 °C
	temperature program	50 °C (2 min) → (10 °C/min) → 280 °C (10 min) → (20 °C/min) → 320 °C (2 min)
	Injector mode	Split (100:1)
	Carrier gas	Helium, 1.0 mL/min, constant linear velocity
Mass Spectrometer	Ion source temperature	300 °C
	Scan range	35 to 500 m/z

Table S3. Overview of the included trace metals, the analyzed wavelengths and their detection limits. The underlined wavelength was used for quantification.

Trace metal	Abb.	CAS N°	Wavelengths (nm)			LOD* (µg/L)	LOQ* (µg/L)
Aluminum	Al	7429-90-5	<u>309.271</u>	167.079	308.215	20	40
Cadmium	Cd	7440-43-9	<u>214.438</u>	228.802	226.502	1.0	1.2
Chromium	Cr	7440-47-3	<u>267.716</u>	283.563	284.325	1.5	5.1
Copper	Cu	7440-50-8	<u>324.754</u>	327.396	224.700	1.0	2.5
Iron	Fe	7439-89-6	<u>259.940</u>	238.204	239.562	1.1	3.5
Manganese	Mn	7439-96-5	<u>257.610</u>	259.373	260.569	1.0	1.9
Nickel	Ni	7440-02-0	<u>231.604</u>	221.647	341.476	1.0	2.4
Lead	Pb	7439-92-1	<u>220.353</u>	216.999	261.418	3.0	10
Strontium	Sr	7440-24-6	<u>407.771</u>	421.552	346.446	1.0	1.2
Zinc	Zn	7440-66-6	<u>202.548</u>	213.856	206.200	1.0	1.4

*LOD and LQD are the limit of detection (LOD)and the limit of quantification (LOQ), respectively. The detection limits were estimated based on the standard deviation of six repeated measurements of the lowest measurable standard.

* The underlined wavelength was used for quantification.

Table S4. List of concentrations of main heavy metals detected in the particles of prepared TWP (mg/kg). Standard deviation analysis is based on triplicate tests.

Sample	Cd	Co	Cr	Cu*	Mn	Ni	Pb	Fe	Zn
Particles	1.5±0.3	0.6±0.1	23.9±6.6	34.9±5.4	6.6±1.1	20.5±4.1	14.4±5.1	12.6±3.7	75.3±7.1

Table S5. Parameters of EPR analysis for identifying EPFRs and ROS on particles of TWP [*].

Parameters	EPFRs identification	ROS identification
Microwave frequency	9.8 GHz (X-band)	9.8 GHz (X-band)
Center field	3504.3 G	3500 G
Microwave power	0.5024 mW	2.0 mW
Modulation frequency	1.0 G	1.0 G
Sweep width	80 G	100 G
Time constant	0.01 ms	15.0 ms
Sweep time	30.0 s	10 s
Scanning times	5	20

* SpinFit (Bruker's Xenon program) was used to simulate solution spectra, since this software provided an automatic fit to the experimental spectrum and determined the relative intensity of each spin adducts.

Table S6. Description of primers used in the PCRs

Gene	primers
IL6-F	CTGCAAGAGACTTCCATCCAG
IL6-R	AGTGGTATAGACAGGTCTGTTGG
TNF-α-F	CAGGCGGTGCCTATGTCTC
TNF-α-R	CGATCACCCCGAAGTTCAGTAG
iNOS-F	GTTCTCAGCCCAACAATACAAGA
iNOS-R	GTGGACGGGTCGATGTCAC

Table S7. EPR spectral characteristics of EPFRs detected on TWP under light irradiation for different days.

light irradiation time (d)	g-factors	line width ($\Delta H_{p\text{-}p}$, Gauss)
0	2.00308	5.93
5	2.00308	5.85
10	2.0031	5.71
15	2.0031	5.94
20	2.00312	5.94
25	2.00313	5.91
30	2.00311	6.03
40	2.00311	6.02
50	2.00308	5.95
60	2.00309	5.95

Table S8. EPFRs concentrations for radical quenching experiments by EPR analysis.

