Electronic Supplementary Material (ESI) for RSC Advances.

ELECTRONIC SUPPLEMENTARY MATERIAL

Pro-oxidant effects of nano-TiO2 on Chlamydomonas reinhardtii during short-term exposure

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CONTENTS

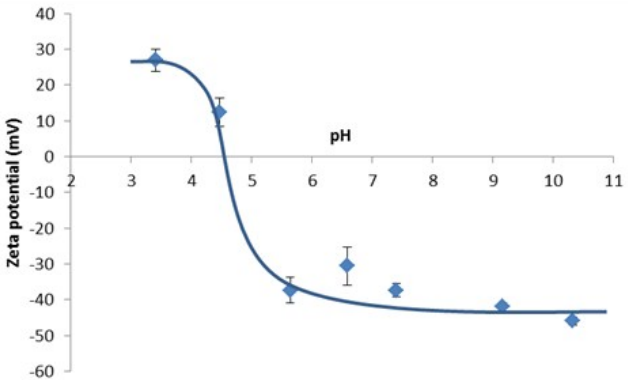
1. MATERIALS AND METHODS

Table S1. Composition of lake Geneva water at the sampling station GE 3 (during sampling period), as provided by the « Département de l'environnement, des transports et de l'agriculture (DETA), Direction Générale de l'Eau" of Canton Geneva, Switzerland. The water used for exposure experiments was sampled at ca. 1.5 m depth.

Parameter		
DEPTH (m)	0	2.5
DATE	28.01.2014	28.01.2014
TEMPERATURE (°C)	6.6	6.5
CONDUCTIVITY (uS*cm^{-1})	306	305
pH	7.95	8.08
OXYGEN (mg/L)	10.69	10.75
SATURATION (%)	93.3	93.6
DOC (mg/L)	1.18	1.04
N NH_4 (mg/L)	0.004	0.003
N NO_2 (mg/L)	0.004	0.002
N NO_3 (mg/L)	0.59	0.62
N MIN.total (mg/L)	0.60	0.63
total N (mg/L)	0.63	0.63
soluble P (mg/L)	0.008	0.009
total P (mg/L)	0.014	0.013
Silica (mg/L)	1.056	1.262
CHLORIDE (mg/L)	9.46	9.36
SULFATE (mg/L)	44.90	45.52
SODIUM (mg/L)	6.96	6.70
POTASSIUM (mg/L)	1.42	1.58
CALCIUM (mEq/L)	2.32	2.32
MAGNESIUM (mEq/L)	0.52	0.52
ALCALINITY (mEq/L)	1.84	1.84

A

- Average primary particle size (TEM): 25 nm (S.D. = 6 nm)
- D_v50 (Malvern) ≈ 75 nm
- SSA (BET) ≈ 35m^2 g^{-1}
- pH isoelectric point ≈ 4.5



B

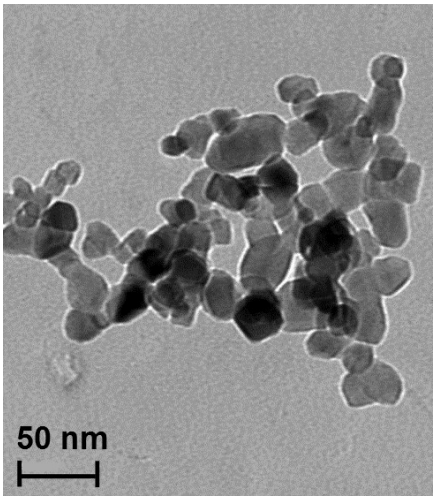


Figure S1. (A) Primary particle properties of nano-TiO_2 P25, Evonik (Degussa, lot number 1837), (B) TEM image of primary particles.

Table S2. Input parameters for the ISDD model (biosensor exposure setup). Values for fractal dimension, packing factor, dynamic media viscosity and density were taken from literature [1, 2].

1) primary NP properties	**lake biosensor**	**lake biosensor**	**MOPS biosensor**	**MOPS biosensor**
1.a) Diameter [nm]	25	25	25	25
1.b) Density [g/mL]	4.26	4.26	4.26	4.26
1.c) nominal concentration [mg/L]	10	200	10	200
2) Agglomerate characteristics				
2.a) Diameter [nm]	566	2912	1374	2752
2.b) Density [g/mL]	model estimate	model estimate	model estimate	model estimate
2.c) Fractal dimension	2.3	2.3	2.3	2.3
2.d) Packing factor	0.637	0.637	0.637	0.637
3) Liquid medium conditions				
3.a) Dish depth [m]	0.0012	0.0012	0.0012	0.0012
3.b) Volume [mL]	0.06	0.06	0.06	0.06
3.c) Temperature [K]	310	310	310	310
3.d) Viscosity [Ns/m^2]	0.00074	0.00074	0.00074	0.00074
3.e) Density [g/mL]	1.00	1.00	1.00	1.00

Table S3. Input parameters for the ISDD model (FCM exposure setup). Values for fractal dimension, packing factor, dynamic media viscosity and density were taken from literature [1, 2].

1) primary NP properties	**lake FCM**	**lake FCM**	**MOPS FCM**	**MOPS FCM**
1.a) Diameter [nm]	25	25	25	25
1.b) Density [g/mL]	4.26	4.26	4.26	4.26
1.c) nominal concentration [mg/L]	10	200	10	200
2) Agglomerate characteristics				
2.a) Diameter [nm]	566	2912	1374	2752
2.b) Density [g/mL]	model estimate	model estimate	model estimate	model estimate
2.c) Fractal dimension	2.3	2.3	2.3	2.3
2.d) Packing factor	0.637	0.637	0.637	0.637
3) Liquid medium conditions				
3.a) Dish depth [m]	0.00588	0.00588	0.00588	0.00588
3.b) Volume [mL]	0.2	0.2	0.2	0.2
3.c) Temperature [K]	310	310	310	310
3.d) Viscosity [Ns/m^2]	0.00074	0.00074	0.00074	0.00074
3.e) Density [g/mL]	1.00	1.00	1.00	1.00

