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Electronic Supplementary Material

Influence of chemical speciation and biofilm composition on mercury accumulation by freshwater biofilms

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Summary:

ESI contains 10 pages, including 2 tables and 3 figures.

Table of content

Section 1 Material preparation for Hg sampling

Teflon bottles and syringes (PP cylinder and PE piston, without latex, VWR, Nyon, Switzerland) used to collect water samples were cleaned according to the following procedure, i.e. soaking in 1) a detergent bath for one week, 2) two successive baths of 10% nitric acid (HNO_3, pro-analysis, Merck, Darmstad, Germany) for one week each and 3) a 10 % HCl (pro-analysis, Merck, Darmstad, Germany) bath for another week. The materials were thoroughly rinsed with ultrapure water (resistivity=18.2 MΩ cm, total organic carbon < 5 ppb, Millipore Direct Q-8, Merck, Schaffhausen, Switzerland) between each bath. The syringes were dried under a laminar flow hood and double-bagged. The Teflon bottles were stored in 0.5% HCl (Ultrex, J.T. Baker®, VWR, Nyon, Switzerland) and double-bagged to be rinsed again twice with ultrapure water and with filtered water at the sampling site prior filling with filtered water samples. Sterivex filters were rinsed prior water sampling by filtering 10% HNO_3 (suprapur, Merck, Darmstadt, Germany), 10% HCl (Ultrex), ultrapure water and sampling site water. Field blanks were performed regularly by filtration of ultrapure water in the field and along the sampling period.

Table S1 Summary of the binding constants used for Hg speciation calculation in ambient waters (I==0; T=298.25K)[1]

Reaction	Log βn
$Hg^{2+} + OH^- \leftrightarrow HgOH^+$	10.6
$Hg^{2+} + 2OH^- \leftrightarrow Hg(OH)_2^0$	22.01
$Hg^{2+} + 3OH^- \leftrightarrow Hg(OH)_3^-$	20.9
$Hg^{2+} + Cl^- \leftrightarrow HgCl^+$	7.31
$Hg^{2+} + 2Cl^- \leftrightarrow HgCl_2^0$	14
$Hg^{2+} + 3Cl^- \leftrightarrow HgCl_3^-$	14.92
$Hg^{2+} + 4Cl^- \leftrightarrow HgCl_4^{2-}$	15.53
$Hg^{2+} + OH^- + Cl^- \leftrightarrow HgOHCl^0$	18.27
$Hg^{2+} + SO_4^{2-} \leftrightarrow HgSO_4^0$	2
$Hg^{2+} + CO_3^{2-} \leftrightarrow HgCO_3^0$	11.51
$Hg^{2+} + OH^- + CO_3^{2-} \leftrightarrow Hg(OH)CO_3^-$	19.34
$Hg^{2+} + PO_4^{3-} \leftrightarrow HgPO_4^-$	15.6
$Hg^{2+} + H^+ + PO_4^{3-} \leftrightarrow HgHPO_4^0$	21.15
$Hg^{2+} + NH_4^+ + OH^- \leftrightarrow HgNH_3^{2+}$	13.76
$Hg^{2+} + 2NH_4^+ + 2OH^- \leftrightarrow Hg(NH_3)_2^{2+}$	27.52
$Hg^{2+} + 3NH_4^+ + 3OH^- \leftrightarrow Hg(NH_3)_3^{2+}$	32.87

