Electronic Supporting Information

Detection of Hg^{2+} by Cyanobacteria in Aqueous media.

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Contents

1. Absorption spectrum of C-Phycocyanin.

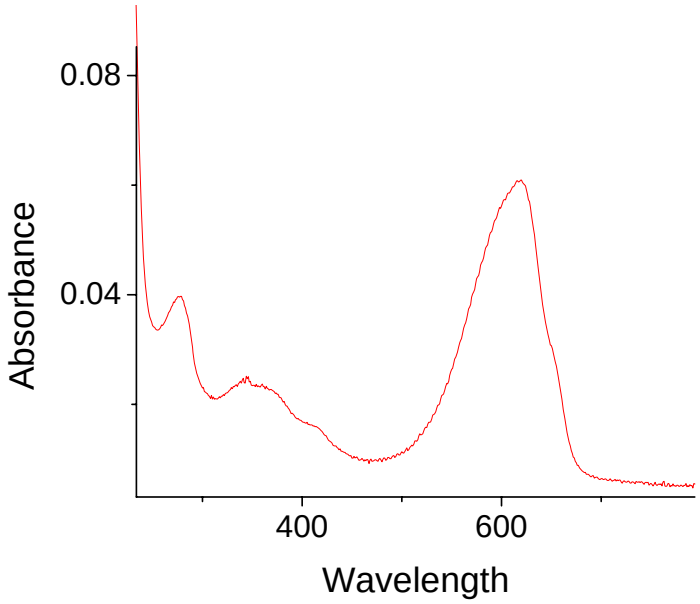


Figure S1 : Absorption spectrum of C-Phycocyanin (2.57 x 10^{-8} M) with phosphate buffer at pH 7.2.

2. Emission Spectrum of C-Phycocyanin.

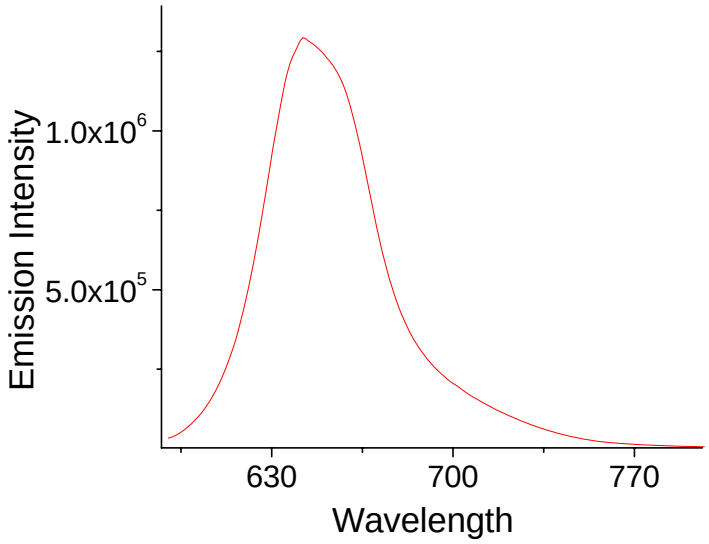


Figure S2 : Fluorescence emission spectrum of C-Phycocyanin (2.57 x 10^{-8} M) with phosphate buffer at pH 7.2. Excitation at 580 nm.

3. Emission spectra with different metal ions.

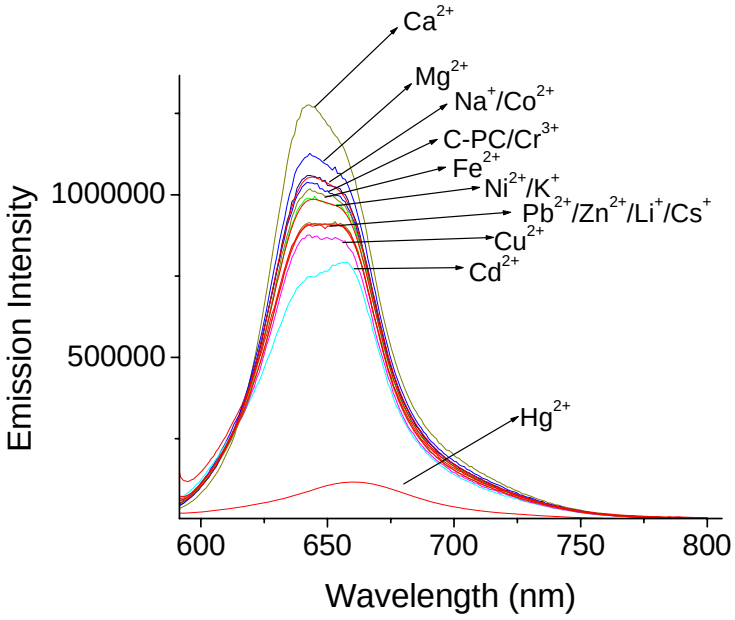


Figure S3: Changes in emission spectra of C-PC upon addition of different metal ions in phosphate buffer at pH 7.2. Excitation wavelength that was used for studies is 580 nm.