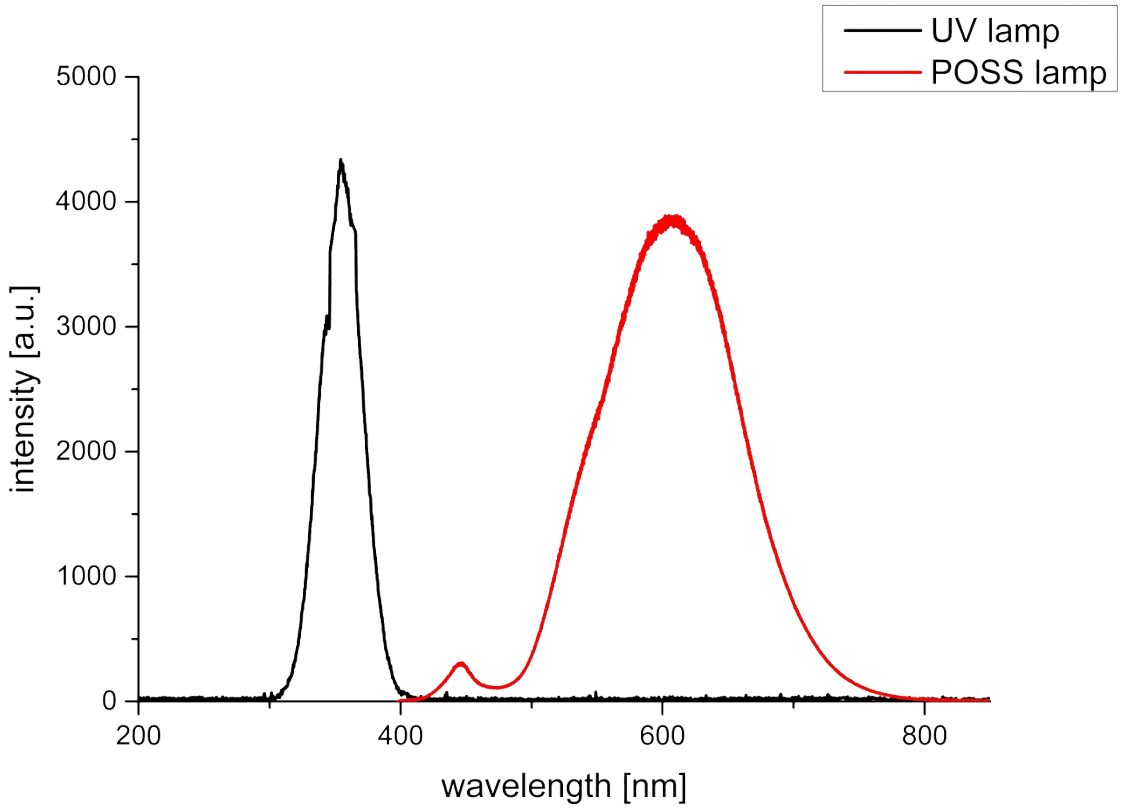


Figure S2. Intensity spectra of longwave UVA irradiation provided by the UV lamp (Waldmann Typ 602352 lamp, 230V, 50Hz, 2x4W) and of the LED lamp (R11/D3/N/B, Relco Group Ltd, 3W) of the portable oxidative stress sensor used in the experiments. At a wavelength of 350 nm the power of the UV lamp at the sample was 60 µW cm^{-2}. The power of the LED diodes at the sample was 25 mW cm^{-2} at a wavelength of 550 nm.

1.1 Controls TiO_2 spectra

Normalized transmission measurements of the TiO_2 suspensions confirmed the rapid aggregation of particles. All TiO_2 exposure concentrations used in both lake and MOPS media exhibited similar scattering profiles (data not shown) and signal distortion did not exceed 1% in the wavelength region of interest (λ = 542 – 550 nm). Below, we provide an example of how Rayleigh scattering of a 200 mg L^{-1} nano-TiO_2 suspension in MOPS remains roughly unchanged after 1 h (Fig. S3).

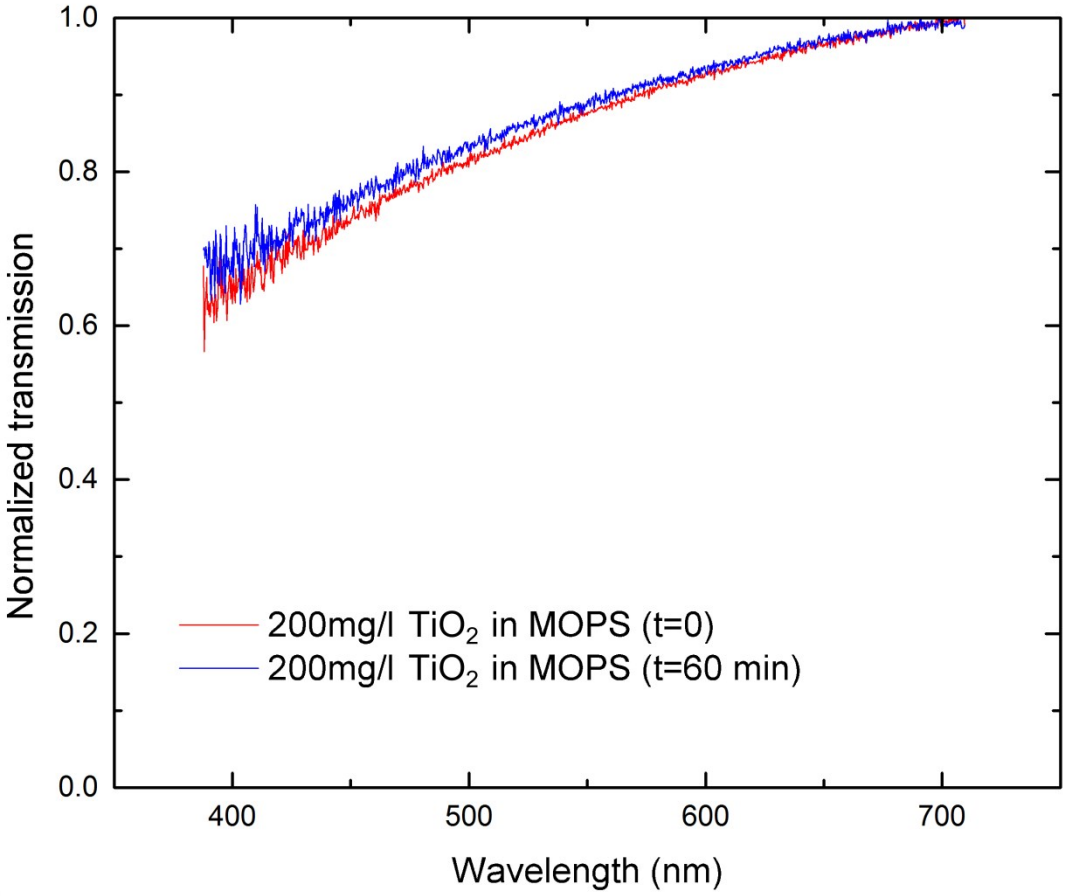


Figure S3. Normalized transmission of a 200 mg L^{-1} nano-TiO_2 suspension in MOPS, immediately after suspension preparation at t = 0 h and after 1 h.

1.2 H_2O_2 calibration in lake water and MOPS buffer

The interaction constant *k* was determined from the calibration curves below (Fig. S4), as described by Koman et al.[3]. *k* values were 0.096 ± 0.031 $min^{-1}·\mu M^{-1}$ for lake and 0.067 ± 0.020 $min^{-1}·\mu M^{-1}$ for MOPS. The difference of the *k* values from those stated by Koman et al. [3] can be due to two reasons. Firstly, a modified experimental protocol in which the cytochrome *c* sensing spots were stored in the frozen state might have changed the activity of cyt *c*. Secondly, the complex biological exposure media studied here may also affect the availability of H_2O_2.