TWP samples	Spin density on (10^{17} spins/g)			Decay efficiency of EFPRs concentrations for radicals quenching tests by water
	Before treated by O_2-purged water	After treated by O_2-purged water	Concentrations of reactive EPFRs	
Without irradiation	3.04	2.87	0.17	5.5%
With 60 d-irradiation	3.97	2.82	1.15	29.0%

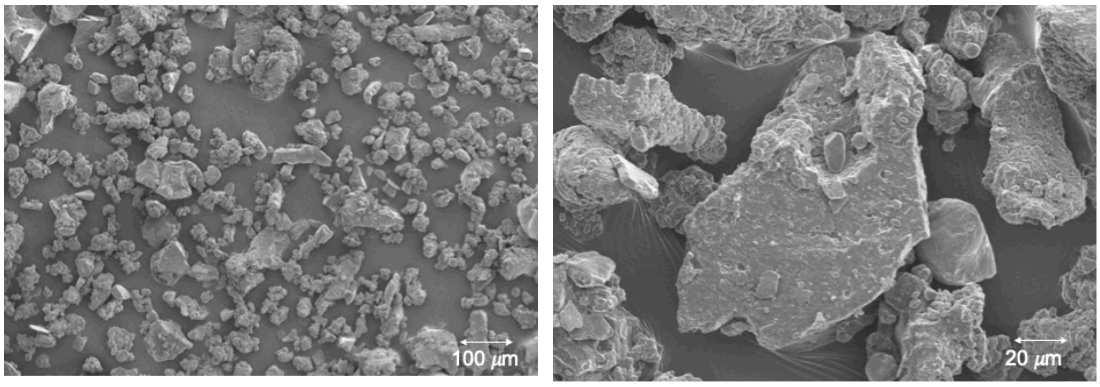


Figure S1. Scanning electron microscope pictures of particles of prepared TWP at magnification of 300× (left) and 2000× (right).

