Electronic Supporting Information for

Non-enzymatic portable optical sensor for Microcystin-LR

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The PVC-based optode sensing films were prepared by dissolving all the membrane components (PVC 33 wt%, DOS plasticizer 60-66 wt%, lipophilic cation exchanger TpClPBK 10 wt% and 1 wt% of DCHQ-Ph chromophore) inside in 1 mL of THF. Membranes were casted, in four replicated spots, onto transparent glass slides for optical tests.

The 0.01M solutions of MES (pH 5.5), HEPES (pH 7.4) and TRIS (pH 8.6) were tested as buffer background solutions. Prior to use the optodes were conditioned for 15 min in one of the above-mentioned buffer solutions containing known amount of $MgCl_2$ to achieve membrane saturation with Mg^{2+}-ions. The indirect optode response to MC-LR was evaluated in the concentration range from 1.7×10^{-11} to 1.5×10^{-8} mol/L.

MC-LR CH_3OH-aqueous calibration solutions were obtained by dilution of standard stock solution of MC-LR in water- CH_3OH, 1:1 v/v (10.2 µM/l, Aldrich).

Natural water samples were tested with standard addition method. Double addition method was employed: for this, two injections of 60 µL each of toxic SAG 17.75 strain filtrate with total concentration of microcystin [MC-LR] = 37.9µg/L determined with commercial enzymatic kit (MicroCystest TUBE by ZEU-INMUNOTEC) were added to 3 mL of 1:1 mixture of water sample with 0.1 M TRIS pH 8.6. The influence of MeOH on the fluorescence response of Mg/DCHQ-Ph optode to MC-LR was tested by adding the same volumes of MC-LR calibration solution and (1:1 v/v) pure MeOH in 0.01mol/L TRIS pH 8.6 background.

The toxic *Microcystis aeruginosa* (Kützing) strains were purchased from the SAG Culture Collection of Algae, Gottingen, Germany (SAG17.85, referred, NCBI accession No. KM019998 for 16S ribosomal RNA gene, partial sequence, and SAG 46.80, non-sequenced according to NCBI). Strains were grown in Bold Basal Medium, BBM, for 1 month period in laboratory conditions and treated prior the analysis as reported in reference 9 of the paper.

Fluorescence tests were performed with a Shimadzu RF-1501 (Kyoto, Japan) fluorimeter. For this Mb1 and Mb2 were deposited on glass slide, which was placed at 30° with respect to the light inside a quartz cuvette and, and illuminated with 365 nm light. We have employed for the luminescence measurements the same photometric setup already used in one of our previous research work [10].

The amount of released MCs was detected with UHPLC-DAD technique on UltiMate 3000 LC system (Dionex), equipped with the automated sampling unit and a Diode Array Detector (DAD). A commercial enzymatic kit (MicroCystest TUBE by ZEU-INMUNOTEC) was employed to evaluate MC-LR amount in natural waters. Millipore grade water was used for aqueous solutions preparation. All the other chemicals were of analytical grade and used without further purification.

For the photometric measurements we used a blue-coloured InGaN LED (Roithner LaserTechnik, Austria, model H2A1-H385, λ_{ex} = 380 nm) as an external monochromatic light source. The transparent cuvette was laterally illuminated with a light source and the responses of the optodes upon MC-LR addition were measured with respect to the three main visible spectrum colours: red, green and blue. A frontally placed digital camera (Philips SPC900NC for notebook with a resolution of 352×288 pixels) was used as a signal detector. The RGB signals

were evaluated after background luminosity subtraction. The videos captured by the camera in two regions of interest (ROI), one for the sensing layer and the other for the background area, were transformed into the analytically useful signal by in-house written MATLAB (v.7.0, 2005, The MathWorks, Inc., Natick, USA) codes. The optical intensity of films was obtained as the difference between the two mean normalised ROIs optical intensities and it was plotted in semi logarithmic scale against the – log [MC-LR] value in order to obtain the calibration curves.

