Supporting information

Humic acid mitigated toxicity of graphene-family materials to algae through reducing oxidative stress and heteroaggregation

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Experimental S1. Adsorption of HA on GFMs

The Freundlich and Langmuir models were used to fit the adsorption isotherms:[1]

$$Q_e = K_f C_e^{\ N}$$

$$Q_e = Q_m C_e/(K_L + C_e)$$

where Q_e (mg/kg) is the equilibrium concentration of adsorbed HA, C_e (mg/L) is the equilibrium aqueous concentration of HA, K_f [(mg/kg)/(mg/L)^N] is the Freundlich capacity factor, N is the Freundlich site heterogeneity factor,[2] Q_m (mg/kg) is the maximum adsorption capacity of adsorbates, and K_L (mg/L) is the Langmuir adsorption affinity coefficient, which is related to adsorption energy.

References

1. J. Zhao, Z. Wang, Q. Zhao and B. Xing, Adsorption of phenanthrene on multilayer graphene as affected by surfactant and exfoliation, *Environ. Sci. Technol.*, 2013, **48**, 331-339.
2. B. M. Lee, Y. S. Seo and J. Hur, Investigation of adsorptive fractionation of humic acid on graphene oxide using fluorescence EEM-PARAFAC, *Water Res.*, 2015, **73**, 242-251.

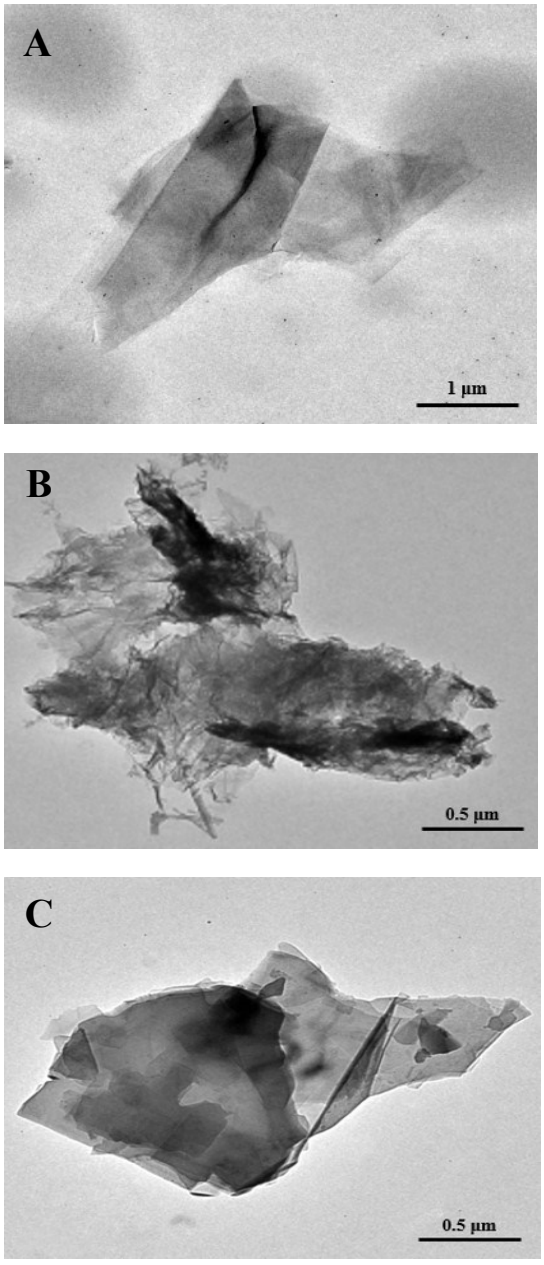


Fig. S1. TEM images of GO (A), rGO (B), and G (C).

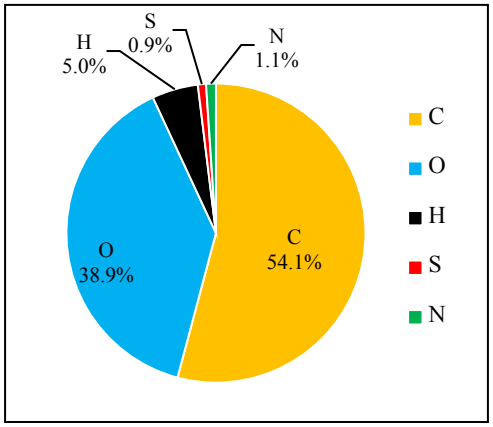
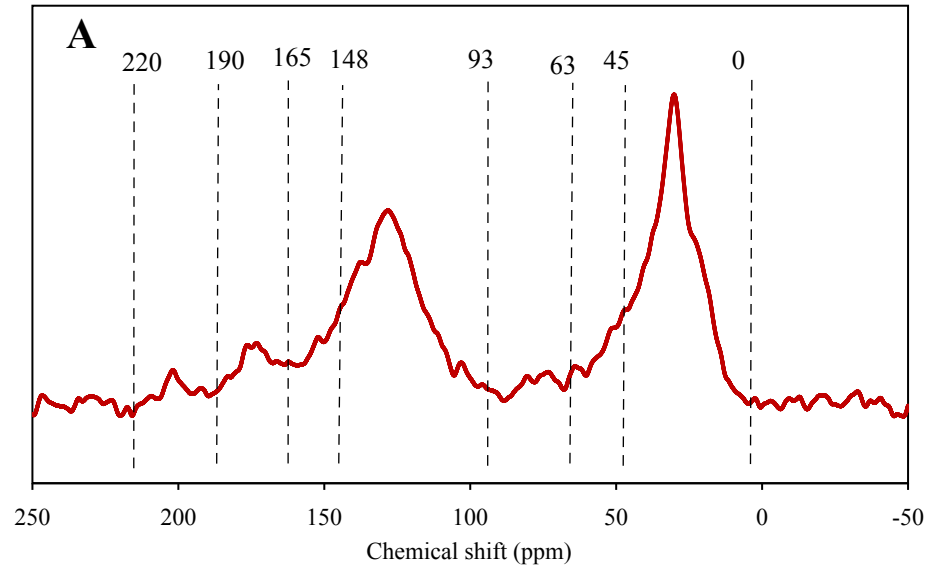