4. Absorption spectrum of Phycocyanobilin in phosphate buffer:

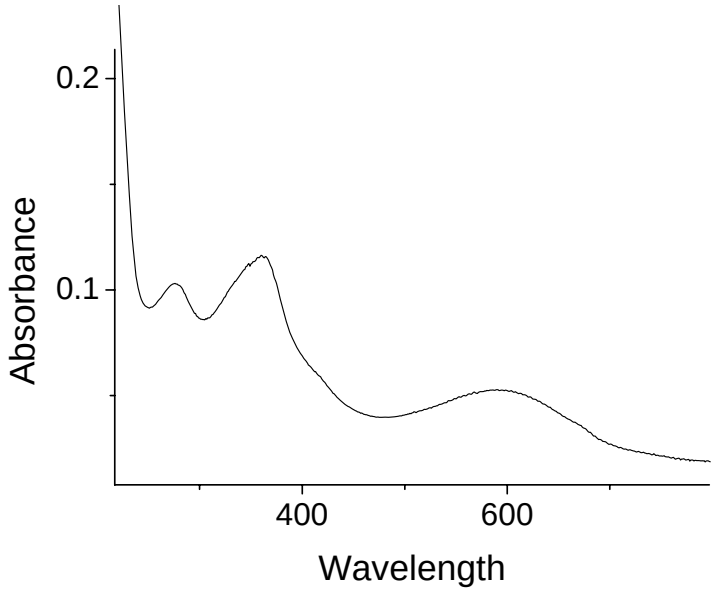


Figure S4 : Absorption spectrum of phycocyanobilin (1 x 10^{-4}M) in phosphate buffer at pH 7.2.

5. Absorption spectrum of Phycocyanobilin in water:

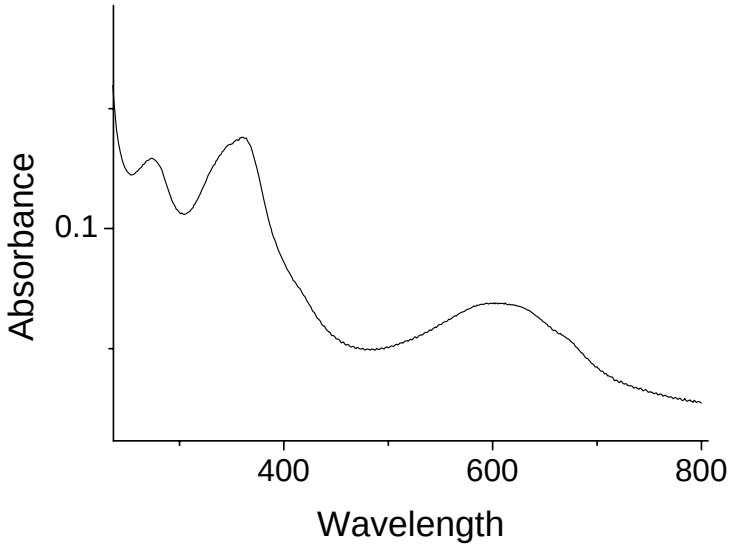


Figure S5 : Absorption spectrum of phycocyanobilin (1.0×10^{-4}M) in water

6. Binding constant for C-PC with various cations in phosphate buffer (pH = 7.2):

Metal ions	Binding constant (K_f)[a]
Na^+	-[b]
K^+	-[b]
Cs^+	-[b]
Ca^{2+}	$(2.5\pm0.10)x10^3M^{-1}$
Mg^{2+}	-[b]
Sr^{2+}	-[b]
Ba^{2+}	-[b]
Cr^{3+}	-[b]
Fe^{3+}	-[b]
Ni^{2+}	-[b]
Cu^{2+}	$(3.0\pm0.15)x10^3M^{-1}$
Hg^{2+}	$(2.7\pm0.2)x10^4M^{-1}$
Cd^{2+}	$(3.1\pm0.2)x10^3M^{-1}$
Co^{2+}	-[b]
Zn^{2+}	-[b]

[a]Binding constant for each metal ions is an average of five independent experiments. Values were evaluated based on the fluorescence titration at room temperature. Spectral changes for Na^+, K^+, Cs^+, Mg^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Fe^{3+}, Ni^{2+}, Co^{2+} and Zn^{2+} were not significant to enable us to evaluate respective binding constant values.

