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Solvent mediated tuning of selectivity in a rhodamine based probe and bio-imaging for Pb(II) detection in plant tissues

Ajoy Pal,[a] Bamaprasad Bag,*[a] M. Thirunavoukkarasu,[b] and Suchismita Pattanaik[b] and Barada K. Mishra[c]

[a]*Colloids and Materials Chemistry Department,* [b]*Bioresource Engineering Department,* [c]Centre for Special Materials; *CSIR-Institute of Minerals and Materials Technology, P.O.: R.R.L., Bhubaneswar-751013, India. Email: bpbag@immt.res.in*

Electronic Supplementary Information

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Measurements and* methods *for determination of quantum yields:

UV-visible spectra were recorded on a Perkin Elmer Lambda 650 UV/VIS spectrophotometer at 298 K in 10^{-4}-10^{-6} M concentration. The absorption spectra were recorded at different concentrations only to estimate its functional range in the medium mentioned. Steady-state fluorescence spectra were obtained with a Fluoromax 4P spectrofluorometer at 298 K with very dilute solutions (1-0.01μM) of the samples to avoid auto quenching. Fluorescence quantum yield was determined in each case by comparing the corrected spectrum with that of Rhodamine G (ϕ_F = 0.95) in EtOH[RS1] by taking the area under total emission using the following equation[RS2]

$$\phi_S = \phi_R \left(\frac{F_S A_R}{F_R A_S}\right).\left(\frac{\eta_S}{\eta_R}\right)^2 \qquad \text{eqn. S1}$$

where ϕ_S and ϕ_R are the radiative quantum yields, F_S and F_R are the area under the fluorescence spectra, A_S and A_R are the absorbance (at the excited wavelength) of the respective sample and the reference; η_S and η_R are the refractive indices of the solvent used for the sample and the reference. The quantum yield of Rhodamine G was measured using quinine sulfate in 1N H_2SO_4 as reference[RS3] excited at (λ_{ex}) 350 nm. The standard quantum yield value thus obtained was used for the calculation of the quantum yield of the samples.

Ref:

[RS1] M. Fischer and J. Georges, *Chem. Phys. Lett.*, 1996, **260**, 115.

[RS2] B. Bag and P. K. Bharadwaj, *J. Phys. Chem. B*, 2005, **109**, 4377.

[RS3] J. B. Birks, *Photophysics of Aromatic Molecules*, 1970, Wiley-Interscience, New York.

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Metal ion induced spectral modulation

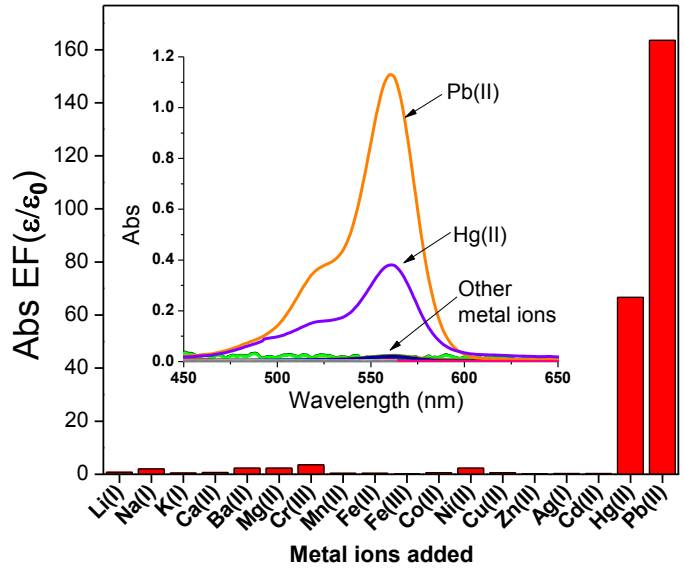

Fig. S1: Absorption enhancement factors of $\mathbf{L_1}$ ([$\mathbf{L_1}$] = 1×10^{-4}M) in presence of various metal ions([M(I/II)] = 1×10^{-5}M) measured immediately (<1 min.) after adding metal ion solution (EtOH-H_2O, HEPES, 1:1 v/v).