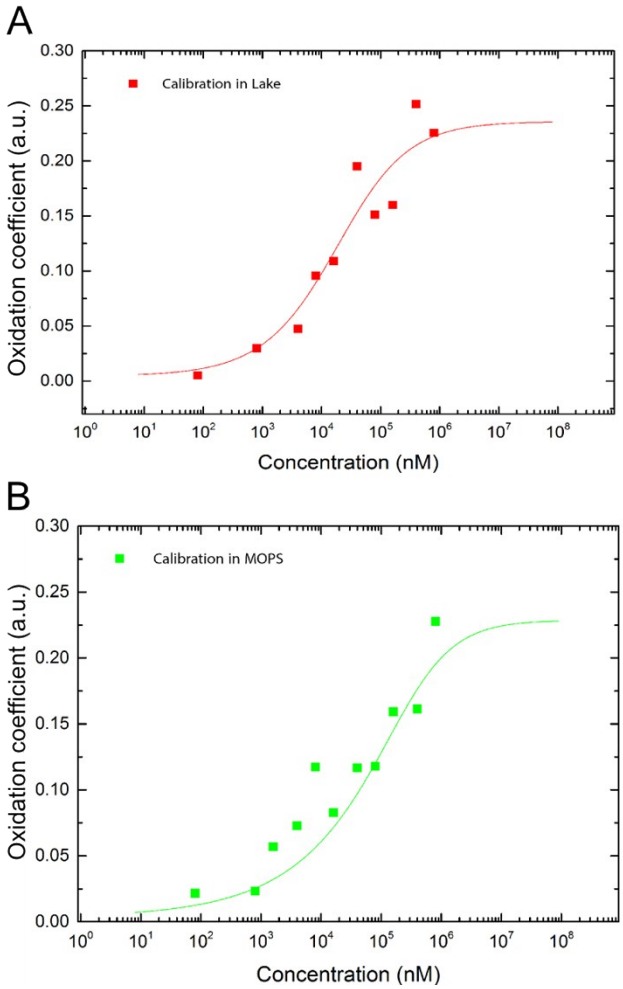


Figure S4. Calibration curves for cyt *c* spots in lake water (A) and MOPS buffer (B) for *t* = 30 min.

1.3 Gating strategy for FCM data analysis

For data analysis the Flow cytometry software provides multiparameter scatter plots in which every dot corresponds to a measured cell/particle. Response variables are obtained in % cells possessing the scatter properties of interest and are extracted by the application of appropriately placed gates (Fig. S5). In all plots, we first remove doublets by applying gate P1 in an FSC-H vs FSC-A plot (Fig. S5A) and define our algae population based on their auto-fluorescence visible in FL3 (Fig. S5B). Next, gates for the fluorescent probes are designed according to the strategy illustrated for CellRox Green reagent (fluorescence in FL1) showing % CellRox Green positive cells (pink gate "CRG pos") for a negative (Fig. S5C) and a positive control (Fig. S5, D) in an FL3 vs FL1 plot. For propidium iodide (fluorescence in FL2) an FL3 vs FL2 plot is used and the gate is adapted where necessary.

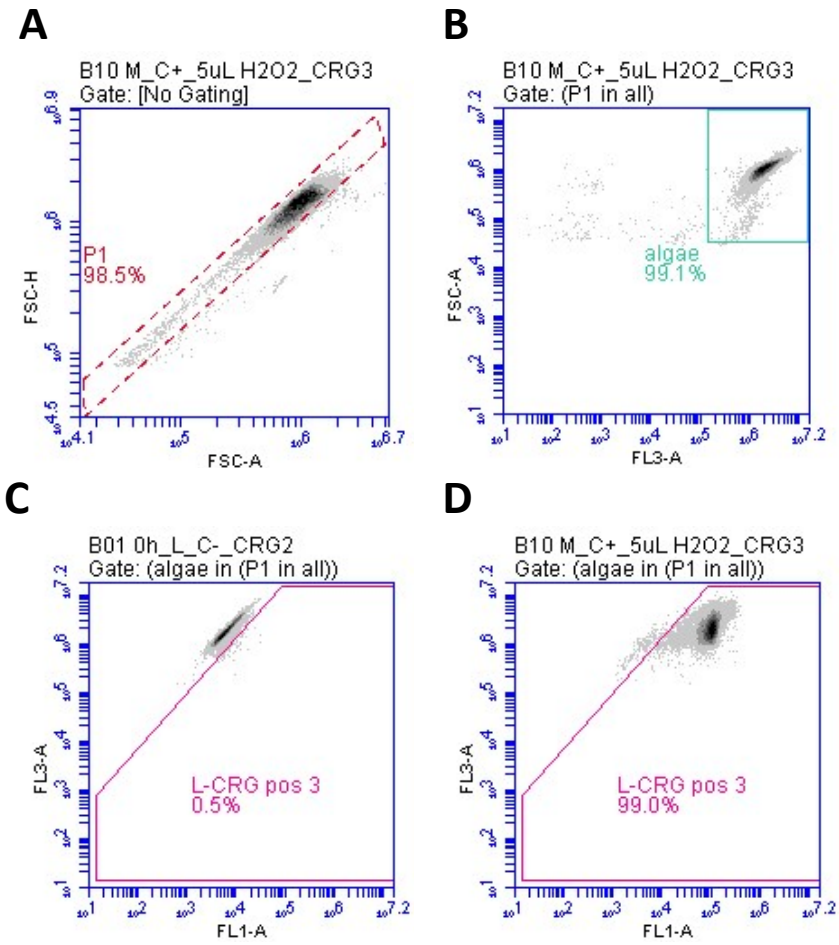


Figure S5. Gating strategy for FCM data analysis. A) Gate P1 to remove doublets. B) Defining the algae population. C) % CellRox Green positive cells in a negative control (0.5 %). D) % CellRox Green positive cells in a positive control (99.0 %).