Principal Component Analysis, PCA was employed for natural water samples and algal strains identification. The PLS regression method was applied to correlate optical sensor array luminescence response to amounts of hepatotoxins (microcystins and nodularin) with UHPLC-DAD data. The RMSEV (Root Mean Square Error of Validation) and the correlation coefficient of predicted versus measured correlation line, R^2, were used to evaluate the efficiency of obtained PLS model. All chemometric treatments were done with the Unscrambler software (v.9.7, 2007, CAMO SoftwareAS,Norway).

MC-LR-PSs were fabricated by drop casting of several spots of 1 mg/ml solution of DCHQ-Ph in THF on Whatman Grade 42 ashless filter paper strips of about 3cm × 0.5 cm size. After solvent evaporation, 0.01 mol/L $MgCl_2$ solution was deposited over DCHQ-Ph spots and dried for 2 hours. The MC-LR-PSs obtained in this way were employed for qualitative analysis of tap water (1:1 v/v mixture with 0.1 M TRIS buffer at pH 8.6) spiked with 0.5 and 20 µg/L of MC-LR.

Acknowledgements

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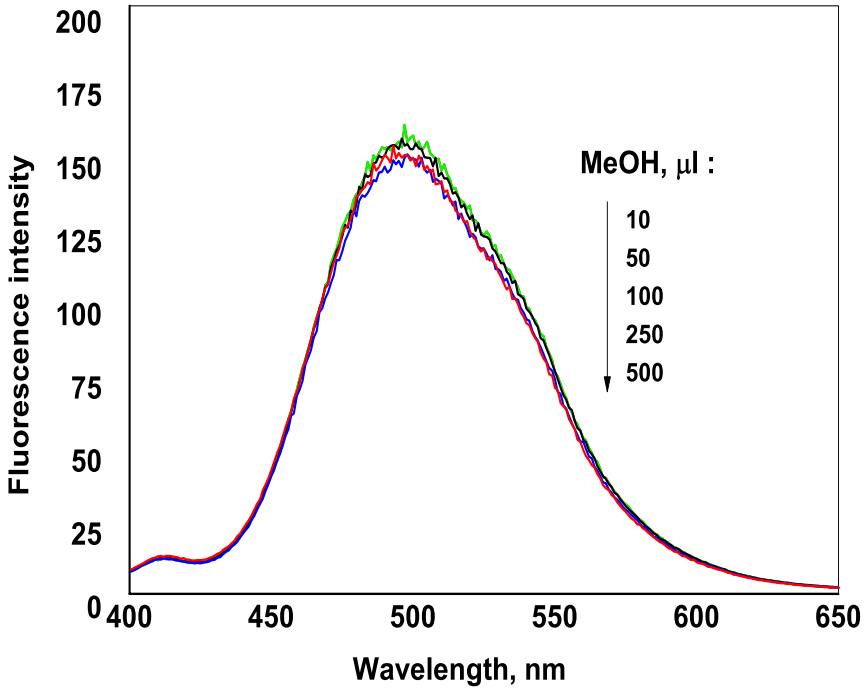

Figure S1: The influence of MeOH on the fluorescence of Mb1 in 0.01 M TRIS at pH 8.6 background, λ_{ex} = 365 nm.

