14TH 2026 SOCRATIC LECTURES
Laboratory of Clinical Biophysics

FarmEVs is funded by the European Union Grant Agreement No. 101131175
Views and opinions expressed are however those of the authors and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them. We thank ARIS for grants P2-0132, P2-0232, P3-0388, J3-60063.

Effect of heavy metals on extracellular particles of *Chlorellae sorokiniana*

Jelena Danilović Luković (1), Anna Romolo (2), Matej Hočevar (3), Darja Feizpour (3), Barbara Šetina Batič (3), Tina Sever (3), Isidora Santrač (1), Marija Tanović (1), Valentina Ćurić (4), Ana Obaha (5), Jan Dolinar (6), Andrej Race (7), Aleš Iglič (8), Venkata Subba Rao Jampani (9), Marko Novinec (5), Ivan Jerman, (7), Veronika Kralj-Iglič (2,*)

(1) Institute of Multidisciplinary Research, University of Belgrade, Belgrade, Serbia, (2) University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia, (3) Institute of Metals and Technology, Ljubljana, Slovenia, (4) University of Belgrade, Faculty of Biology, Belgrade, Serbia, (5) University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia, (6) University of Ljubljana, Faculty of Physics, Ljubljana, Slovenia, (7) National Institute of Chemistry, Ljubljana, Slovenia, (8) University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Slovenia, (9) J Stefan Institute, Ljubljana, Slovenia
* veronika.kralj-iglic@zf.uni-lj.si

Outline

Microalgae are of great importance for ecology and industry; however, the mechanisms of ecosystem resiliency and production of economically relevant compounds should be better understood. Here, we focus on adaptive response of microalgae to heavy metals, specifically, manganese (Mn) and copper (Cu). In line with the observation on human neuroblastoma cells in vitro, which expel the metals outside the cell using extracellular particles (EPs) to resist toxicity, we investigated if this could be applied to microalgae.

Methods

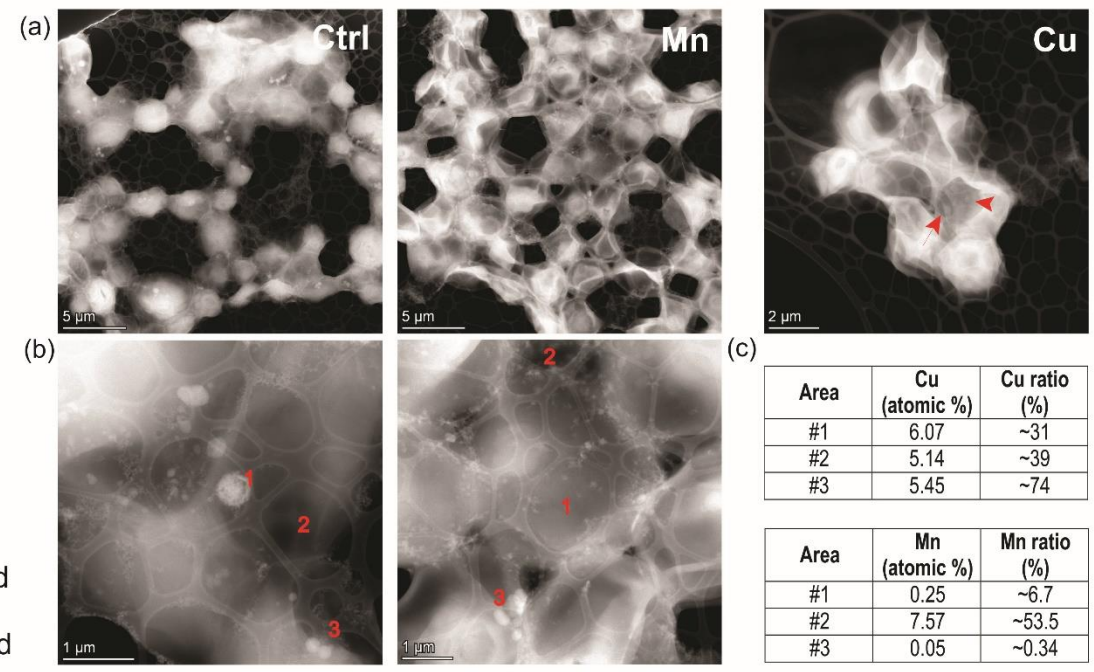
Chlorella sorokiniana cultures were treated with 1mM $MnCl_2$ and $CuCl_2$ at the early stationary phase (20 days old). During 4 days after treatment, supernatants and pellets obtained by differential centrifugation were observed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) with Energy Dispersive Spectroscopy (EDS). We assessed number density and hydrodynamic diameter of EPs by Interferometric Light Microscopy (ILM) (Videodrop, Myriade). Photometry was made on Refeyn Two MP mass photometer. Single spectrum Raman spectroscopy was made on WiTec Alpha 300 RA spectrometer with 532 nm laser and 50x magnification objective.