2. RESULTS

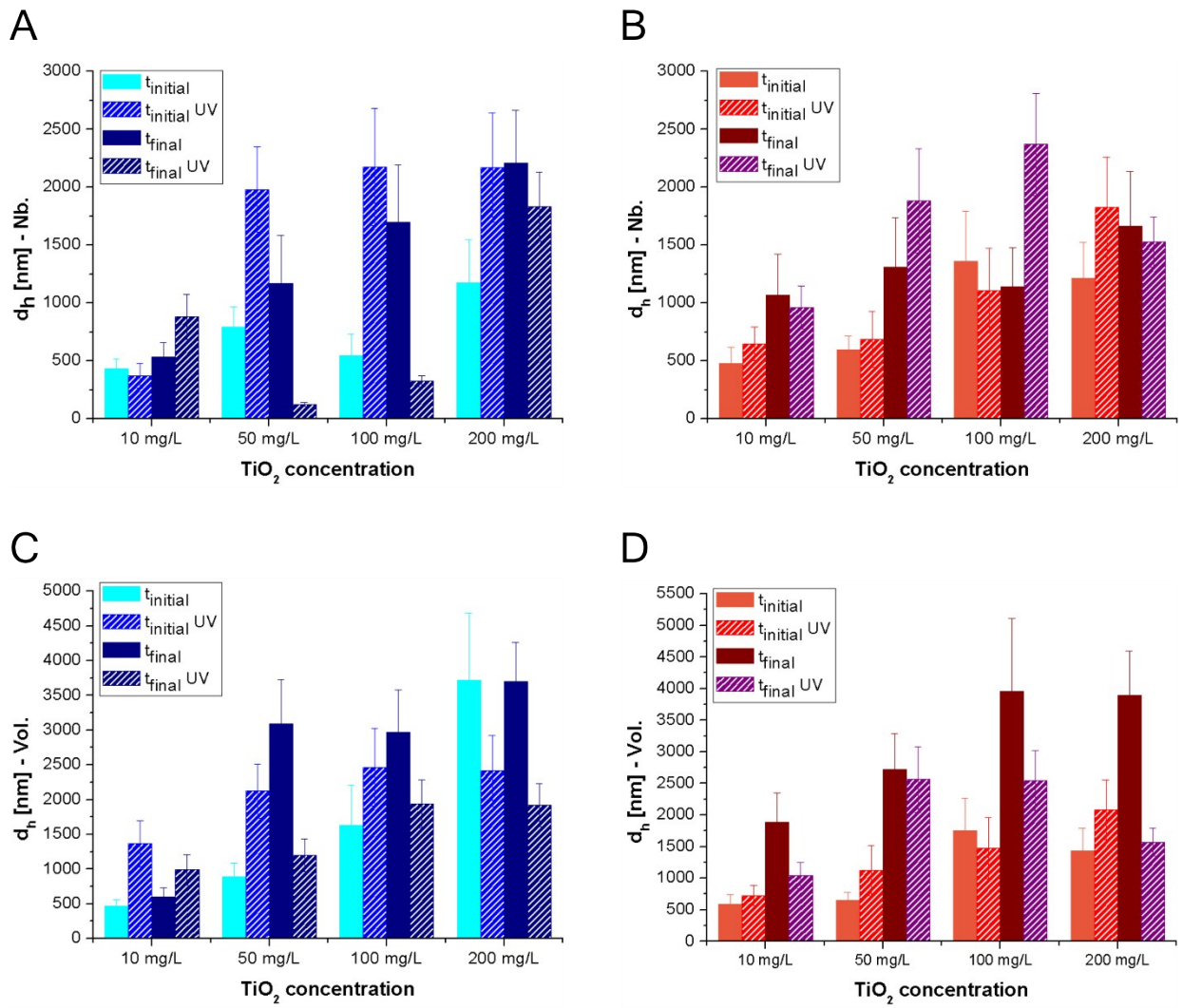


Figure S6. Mean number-weighted (A, B) and volume-weighted (C, D) hydrodynamic diameters (d_H) [nm] of different TiO_2 concentrations in lake water (A, C) and MOPS buffer (B, D). Diameters for untreated samples are provided at times $t_{initial}$ = 0 min and t_{final} = 60 min (min after the preparation of the suspension). Diameters of UV pre-treated samples are provided at times $t_{initial}$ *UV* and t_{final} *UV* (times t = 20 min and t = 80 min after suspension preparation, respectively).

Table S4. Polydispersity indices of nano-TiO_2 suspensions in lake water and MOPS (average values of 3 measurements).

	10 mg/L		50 mg/L		100 mg/L		200 mg/L	
	0 h	**1 h**	**0 h**	**1 h**	**0 h**	**1 h**	**0 h**	**1 h**
Lake water	0.322	0.405	0.212	0.269	0.199	0.942	0.250	0.412
MOPS	0.242	0.315	0.227	0.180	0.228	0.960	0.187	0.528

Table S5. Particle sedimentation after 1 h in the two experimental setups (biosensor and FCM), as measured experimentally by ICP-MS and estimated computationally by ISDD.

initial TiO_2 concentration [nominal, administered dose, mg TiO_2/L]	**medium**	**% administered Ti sedimented after 1 h (ICP-MS)**	**fraction of administered dose deposited after 1 h (ISDD)**	
		FCM	**FCM**	**biosensor**
10	**Lake water**	56	0.015	0.075
	MOPS	52	0.014	0.069
50	**Lake water**	89	-	-
	MOPS	87	-	-
100	**Lake water**	94	-	-
	MOPS	94	-	-
200	**Lake water**	97	0.017	0.085
	MOPS	94	0.017	0.082

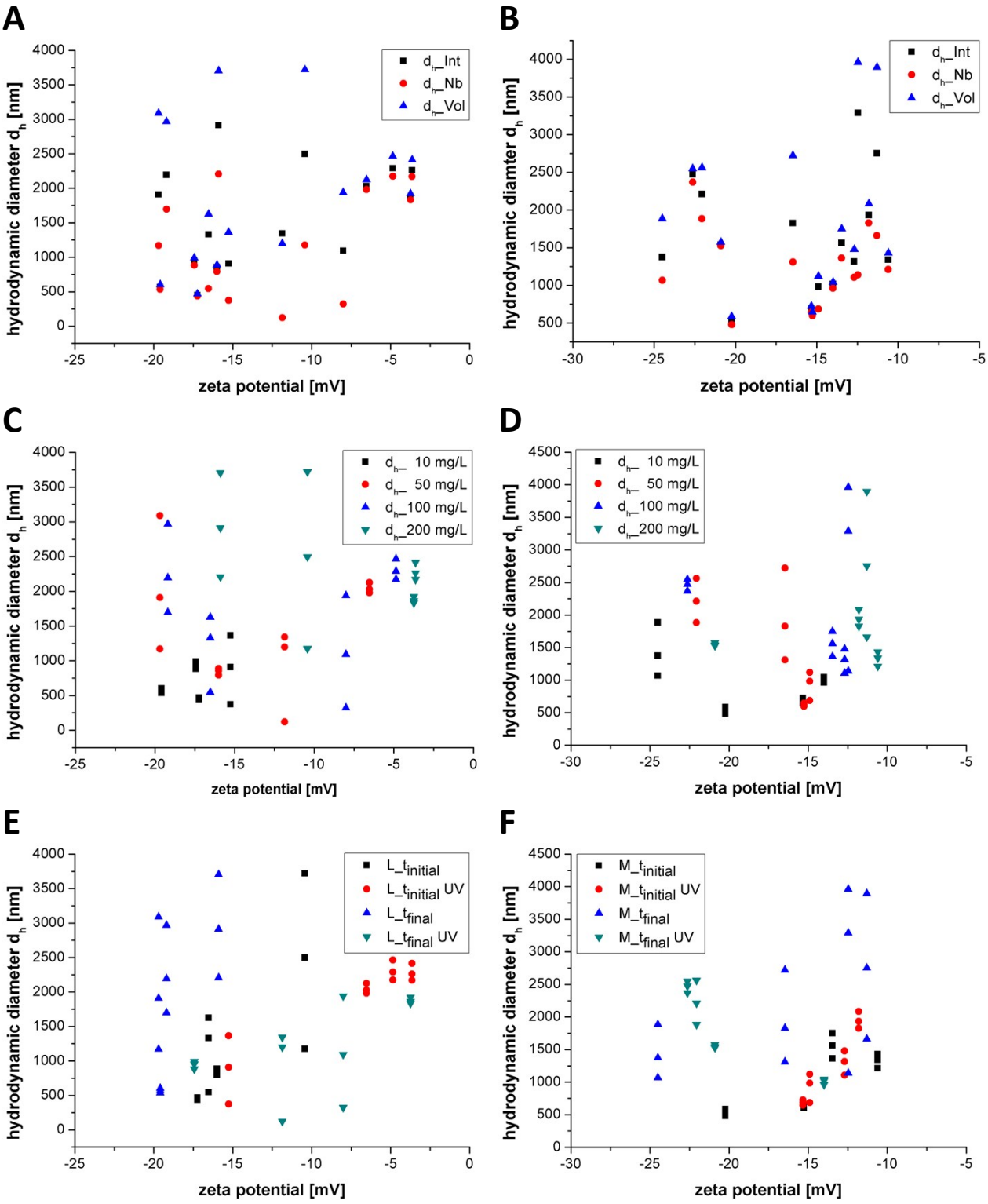


Figure S7. Scatter plots of hydrodynamic particle diameters vs zeta potentials in lake water (left column) and MOPS buffer (right column) as a function of diameter type (A, B), suspension concentrations (C, D) and time (E, F).

