

Supplementary material

Graphene-based materials do not impair physiology, gene expression and growth dynamics of the aeroterrestrial microalga *Trebouxia gelatinosa*

Supplementary Table S1. Limits of Detection of the ICP-OES elemental analysis.

	Wavelength	LOD (mg L ⁻¹)
Cd	228.802	0.005
Cr	267.716	0.005
Cu	327.393	0.010
Pb	220.353	0.010
Mn	257.610	0.005
K	766.490	0.010

Supplementary Table S2. Primers used for quantitative Real-Time PCR analysis.

Gene	Primer ID	Forward sequence	Reverse sequence
Ascorbate peroxidase[#]	APX	CAGGGTTCACAAGGACAGGT	TCAGCAAACAGGCACTCATC
Catalase[#]	CAT	ACTACTTCCCATCCCGCTTT	CCTGGTGATGAACCTGTCCT
Desiccation Related Protein 11[#]	DPR11	CATATGGCGAGGGTATTGCT	TGTGCGATTTTCATTCTCAGC
Glutathione reductase[#]	GR	TTCGAACAGCAGACATCGAC	CCTCCAGTCTTTTCGTCAGC
Heat Shock Cognate 70[#]	HSC70	AGGAGCAGACCTTCTCCACA	GACCACAATTTGGGGAACAC
Heat Shock Protein 70 - 1[#]	HSP70-1	CAGTCACCACTGCCTTCTCA	CAAGTCAGCCAATGCAAAGA
Light Harvesting Complex II[#]	LHCII	CTGATGACCCAGATGCCTTT	GGTCCTTTGCCTGTCACAAT
Mn-superoxide dismutase[#]	MnSOD	CACCCAGCTTGCTGACTACA	GGTCAAACCTGTGCCTGGAAT
Ribosomal protein L6[*]	RPL6	AGGAGCTAGCTAGGGGCATC	TCTCGTGCTTTGGGAACTCT

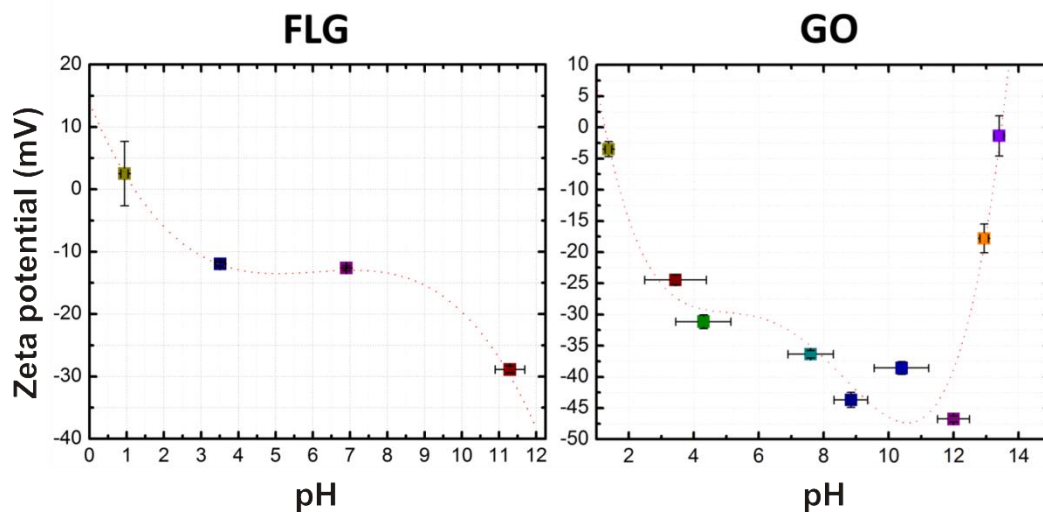
[#] from Montagner et al. (2017); ^{*}from Candotto Carniel et al. (2016)

Supplementary Table S3. Total carotenoids content (mean \pm standard deviation) in *T. gelatinosa* after 4 weeks of growth ($\mu\text{g mL}^{-1}$; n = 7).