7. Mass spectra of Phycocyanobilin:.

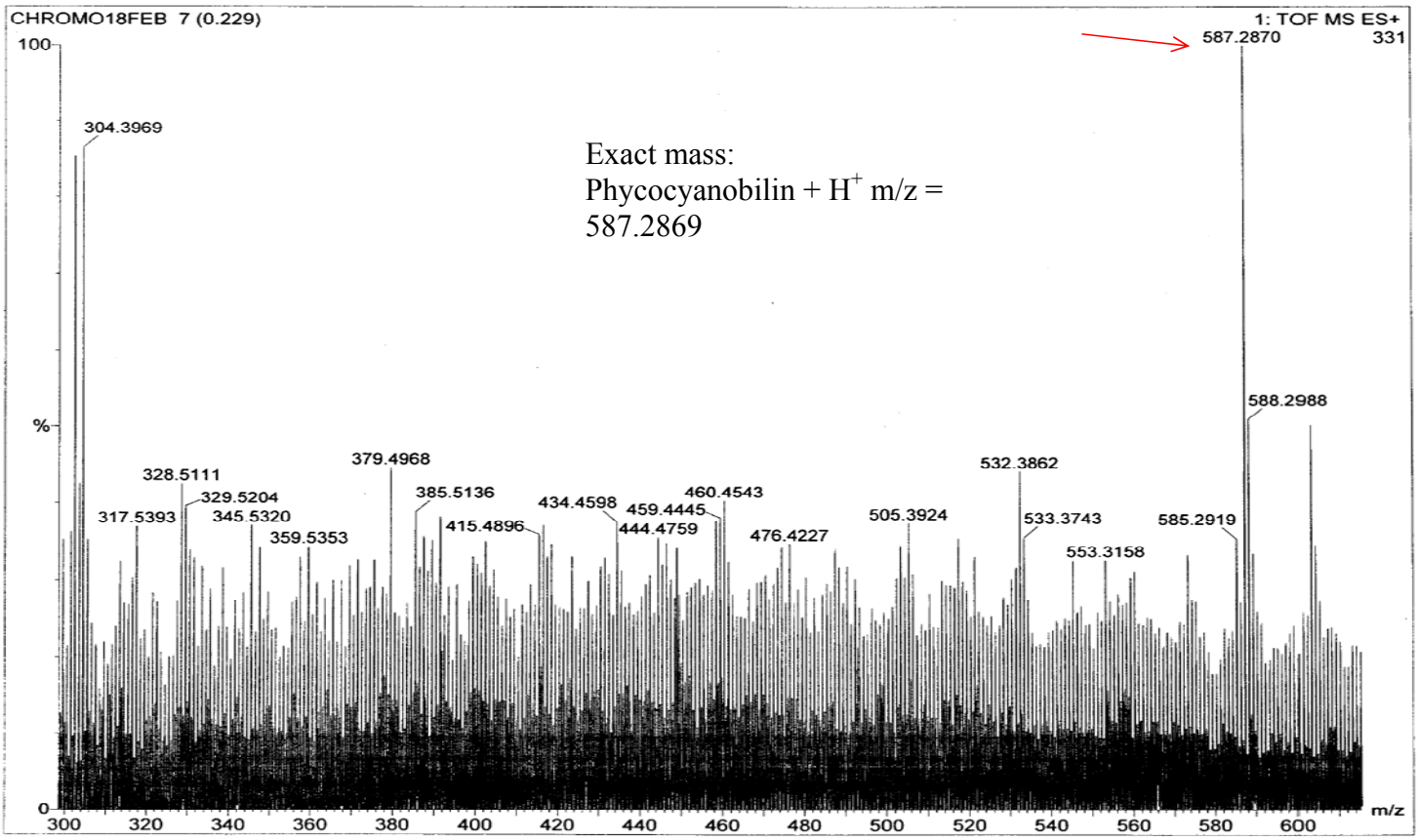
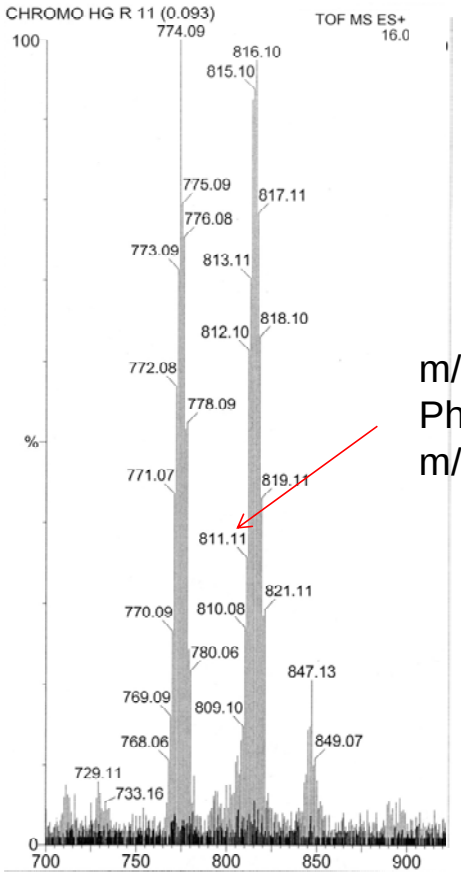