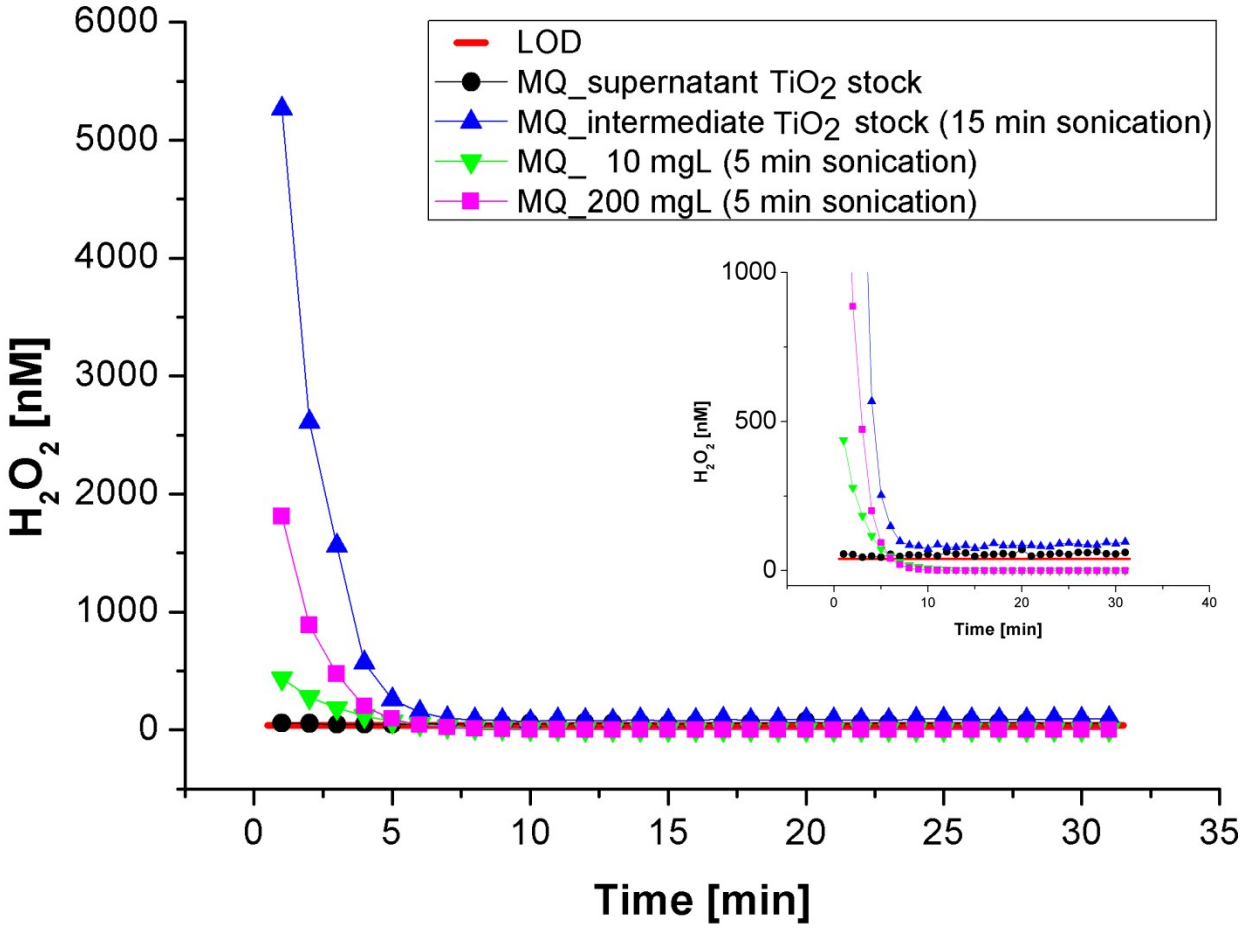


Figure S8. Average (of at least triplicates) H_2O_2 concentrations measured in the supernatants of a) the nano-TiO_2 stock suspension (2 g L^{-1}) in distilled water (as stored), b) nano-TiO_2 stock suspension (2 g L^{-1}) after initial 15 min sonication (= intermediate stock suspension), c) after 5 min sonication of the final nominal concentrations of 10 mg L^{-1} and 200 mg L^{-1} nano-TiO_2 in distilled water.

2.1 Output tables statistical analyses

Model selection is the process of considering all possible subsets of explanatory variables and finding the model that best fits the data according to some criteria (e.g. adjusted R^2, AIC, BIC) used to judge the usefulness of each parameter (www.stat.columbia.edu/~martin/W2024/R10.pdf, 26.01.16). There is always a trade-off between goodness of fit and explanatory power (complexity & bias). The Bayes information criterion (BIC) penalizes models with more parameters more strongly than the Akaike information criterion (AIC) and hence includes fewer parameters in the minimal adequate model[4, 5].

Treatment contrasts used to calculate model coefficients:

```
> contrasts(d.dcf$medium)
      [,1]
lake    1
MOPS   -1
```

Table S6. BIC model selection for abiotic ROS.

```
> summary(dcf.mod.BIC)

Call:
lm(formula = dcf ~ medium + time + UV + conc + medium:UV + medium:conc +
UV:conc + medium:UV:conc, data = d.dcf)

Residuals:
     Min       1Q   Median       3Q      Max
-1031.56  -259.18   -38.11   233.06  3137.47

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          1270.31      63.99  19.851  < 2e-16 ***
medium1               -51.17      60.91  -0.840  0.40134
time1                 465.63      39.23  11.869  < 2e-16 ***
UV1                   228.12      87.68   2.602  0.00958 **
conc10               -236.90      86.14  -2.750  0.00620 **
conc50               -274.60      86.14  -3.188  0.00153 **
conc100               -80.75      86.14  -0.937  0.34905
conc200               553.62      86.14   6.427 3.36e-10 ***
medium1:UV1           -16.33      87.68  -0.186  0.85234
medium1:conc10         17.10      86.14   0.199  0.84270
medium1:conc50         73.48      86.14   0.853  0.39411
medium1:conc100        35.67      86.14   0.414  0.67903
medium1:conc200       124.12      86.14   1.441  0.15030
UV1:conc10           -102.14     123.98  -0.824  0.41045
UV1:conc50             -1.03     123.98  -0.008  0.99338
UV1:conc100           111.56     123.98   0.900  0.36867
UV1:conc200           266.21     123.98   2.147  0.03231 *
medium1:UV1:conc10    -38.19     123.98  -0.308  0.75819
medium1:UV1:conc50   -168.64     123.98  -1.360  0.17445
medium1:UV1:conc100  -310.23     123.98  -2.502  0.01270 *
medium1:UV1:conc200  -644.71     123.98  -5.200 3.04e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 422 on 444 degrees of freedom
  (15 observations deleted due to missingness)
Multiple R-squared:  0.5963,   Adjusted R-squared:  0.5781
F-statistic: 32.79 on 20 and 444 DF,  p-value: < 2.2e-16
```

Table S7. AIC model selection for abiotic ROS.

```
> summary(dcf.mod.AIC)

Call:
lm(formula = dcf ~ medium + time + UV + conc + medium:UV + time:UV +
    medium:conc + UV:conc + medium:UV:conc, data = d.dcf)

Residuals:
     Min       1Q   Median       3Q      Max
-1004.22  -256.64   -52.39   228.27  3107.86

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          1240.69      66.61  18.625  < 2e-16 ***
medium1               -51.17      60.81  -0.841  0.40057
time1                 524.87      54.39   9.650  < 2e-16 ***
UV1                   291.91      96.51   3.025  0.00263 **
conc10               -236.90      86.00  -2.755  0.00612 **
conc50               -274.60      86.00  -3.193  0.00151 **
conc100               -80.75      86.00  -0.939  0.34826
conc200               553.62      86.00   6.438 3.16e-10 ***
medium1:UV1           -18.61      87.54  -0.213  0.83178
time1:UV1            -123.02      78.39  -1.569  0.11725
medium1:conc10         17.10      86.00   0.199  0.84244
medium1:conc50         73.48      86.00   0.854  0.39333
medium1:conc100        35.67      86.00   0.415  0.67854
medium1:conc200       124.12      86.00   1.443  0.14963
UV1:conc10           -102.14     123.77  -0.825  0.40968
UV1:conc50             -1.03     123.77  -0.008  0.99337
UV1:conc100           111.56     123.77   0.901  0.36788
UV1:conc200           266.21     123.77   2.151  0.03203 *
medium1:UV1:conc10    -38.19     123.77  -0.309  0.75781
medium1:UV1:conc50   -168.64     123.77  -1.362  0.17374
medium1:UV1:conc100  -310.23     123.77  -2.506  0.01255 *
medium1:UV1:conc200  -644.71     123.77  -5.209 2.92e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 421.3 on 443 degrees of freedom
  (15 observations deleted due to missingness)
Multiple R-squared:  0.5985,   Adjusted R-squared:  0.5795
F-statistic: 31.45 on 21 and 443 DF,  p-value: < 2.2e-16
```