Fig. S2. Elemental composition of the organic components in HA as detected by an elemental analyzer.



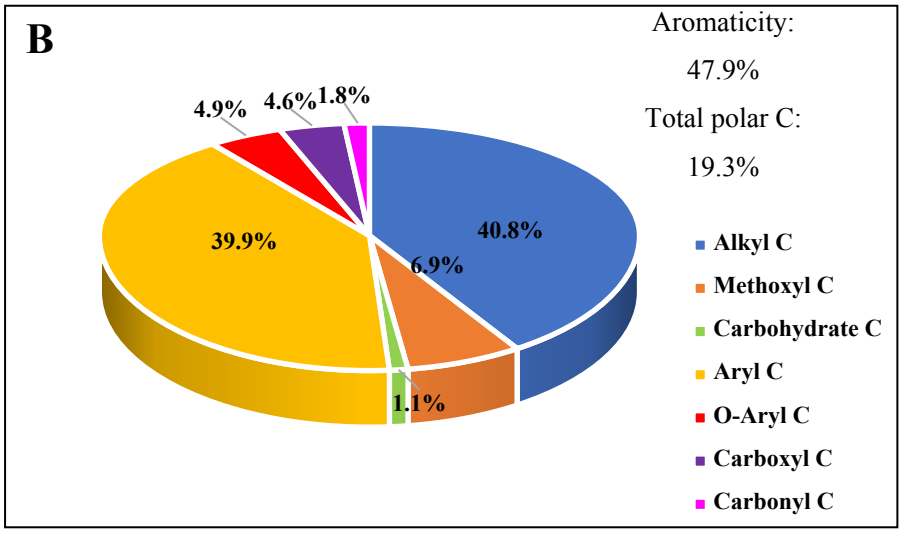


Fig. S3. The C composition of HA by ^{13}C NMR spectra analysis. (A): Cross-polarization magic angle spinning ^{13}C NMR spectra of HA. Chemical shift 0-45 ppm is alkyl C, 45-63 ppm is methoxyl C, 63-93 ppm is carbohydrate C, 93-148 ppm is aryl C, 148-165 ppm is O-aryl C, 165-190 ppm is carboxyl C, and 190-220 ppm is carbonyl C. Therefore, alkyl C, methoxyl C, and carbohydrate C are collectively known as aliphatic C; aryl C and O-aryl C are collectively known as aromatic C; (B): Contents of all types of carbon in HA. Based on above information, aromaticity and total polar C could be calculated by formulas: aromaticity (%) = aromatic C / (aliphatic C + aromatic C); total polar C (%) = polar aliphatic C (methoxyl C and carbohydrate C) + polar aromatic C (O-aryl C) + carboxyl C + carbonyl C.

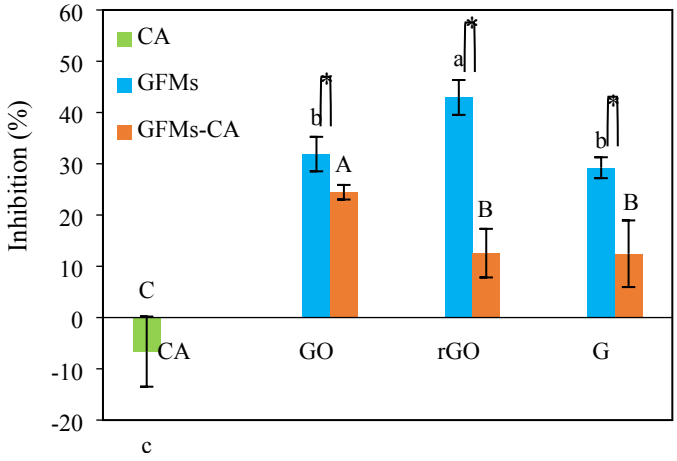


Fig. S4. Growth inhibition of GFMs in the presence of CA. The concentrations of GFMs and CA were 40 and 20 mg/L, respectively. Significant differences among CA, GO, rGO, and G treatments are marked with different lowercase letters (p<0.05, LSD, n=3). Significant difference among CA, GO-CA, rGO-CA, and G-CA treatments are marked with different capital letters (p<0.05, LSD, n=3). For a given GFMs, significant difference between GFMs and GFMs-CA is marked with "*" (p<0.05, T test, n=3).