Fig S6: ESI-mass spectrum of phycocyanobilin extracted from *C-Phycocyanin* using Micromass Q-Tof micro^{TM}, equipped with ESI source and Q-Tof analyzer.

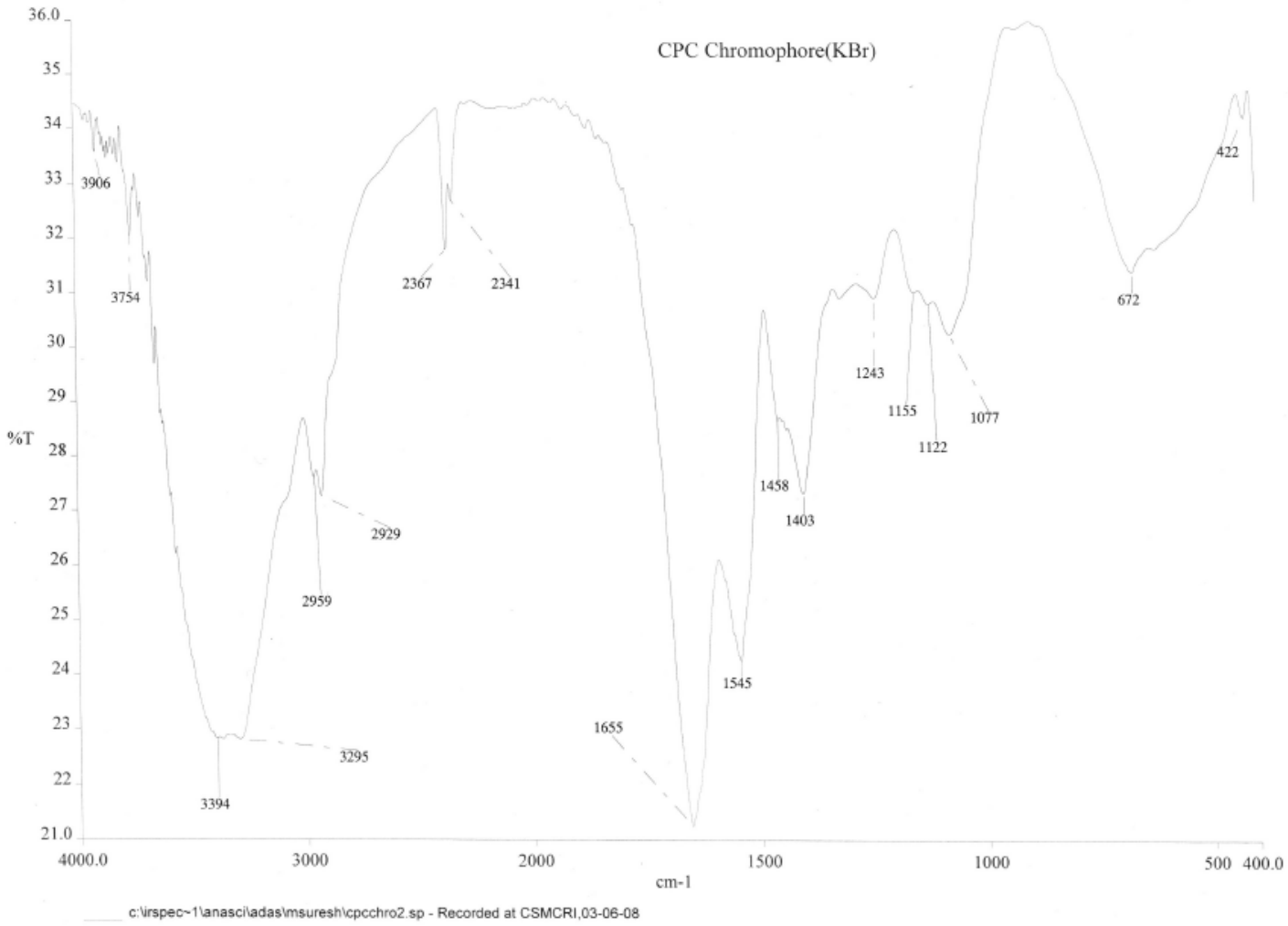
8. Mass spectra of Phycocyanobilin with Hg^{2+}:.