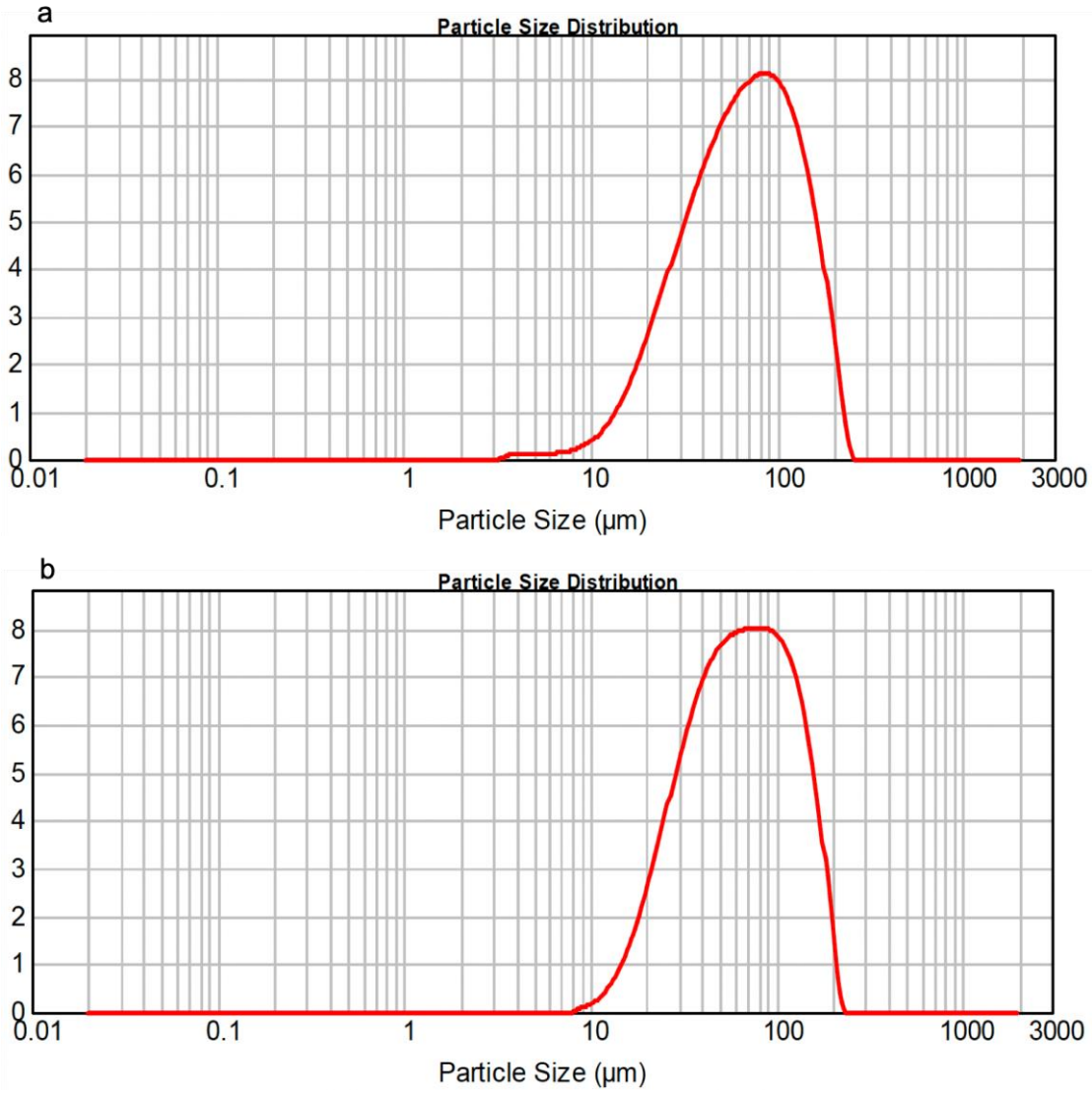


Figure S2. Particle size distribution of (a) pristine and (b) aged TWP determined by volume of particles.

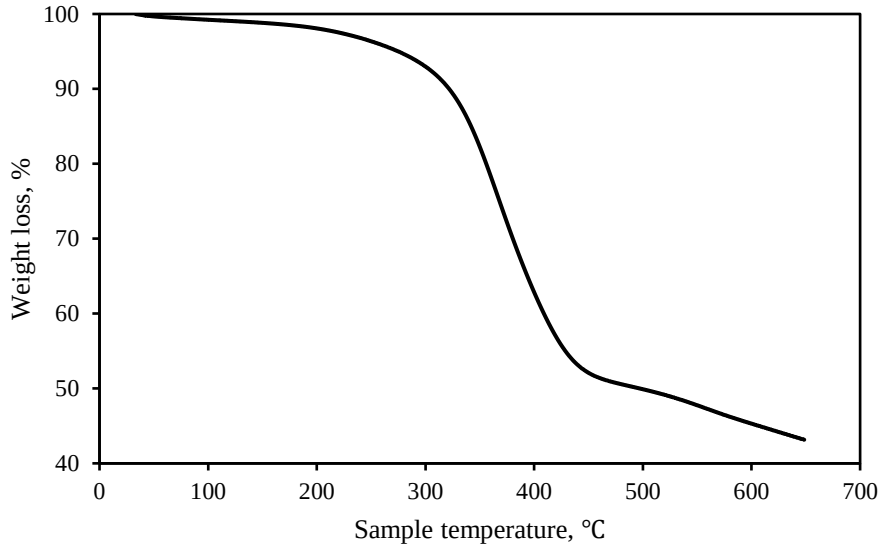


Figure S3. Weight loss (%) of particles of the prepared TWP using thermogravimetric analysis. Volatile substances (vaporize between 30- 300°C), polymers (300- 600°C), carbon black (600- 850°C), and the residual were determined.