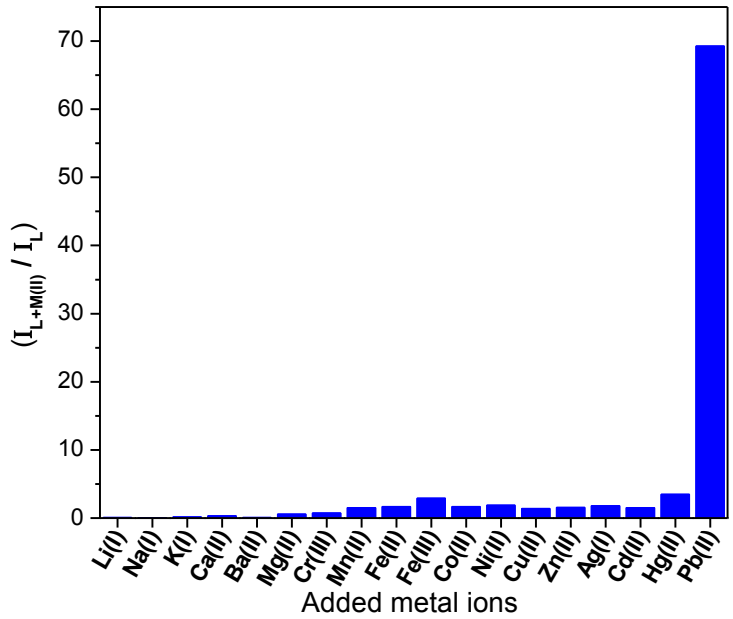

Fig. S2: Fluorescence EF of $\mathbf{L_1}$ ([$\mathbf{L_1}$] = 1μM) in presence of various metal ions (EtOH-H_2O, HEPES, 1:1 v/v), [M(I/II)] = 10μM, spectra taken after 30 min. of mixing, RT, λ_{ex}= 500nm, *ex* and *em* b. p. = 5nm.

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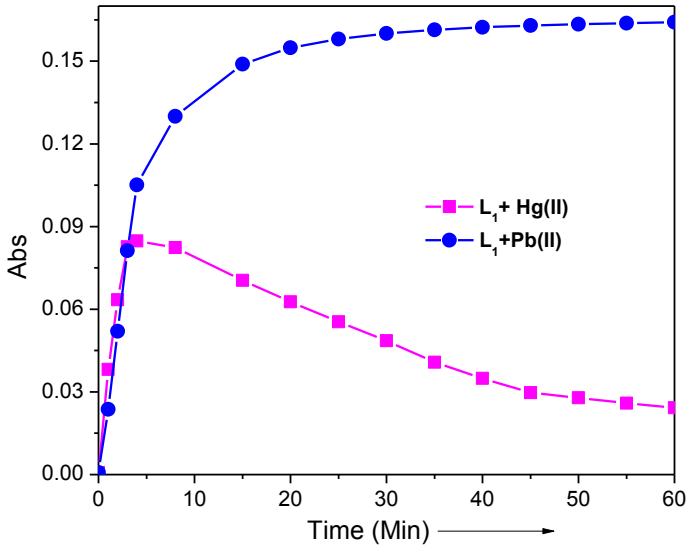

Fig. S3: Plot of absorbance of $\mathbf{L_1}$ ([$\mathbf{L_1}$] = 5μM) in presence of Hg(II) and Pb(II) ions as a function of time (EtOH-H_2O, 1:1 v/v).