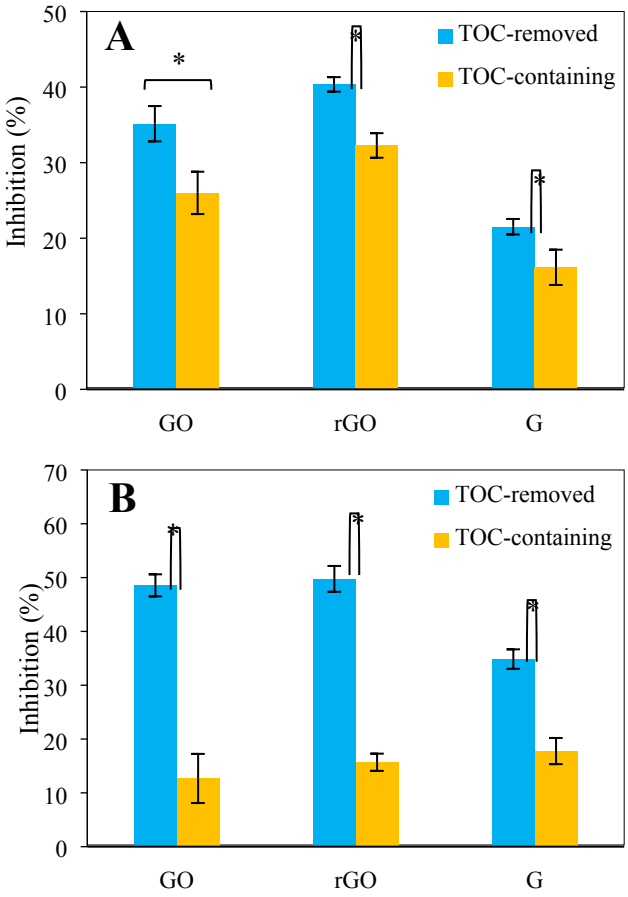
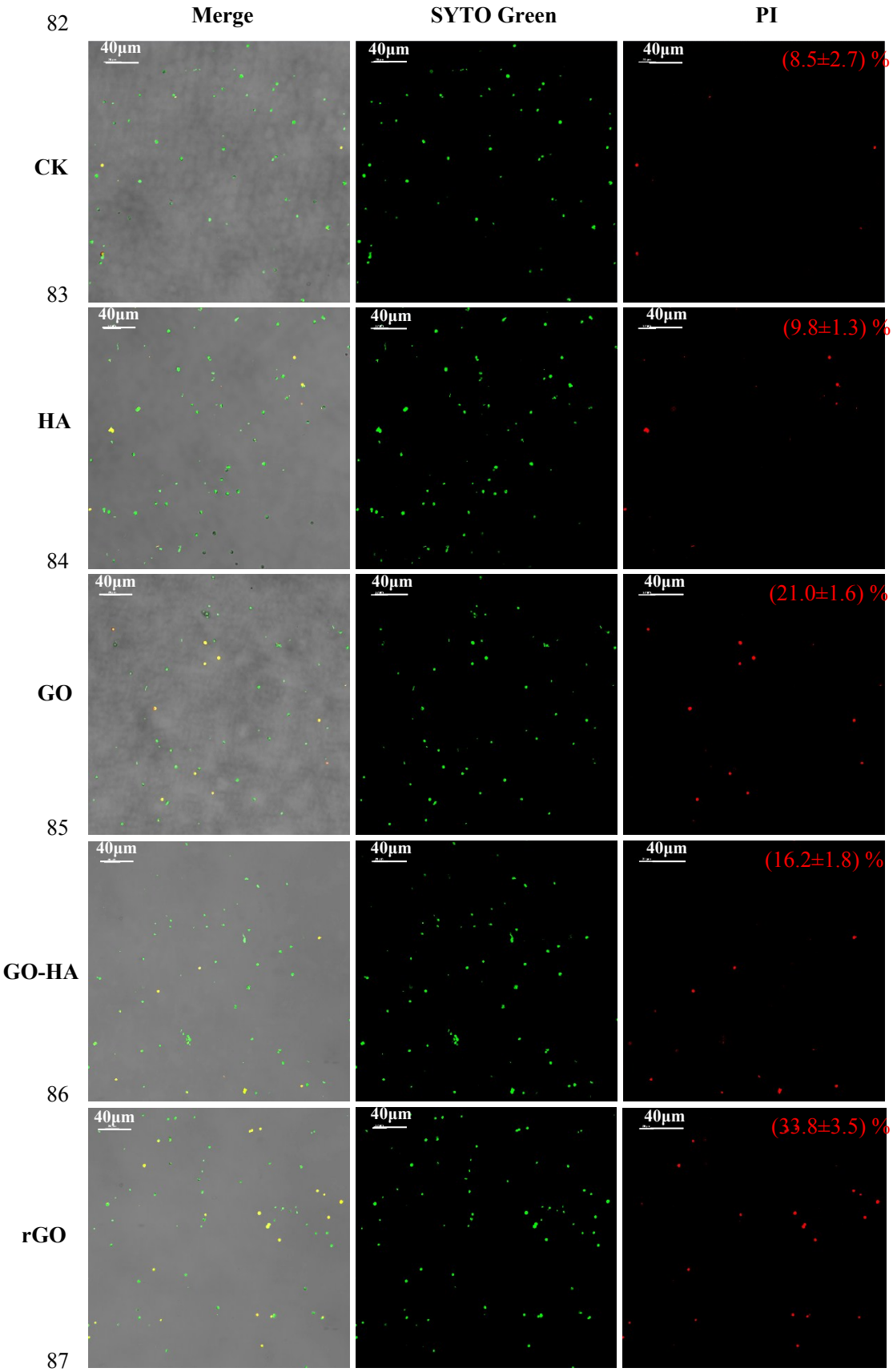


Fig. S5. Growth inhibition of GFMs in natural water. (A): Growth inhibition of GFMs in natural water as obtained in Zhangcun River (TOC: 21.1 mg/L) in Qingdao; (B): Growth inhibition of GFMs in natural water as obtained in the campus lake (TOC: 90.7 mg/L) in Ocean University of China. For a given GFMs, significant difference between TOC-removed and TOC-containing water is marked with "*" (p<0.05, T test, n=3).


