Supplementary Information (SI) for Green Chemistry. This journal is © The Royal Society of Chemistry 2025

Supplementary Material

Nordic microalgae immobilized to a sulfur—cooking oil copolymer form a highly efficient, sustainable and reusable sorbent to remove heavy metals from complex mixtures

Antonio Leon-Vaz^{1,2±}, Martin Plöhn^{1±}, Juan Cubero-Cardoso^{3,4,5}, Juan Urbano³ and Christiane Funk^{1*}

¹Department of Chemistry, Umeå University, 901 87 Umeå, Sweden. martin.ploehn@ezag.com; christiane.funk@umu.se

²Laboratory of Biochemistry. Faculty of Experimental Sciences. Marine International Campus of Excellence and REMSMA. University of Huelva, 210071 Huelva, Spain. antonio.leon@dqcm.uhu.es

³Laboratory of Sustainable and Circular Technology. CIDERTA and Chemistry Department, Faculty of Experimental Sciences. Campus de "El Carmen", University of Huelva, 21071 Huelva, Spain. j.cubero@dqcm.uhu.es; juan.urbano@dqcm.uhu.es

⁴Institute of Water Research, University of Granada, Granada 18071, Spain.

⁵Department of Microbiology, Pharmacy Faculty, University of Granada, Campus de Cartuja s/n, Granada 18011, Spain.

[±] These authors contributed equally to the work.

^{*}Corresponding author: Christiane Funk christiane.funk@umu.se

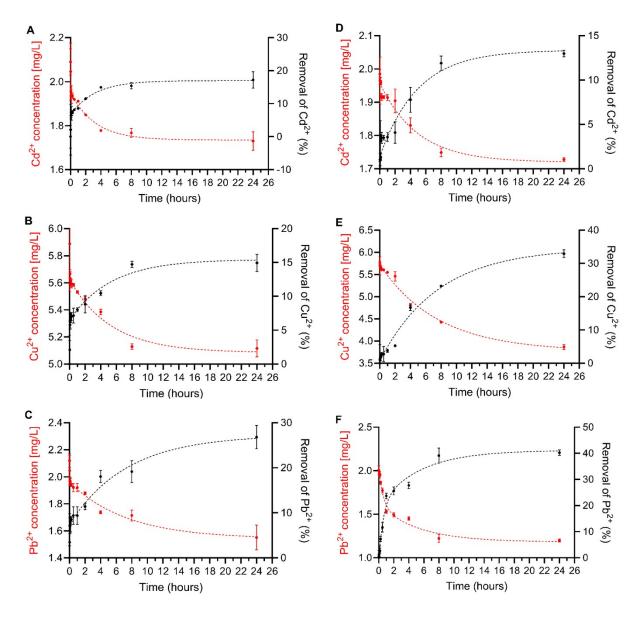


Fig. S1. Removal of 5 mg L⁻¹ Cd²⁺, 2 mg L⁻¹ Cu²⁺ and 1.8 mg L⁻¹ Pb²⁺ in a multi-elemental mixture by *Scotelliopsis reticulata* (UFA-2) in the absence of copolymer (A-C) and after immobilization to the copolymer (D-F). Mean \pm SD of three biological replicates. Dashed lines represent the fitted curves of two-phase association (black) and decay (red). Solid lines in D and E show the sigmoidal curve fitting for removal of the corresponding elements.