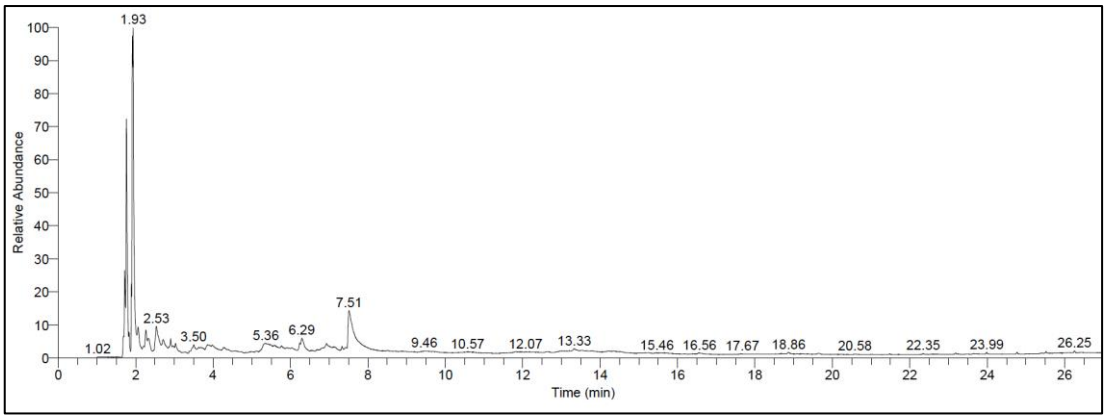


Figure S4. Example of pyrolysis-GC/MS data obtained from the sample of particles of the prepared TWP.