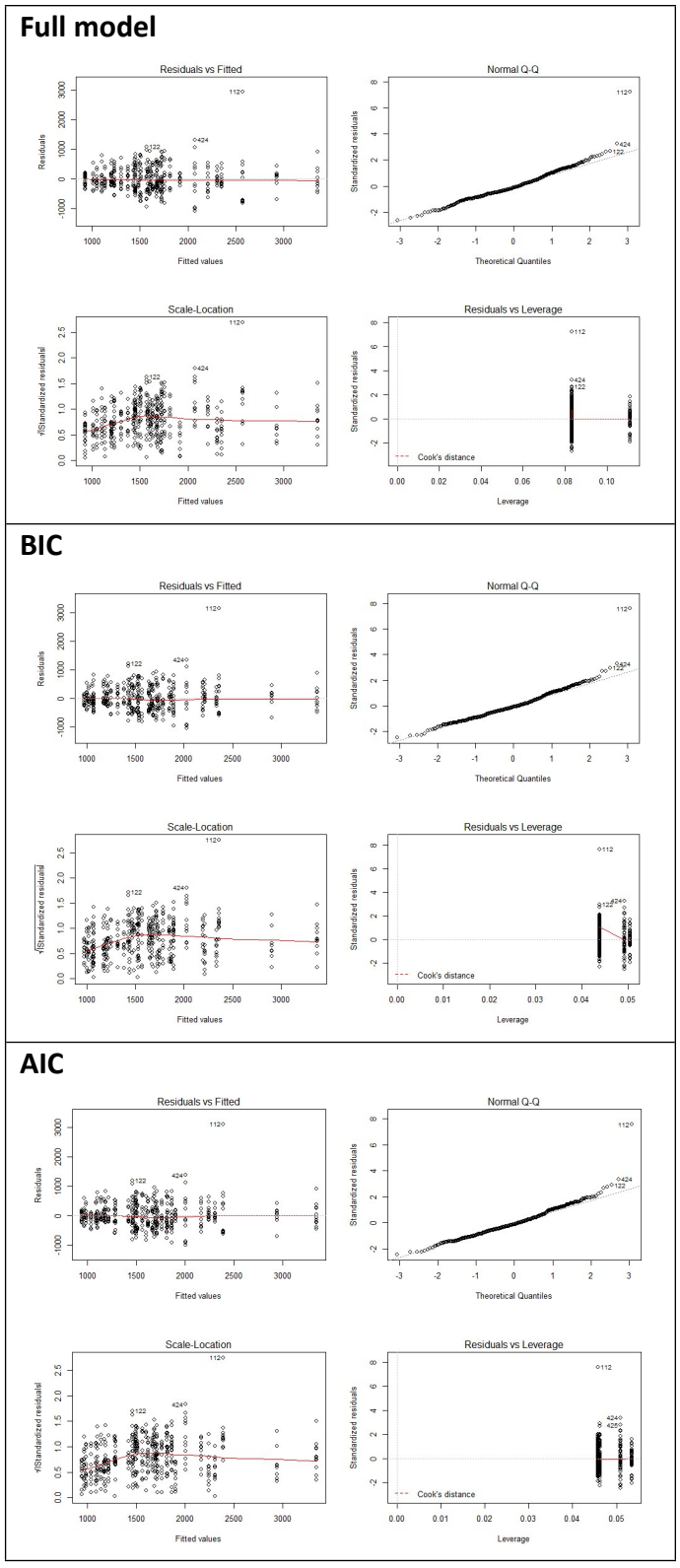


Figure S9. Residual analyses (R output) of the full linear model (1st row), models selected by BIC (2nd row) and by AIC (3rd row) for abiotic ROS.

Table S8. Intracellular ROS/oxidative stress – summary lake water (Fig. 5A).

	conc	time	UV	Min.	X1st.Qu.	Median	Mean	X3rd.Qu.	Max.
1	0	0	0	0.07	0.1400	0.140	0.2440	0.1400	0.73
2	10	0	0	0.10	0.1000	0.130	0.2260	0.2100	0.59
3	50	0	0	0.10	0.1000	0.200	0.2780	0.3600	0.63
4	100	0	0	0.26	0.2700	0.270	0.5340	0.4000	1.47
5	200	0	0	0.00	0.2200	0.220	0.3560	0.5600	0.78
6	0	1	0	0.22	0.2650	0.380	0.4600	0.5750	0.86
7	10	1	0	0.22	0.2575	0.390	0.4800	0.6125	0.92
8	50	1	0	0.21	0.4575	0.635	0.8050	0.9825	1.74
9	100	1	0	0.24	1.0350	1.405	1.4220	1.7920	2.64
10	200	1	0	0.65	3.6800	5.235	5.6200	7.1750	11.36
11	0	0	1	0.09	0.1100	0.170	0.2580	0.3900	0.53
12	10	0	1	0.09	0.0900	0.100	0.3900	0.3400	1.33
13	50	0	1	0.18	0.2500	0.300	0.5480	0.9700	1.04
14	100	0	1	0.17	0.2225	0.485	0.5900	0.8525	1.22
15	200	0	1	0.34	0.4750	1.020	0.9825	1.5280	1.55
16	0	1	1	0.39	0.7700	0.980	0.9040	1.1200	1.26
17	10	1	1	0.73	0.9025	1.070	1.0120	1.1800	1.18
18	50	1	1	0.88	1.0450	1.180	1.2220	1.3580	1.65
19	100	1	1	1.46	1.6930	1.835	1.8380	1.9800	2.22
20	200	1	1	1.67	2.2100	2.865	2.8450	3.5000	3.98

Table S9. Intracellular ROS/oxidative stress – summary MOPS buffer (Fig. 6A).