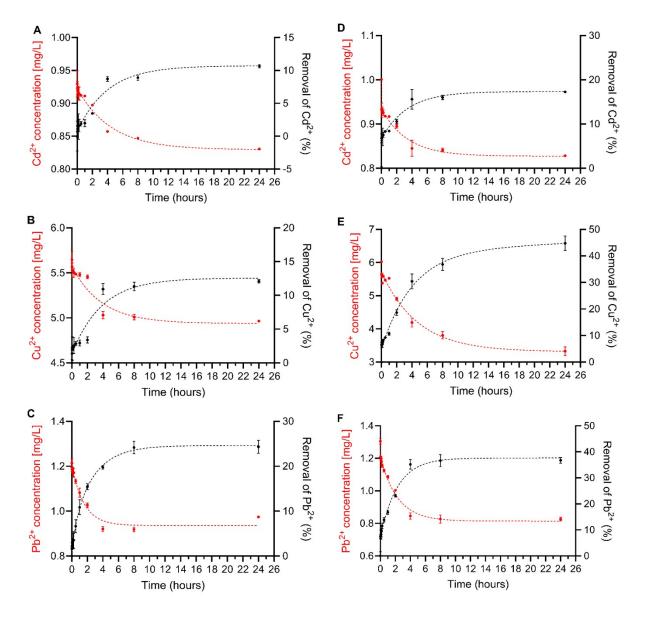


Fig. S2. Removal of 5 mg L^{-1} Cd^{2+} , 1 mg L^{-1} Cu^{2+} and 1 mg L^{-1} Pb^{2+} in a multi-elemental setup by *Scenedesmus obliquus* (13-8) in the absence of copolymer (A-C) and after immobilization on the copolymer (D-F). Mean \pm SD of three biological replicates. Dashed lines represent the fitted curves of two-phase association (black) and decay (red). Solid lines in D and E show the sigmoidal curve fitting for removal of the corresponding elements.

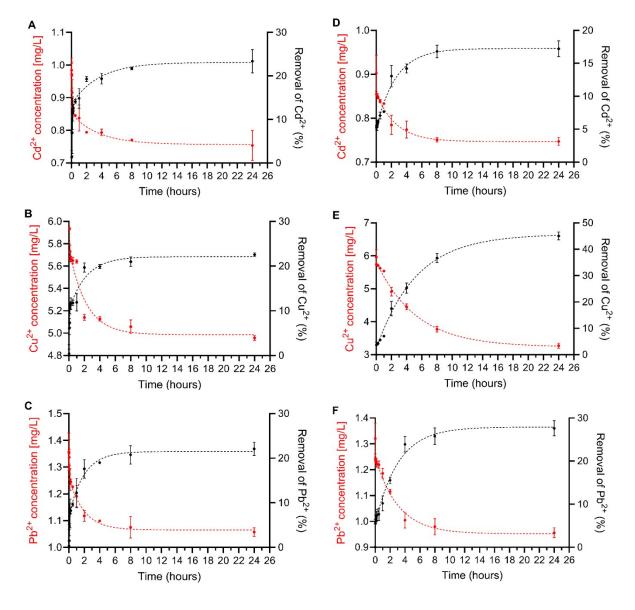


Fig. S3. Removal of 5 mg L^{-1} Cd²⁺, 1 mg L^{-1} Cu²⁺ and 1 mg L^{-1} Pb²⁺ in a multi-elemental setup by *Micractinium* sp. (P9-1) in the absence of copolymer (A-C) and after immobilization on the copolymer (D-F). Mean \pm SD of three biological replicates. Dashed lines represent the fitted curves of two-phase association (black) and decay (red). Solid lines in D and E show the sigmoidal curve fitting for removal of the corresponding elements.