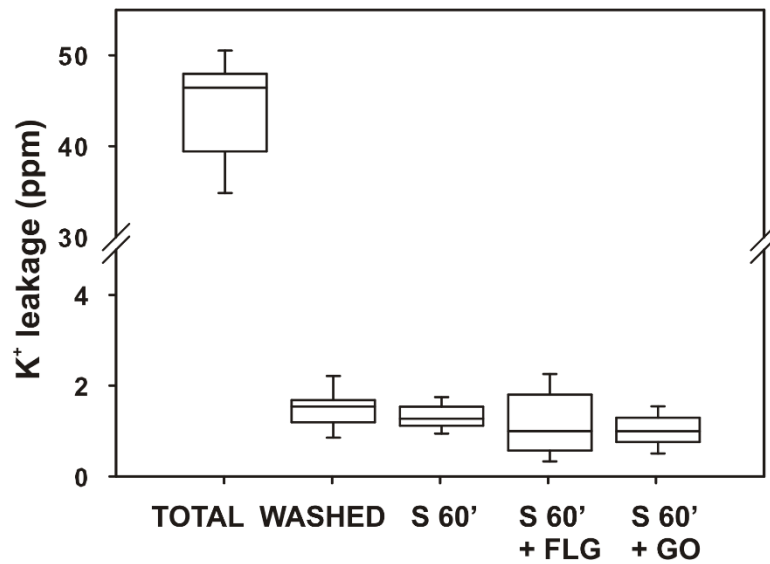
	FLG	GO
CTRL	5.7 \pm 0.8	6.3 \pm 0.8
0.01 S	6.5 \pm 0.8	7.1 \pm 0.7
1 S	6.3 \pm 0.6	7.6 \pm 0.4
50 S	6.3 \pm 0.7	6.6 \pm 0.5
0.01 D	6.4 \pm 0.7	7.1 \pm 0.2
1 D	6.1 \pm 0.8	7.2 \pm 0.8
50 D	6.5 \pm 0.8	6.5 \pm 0.7

Supplementary Table S4. Total chlorophylls and carotenoids content (mean \pm standard deviation) in *T. gelatinosa* treated with melamine after 4 weeks of growth ($\mu\text{g mL}^{-1}$; n = 7).

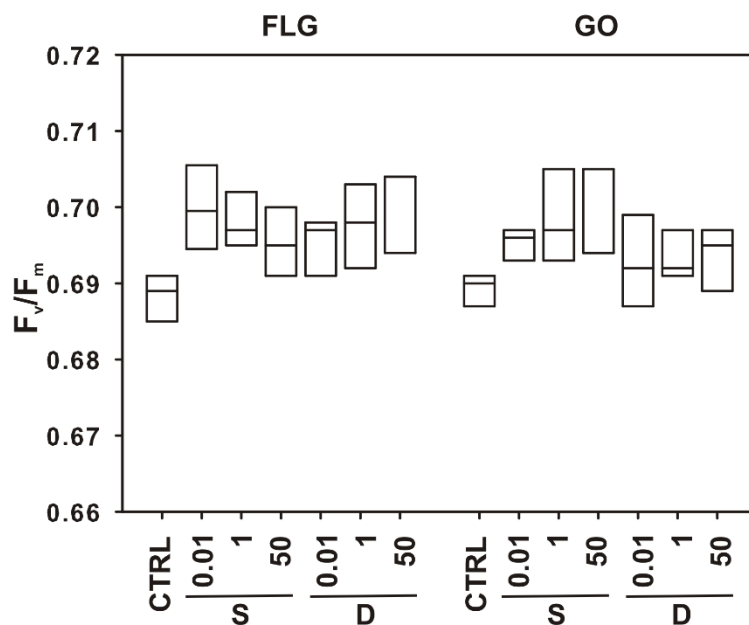
	Chl a	Chl b	Car
CTRL	28.90 \pm 3.65	8.25 \pm 2.18	8.50 \pm 1.27
0.01	31.13 \pm 0.76	9.08 \pm 0.31	9.06 \pm 0.21
0.5	28.55 \pm 4.16	7.69 \pm 1.87	8.60 \pm 1.10



Supplementary Figure S1. Zeta potential of few-layer graphene (FLG) and graphene oxide (GO) in MilliQ water at different pH levels.



Supplementary Figure S2. Potassium (K⁺) leakage in *Trebouxia gelatinosa* samples. Total K⁺ content of pre-exposure samples (TOTAL); K⁺ leakage from control samples washed but not shaken (WASHED); samples washed and shaken for 60' without GBMs (S 30') and with 50 μg mL⁻¹ FLG (S 60' + FLG) or GO (S 60' + GO) (n = 9). Boxplots report median, 25°–75° percentiles (boxes) and non-outlier minimum and maximum (whiskers).



Supplementary Figure S3. Maximum quantum efficiency of PSII photochemistry (F_v/F_m) measured in 4-week old axenic cultures of *Trebouxia gelatinosa*. The cells were exposed to FLG (a) and GO (b) at 0.01, 1, 50 $\mu\text{g mL}^{-1}$ (0.01, 1, 50 respectively), through the “shaken” and “deposit” treatments (S and D, respectively) ($n = 7$). Boxplots report median and 25°–75° percentiles.

Reference : Montagner, A. 2017. Ecotoxicological effects of Graphene-Based Materials. Thesis (PhD). University of Trieste.