Results

SEM revealed numerous microalgae surrounded by amorphous mucilage. In supernatant we observed fractal structures, which were particularly abundant in metal-treated samples. In the pellet of control and Cu-treated samples, SEM and TEM revealed small amount of globular particles. TEM EDS of Cu-treated samples exhibited in the globular particles an increased content of metals with respect to the surroundings. Controls composed of water-added phosphates and copper in the same concentrations as in the culture showed the presence of similarly shaped, albeit larger globular structures. ILM showed the presence of numerous particles (in the range of 10^9 per mL in the culture and up to 10 times larger in the supernatant), while the detected number density in the culture was under the detection limit. Measurements of the pellets were not feasible due to the presence of the mucilage. Mass photometry and Raman spectroscopy results are given in Figures 3 and 2, respectively.

Conclusions

Extracellular particles observed in microalgae samples may be formed by phosphates contained in the mucilage shed by microalgae. Our results support the assumption of accumulation of copper by EPs composed by extracellular material shed by microalgae.



Area	Cu (atomic %)	Cu ratio (%)
#1	6.07	~31
#2	5.14	~39
#3	5.45	~74

Area	Mn (atomic %)	Mn ratio (%)
#1	0.25	~6.7
#2	7.57	~53.5
#3	0.05	~0.34

Figure 1. (a): Representative TEM micrographs of *Chlorellae sorokiniana* cells under control conditions and after treatment with 1 mM $MnCl_2$ or 1 mM $CuCl_2$ for 72h, recorded using the HAADF-STEM imaging mode, (b): EDS analysis regions are marked with numbers. (c): The table summarizes the atomic fraction (%) of Mn or Cu within the overall elemental composition and the calculated Mn or Cu ratio relative to the biologically relevant intracellular metals (Na, K, Mg, Ca).

Table 1. Interferometric light microscopy measurement of number density and hydrodynemic diameter of nanoparticles (between 80 and 500 nm).

Time	Sample	Dil.	S5000g AV.C . 10^9/mL	S5000g corr AV.C . 10^9/mL	S5000g D_h (nm)	Dil.	Culture AV.C 10^9/mL	Culture corr AV.C 10^9/mL	Culture D_h (nm)
15'	Algae	1	1.04	1.04±0.07	187±14	10	0.10	1.00±0.20	233±33
	Algae+Cu	10	2.85	28.50±01.70	204±4	10	2.74	27.37±1.10	225±5
	Algae+Mn	10	2.71	2.87±0.31	205±12	10	0.41	4.10±0.40	211±16
60'	Algae	1	1.10	1.10±0.70	190±7	10	0.09	0.87±0.00	184±29
	Algae+Cu	10	2.59	25.90±0.80	213±8	10	2.53	25.33±2.80	230±4
	Algae+Mn	10	2.56	25.60±0.10	202±5	10	0.39	3.90±0.90	232±7
24h	Algae	10	0.11	0.97±0.30	206±48	10	0.57	0.57±0.10	260±40
	Algae+Cu	1	3.24	3.34±0.15	193±7	10	2.79	27.89±1.50	234±23
	Algae+Mn	20	3.79	75.87±3.60	167±4	10	6.29	62.90±4.30	291±22
		30	2.78	83.4±0.90	179±7	20	0.35	7.00±0.30	195±10
48h	Algae	1	2.34	2.34±0.15	187±20	10	0.17	1.70±0.20	209±24
	Algae+Cu	1	10.50	10.50±0.06	207±7	10	2.64	26.37±1.30	202±64
		2.5	5.44	13.59±1.02	208±4				
		5	2.53	12.67±1.25	205±5				
		7.5	1.64	12.30±1.05	201±4				
		10	0.98	9.83±0.60	211±4				
		15	1.02	15.35±0.90	207±7				
	Algae+Mn	1	7.14	7.14±0.36	244±3	10	0.93	9.33±0.60	282±15
		2.5	1.51	3.78±0.14	208±7				
		5	1.77	8.83±0.70	205±9				
		7.5	1.77	13.3±1.28	167±24				
		10	1.12	11.17±0.20	226±3				
		15	0.53	7.9±0.30	219±2				
72h	Algae	1	1.46	1.46±0.05	225±4	10	0.20	2.00±0.20	278±39
	Algae+Cu	10	3.29	32.93±11.12	225±24	10	8.29	82.93±0.00	192±9
	Algae+Mn	30	2.71	81.20±2.70	203±6	10	8.84	88.40±0.80	193±11
						20	4.26	85.2±0.50	204±10
						30	3.08	92.50±1.30	198±3

Dil.: dilution, corr: corrected for dilution, S: supernatant, AV.C.: average number density, g: gravity acceleration of Earth. Time: time after treatment with metals. Results are given +/- standard deviations.