Merge
SYTO Green
PI
CK
HA
GO
GO-HA
rGO
40μm
(8.5±2.7) %
(9.8±1.3) %
(21.0±1.6) %
(16.2±1.8) %
(33.8±3.5) %

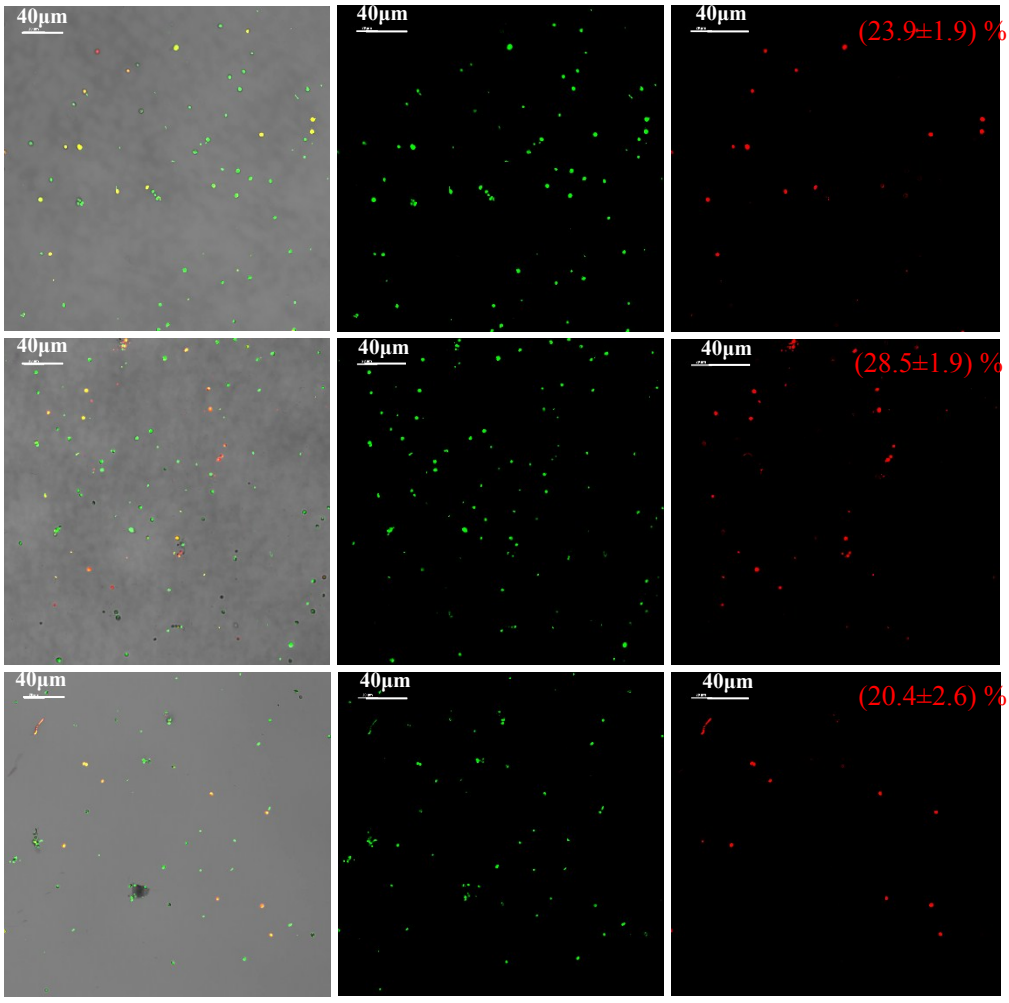


Fig. S6. GFMs-induced membrane damage of algal cells in the presence of HA as detected by CLSM. Algal cells were exposed to GFMs (40 mg/L) in the absence and presence of HA (20 mg/L) for 96 h. The membrane damage was detected by SYTO Green and PI staining. In each group of CLSM images, green and red dots indicate total cells and membrane-damaged cells, respectively. "CK" represents the un-exposed algal cells.