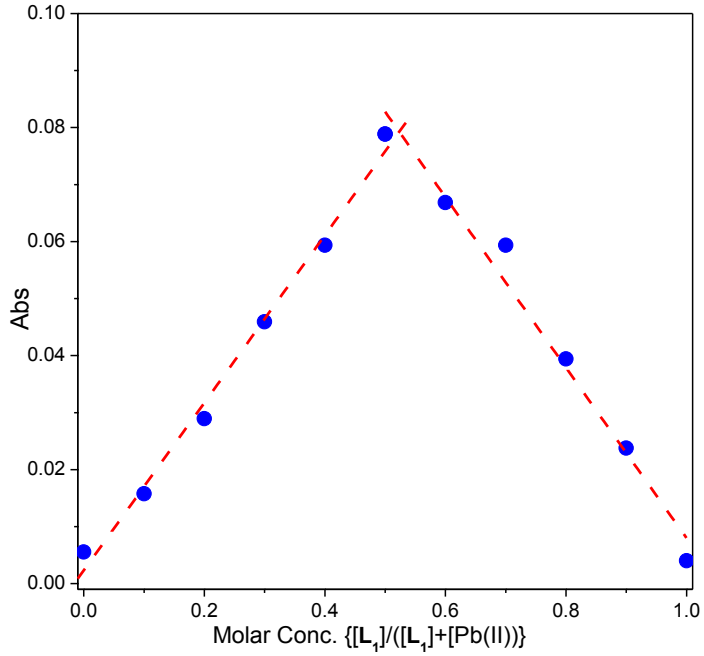

Fig. S4: Plot of absorbance of $\mathbf{L_1}$ ([$\mathbf{L_1}$] = 5μM) against its molar concentration as a function of Pb(II) ion added (Job's Plot) depicting the stoichiometry of complexation to be 1:1 (L:M).

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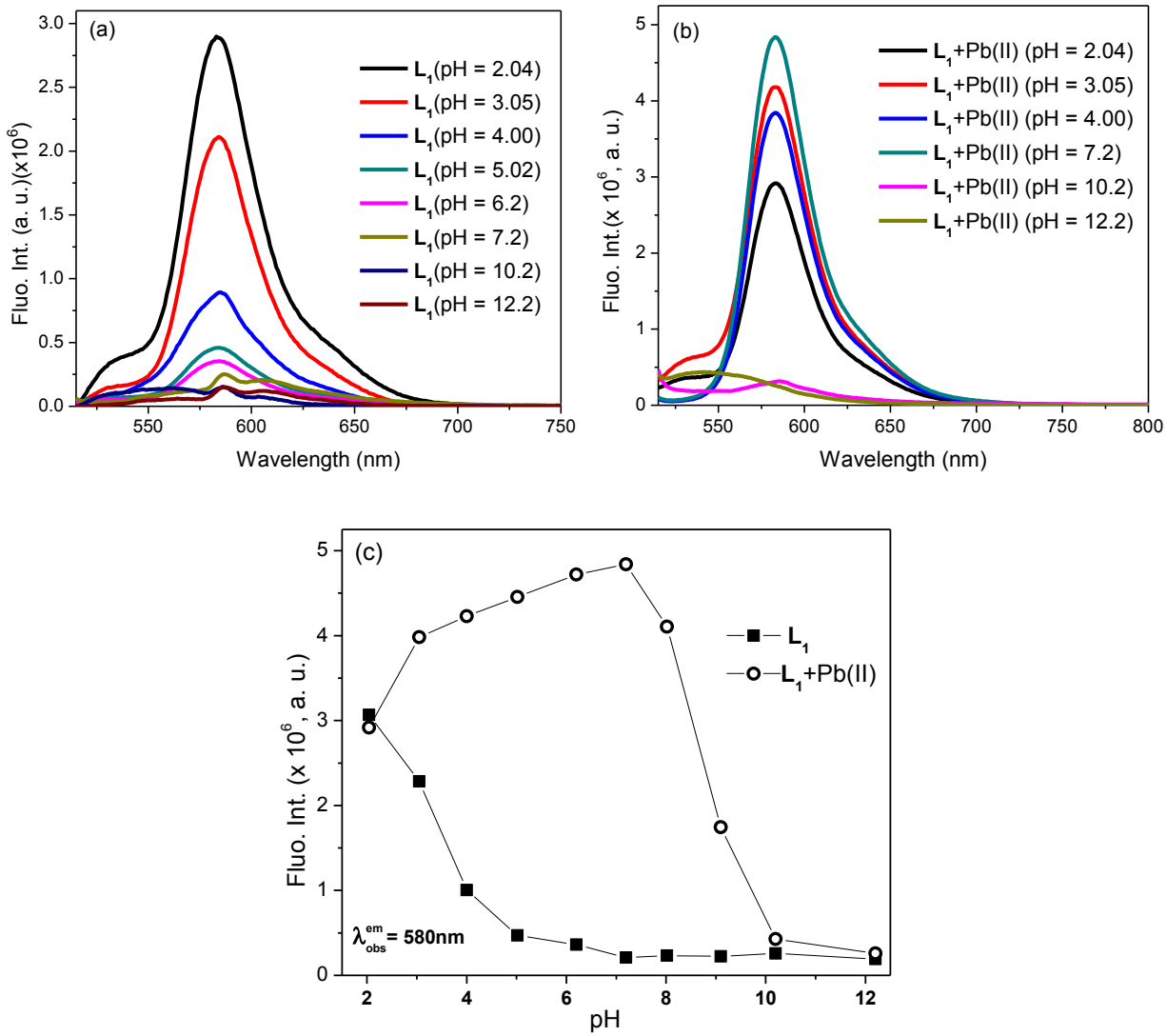