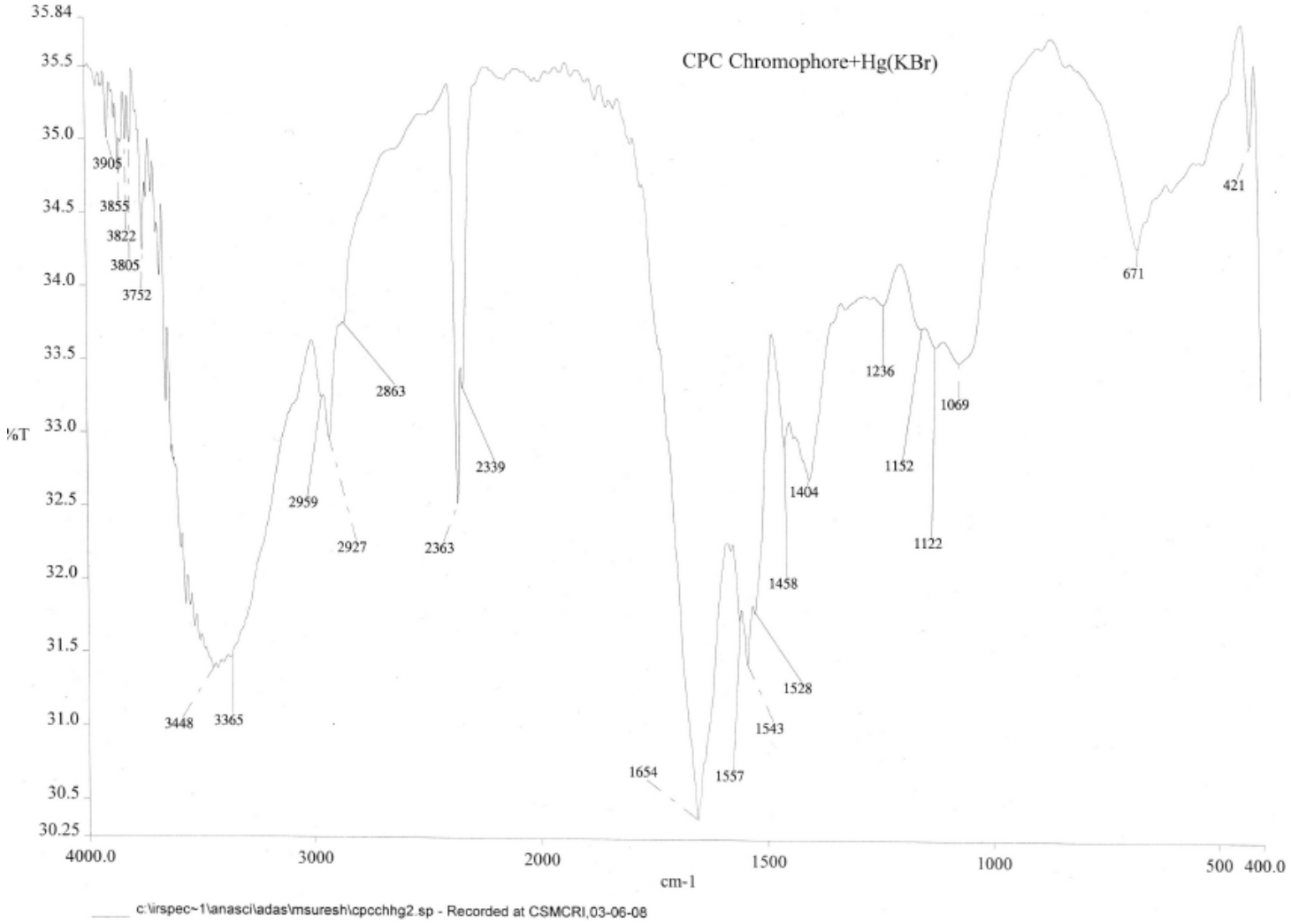
m/z = 811 Corresponds to Phycocyanobilin + Hg^{2+} + 23.
m/z for Na^{+} ion = 23

Fig S7: ESI-mass spectrum of ESI-mass spectrum of phycocyanobilin with Hg2+using Micromass Q-Tof micro™, equipped with ESI source and Q-Tof analyzer.

