

Supplementary materials

Biofilm and rivers: the natural association to reduce metals in waters.

Method M1. Quantification of carotenoids and chlorophylls in biofilm

Algal and bacterial pigments are extracted (overnight, at 4 °C, in the dark and under nitrogen) from ca. 1-2 g wet biofilm using 90% acetone and then centrifuged at 4000 rpm for 10 min in 15 ml glass centrifuge tubes. The supernatant is used to measure total pigment concentration (UV-VIS spectrophotometer, SAFAS UVmc2) and specific carotenoids (HPLC Ultimate 3000, Thermo Fisher Scientific).

SAFS UV-Vis mc2 is a dual-band spectrophotometer, the spectra are recorded in the range 350-800nm and the Chlorophyll Derivatives (CD) and Total Carotenoids (TC) are calculated as reported in Lami et.al [32]. Specific carotenoids and chlorophylls are determined by ion pairing and reverse-phase HPLC. Pigment concentrations are expressed as nanomoles per gram of organic matter. The ion pairing (PIC A) allows for greater resolution of the dephytolated acidic chloropigments (Chl c, chlorophyllide a, and pheophorbide a).

The HPLC system consists of an autosampler (Ultimate 3000), a quaternary pump (P680), a thermostated column oven (TCC100), and a DAD detector (Ultimate 3000). The chromatogram is acquired at 460 nm and 656 nm for carotenoids and chloropigments, respectively. The whole spectra, for peak identification, are also recorded. The column is a C18 ODS column (5 µm particle size; 250 mm x 4.6 mm i.d.). After sample injection (100 µl of acetone extract, a gradient program that ramped from 85% mobile-phase A (80:20, by vol. methanol: aqueous solution of 0.001 M PIC A ion-pairing and 0.001 M propionic acid) to 100% mobile-phase B (60:40, acetone: methanol) in 30 min with a hold for 20 min provided sufficient resolution of all pigments of interest. Flow rates from 1 ml min⁻¹ to 2 ml min⁻¹. The column is re-equilibrated between samples by linear ramping to 85% mobile-phase A for 5 min and maintenance for 10 min before sample injection. With this procedure, we are able to separate zeaxanthin from lutein and β-carotene from pheophytin a. Analysis of replicates samples yielded a C.V. of 4.5%- 11.5%, depending on pigments.

Table S1. List of diatomic species. Species expressed as presence/absence identified in the 6 samples analyzed and risk status according to the Red List (Hofmann et al., 2018). 0-not classified, 3-Highly endangered, 4-Threatened of extinction, 5-Risk not estimated, 7-Early alert, 8-Not endangered, 9-Not enough data.

Species	Red List	March			October		
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Achnantheidium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot	8	x	x				
<i>Achnantheidium lineare</i> W.Smith	3		x	x	x	x	
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	0	x	x	x	x	x	x
<i>Achnantheidium pyrenaicum</i> (Hustedt) Kobayasi	8	x	x		x	x	
<i>Achnantheidium straubianum</i> (Lange-Bertalot) Lange-Bertalot	8				x		
<i>Achnantheidium subatomus</i> (Hustedt) Lange-Bertalot	7	x			x	x	x
<i>Adlafia minuscula</i> var. <i>minuscula</i> (Grunow) Lange-Bertalot	8					x	x
<i>Adlafia muralis</i> (Grunow) Li & Qi	8		x				
<i>Amphora aequalis</i> Krammer	4			x	x		x
<i>Amphora inariensis</i> Krammer	8		x				
<i>Amphora pediculus</i> (Kützing) Grunow	8	x		x	x	x	x
<i>Caloneis silicula</i> (Ehrenberg) Cleve	8						x
<i>Cocconeis euglypta</i> Ehrenberg	8				x	x	x
<i>Cocconeis placentula</i> Ehrenberg	9	x	x		x	x	
<i>Cocconeis pseudolineata</i> (Geitler) Lange-Bertalot	8	x	x		x	x	
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	8						x
<i>Cyclotella distinguenda</i> Hustedt	8					x	
<i>Cymatopleura solea</i> (Brébisson) W. Smith	8						x
<i>Cymbella</i> C.Agardh	0	x				x	
<i>Cymbella excisa</i> Kützing	8	x			x	x	x
<i>Cymboppleura subaequalis</i> var. <i>subaequalis</i> (Grunow) Krammer	4				x		
<i>Denticula tenuis</i> var. <i>tenuis</i> Kützing	0				x		
<i>Diatoma ehrenbergii</i> Kützing	8	x	x		x	x	x
<i>Diatoma mesodon</i> (Ehrenberg) Kützing	8	x	x	x	x		
<i>Diatoma moniliformis</i> Kützing	8	x				x	
<i>Encyonema minutum</i> (Hilse in Rabh.) D.G. Mann	8	x	x	x	x	x	x
<i>Encyonema reichardtii</i> (Krammer) D.G. Mann	8	x					
<i>Encyonema silesiacum</i> (Bleisch) D.G. Mann	8	x	x	x		x	x
<i>Encyonema ventricosum</i> (Kützing) Grunow	8				x		
<i>Epithemia</i> F.T. Kützing	0					x	
<i>Eunotia</i> C.G. Ehrenberg	0					x	
<i>Eucoconeis laevis</i> (Østrup) Lange-Bertalot	7		x		x		
<i>Fragilaria gracilis</i> Østrup	8		x	x		x	
<i>Fragilaria pararumpens</i> Lange-Bertalot, G.Hofmann & Werum	8						x
<i>Fragilaria pectinalis</i> (O.F.Müller) Lyngbye	9		x	x			x
<i>Fragilaria perdelicatissima</i> (W.Smith) Lange-Bertalot & Van de Vijver	7			x			
<i>Fragilaria perminuta</i> (Grunow) Lange-Bertalot	8	x		x			
<i>Fragilaria rumpens</i> (Kützing) G.W.F.Carlson	8			x			
<i>Fragilaria vaucheriae</i> (Kützing) Petersen	8	x	x	x		x	
<i>Gomphonema</i> C.G. Ehrenberg	0	x	x			x	x
<i>Gomphonema angustivalva</i> E. Reichardt	9				x		x
<i>Gomphonema elegantissimum</i> Reichardt & Lange-Bertalot	8				x		