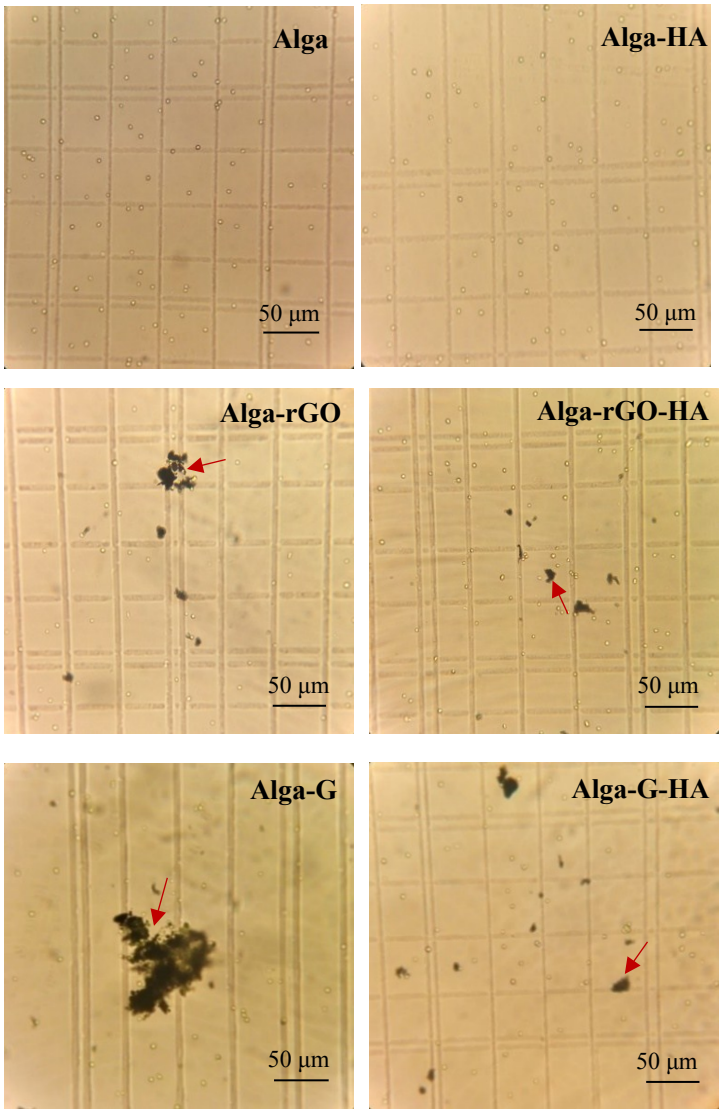


Fig. S7. Light microscope photos on the aggregation of algal cells with GFMs (e.g., rGO, G) as affected by HA (20 mg/L) in algal medium. Red arrows indicate the heteroaggregates of algal cells and GFMs.

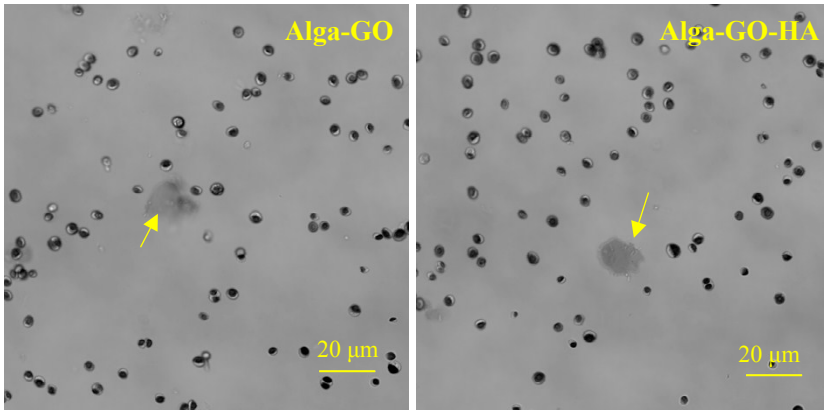
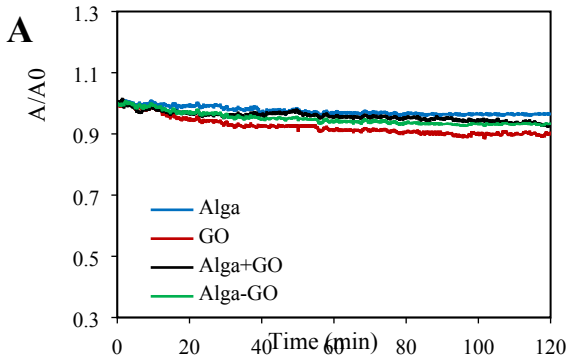


Fig. S8. Microscope photos in light field of algal cells exposed to GO (40 mg/L) in the absence and presence of HA (20 mg/L) using confocal microscope. Yellow arrows indicate GO sheets in the images.



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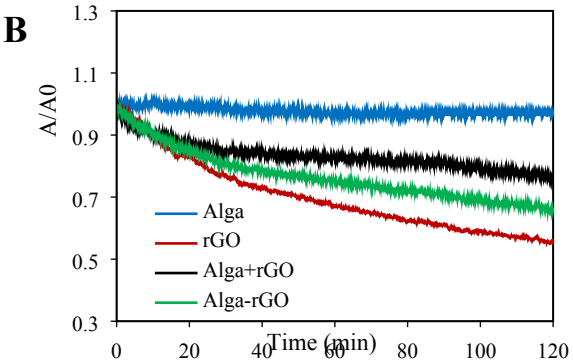

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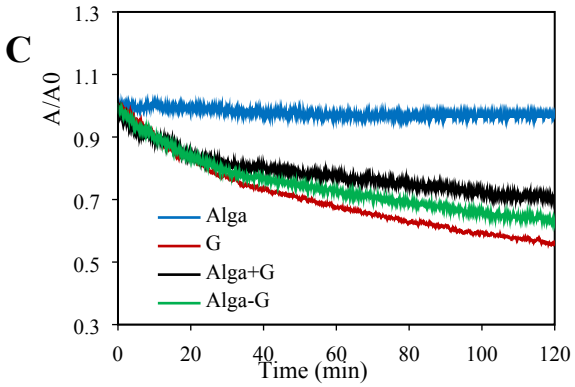

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113 Fig. S9. The settling curves of algal cells and GFMs. (A), (B), and (C): Settling curves
114 of algal cells with GO, rGO, and G, respectively. For each panel, “Alga+GFMs”
115 represents the theoretically additive settling curves, while “Alga-GFMs” represents
116 the actual settling curves between algal cells and GFM. The settling curves were
117 detected for 2 h after the incubation of algal cells with GFMs in algal medium.