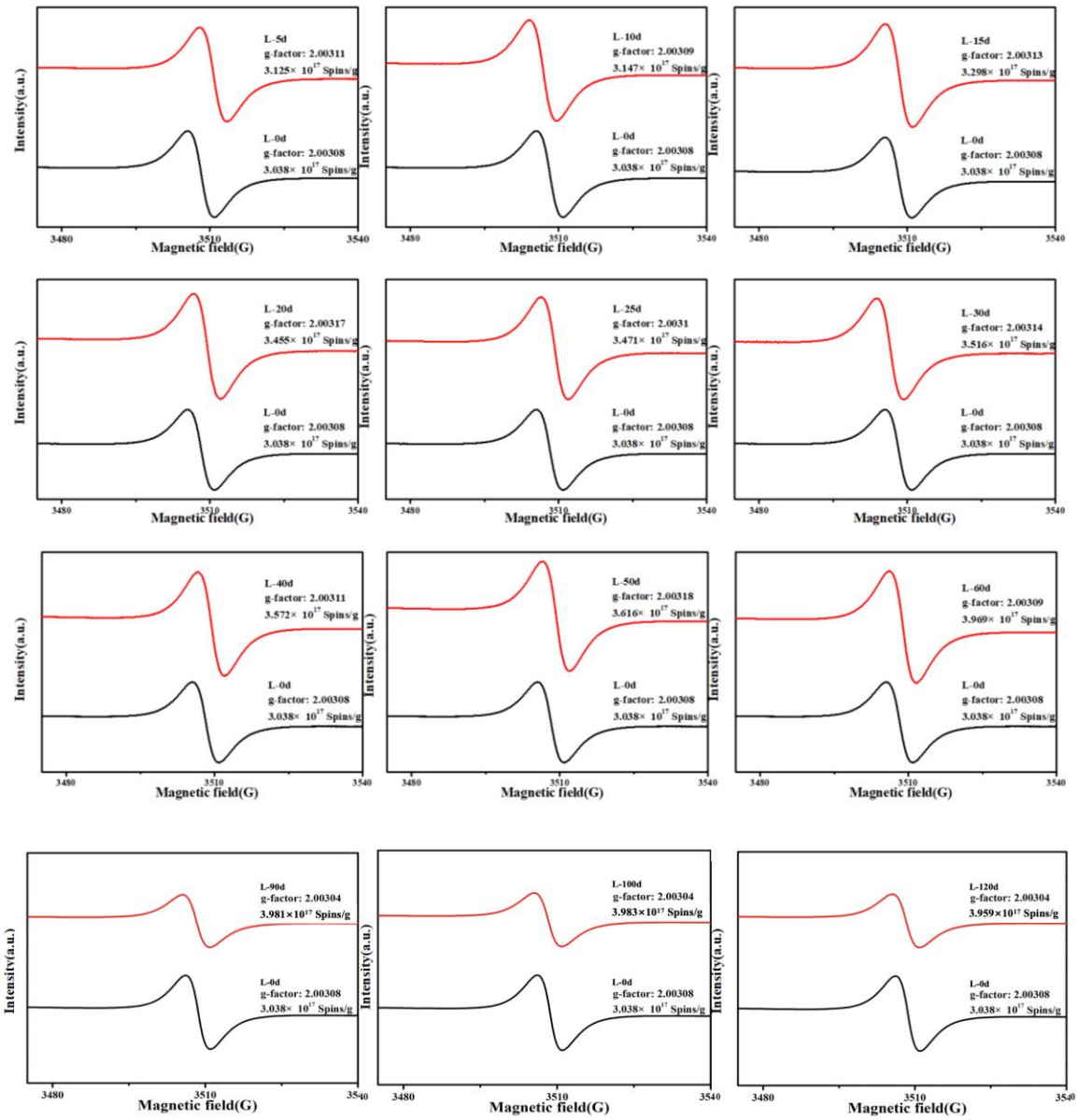


Figure S5. EPR spectra obtained from the EPR analysis of TWP with different light irradiation (from 0 d to 120 d, red lines). EPR analysis of TWP without light irradiation was the controls (black lines). TWP without light irradiation were regarded as the controls. The values in the figure are the g-factors and EPFRs concentrations for EPR spectra of photoaged TWP.

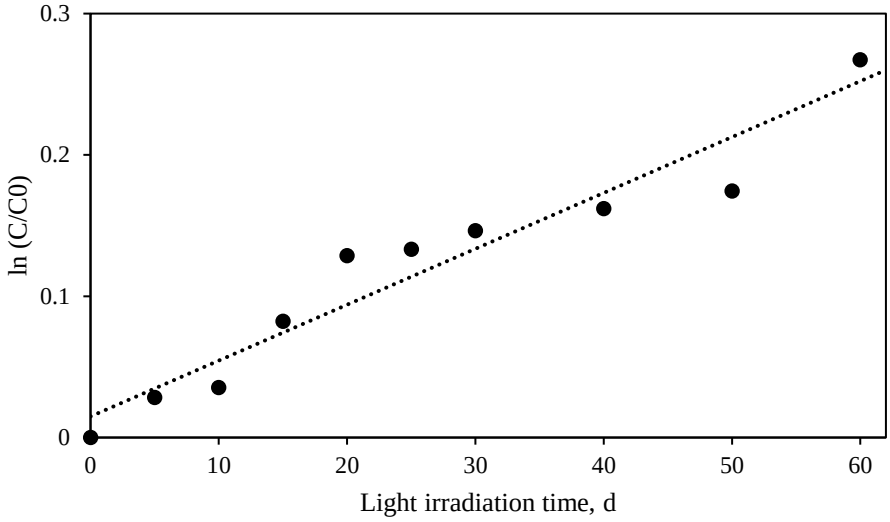


Figure S6. Generation kinetics for EPFRs on TWP with light irradiation as function of time. Experimental results are shown by the data points, whereas the dashed lines represent the pseudo-first order kinetics, obtained by least-squares fitting (R^2=0.9275, k value =0.004 d^{-1}).

