

*Supplementary Materials*

# Effects of Humic Acids on the Ecotoxicity of Fe<sub>3</sub>O<sub>4</sub> Nanoparticles and Fe-Ions: Impact of Oxidation and Aging

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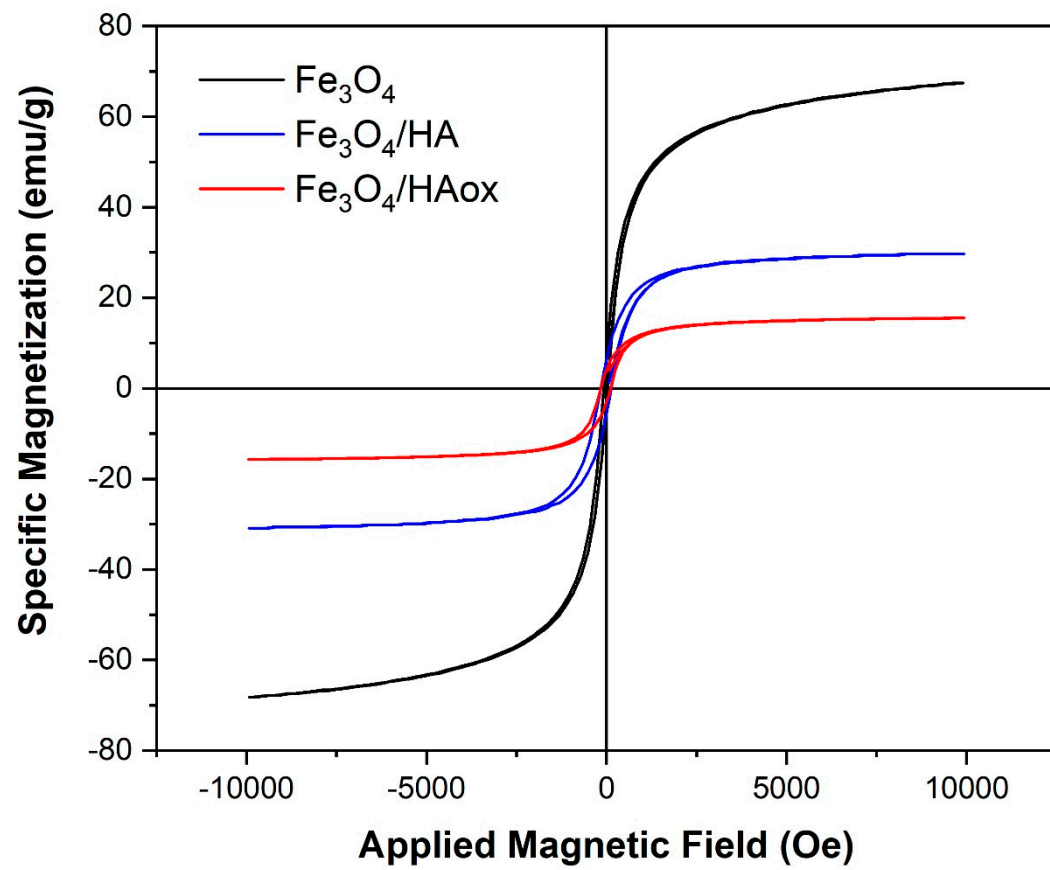


Figure S1.

**Table S1.** Exposure concentration (mg Fe/L) and concentration for soluble Fe-salt and studied MNPs (mg/L, without color).

Samples	Ciliates							
	0.25%	0.05%	0.025%	0.005%	0.0025%	0.0005%	0.00025%	0.00005%
Fe <sup>2+</sup>	745	149	74.5	14.9	7.45	1.49	0.745	0.149
Fe <sup>3+</sup>	770	154	77	15.4	7.7	1.54	0.77	0.154
FeCl <sub>2</sub> , FeCl <sub>3</sub>	1650	330	165	33	16.5	3.3	1.65	0.33
Fe <sup>2+</sup> /Fe <sup>3+</sup>	-	-	-	15.1	7.55	1.51	0.76	0.151
FeCl <sub>2</sub> /FeCl <sub>3</sub>				33	16.5	3.3	1.65	0.33
Fe(II)HA	-	-	-	14.9	7.45	1.49	0.745	0.149
Fe(III)HA	-	-	-	15.4	7.7	1.54	0.77	0.154
Fe(II,III)HA	-	-	-	15.1	7.55	1.51	0.76	0.151
Salt with HA	-	-	-	33	16.5	3.3	1.65	0.33
Fe <sup>2+</sup> /Fe <sup>3+</sup> (90 days)	755	151	75.5	15.1	7.55	1.51	0.76	0.151
Fe(II,III)HA (90 days)	755	151	75.5	15.1	7.55	1.51	0.76	0.151
FeCl <sub>2</sub> /FeCl <sub>3</sub> (90 days), FeCl <sub>2</sub> /FeCl <sub>3</sub> /HA (90 days)	1650	330	165	33	16.5	3.3	1.65	0.33
Fe <sub>3</sub> O <sub>4</sub> , Fe <sub>3</sub> O <sub>4</sub> /HA, Fe <sub>3</sub> O <sub>4</sub> (90 days), Fe <sub>3</sub> O <sub>4</sub> /HA (90 days), Fe <sub>3</sub> O <sub>4</sub> /HAox	-	-	-	33	16.5	3.3	1.65	0.33
HA, HA (90 days)	-	-	-	33	16.5	3.3	1.65	-
Samples	The higher plants							
	0.5%	0.25%	0.05%	0.025%	0.005%		0.0005%	
Fe <sup>2+</sup>	1490	745	149	74.5	14.9		1.49	
Fe <sup>3+</sup>	1540	770	154	77	15.4		1.54	
Fe <sup>2+</sup> /Fe <sup>3+</sup>	1510	755	151	75.5	15.1		1.51	
Fe(II)HA	1490	745	149	74.5	14.9		1.49	
Fe(III)HA	1540	770	154	77	15.4		1.54	
Fe(II,III)HA	1510	755	151	75.5	15.1		1.51	
Fe <sup>2+</sup> /Fe <sup>3+</sup> (90 days), Fe(II,III)HA (90 days)	1510	755	151	75.5	15.1		1.51	
Salts and others samples	3300	1650	330	165	33		16.5	