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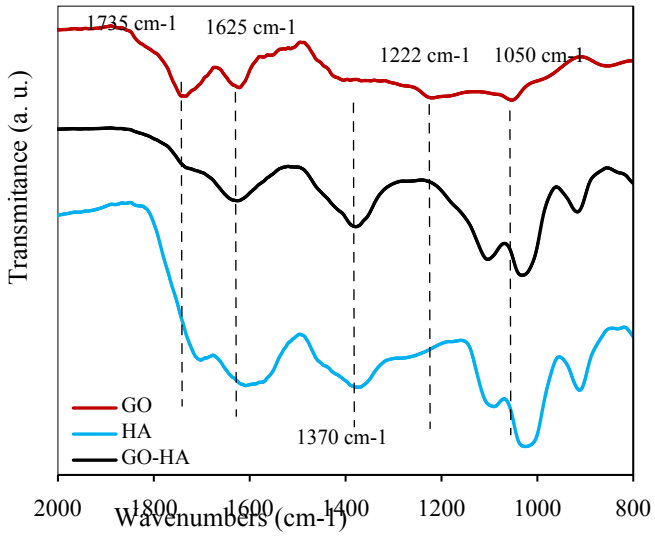

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120 Fig. S10. FTIR spectra of HA, GO, and GO after the adsorption of HA. In the figure,
121 "GO-HA" represents HA-adsorbed GO after incubation for 96 h.

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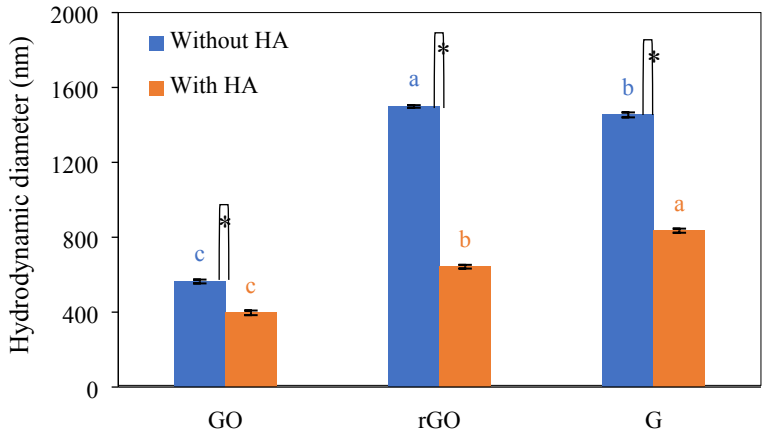

Fig. S11. Hydrodynamic diameters of GFMs in the absence and presence of HA in algal medium (pH, 7; GFMs, 40 mg/L; HA, 20 mg/L). For a given treatment (with or without HA), significant differences among GFMs are marked with different letters (p<0.05, LSD, n=3). For a given GFMs, significant difference between GFMs and GFMs-HA is marked with "*" (p<0.05, T test, n=3).

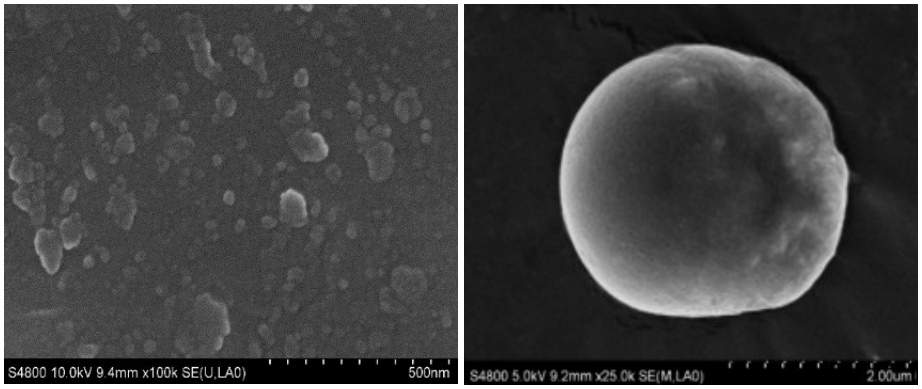

Fig. S12. SEM image of HA and normal algal cell in the algal medium.

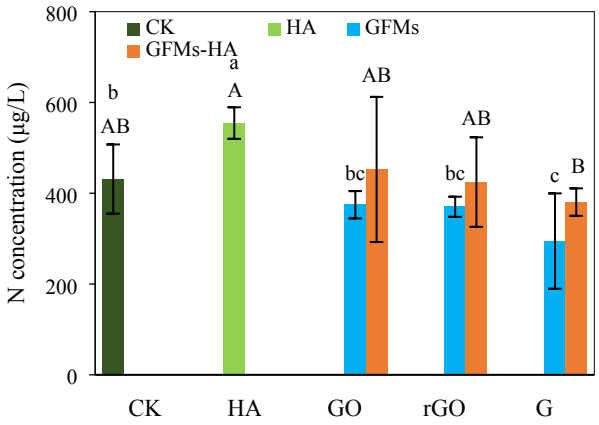

Fig. S13. Concentration of N in the supernatants after adsorption. "CK" represents the pristine algal medium. Significant differences among CK, HA, GO, rGO, and G treatments are marked with different lowercase letters (p<0.05, LSD, n=3). Significant difference among CK, HA, GO-HA, rGO-HA, and G-HA treatments are marked with different capital letters (p<0.05, LSD, n=3). For a given GFMs, significant difference between GFMs and GFMs-HA is marked with "*" (p<0.05, T test, n=3).