<i>Gomphonema innocens</i> Reichardt	8					x	
<i>Gomphonema pseudotenellum</i> Lange-Bertalot	5		x				
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	8	x	x				x
<i>Gomphonema saprophilum</i> (Lange-Bertalot & Reichardt) Abarca, R. Jahn, J. Zimmermann & Enke	8	x	x				
<i>Hannaea arcus</i> (Ehr.) Patrick	7		x	x			
<i>Hippodonta capitata</i> (Ehr.) Lange-BertalotMetzeltin & Witkowski	8						x
<i>Hippodonta pumila</i> Lange-Bertalot, Hofmann & Metzeltin	8					x	
<i>Melosira varians</i> Agardh	8			x		x	x
<i>Navicula</i> J.B.M. Bory de St. Vincent	0					x	
<i>Navicula antonii</i> Lange-Bertalot	8				x	x	x
<i>Navicula caterva</i> Hohn & Hellerman	8				x		
<i>Navicula cryptotenella</i> Lange-Bertalot	8		x	x	x	x	x
<i>Navicula radiosa</i> var. <i>radiosa</i> Kützing	8				x	x	x
<i>Navicula reinhardtii</i> (Grunow) Grunow	8	x					
<i>Navicula splendicula</i> Van Landingham	5				x		
<i>Navicula subalpina</i> Reichardt	4		x	x	x	x	x
<i>Nitzschia</i> A.H. Hassall	0		x				x
<i>Nitzschia acidoclinata</i> Lange-Bertalot	7					x	
<i>Nitzschia alpinobacillum</i> Lange-Bertalot	3			x			
<i>Nitzschia archibaldii</i> Lange-Bertalot	8						x
<i>Nitzschia dealpina</i> Lange-Bertalot & Hofmann	4			x	x		
<i>Nitzschia dissipata</i> subsp. <i>dissipata</i> (Kützing) Grunow	8	x	x	x	x		x
<i>Nitzschia fonticola</i> Grunow	8	x	x	x		x	x
<i>Nitzschia heufleriana</i> var. <i>heufleriana</i> Grunow	8	x	x	x	x		x
<i>Nitzschia inconspicua</i> Grunow	9			x			x
<i>Nitzschia lacuum</i> Lange-Bertalot	5					x	
<i>Nitzschia linearis</i> (Agardh) W.M.Smith	8				x		
<i>Nitzschia palea</i> var. <i>palea</i> (Kützing) W.Smith	8		x	x	x		x
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützing) Grunow	8			x	x	x	x
<i>Nitzschia palea</i> var. <i>tenuirostris</i> Grunow	8				x	x	x
<i>Nitzschia recta</i> Hantzsch ex Rabenhorst	8						x
<i>Pantocsekiella comensis</i> (Grunow) K.T. Kiss & Ács	5					x	
<i>Placoneis pseudanglica</i> (Lange-Bertalot) Cox	8					x	
<i>Planothidium dubium</i> (Grunow) Round & Bukhtiyarova	8		x	x			x
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Round & Bukhtiyarova	8					x	
<i>Planothidium lanceolatum</i> (Brébisson) Lange-Bertalot	8				x		
<i>Psammothidium bioretii</i> (Germain) Bukhtiyarova & Round	8			x	x	x	
<i>Reimeria sinuata</i> (Gregory) Kociolek & Stoermer	8				x	x	x
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	8	x					
<i>Rossithidium pusillum</i> (Grunow) F.E.Round & Bukhtiyarova	3	x					
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky	9						x
<i>Staurosira</i> (C.G. Ehrenberg) D.M. Williams & F.E. Round	0			x			
<i>Staurosira venter</i> (Ehrenberg) Cleve et Moeller	8					x	
<i>Staurosirella</i> D.M. Williams & F.E. Round	0			x	x	x	
<i>Stephanodiscus alpinus</i> Hustedt	8					x	
<i>Tabellaria ventricosa</i> Kützing	4					x	
<i>Ulnaria acus</i> (Kützing) Aboal	8			x			