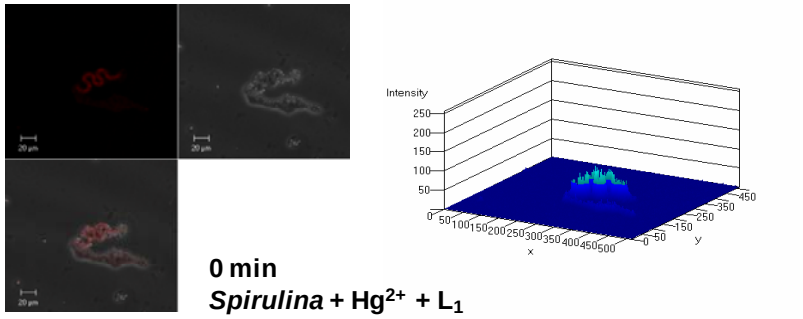
9. IR spectra of Phycocynobilin:



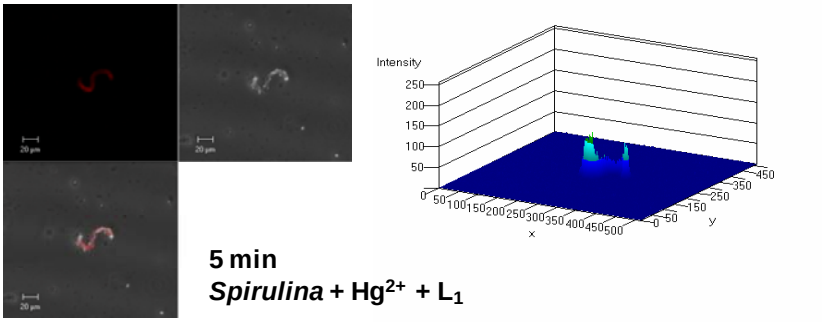
10. IR spectra of Phycocynobilin with Hg^{2+}:



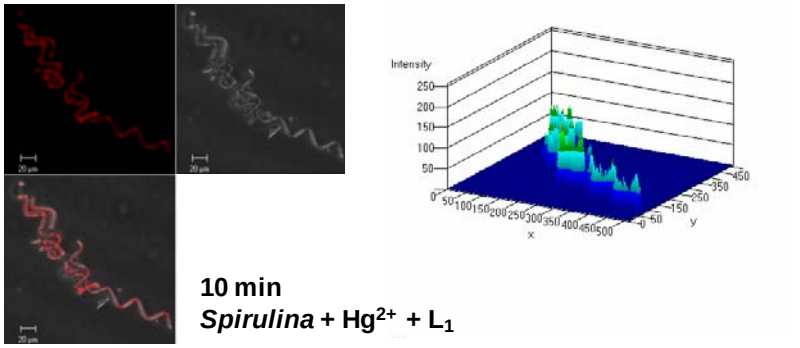
11. Confocal images at different time intervels:



0 min
Spirulina **+ Hg^{2+} + L_1**



5 min
Spirulina **+ Hg^{2+} + L_1**



10 min
Spirulina **+ Hg^{2+} + L_1**

Fig S8. Confocal images of *spirulina platensis* with 10 μM Hg^{2+} in 1:1 water / ethanol when exposed to 20 μM $\mathbf{L_1}$ in a different time interval.