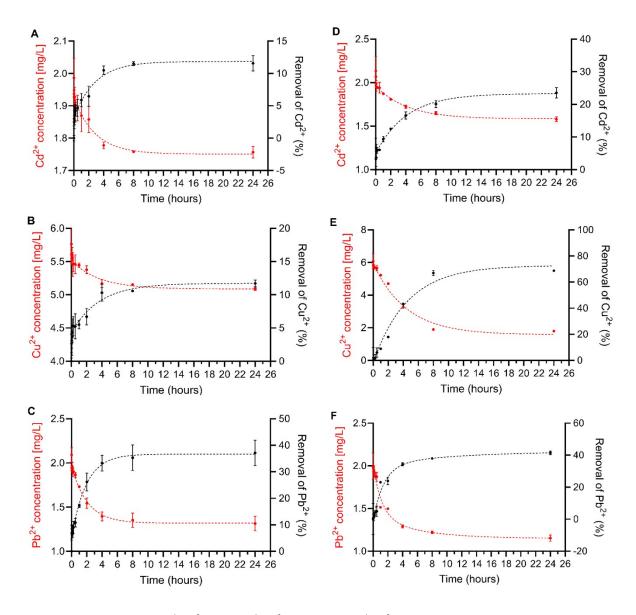


Fig. S4. Removal of 5 mg L^{-1} Cd^{2+} , 2 mg L^{-1} Cu^{2+} and 1.8 mg L^{-1} Pb^{2+} in a multi-elemental setup by *Coelastrella* sp. (3-4) in the absence of copolymer (A-C) and after immobilization on the copolymer (D-F). Mean \pm SD of three biological replicates. Dashed lines represent the fitted curves of two-phase association (black) and decay (red). Solid lines in D and E show the sigmoidal curve fitting for removal of the corresponding elements.

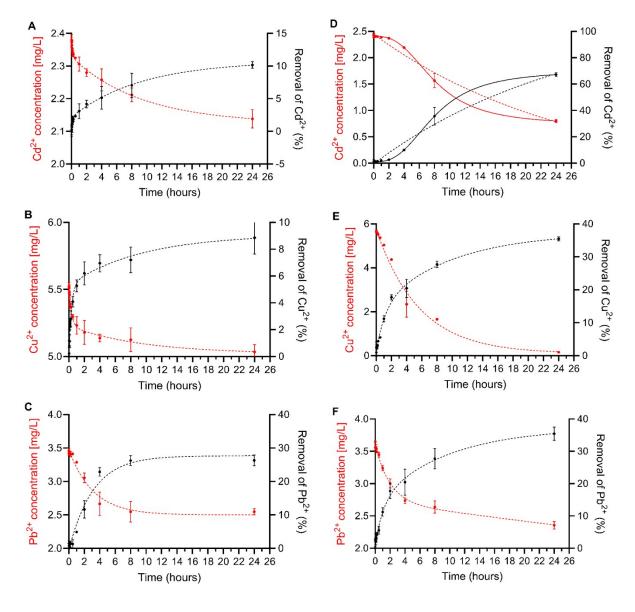


Fig. S5. Removal of 5 mg L⁻¹ Cd²⁺, 2.5 mg L⁻¹ Cu²⁺ and 3.6 mg L⁻¹ Pb²⁺ in a multi-elemental setup by *Chlorella sorokiniana* (2-21-1) in the absence of copolymer (A-C) and after immobilization on the copolymer (D-F). Mean \pm SD of three biological replicates. Dashed lines represent the fitted curves of two-phase association (black) and decay (red). Solid lines in D and E show the sigmoidal curve fitting for removal of the corresponding elements.

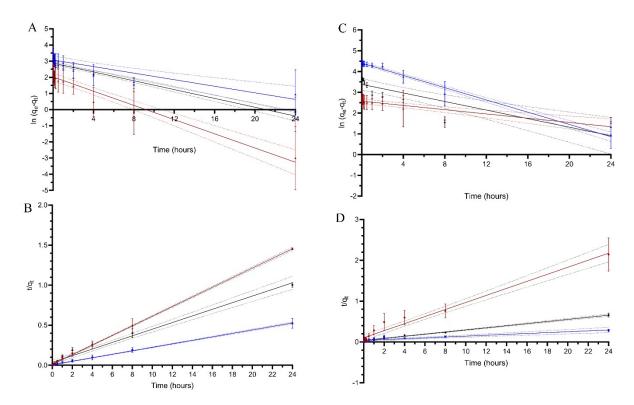


Fig. S6. Kinetic modeling of heavy metal removal for *Scotelliopsis reticulata* (UFA-2) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (**A**) Pseudo-first-order kinetics before immobilization; (**B**) pseudo-second order kinetics before immobilization. (**C**) Pseudo-first-order kinetics after immobilization; (**D**) pseudo-second order kinetics after immobilization.