$$Hg^{2+} + 4NH_4^+ + 4OH^- \leftrightarrow Hg(NH_3)_4^{2+} \qquad 38.52$$

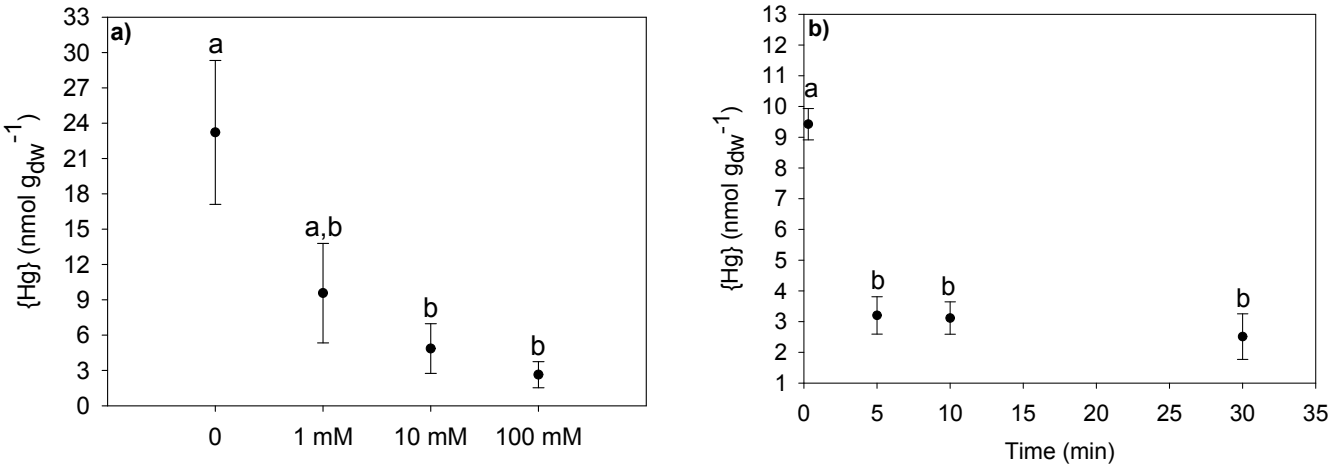

Fig. S1 Mercury content in biofilms exposed to 2 µg L^{-1} of IHg for 24h and then rinsed with (a) different cysteine concentration and at (b) different exposure time with 100 mM cysteine.

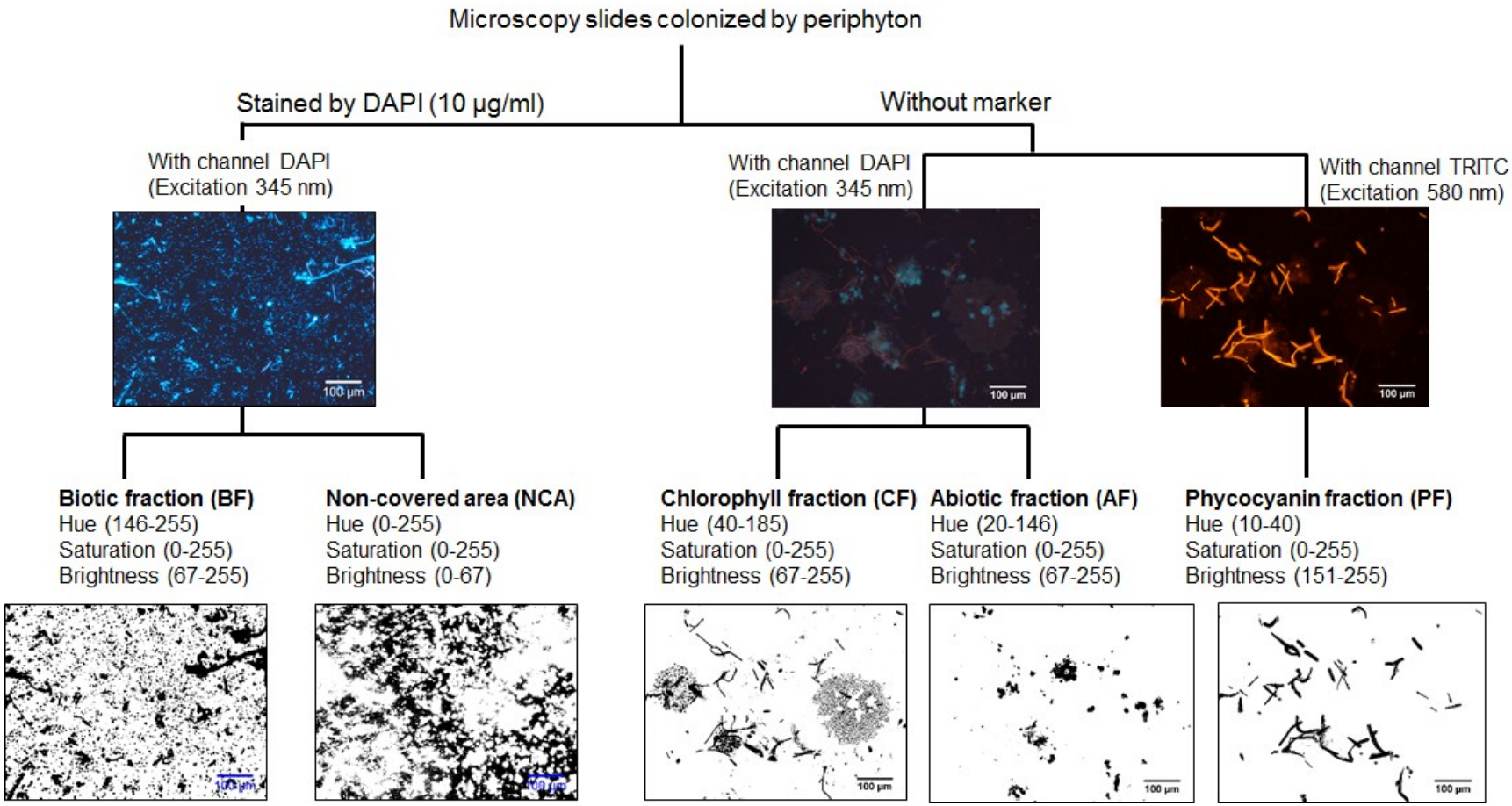

Fig. S2 Microscopy method used to determine different fractions of the biofilms collected at each studied site.