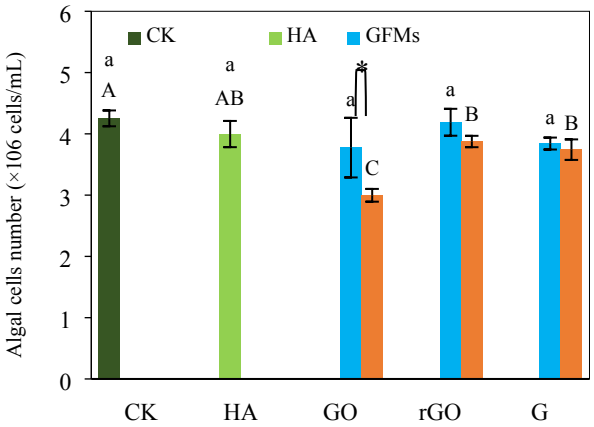

Fig. S14. The shading effect induced by HA (20 mg/L), GFMs (40 mg/L) and GFMs-HA (GFMs, 40 mg/L; HA, 20 mg/L) on algal growth after exposure for 96 h. "CK" represents un-exposed algal cells without HA or GFMs exposure. Significant differences among CK, HA, GO, rGO, and G treatments are marked with different lowercase letters (p<0.05, LSD, n=3). Significant difference among CK, HA, GO-HA, rGO-HA, and G-HA treatments are marked with different capital letters (p<0.05, LSD, n=3). For a given GFMs, significant difference between GFMs and GFMs-HA is marked with "*" (p<0.05, T test, n=3).

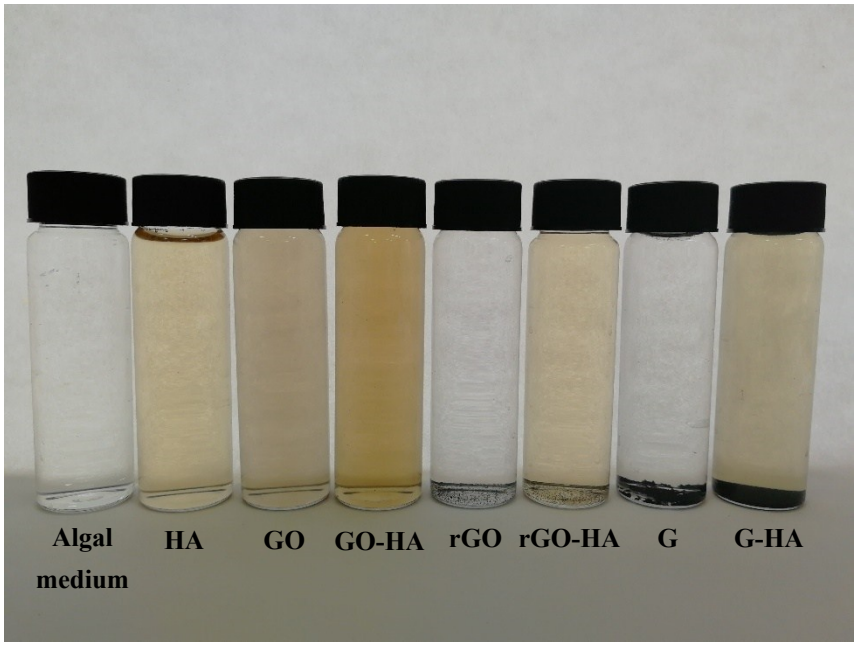

Fig. S15. Images of algal medium in the presence of HA (20 mg/L), GFMs (40 mg/L), and GFMs-HA (GFMs, 40 mg/L; HA, 20 mg/L) after settling for 12 h.

Table S1. Components of SE medium.

Components	Content	Components	Content
$NaNO_3$	250 mg/L	EDTA-Fe[a]	1 mL/L
$K_2HPO_4 \cdot 3H_2O$	75 mg/L	H_3BO_3	2.86 mg/L
$MgSO_4 \cdot 7H_2O$	75 mg/L	$MnCl_2 \cdot 4H_2O$	1.86 mg/L
$CaCl_2 \cdot 2H_2O$	25 mg/L	$ZnSO_4 \cdot 7H_2O$	0.22 mg/L
KH_2PO_4	175 mg/L	$CuSO_4 \cdot 5H_2O$	0.079 mg/L
NaCl	25 mg/L	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.039 mg/L
$FeCl_3 \cdot 6H_2O$	5 mg/L		

[a] Preparation of EDTA-Fe (0.1861 g EDTA-Na_2 was dissolved into 10 mL ultrapure water and 0.901 g $FeCl_3 \cdot 6H_2O$ was dissolved in 10 mL 1M HCl, then the two parts were mixed and the mixture solution was diluted to 1000 mL by ultrapure water).

160 Table S2. Selected properties of the natural water samples.

Water sample	pH[a]	Conductivity[b] (ms/cm)	Salinity[c]	TOC[d] (mg/L)
Zhangcun River	8.24	0.90	0.4	21.1
Campus lake in Ocean University of China	8.29	0.95	0.4	90.7

161 [a] was measured by the pH meter (AB150, Accumet, USA).

162 [b] was determined by the conductivity meter (Cond 3210 SET 1, WTW, Germany).