12. Fluorescence competitive metal ion study of C-PC with Hg^{2+}:

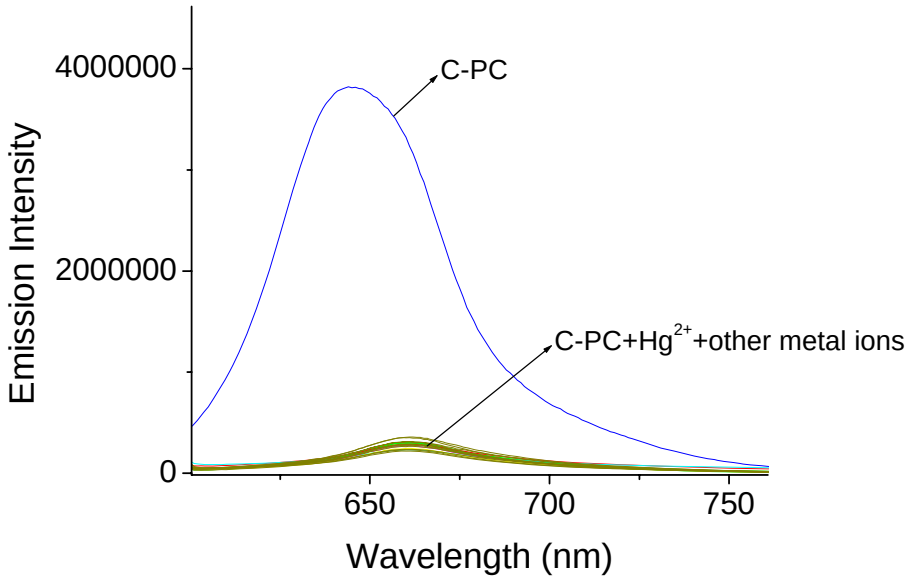
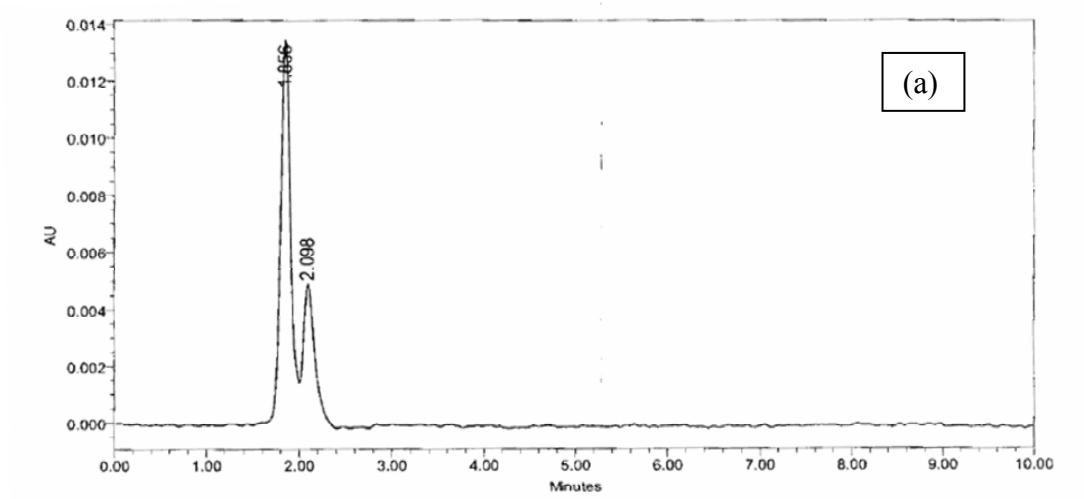


Fig S9: Change in the emission spectra of C-PC (4.0×10^{-8}M) in the presence of Hg^{2+} (8.0×10^{-4}M) with various other metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Ca^{2+}, Cd^{2+}, Ca^{2+}, Sr^{2+} Mg^{2+}, Zn^{2+}, Na^{+}, K^{+}, Li^{+}, Fe^{3+}) (8.0×10^{-3}M) in phosphate buffer at pH 7.2. Excitation at 580 nm.

13. HPLC study:

Purity of phycocyanobilin sample is further demonstrated by HPLC studies. A Waters HPLC system 2695 separation module (Alliance) coupled with Waters 2696 photodiode array UV-vis detector (PDA) was used for the HPLC separations on analytical C_8 column, 25cm x 4.6mm, 5µm. The column is equilibrated with mobile combination of 95% water and 5% methanol (0.1% acetic acid). The flow rate was 0.5 ml/min. The PDA detector was set to monitor the absorbance of eluent at 590 nm. Two fractions of phycocyanobilin chromophore were found in HPLC chromatogram at t_r= 1.8 min, (69.37%) and t_r = 2.0 min (30.63%) which corresponds to the existence of cis-trans isomers of phycocyanobilin.