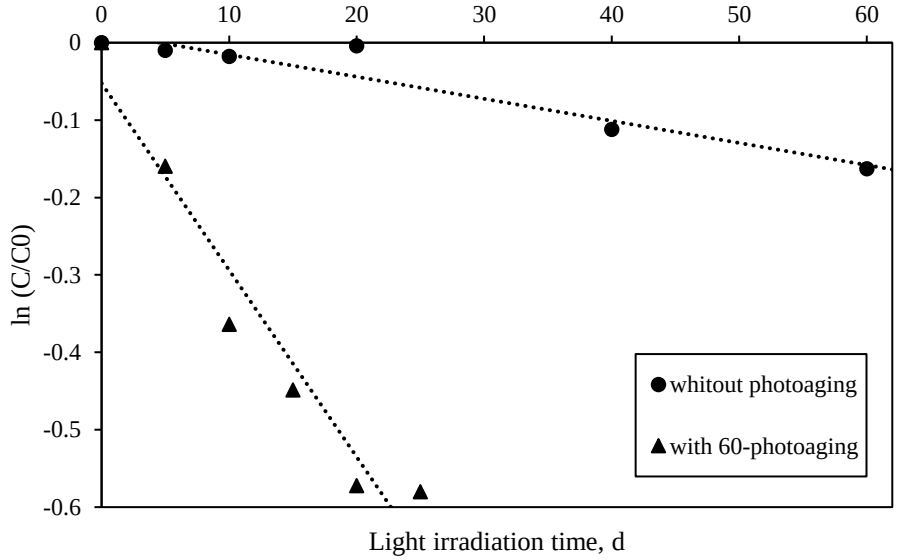


Figure S7. Decay kinetics for EPFRs on TWP (without photoaging and with 60 d-photoaging) as function of time. Experimental results are shown by the data points, whereas the dashed lines represent the pseudo-first order kinetics, obtained by least-squares fitting (for TWP without photoaging: R^2=0.9404, k value =0.0241 d^{-1}; TWP with 60 d-photoaging: R^2=0.9175, k value =0.0029 d^{-1}).

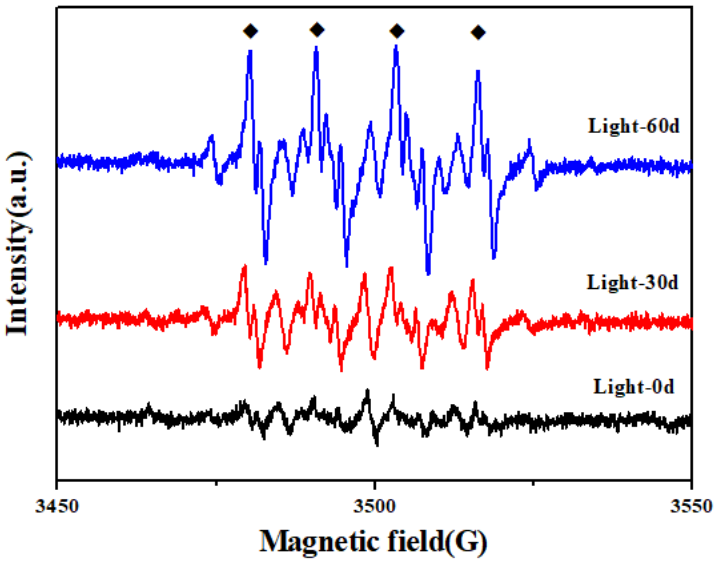
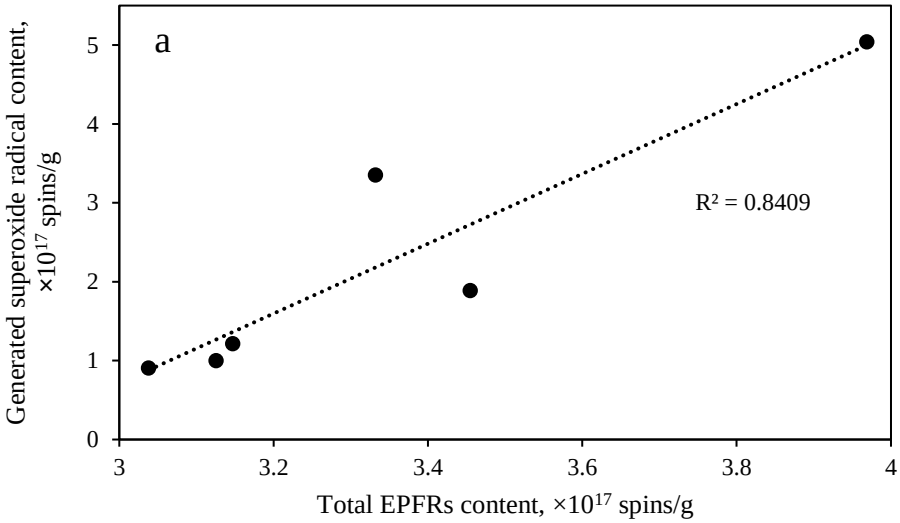
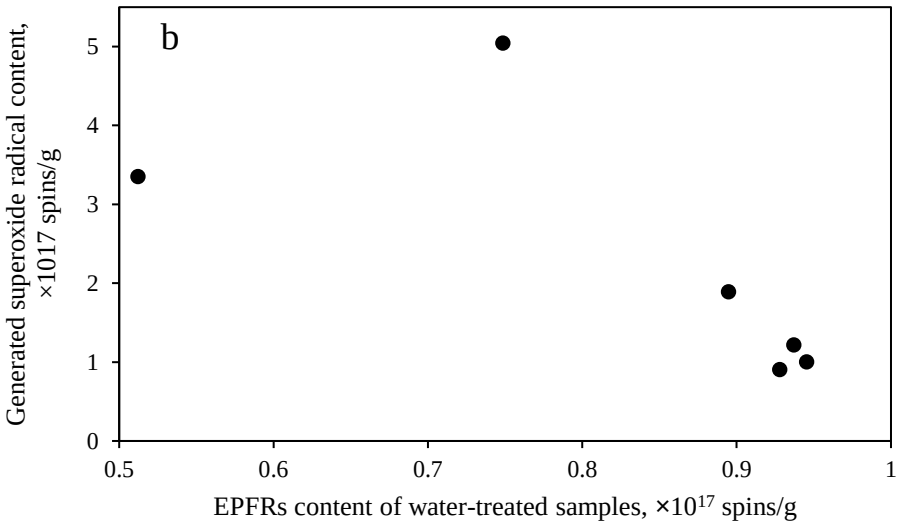