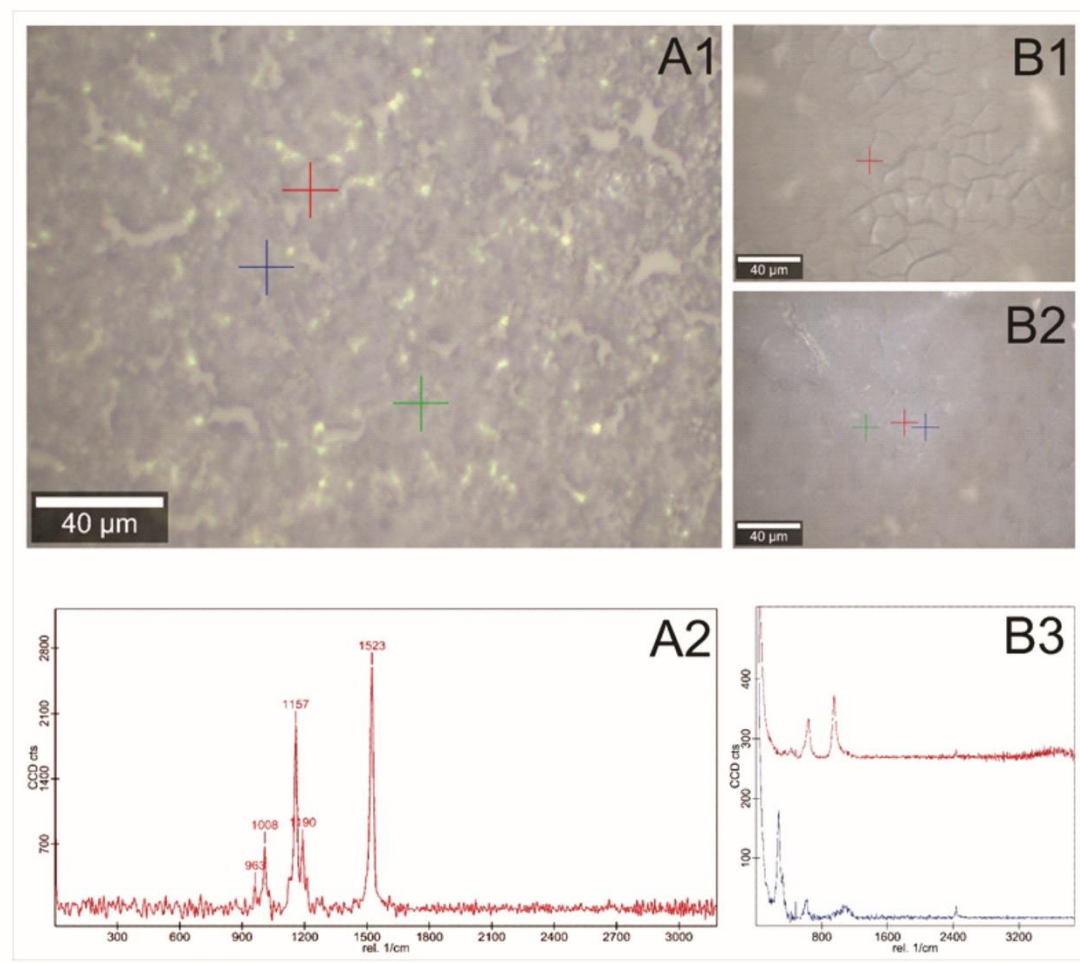


Figure 2. A1: Raman micrograph of a representative sample of microalgae culture and A2: the corresponding spectrum pertaining to the red cross in A1. B1 and B2: Raman micrographs of microalgae samples treated with metals. Samples had carotene signal. B3: Microalgae - blind measurements.

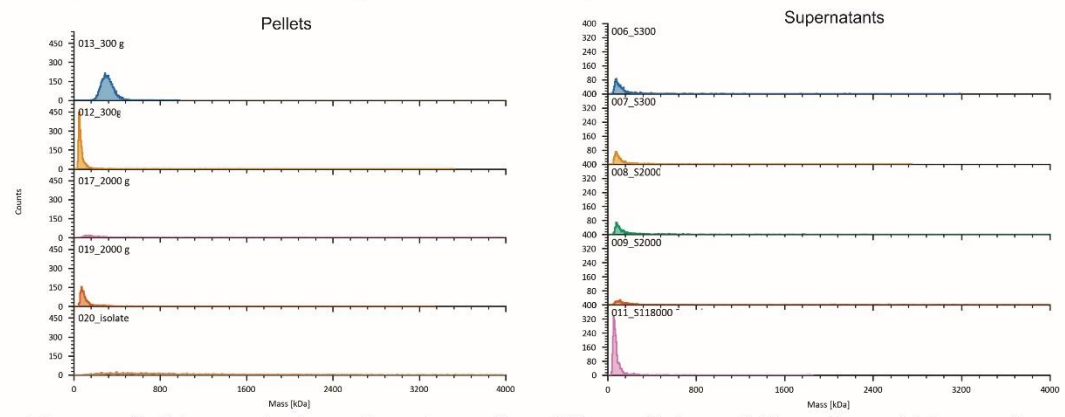


Figure 3. Mass photometry along the differential centrifugation. At lower forces, pellets were richer than supernatants. In particular at the last step, the supernatant was richer than the isolate. Most of the molecules were in the range (100 -200)kDa. There were also larger particles present.