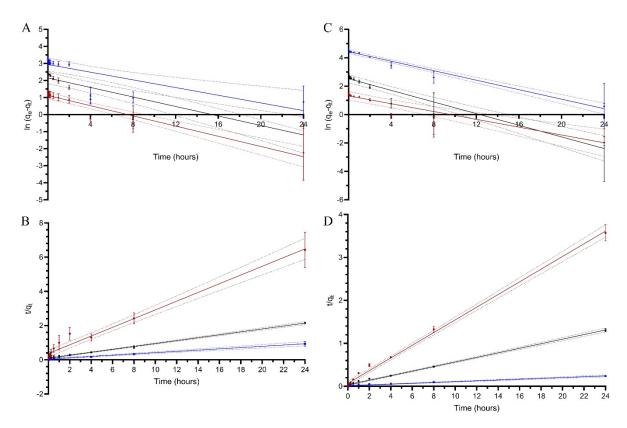


Fig. S7. Kinetic modeling of heavy metal removal for *Scenedesmus obliquus* (13-8) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (**A**) Pseudo-first-order kinetics before immobilization; (**B**) pseudo-second order kinetics before immobilization. (**C**) Pseudo-first-order kinetics after immobilization; (**D**) pseudo-second order kinetics after immobilization.

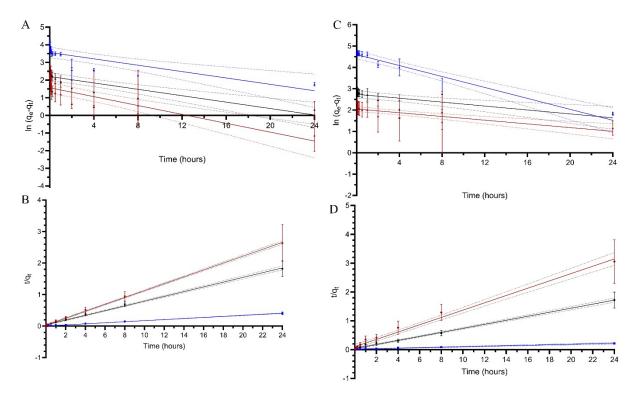


Fig. S8. Kinetic modeling of heavy metal removal for *Micractinium* sp. (P9-1) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (A) Pseudo-first-order kinetics before immobilization; (B) pseudo-second order kinetics before immobilization. (C) Pseudo-first-order kinetics after immobilization; (D) pseudo-second order kinetics after immobilization.

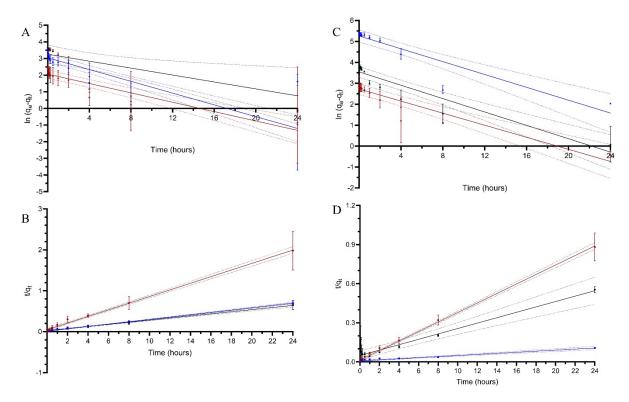


Fig. S9. Kinetic modeling of heavy metal removal for *Coelastrella* sp. (3-4) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (**A**) Pseudo-first-order kinetics before immobilization; (**B**) pseudo-second order kinetics before immobilization. (**C**) Pseudo-first-order kinetics after immobilization; (**D**) pseudo-second order kinetics after immobilization.