Figure S8. EPR spectra obtained from TWP samples with DMPO/DMSO (100 mM, 500 μL). Black lines represent 0 d-samples, red lines represent 7 d-samples and blue lines represent 14 d-samples.


a
Generated superoxide radical content, ×10^17 spins/g
R² = 0.8409
Total EPFRs content, ×10^17 spins/g


b
Generated superoxide radical content, ×1017 spins/g
EPFRs content of water-treated samples, ×10^17 spins/g

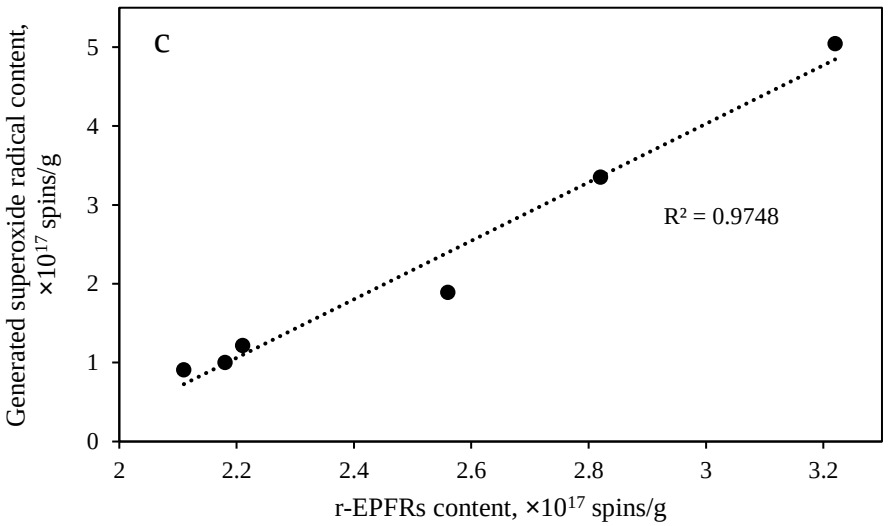

c
Generated superoxide radical content, ×10^17 spins/g
R² = 0.9748
r-EPFRs content, ×10^17 spins/g

Figure S9. Relationships between generated superoxide radicals and different free radical contents on TWP samples. Relationship between generated superoxide radicals and (a) total EPFR content of TWP samples, (b) EPFR content of TWP samples after being treated in O_2-purged water, called water-treated samples, and (c) t-EPFR content of TWP samples, which is the difference of between TWP samples and water-treated TWP samples. Each data point is the mean value obtained from three independent tests, and data are shown as mean ± SD.