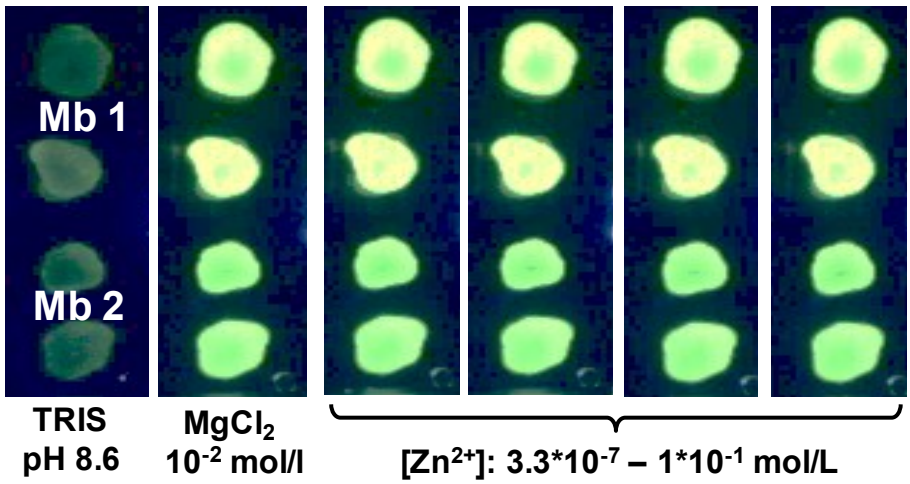

Figure S2: The influence of Zn^{2+}- ions on PT-optical response of DCHQ-Ph-based Mb1, Mb2 (preliminary saturated with Mg^{2+}-ions in 0.01 mol/l $MgCl_2$ for 15 min) at 0.01 mol/L TRIS buffer at pH 8.6, λ_{ex} = 380 nm LED illumination.

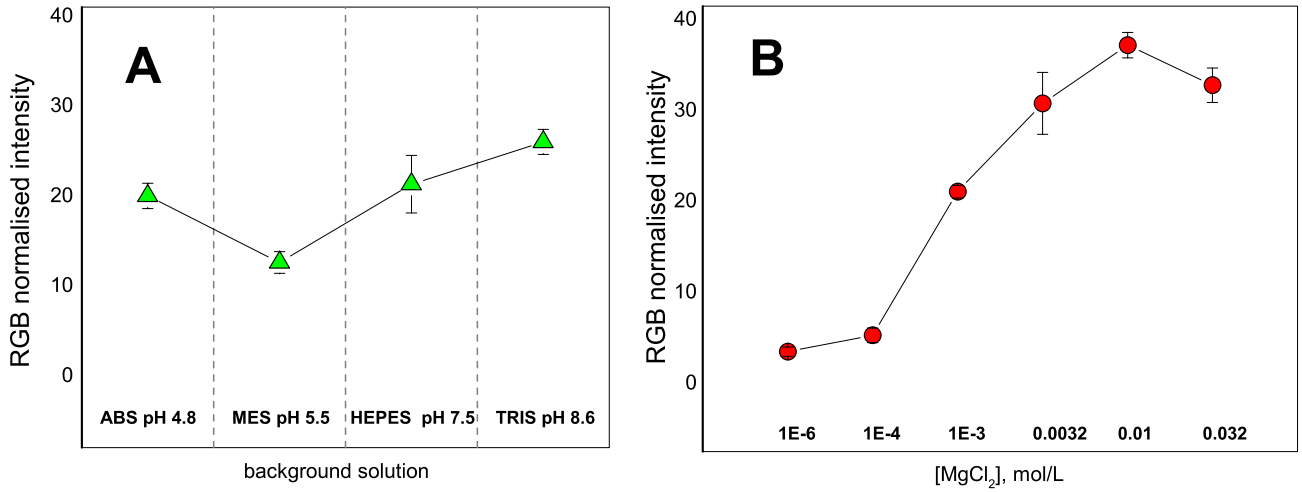

Figure S3: The influence of the: (A) buffer nature and pH (conditioning in 0.01 mol/L $MgCl_2$) ; (B) $MgCl_2$ conditioning solution concentration (in 0.01 mol/L TRIS pH 8.6) on PT-optical response of DCHQ-Ph-based Mb1 in the presence of 0.03 µg/L of MC-LR . λ_{ex} = 380 nm LED illumination.