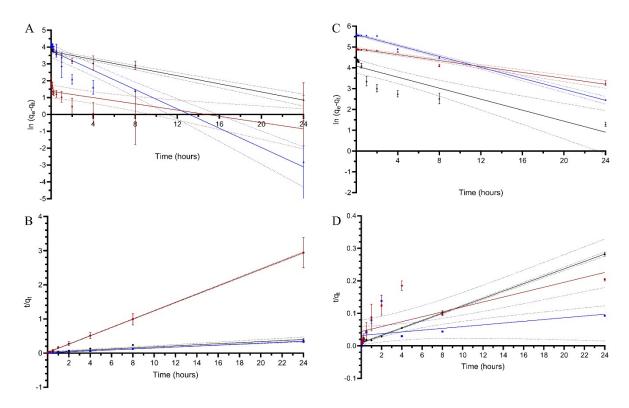


Fig. S10. Kinetic modeling of heavy metal removal by *Chlorella vulgaris* (13-1) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (**A**) Pseudo-first-order kinetics before immobilization; (**B**) pseudo-second order kinetics before immobilization. (**C**) Pseudo-first-order kinetics after immobilization; (**D**) pseudo-second order kinetics after immobilization.

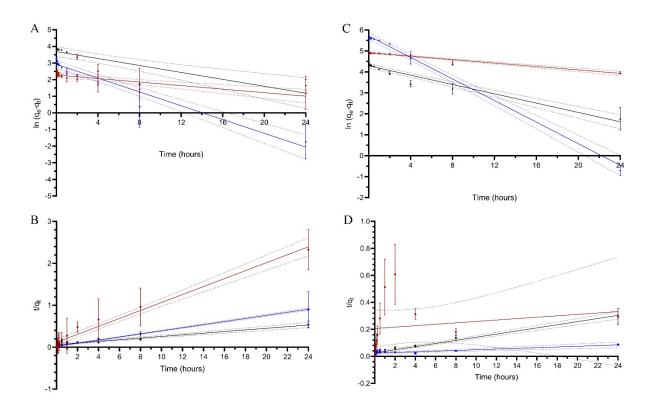


Fig. S11. Kinetic modeling of heavy metal removal for *Chlorella sorokiniana* (2-21-1) (red: Cd^{2+} ; blue: Cu^{2+} , black: Pb^{2+}); data is presented as Mean \pm SD of three biological replicates. (**A**) Pseudo-first-order kinetics before immobilization; (**B**) pseudo-second order kinetics before immobilization. (**C**) Pseudo-first-order kinetics after immobilization; (**D**) pseudo-second order kinetics after immobilization.

Table S1. Heavy metal concentrations in the mixtures applied to different microalgal strains

		Concentration [mg L-1]			
Heavy metal mix	Applied to strain	Cu ²⁺	Cd ²⁺	Pb ²⁺	
High (H)	Chlorella vulgaris (13-1) Chlorella sorokiniana (2-21-1)	5	2.5	3.6	
Medium (M)	Coelastrella sp. (3-4) Scotelliopsis reticulata (UFA-2)	5	2	1.8	
Low (L)	Scenedesmus obliquus (13-8) Micractinium sp. (P9-1)	5	1	1	

Table S2. Removal of Cd²⁺, Cu²⁺ and Pb²⁺ in a multi-elemental mixture under equilibrium conditions calculated with the Freundlich and the Dubinin-Radushkevich model. Data presented for 5 Nordic microalgae and the copolymer individually as well as after immobilization.

