

## *Supplementary Material*

# **Response of Freshwater Biofilms to Antibiotic Florfenicol and Ofloxacin Stress: Role of Extracellular Polymeric Substances**

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## Information S1 Spectral measurements of extracellular polymeric substances

A part of the above supernatants purified with an 8000-Da semipermeable membrane to remove residues and salt was lyophilized to obtain EPS samples for spectral analysis. The freeze-dried EPS samples were dissolved in Milli Q water, and a series of solutions containing 100 mg·L<sup>-1</sup> of EPS and 0 to 2.5 mg·L<sup>-1</sup> of antibiotics were prepared in 40-mL vials. The vials were kept in dark and shaken in a water-bath shaker at 150 rpm for 24 h at 25 °C. After that, 5 mL of mixture was collected and passed through a 0.45- $\mu$ m filter for three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectra analysis. The 3D-EEM fluorescence spectra (F-2700, Hitachi, Japan) was measured with subsequent scanning emission wavelength of 300 to 600 nm at 0.5-nm increments by excitation wavelengths of 250 to 450 nm at 10-nm increments. The slits for both excitation and emission were 5 nm, and the scan speed was 1200 nm·min<sup>-1</sup>.

The freeze-dried EPS samples and KBr were mixed at a ratio of 1:100 and homogenized in an agate grinder. The mixture was compressed into tablets. The interactions between the EPS and the two antibiotics were measured with an infrared spectrometer (IR, IRAffinity-1S, Shimadzu, Japan), and the spectra in the range of 4000 to 400 cm<sup>-1</sup> were recorded with 20 replications at a resolution of 2 cm<sup>-1</sup>.

Table S1. Basic information on florfenicol and ofloxacin.

Antibiotic	Molecular structure	CAS Number	Molecular weight	Dissociation Constant	Partition Coefficient	Water solubility
Florfenicol		73231-34-2	358.21	9.03	-0.04	1.32
Ofloxacin		82419-36-1	361.37	6.10/8.28	-0.39	2.55

Table S2. Information on the Nanhu Lake and some physicochemical properties of the lake water.

Latitude	Longitude	Depth Max.	DOC <sup>a</sup>	TN <sup>b</sup>	TP <sup>c</sup>	pH
(North)	(East)	(m)	(mg·L <sup>-1</sup> )	(mg·L <sup>-1</sup> )	(mg·L <sup>-1</sup> )	
43°51'	125°18'	6.5	8.61	1.16	0.15	7.4-7.6

<sup>a</sup> DOC is the dissolved organic carbon.

<sup>b</sup> TN is the total nitrogen.

<sup>c</sup> TP is the total phosphorus.