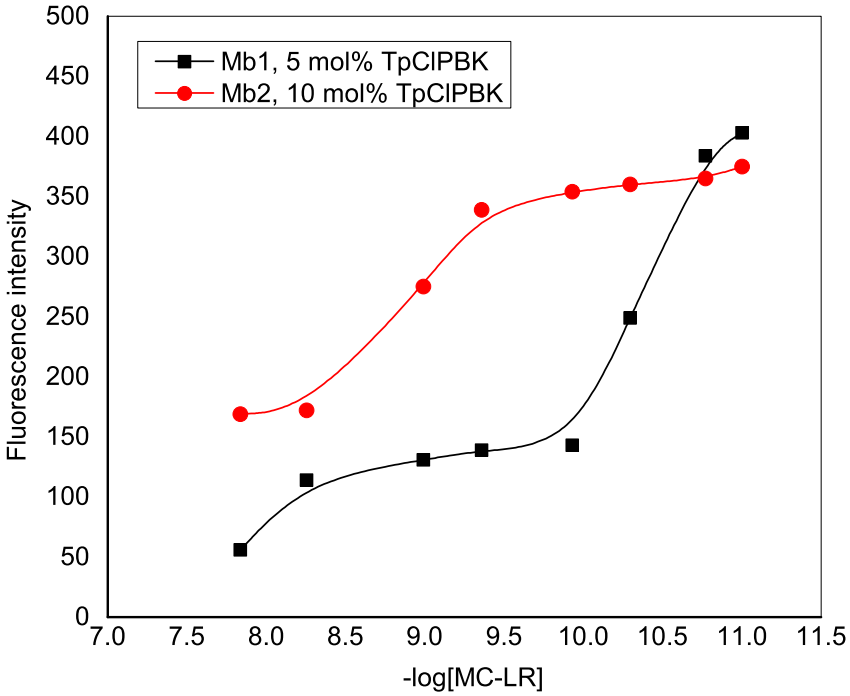

Figure S4: Fluorescence response (intensity at 502 nm) of Mb1, Mb2 (with various amount of TpClPBK cation exchanger) upon increasing amounts of MC-LR.

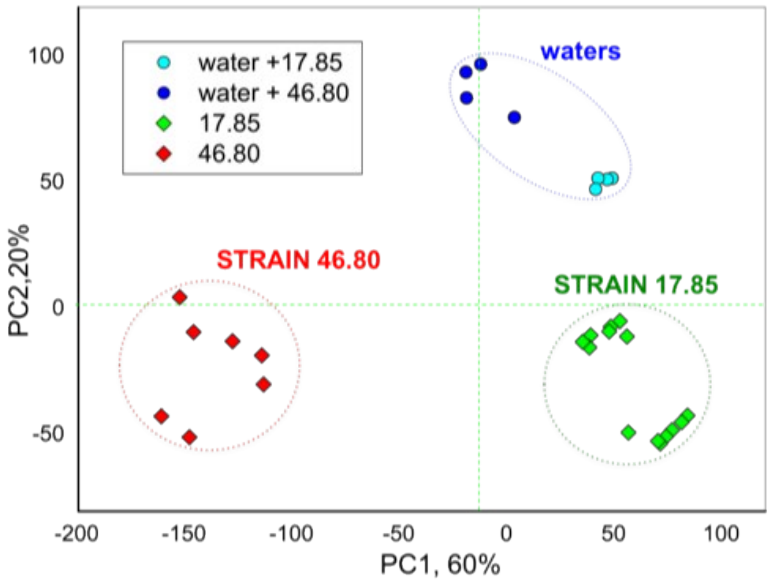

Figure S5: A PCA score plot of the DCHQ-Ph based array response in natural waters and waters spiked with toxic *Microcystis aeruginosa* filtrates.