	RT	Area	% Area	Height
1	1.856	102081	69.37	13530
2	2.098	45077	30.63	5012

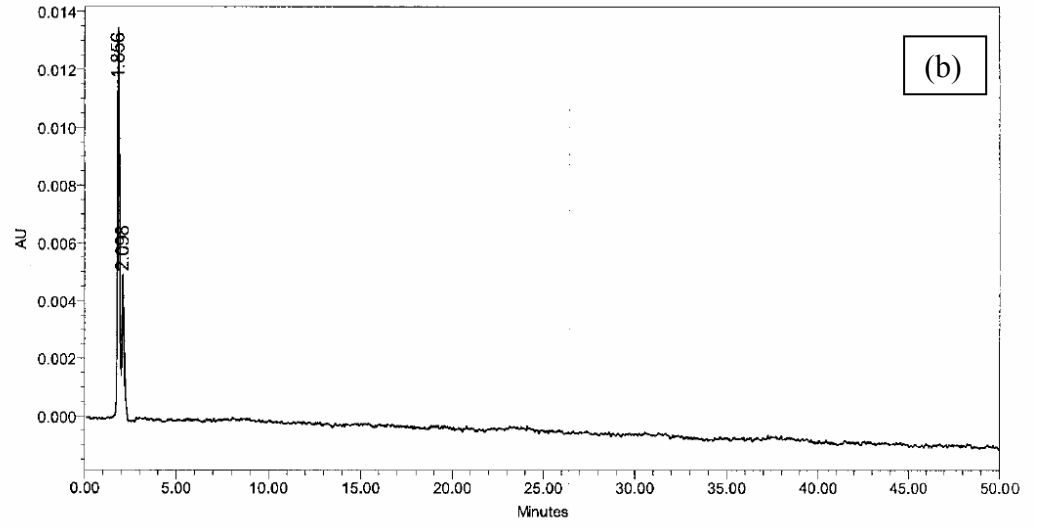


Fig S10: HPLC profile of phycocyanobilin using analytical C_8 column (25cm x 4.6mm) and water/CH_3OH (95:5, v/v). Figure (a) shows the profile with 10 min retention time, while (b) shows retention time upto 50 minutes.

14. ^{1}H NMR spectra for phycocyanobilin in $CDCl_3$:

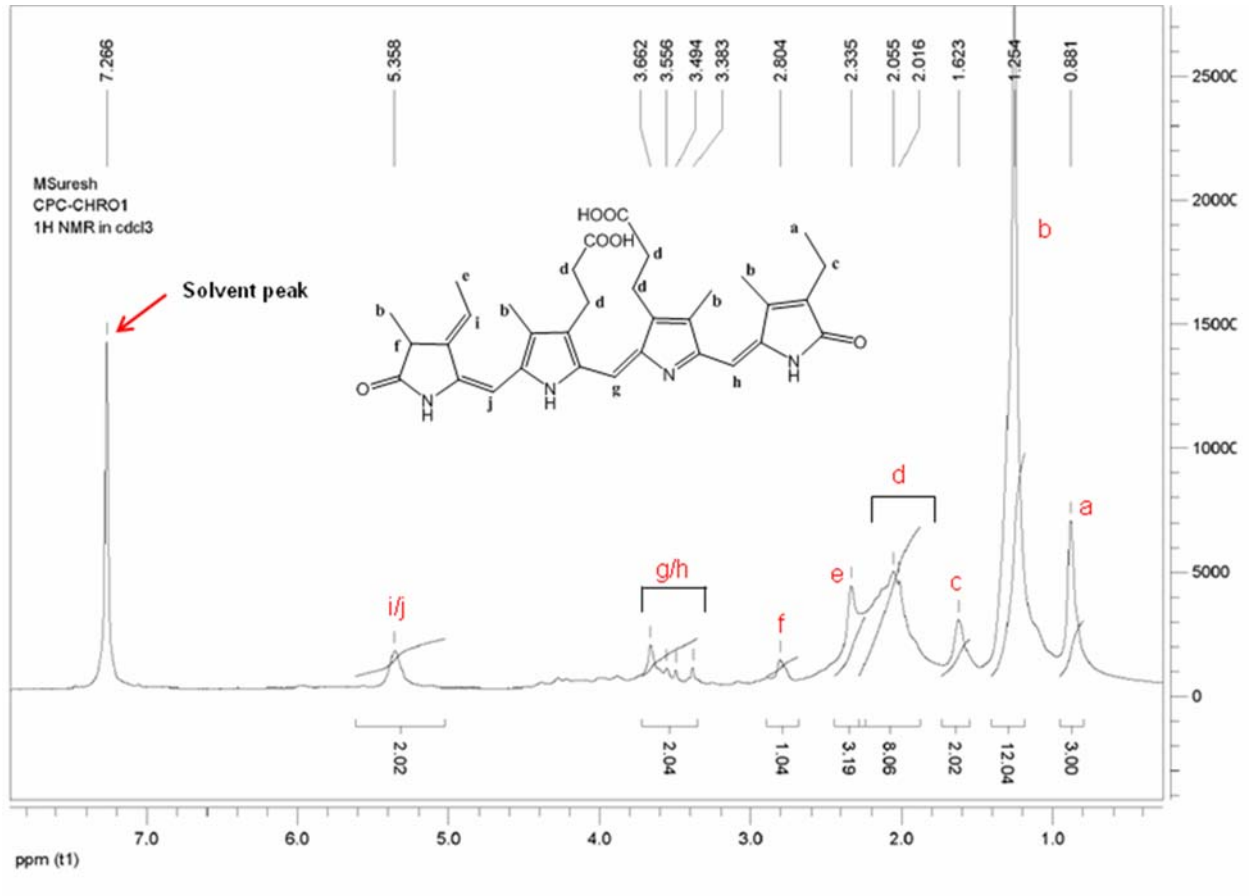


Fig S11: ^{1}H NMR spectra for phycocyanobilin in $CDCl_3$