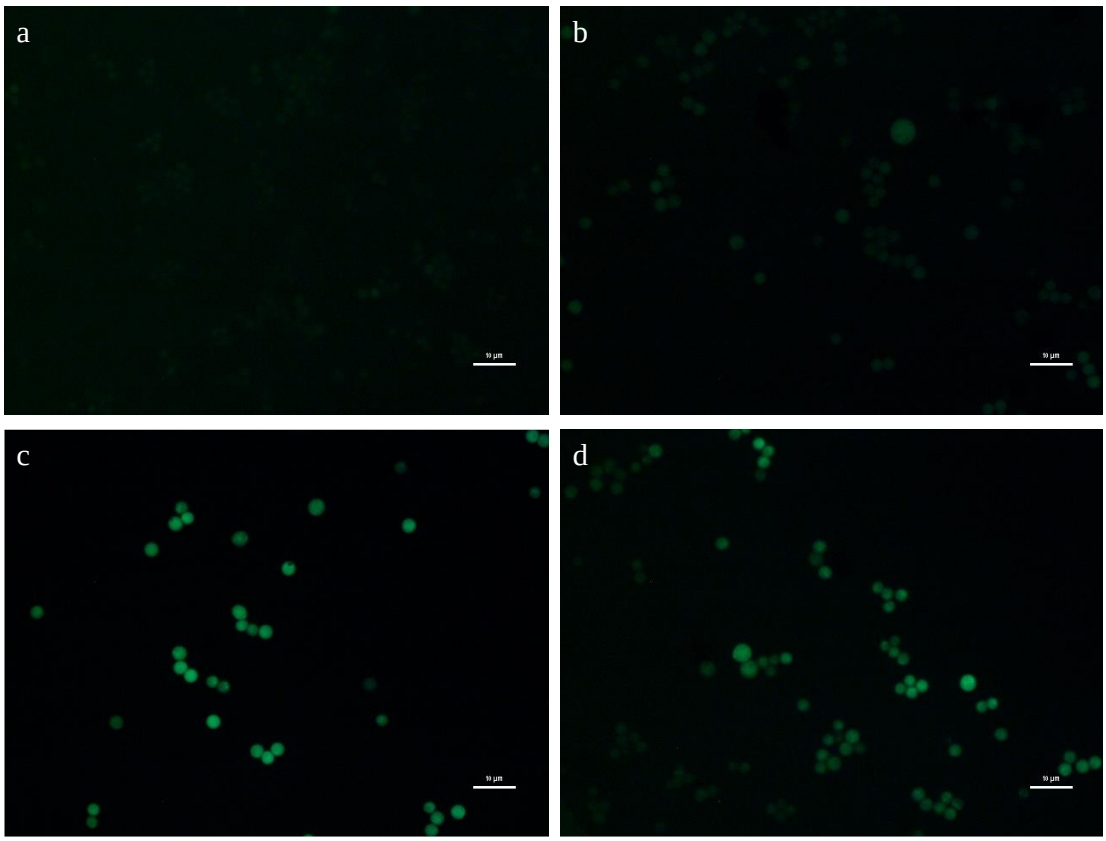


Figure S10. Fluorescence images of macrophages in the cell culture supernatants following exposure to (b) 10 d-, (c) 30 d- and (d) 60-d photoaged TWP with adding 5 mM NAC. (a) Macrophages unexposed to TWP were regarded as the controls. The exposure time was 300 ms.

References

(1) Redondo-Hasselerharm, P. E.; de Ruijter, V. N.; Mintenig, S. M.; Verschoor, A.; Koelmans, A. A. Ingestion and Chronic Effects of Car Tire Tread Particles on Freshwater Benthic Macroinvertebrates. *Environ. Sci. Technol.* **2018**, *52* (23), 13986–13994. https://doi.org/10.1021/acs.est.8b05035.

(2) Zhu, K.; Jia, H.; Zhao, S.; Xia, T.; Guo, X.; Wang, T.; Zhu, L. Formation of Environmentally Persistent Free Radicals on Microplastics under Light Irradiation. *Environ. Sci. Technol.* **2019**. https://doi.org/10.1021/acs.est.9b01474.