Table S2. List of non-diatom taxa. Data collected from 3 sample sites in the Toce River in March and October 2019.

Species	March			October		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Cyanobacteria						
<i>Aphanocapsa</i> sp.		X		X		X
<i>Aphanocapsa muscicola</i> (Meneghini) Wille	X					
<i>Aphanothece</i> sp.	X	X			X	
<i>Aphanothece minutissima</i> (West) J.Komárková-Legnerová & G.Cronberg	X	X	X	X	X	X
<i>Aphanothece stagnina</i> (Sprengel) A.Braun		X	X	X	X	X
<i>Bacularia</i> cfr. <i>gracilis</i> (Komárek)					X	
<i>Chamaecalyx swirenkoi</i> (Schirshoeff) Komárek & Anagnostidis					X	
<i>Chamaesiphon</i> cfr. <i>polonicus</i> (Rostafinski) Hansgirg	X	X			X	X
<i>Chamaesiphon</i> sp.		X				
<i>Chlorogloea</i> sp.		X			X	
<i>Chroococcus</i> sp.		X		X	X	
<i>Gloeocapsa</i> cfr. <i>sanguinea</i> (C.Agardh) Kützing		X				
<i>Gloeocapsa</i> sp.	X	X	X		X	X
<i>Gloeocapsopsis</i> sp.		X	X	X		
<i>Komvophoron</i> sp.			X			
<i>Leptolyngbya</i> sp.	X	X		X	X	
<i>Leptolyngbya olivacea</i> (Kützing ex Hansgirg) Anagnostidis					X	
<i>Merismopedia minima</i> Beck	X	X			X	
<i>Phormidium</i> sp.	X	X	X		X	
<i>Phormidium autumnale</i> Gomont						X
<i>Pseudanabaena</i> sp.	X	X		X	X	
<i>Pseudanabaena mucicola</i> (Naumann & Huber-Pestalozzi) Schwabe		X			X	
Chlorophyceae (sensu lato)						
<i>Characium</i> sp.		X			X	
<i>Chlamydocapsa</i> sp.		X			X	
<i>Chlorella</i> sp.					X	X
<i>Cladophora glomerata</i> (Linnaeus) Kützing			X			
<i>Closterium diana</i> Ehrenberg ex Ralfs		X				
<i>Coelastrum cambricum</i> W.Archer						X
<i>Cosmarium lobatum</i> Börgesen			X			
<i>Cylindrocystis brebissonii</i> (Ralfs) De Bary					X	
<i>Hyalotheca</i> sp.		X	X	X		
<i>Microspora</i> sp.			X			
<i>Pediastrum boryanum</i> (Trup.) Menegh.					X	
<i>Scenedesmus ecornis</i> (Ehrenberg) Chodat		X				X
<i>Scenedesmus aculeolatus</i> Reinsch		X				
<i>Schizomeris</i> sp.			X		X	
<i>Spirogyra</i> sp.			X	X		X
<i>Staurastrum punctulatum</i> Brébisson					X	X
<i>Tetraselmis cordiformis</i> (H.J.Carter) F.Stein					X	
<i>Ulothrix</i> sp.			X	X		
<i>Ulothrix zonata</i> (F.Weber & Mohr) Kützing	X	X	X		X	X
<i>Ulothrix variabilis</i> Kützing		X		X		

Chryptophyceae				
<i>Rhodomonas</i> sp.	X	X		X
Xanthophyceae				
<i>Tribonema</i> sp.	X		X	
Euglenophyceae				
<i>Phacus caudatus</i> Hübner			X	
Chrysophyta				
<i>Hydrurus foetidus</i> (Villars) Trevisan		X	X	X
Amoebozoa				
Tubulinea				
<i>Galeripora discoides</i> (Ehrenberg, 1871) González-Miguéns et al., 2021			X	
<i>Euglypha crenulata</i> Wailes			X	