163 [c] was determined by the conductivity meter (Cond 3210 SET 1, WTW, Germany).

164 [d] was measured by the TOC analyzer (TOC-VCPH, Shimadu, Japan).

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Table S3. Individual and joint toxicity of GFMs and HA on algal cells. Algal cells were exposed to HA, GFMs, and GFMs-HA for 96 h before detection.

Materials	96 h EC_{50} (mg/L)		*S*[c]	*AI*[d]	Joint toxicity
	Individual[a]	Mixture[b]			
HA	1093.20	114.82	6.25	-5.25	Less than additive toxicity (antagonism)
GO	37.30	229.09			
HA	1093.20	126.47	7.56	-6.56	Less than additive toxicity (antagonism)
rGO	34.00	252.93			
HA	1093.20	53.09	1.76	-0.76	Less than additive toxicity (antagonism)
G	62.20	106.17			

[a] refers to the EC_{50} of GFMs or HA when exposed to algal cells individually.

[b] refers to the EC_{50} of GFMs or HA when exposed to algal cells in mixture.

[c] refers to the sum of biological contributions.

[d] refers to the additive index. "*AI*=0" indicates the simply additive toxicity between two materials; "*AI*>0" indicates the synergistic effect between the two materials; "*AI*<0" indicates the antagonistic effect between two materials. The absolute value of *AI* reflects the level of synergistic or antagonistic effect. The higher value, the greater effect.

Table S4. The exponential-model-fitted parameters of the settling curves as presented in Fig. 4 and Fig. S9.

Test substances	$OD_{plateau}$[a]	$OD_{reduced}$[b]	v[c] (OD/min)	R^2
Alga	0.959±0.000	0.047±0.000	0.022±0.000	0.880
GO	0.897±0.000	0.105±0.000	0.032±0.000	0.957
rGO	0.530±0.001	0.455±0.001	0.020±0.000	0.994
G	0.534±0.001	0.455±0.001	0.020±0.000	0.994
Alga-GO	0.928±0.000	0.075±0.000	0.028±0.000	0.959
Alga+GO	0.826±0.017	0.157±0.017	0.003±0.000	0.807
Alga-rGO	0.652±0.001	0.310±0.001	0.021±0.000	0.986
Alga+rGO	0.776±0.001	0.166±0.001	0.023±0.000	0.936
Alga-G	0.616±0.001	0.351±0.001	0.021±0.000	0.990
Alga+G	0.699±0.001	0.247±0.001	0.022±0.000	0.974
Alga-HA	0.949±0.000	0.047±0.000	0.022±0.000	0.880
Alga-GO-HA	0.950±0.000	0.072±0.000	0.022±0.000	0.938
Alga-GO+HA	0.939±0.000	0.061±0.000	0.025±0.000	0.941
Alga-rGO-HA	0.870±0.001	0.135±0.000	0.020±0.000	0.955
Alga-rGO+HA	0.859±0.000	0.106±0.000	0.022±0.000	0.964
Alga-G-HA	0.852±0.000	0.120±0.000	0.021±0.000	0.989
Alga-G+HA	0.775±0.000	0.197±0.000	0.021±0.000	0.993

[a] refers to the optical density at the plateau of the settling curves;

[b] refers to the reduced optical density from the initial time to the plateau;

[c] refers to sedimentation rate.

Table S5. Fitting parameters of the isotherms on HA adsorption by GFMs in the algal medium as fitted by Langmuir and Freundlich models.

	Adsorbent	Langmuir model			Freundlich model			Q_{20}[b] (mg/g)
		Q_m	K_L	R_{adj}^{2a}	K_f	N	R_{adj}^2	
HA	GO	200.06±35.59	50.47±16.80	0.963	7.71±3.09	0.64±0.10	0.919	39.22
	rGO	51.33±11.68	42.63±21.10	0.853	3.82±0.93	0.50±0.06	0.941	14.14
	G	63.18±13.06	24.72±13.68	0.765	8.09±1.78	0.41±0.06	0.927	23.89

[a]: The correlation coefficient square corrected by degree of freedom.

[b]: The equilibrium concentration of adsorbed HA on GFMs when the initial aqueous concentration of HA was 20 mg/L.

185 Table S6. HA adsorption on GFMs as normalized by surface area and pore volume.

	Surface area[a] (m^2/g)	Q_{20}/A_{surf}[b] (mg/m^2)	Total pore volume[a] (cm^3/g)	Q_{20}/V_{total}[c] (mg/cm^3)	Micropore volume[d] (cm^3/g)	Q_{20}/V_{micro}[e] (mg/cm^3)
GO	23.3	5.32	0.029	4271.38	3.62×10^{-4}	3.42×10^{5}
rGO	194.0	0.29	0.273	208.17	5.89×10^{-2}	9.65×10^{2}
G	31.7	2.12	0.162	415.74	4.06×10^{-3}	1.66×10^{4}

186 [a] measured by multipoint N_2 adsorption-desorption method by Autosorb-1 (Quantachrome, USA).

187 [b] refers to the adsorbed equilibrium amount of HA (initial concentration, 20 mg/L) on GFMs as
188 normalized by surface area.

189 [c] refers to the adsorbed equilibrium amount of HA (initial concentration, 20 mg/L) as normalized
190 by total pore volume.

191 [d] measured by DR method.

192 [e] refers to the adsorbed equilibrium amount of HA (initial concentration, 20 mg/L) as normalized
193 by micropore volume.

194