Section S2 Details on DNA extraction and qPCR analysis

DNA integrity, purity and amount was visualized on 1% agarose gel, by measuring the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280} between 1.8-2.0) using a spectrophotometer HeλIOS γ (Thermo Scientific, Reinach, Switzerland), and the Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). All PCR reactions were set up in a sterile PCR hood to limit potential contamination and performed with 1 µL of sample, control or standard DNA in 20 µL final volume according to the supplier's protocol (200 nM of each primer, enzyme activation 3 min at 95ºC, denaturation 3 s at 95ºC, annealing at 55ºC for 20 s) with 40 amplification cycles and quantitative data acquired during the annealing step of each cycle. Finally, one cycle of denaturation at 95°C for 15 s, annealing at 55°C for 15 s and a ramp up to 95°C for 15 s was applied to generate the melting curve. Positive (standard samples) and non-template controls were run with each qPCR reaction and the numbers of targets per sample were calculated. The quality of standard curve and melting curves were tested with qpcR package (www.dr-speciess.de/qpcR.html)[2, 3] using the freely available R software (http://www.r-project.org/). Standard curves were performed as previously described[4] with PCR amplicons for each qPCR primer set with genomic DNA extracted from *Escherichia coli* DH5α, *Geobacter sulferreducens, Desulfovibrio vulgaris* subsp. *Vulgaris* and the primer pJMY2. Standard curves had an amplification efficiency ranging between 1.41 and 1.93, a $R^2 > 0.980$ and a minimum sensitivity of 10^2 target molecules per reaction. In addition, the absence of primer dimers or other non-specific amplicons in these reactions were further confirmed by visualizing the PCR samples on an agarose gel.

Table S2 Sequence and target of the primers and characteristics of the standard curves used to characterize the microbial munity by qPCR.

Target	Gene	5'→ 3' sequence	References	Standard curve	Process/function	Efficiency[¥]	R^2
All bacteria	*16S rRNA*	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	[5]	*Escherichia coli* DH5α	Universal primer	1.75±0.12	0.99
SRB	*dsrA*	ACSCACTGGAAGCACG GTGTAGCAGTTACCGCA	[6]	*Desulfovibrio vulgaris*	Reduction of sulfite: $SO_3^{2-} \rightarrow H_2S$	1.93±0.16	0.99
Geobacteraceae	*gcs (gltA)*	TTCCGYGGYAWGACMATTCC TCCCARGTGATGTTBGCCAWGC	[7]	*Geobacter sulferreducens*	Citrate synthase gene	1.71±0.09	0.96
Hg methylating bacteria	*hgcA*	GGNRTYAAYRTNTGGTGYGC GGTGTAGGGGGTGCAGCCSGTRWARKT	[8] [9]	*Desulfovibrio vulgaris*	Hg methylation: IHg → MeHg	1.41±0.03	0.99
Hg resistance	*merA*	CGATCCGCAAGTGGCIACBGT ACCATCGTCAGRTARGGRAAVA	[10]	pJMY2 plasmid	Hg reduction: IHg → Hg^0	1.84±0.12	1

[¥]Amplification efficiency (average ± SD; n= 27) was determined on amplification curves as described in elsewhere [2]

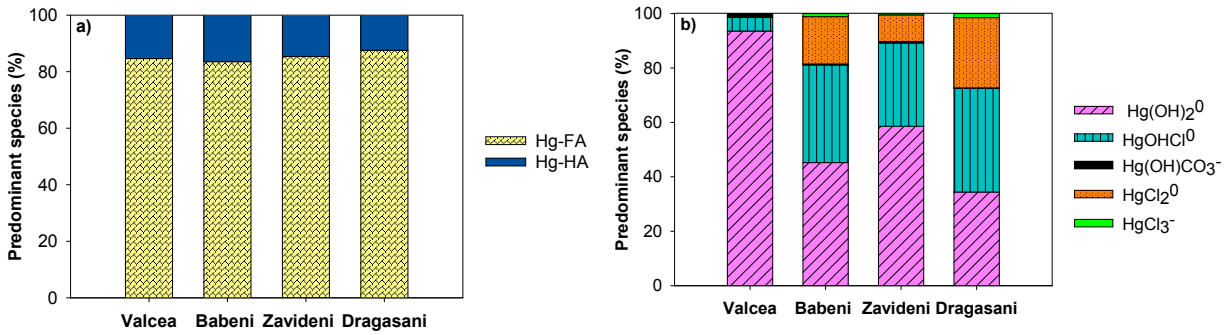

Fig. S3 Distribution of IHg in the four studied reservoirs of the Olt River where IHg was estimated at 80% of the total Hg (a) IHg bound to humic (HA) and fulvic (FA) acids (99.99% of the IHg complexes) and (b) IHg not bound to DOM (0.01% of the IHg complexes).

Reference

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