		Freundlich			Dubinin-Radushkevich			
Strain	нм	K _F (mg g ⁻¹)	n _F	R^2	q _{max} (mg g ⁻¹)	K _{DR} (mol ² J ⁻²)	\mathbb{R}^2	
	Cd ²⁺	0.14	1.80	0.991	0.76	3.83E-06	0.869	
Copolymer alone (CP)	Cu ²⁺	0.09	1.02	0.930	1.11	4.34E-06	0.957	
	Pb ²⁺	0.13	1.24	0.973	1.82	9.61E-06	0.967	
Scotelliopsis reticulata	Cd ²⁺	9.51	2.61	0.887	27.39	6.01E-06	0.955	
(UFA-2)	Cu ²⁺	9.37	1.40	0.944	57.86	2.54E-06	0.837	
	Pb ²⁺	31.14	1.24	0.936	431.80	9.43E-06	0.975	
Scotelliopsis reticulata	Cd ²⁺	36.59	1.69	0.895	243.20	4.59E-06	0.977	
(UFA-2)	Cu ²⁺	35.58	1.05	0.956	372.30	3.03E-06	0.978	
+ CP	Pb ²⁺	*	*	*	1044.00	3.67E-06	0.961	
Scenedesmus obliquus	Cd ²⁺	18.96	3.60	0.962	36.62	1.86E-07	0.658	
(13-8)	Cu ²⁺	17.25	1.16	0.947	153.00	2.91E-06	0.963	
	Pb ²⁺	17.89	1.08	0.971	368.40	1.22E-05	0.979	
Scenedesmus obliquus	Cd ²⁺	13.10	1.84	0.975	71.38	3.87E-06	0.891	
(13-8)	Cu ²⁺	*	*	*	171.20	3.53E-07	0.976	
+ CP	Pb ²⁺	33.00	1.27	0.899	426.40	8.68E-06	0.969	
Coelastrella sp.	Cd ²⁺	11.17	2.51	0.965	33.81	8.88E-07	0.850	
(3-4)	Cu ²⁺	14.72	1.06	0.919	171.10	3.99E-06	0.946	
	Pb ²⁺	26.30	1.52	0.994	202.50	4.99E-06	0.912	
Coelastrella sp.	Cd ²⁺	30.75	2.61	0.988	94.48	1.59E-06	0.735	
(3-4)	Cu ²⁺	*	*	*	278.50	5.35E-07	0.963	
+ CP	Pb ²⁺	37.00	1.29	0.941	412.00	6.66E-06	0.982	
Chlorella vulgaris	Cd ²⁺	18.41	2.93	0.960	44.04	3.35E-07	0.877	
(13-1)	Cu ²⁺	*	*	*	169.80	7.83E-06	0.883	
	Pb ²⁺	24.00	2.05	0.958	101.10	2.24E-06	0.868	
Chlorella vulgaris	Cd ²⁺	36.55	3.19	0.959	78.48	2.17E-07	0.826	
(13-1)	Cu ²⁺	*	*	*	491.70	6.51E-06	0.947	
+ CP	Pb ²⁺	*	*	*	446.60	7.59E-06	0.954	
Chlorella sorokiniana	Cd ²⁺	21.33	2.58	0.929	60.76	5.73E-07	0.921	
(2-21-1)	Cu ²⁺	13.62	1.76	0.978	47.80	6.81E-07	0.892	
	Pb ²⁺	27.97	1.32	0.906	333.20	7.89E-06	0.984	
Chlorella sorokiniana	Cd ²⁺	19.19	1.81	0.938	105.40	2.97E-06	0.972	
(2-21-1)	Cu ²⁺	*	*	*	230.50	6.34E-07	0.930	
+ CP	Pb ²⁺	29.00	1 11	0.928	505.30	9.76E-06	0.994	

^{*} prediction not possible

Table S3. Remaining removal rate of microalgal/copolymer sorbent after desorption of heavy metals with HNO_3 or EDTA (given as % of initial removal rate).

	0.1M HNO ₃ % of previous removal rate			0.1M EDTA % of previous removal rate		
	Cd^{2+}	Cu ²⁺	Pb ²⁺	Cd^{2+}	Cu ²⁺	Pb ²⁺
Copolymer (CP) alone	62	91	94	110	90	94
Scotelliopsis reticulata (UFA-2) + CP	72	94	60	115	99	98
Scenedesmus obliquus (13-8) + CP	59	80	39	94	99	82
Coelastrella sp. (3-4) + CP	90	104	94	104	106	105
Chlorella vulgaris (13-1) + CP	41	71	64	84	78	83
Chlorella sorokiniana (2-21-1) + CP	60	73	97	84	86	103