	conc	time	UV	Min.	X1st.Qu.	Median	Mean	X3rd.Qu.	Max.
1	0	0	0	2.52	3.890	3.89	4.452	5.090	6.87
2	10	0	0	2.77	4.760	4.76	5.058	6.010	6.99
3	50	0	0	3.56	5.210	6.01	5.886	6.010	8.64
4	100	0	0	4.00	5.800	6.19	5.908	6.190	7.36
5	200	0	0	3.32	3.540	3.54	4.198	4.720	5.87
6	0	1	0	4.85	8.160	16.59	20.850	26.010	48.63
7	10	1	0	6.27	9.230	16.80	21.240	24.130	49.79
8	50	1	0	7.25	11.750	19.17	20.030	21.240	40.73
9	100	1	0	9.12	14.590	20.41	21.200	23.780	38.09
10	200	1	0	6.25	10.930	14.61	13.810	17.070	20.17
11	0	0	1	1.83	3.825	5.05	4.688	5.912	6.82
12	10	0	1	1.78	3.450	4.22	4.258	4.820	7.02
13	50	0	1	2.17	3.930	5.48	4.946	6.370	6.78
14	100	0	1	2.56	5.100	5.93	5.830	7.060	8.50
15	200	0	1	2.25	3.740	4.61	5.262	7.120	8.59
16	0	1	1	4.50	4.710	12.07	13.150	20.510	23.95
17	10	1	1	3.48	4.080	11.48	11.860	19.250	20.99
18	50	1	1	5.35	8.230	15.85	15.540	23.160	25.12
19	100	1	1	5.75	9.605	15.42	15.520	21.340	25.47
20	200	1	1	5.04	5.408	11.79	12.000	18.380	19.37

Table S10. BIC model selection for intracellular ROS/oxidative stress.

```
> contrasts(d.mem$medium) <- contr.sum(2)
> contrasts(d.mem$medium)
     [,1]
lake    1
MOPS   -1

Call:
lm(formula = log(ox) ~ medium + time + UV + medium:UV, data = d.ox)

Residuals:
     Min       1Q   Median       3Q      Max
-1.50586 -0.46791 -0.00533  0.43828  2.51000

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.24643    0.08394   2.936  0.00376 **
medium1     -1.39984    0.06942 -20.165  < 2e-16 ***
time1        1.07964    0.10009  10.787  < 2e-16 ***
UV1          0.08336    0.09996   0.834  0.40541
medium1:UV1  0.29780    0.10003   2.977  0.00332 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6751 on 178 degrees of freedom
Multiple R-squared:  0.8123,   Adjusted R-squared:  0.8081
F-statistic: 192.6 on 4 and 178 DF,  p-value: < 2.2e-16
```

Table S11. AIC model selection for intracellular ROS/oxidative stress.

```
> contrasts(d.ox$medium) <- contr.sum(2)
> contrasts(d.ox$medium)
      [,1]
lake    1
MOPS   -1

Call:
lm(formula = log(ox) ~ medium + time + UV + conc + medium:UV +
    medium:conc, data = d.ox)

Residuals:
     Min       1Q   Median       3Q      Max
-2.15191 -0.42634  0.03094  0.38785  1.86758

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)     -0.01893    0.11969  -0.158 0.874540
medium1         -1.57796    0.11106 -14.209  < 2e-16 ***
time1            1.07601    0.09164  11.742  < 2e-16 ***
UV1              0.09782    0.09154   1.069 0.286783
conc10           0.03191    0.14377   0.222 0.824626
conc50           0.27378    0.14377   1.904 0.058554 .
conc100          0.49466    0.14487   3.415 0.000799 ***
conc200          0.53503    0.14487   3.693 0.000298 ***
medium1:UV1      0.31444    0.09161   3.432 0.000751 ***
medium1:conc10   0.02213    0.14374   0.154 0.877831
medium1:conc50   0.08907    0.14374   0.620 0.536308
medium1:conc100  0.22441    0.14487   1.549 0.123219
medium1:conc200  0.55451    0.14487   3.828 0.000181 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6179 on 170 degrees of freedom
Multiple R-squared:  0.8499,   Adjusted R-squared:  0.8393
F-statistic: 80.19 on 12 and 170 DF,  p-value: < 2.2e-16
```

Tabls S12. Membrane damage – summary lake water (Fig. 5B).

	conc	time	UV	Min.	X1st.Qu.	Median	Mean	X3rd.Qu.	Max.
1	0	0	0	1.67	2.09	2.36	3.182	2.98	6.81
2	10	0	0	1.53	1.91	2.69	2.980	3.21	5.56
3	50	0	0	1.51	1.81	2.54	2.242	2.65	2.70
4	100	0	0	1.98	2.15	2.75	2.564	2.91	3.03
5	200	0	0	1.97	2.97	6.32	6.122	7.41	11.94
6	0	1	0	5.03	5.04	9.66	7.970	9.80	10.32
7	10	1	0	3.57	8.23	9.83	8.700	10.58	11.29
8	50	1	0	3.71	9.17	9.41	9.152	10.23	13.24
9	100	1	0	4.54	12.84	12.89	12.630	13.01	19.89
10	200	1	0	5.22	16.56	17.40	19.890	28.44	31.82
11	0	0	1	0.50	1.18	1.64	1.478	1.88	2.19
12	10	0	1	0.83	1.84	2.05	1.968	2.50	2.62
13	50	0	1	0.77	1.83	2.40	2.078	2.41	2.98
14	100	0	1	0.58	2.05	2.44	2.732	3.90	4.69
15	200	0	1	1.41	2.14	3.39	3.236	4.05	5.19
16	0	1	1	3.10	3.65	4.14	4.190	4.37	5.69
17	10	1	1	0.10	3.37	5.39	5.404	6.74	11.42
18	50	1	1	0.07	2.36	3.80	4.354	4.24	11.30
19	100	1	1	0.32	2.82	4.21	4.718	5.52	10.72
20	200	1	1	1.09	2.64	7.06	6.132	9.34	10.53

Table S13. Membrane damage – summary MOPS buffer (Fig. 6B).

	conc	time	UV	Min.	X1st.Qu.	Median	Mean	X3rd.Qu.	Max.
1	0	0	0	0.06	0.08	0.13	0.164	0.26	0.29
2	10	0	0	0.06	0.08	0.11	0.174	0.27	0.35
3	50	0	0	0.07	0.11	0.12	0.156	0.19	0.29
4	100	0	0	0.12	0.13	0.15	0.188	0.25	0.29
5	200	0	0	0.20	0.24	0.24	0.256	0.30	0.30
6	0	1	0	0.45	0.47	0.63	1.192	1.45	2.96
7	10	1	0	0.28	0.42	0.46	0.646	0.60	1.47
8	50	1	0	0.16	0.33	0.47	0.540	0.49	1.25
9	100	1	0	0.11	0.20	0.23	0.614	0.74	1.79
10	200	1	0	0.33	0.41	0.43	0.730	0.57	1.91
11	0	0	1	0.04	0.06	0.07	0.078	0.10	0.12
12	10	0	1	0.02	0.06	0.06	0.070	0.08	0.13
13	50	0	1	0.03	0.04	0.06	0.068	0.09	0.12
14	100	0	1	0.03	0.08	0.11	0.096	0.12	0.14
15	200	0	1	0.00	0.16	0.19	0.166	0.23	0.25
16	0	1	1	0.11	0.17	0.34	0.298	0.42	0.45
17	10	1	1	0.07	0.09	0.10	0.196	0.36	0.36
18	50	1	1	0.14	0.19	0.24	0.274	0.32	0.48
19	100	1	1	0.16	0.16	0.33	0.316	0.43	0.50
20	200	1	1	0.25	0.28	0.62	0.580	0.66	1.09

Table S14. BIC model selection for membrane damage.

```
> contrasts(d.mem$medium) <- contr.sum(2)
> contrasts(d.mem$medium)
      [,1]
lake    1
MOPS   -1

Call:
lm(formula = log(mem) ~ medium + time + UV, data = d.mem)

Residuals:
    Min      1Q  Median      3Q     Max
-3.8505 -0.4012  0.0372  0.4193  1.7022

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.20551    0.08992  -2.285   0.0234 *
medium1      1.37810    0.05192  26.545  < 2e-16 ***
time1        0.97336    0.10383   9.374  < 2e-16 ***
UV1         -0.70338    0.10383  -6.774 1.42e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7342 on 196 degrees of freedom
Multiple R-squared:  0.8105,   Adjusted R-squared:  0.8076
F-statistic: 279.5 on 3 and 196 DF,  p-value: < 2.2e-16
```