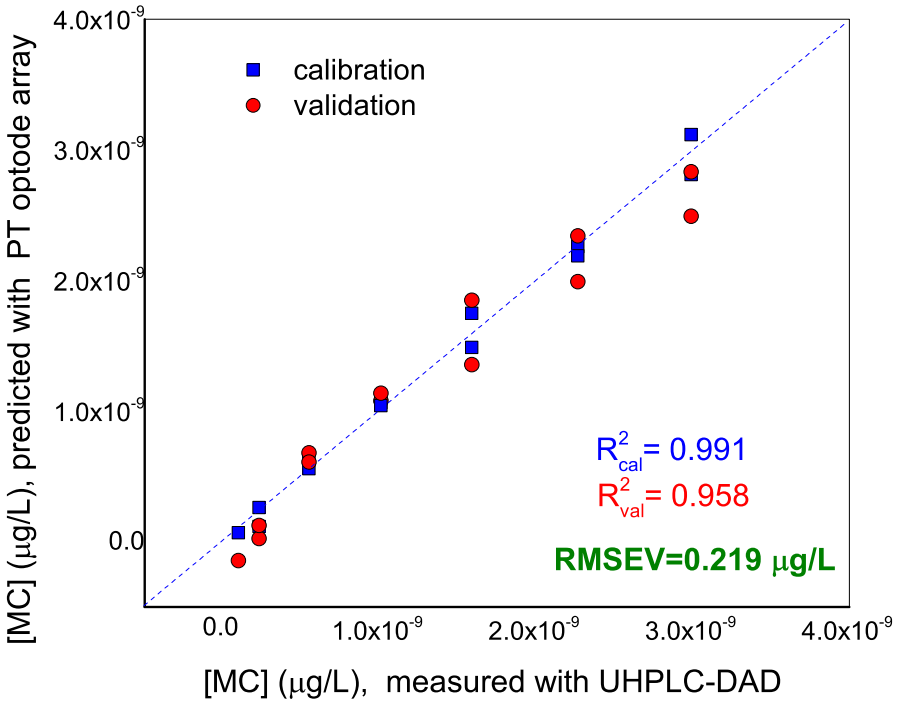

Figure S6: Result of PLS1 prediction of MC-LR concentration in *Microcystis aeruginosa* 17.85 toxic strain filtrate by DCHQ-Ph based optode array.

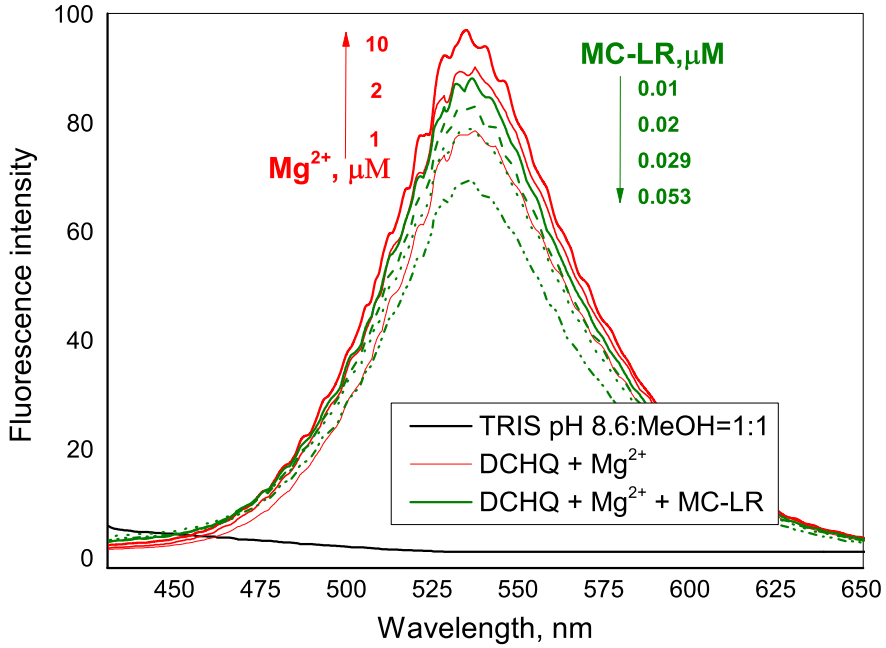

Figure S7: Emission spectra (λ_{ex} = 365 nm) of DCHQ-Ph (0.03 µM) in TRIS:MeOH=1:1 at pH 8.6 upon addition of increasing amounts of Mg^{2+} (1, 2 and 10 µM, red lines) and subsequent fluorescence quenching upon further additions of MC-LR (0.01, 0.02, 0.029 and 0.053 µM, green lines) in the same solution.

Table S1.

Comparison of the data obtained for the detection if MC-LR in surface waters with our indirect optical method employing DCHQ-Ph based Mg-selective optode and Photoassisted Technique (PT) and a commercial enzymatic kit.

Sample	Indirect optical method				Commercial Enzymatic kit, added 1 µg/L		
	Added, µg/L	Found, µg/L	Recovery,%	RSD, %	Found, µg/L	Recovery,%	RSD, %
Albano Lake	0.74	0.75	98.6	1.4	1.213	121.3	23.1
	1.46	1.23	118.9	15.8			
Fountain Ciampino	0.74	0.87	85.1	17.4	1.133	113.3	16.3
	1.46	1.23	115.3	13.1			