**Table S2.** p-Values calculated with R using ANOVA for the toxicity of MNPs and Fe-ions to *P. caudatum* and *S. alba*.

Sample	concentration	p-value			
		samples			
Toxicity to <i>P. caudatum</i> (mg/L)					
Fe <sup>2+</sup>	0.0001	Fe <sup>2+</sup>	>0.05	Fe <sup>2+</sup>	0.0001
Fe <sup>3+</sup>	0.0001	Fe <sup>3+</sup>		HA	
Fe <sup>2+</sup> /Fe <sup>3+</sup>	>0.05	Fe <sup>2+</sup> /Fe <sup>3+</sup>	0.0001	Fe(II)HA	
Fe <sub>3</sub> O <sub>4</sub>	0.001	Fe <sub>3</sub> O <sub>4</sub>		Fe <sup>3+</sup>	
HA	0.01	Fe <sub>3</sub> O <sub>4</sub>		HA	0.0001
Fe(II)HA	0.001	HA	0.01	Fe(III)HA	
Fe(III)HA	0.001	Fe <sub>3</sub> O <sub>4</sub> /HA		Fe <sup>2+</sup> /Fe <sup>3+</sup>	
Fe(II,III)HA	>0.05	Fe(II,III)HA	>0.05	HA	0.0001
Fe <sub>3</sub> O <sub>4</sub> /HA	0.0001	Fe <sub>3</sub> O <sub>4</sub> /HA		Fe(II,III)HA	
Toxicity to <i>S. alba</i> (mg/L)					
Fe <sup>2+</sup>	0.0001	Fe <sup>2+</sup>	0.001	Fe <sup>2+</sup>	0.0001
Fe <sup>3+</sup>	0.0001	Fe <sup>3+</sup>		HA	
Fe <sup>2+</sup> /Fe <sup>3+</sup>	0.0001	Fe <sup>2+</sup> /Fe <sup>3+</sup>	0.0001	Fe(II)HA	
Fe <sub>3</sub> O <sub>4</sub>	>0.05	Fe <sub>3</sub> O <sub>4</sub>		Fe <sup>3+</sup>	
HA	0.0001	Fe <sub>3</sub> O <sub>4</sub>	0.0001	HA	0.0001
HA/Fe <sup>2+</sup>	0.001	HA		HA/Fe <sup>3+</sup>	
HA/Fe <sup>3+</sup>	0.0001	Fe <sub>3</sub> O <sub>4</sub> /HA		Fe <sup>2+/3+</sup>	
Fe(II,III)HA	0.0001	HA/Fe <sup>2+/3+</sup>	0.01	HA	0.0001
Fe <sub>3</sub> O <sub>4</sub> /HA	0.0001	Fe <sub>3</sub> O <sub>4</sub> /HA		Fe(II,III)HA	

**Table S3.** p-Values for the toxicity (EC50) of humic acids, as prepared and aged Fe<sup>2+</sup>/Fe<sup>3+</sup> ions to *P. caudatum* and *S. alba*.

Sample	p-value	
	concentrations	samples
Toxicity to ciliates <i>P. caudatum</i> (24 h EC50)		
HA*	0.01	0.0001
HA* (90 days)	0.001	
Fe <sup>2+</sup> /Fe <sup>3+</sup>	>0.05	>0.05
Fe <sup>2+</sup> /Fe <sup>3+</sup> (90 days)	0.0001	
Fe(II,III)HA	>0.05	0.0001
Fe(II,III)HA (90 days)	0.05	
Toxicity to plants <i>S. alba</i> (96 h EC50)		
HA*	0.0001	0.0001
HA* (90 days)	0.0001	
Fe <sup>2+</sup> /Fe <sup>3+</sup>	0.0001	0.001
Fe <sup>2+</sup> /Fe <sup>3+</sup> (90 days)	0.0001	
Fe(II,III)HA	0.0001	>0.05
Fe(II,III)HA (90 days)	0.001	