Table S15. AIC model selection for membrane damage.

```
> contrasts(d.mem$medium) <- contr.sum(2)
> contrasts(d.mem$medium)
      [,1]
lake    1
MOPS   -1

Call:
lm(formula = log(mem) ~ medium + time + UV + conc + medium:time +
    medium:UV + time:UV + medium:time:UV, data = d.mem)

Residuals:
    Min      1Q  Median      3Q     Max
-3.3766 -0.3697  0.0945  0.3983  1.6926

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       -0.32904    0.14003  -2.350 0.019825 *
medium1            1.37817    0.09902  13.918  < 2e-16 ***
time1              1.16753    0.14003   8.337 1.58e-14 ***
UV1               -0.50921    0.14003  -3.636 0.000357 ***
conc10            -0.13841    0.15656  -0.884 0.377798
conc50            -0.16581    0.15656  -1.059 0.290929
conc100            0.01157    0.15656   0.074 0.941164
conc200            0.42488    0.15656   2.714 0.007270 **
medium1:time1      0.06103    0.14003   0.436 0.663479
medium1:UV1        0.12331    0.14003   0.881 0.379691
time1:UV1         -0.38835    0.19804  -1.961 0.051358 .
medium1:time1:UV1 -0.36896    0.19804  -1.863 0.064012 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7002 on 188 degrees of freedom
Multiple R-squared:  0.8347,   Adjusted R-squared:  0.825
F-statistic: 86.31 on 11 and 188 DF,  p-value: < 2.2e-16
```

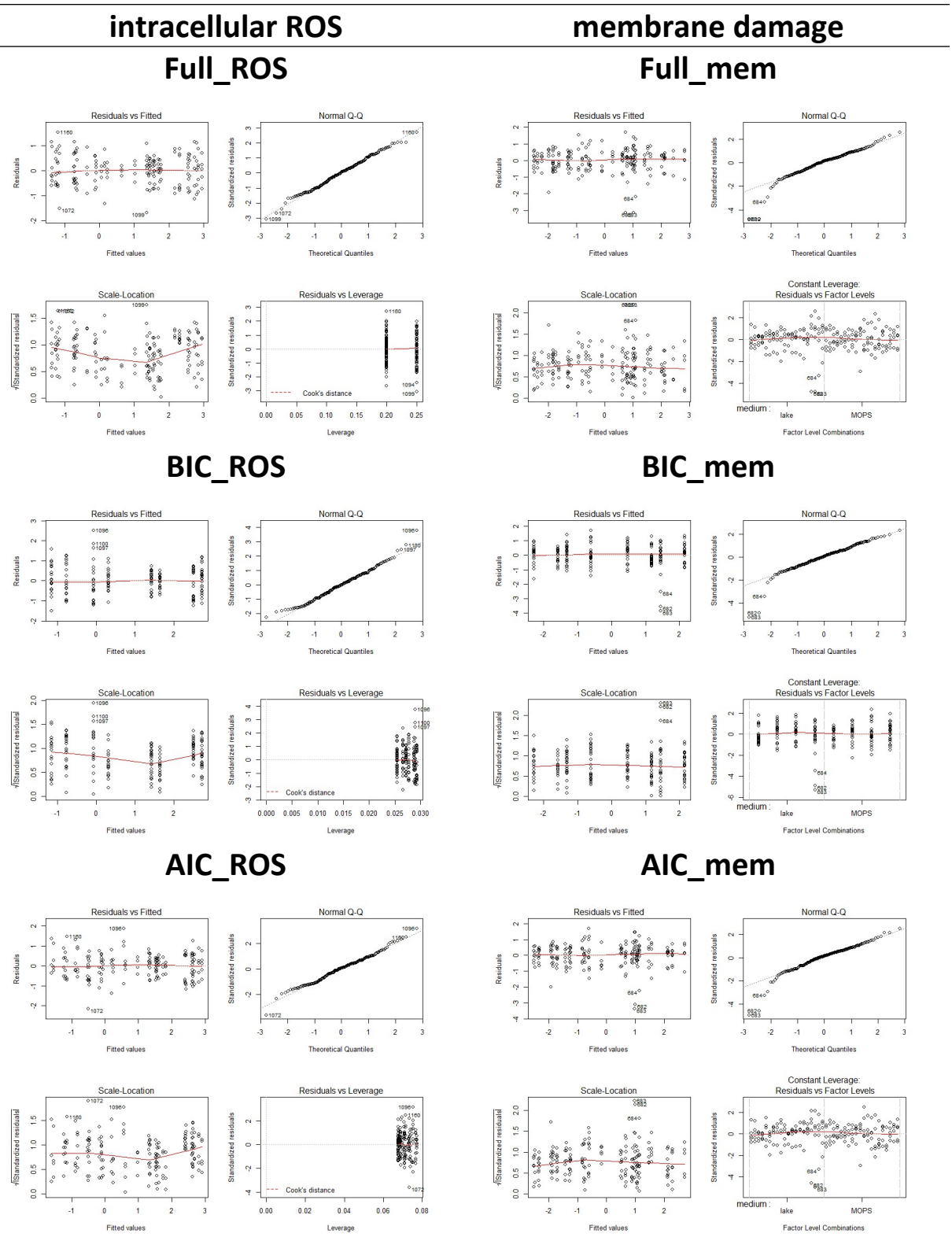


Figure S10. Residual analyses (R output) of the full linear models (1st row), models selected by BIC (2nd row) and by AIC (3rd row) for intracellular ROS (1st column) and membrane damage (2nd column).

Table S16. Summary of linear models fitted to data for abiotic ROS and cellular endpoints of stress (Tables S2-S11, Figs. S10 and S11).

Endpoint: abiotic ROS (H_2DCF-DA)	
BIC model:	`medium + time + UV + conc + medium:UV + medium:conc + UV:conc + medium:UV:conc`
AIC model:	`medium + time + UV + conc + medium:UV + time:UV + medium:conc + UV:conc + medium:UV:conc`
Endpoint: oxidative stress (CellRox Green)	
BIC model:	`medium + time + UV + medium:UV`
AIC model:	`medium + time + UV + conc + medium:UV + medium:conc`
Endpoint: membrane damage (propidium iodide)	
BIC model:	`medium + time + UV`
AIC model:	`medium + time + UV + conc + medium:UV + time:UV + medium:time + medium:time:UV`

REFERENCES

1. P. Hinderliter, K. Minard, G. Orr, W. Chrisler, B. Thrall, J. Pounds and J. Teeguarden, *Particle and Fibre Toxicology*, 2010, **7**, 36.
2. G. DeLoid, J. M. Cohen, T. Darrah, R. Derk, L. Rojanasakul, G. Pyrgiotakis, W. Wohlleben and P. Demokritou, *Nature Communications*, 2014, **5**, 10.
3. V. B. Koman, C. Santschi, N. R. von Moos, V. I. Slaveykova and O. J. F. Martin, *Biosensors and Bioelectronics*, 2015, **68**, 245-252.
4. M. J. Crawley, *Statistics: an introduction using R*, John Wiley & Sons, 2014.
5. K. Aho, D. Derryberry and T. Peterson, *Ecology*, 2014, **95**, 631-636.