Fig. S5: Change in fluorescence intensity of $\mathbf{L_1}$ alone(a) and in presence of Pb(II) ion(b) in different pH. [$\mathbf{L_1}$] = 1.0μM, [Pb(II)] = 10μM, λ_{ex} = 500nm, RT, *ex* and *em* b. p. = 5nm. (c) Fluorescence Intensity (a. u.) at λ_{580} nm of the samples as a function of pH depicting optimal observable change at pH 7.2.

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Determination of association constant (K_a):

The association constant (K_a) of the complex were determined from the change in absorbance or fluorescence resulted from titration of dilute solutions (~10^{-5}-10^{-7} M) of $\mathbf{L_1}$ against Pb(II) ion solution following the Benesi-Hildebrand method for a complexation of 1:1 (ligand: metal) stoichiometry as depicted in the eqn.(S2)

$$1/(X-X_0) = 1/\{K_a(X_{max}-X_0)[M(II)]\} + 1/(X_{max}-X_0) \quad \ldots\ldots\ldots\ldots \text{ eqn. (S2)}$$

where X_0 is the absorbance or fluorescence of $\mathbf{L_1}$ at a particular wavelength, X is absorbance or fluorescence intensity obtained with added [M(II)], X_{max} is the absorbance or fluorescence obtained with excess amount of metal ion added and [M(II)] is the concentration of metal ion added. The double reciprocal plot of absorption or fluorescence spectral change $\{1/(X-X_0)\}$ as a function of added metal ion concentration (1/ [Pb(II)]) results in a linear regression and its slope determines K_a.

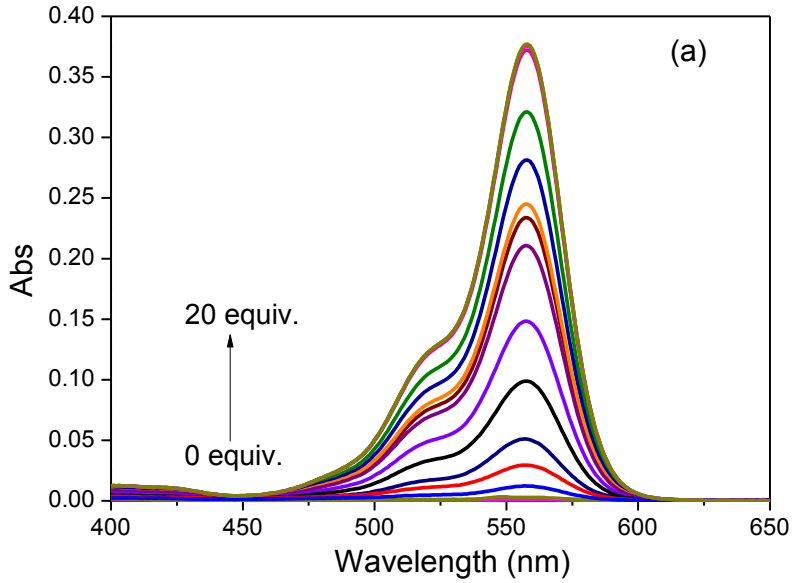

Fig. S6: Absorption spectral change in $\mathbf{L_1}$ as a function of various equivalents of added Pb(II) metal ions in EtOH-H_2O(1:1 v/v). [$\mathbf{L_1}$] = 10 μM.

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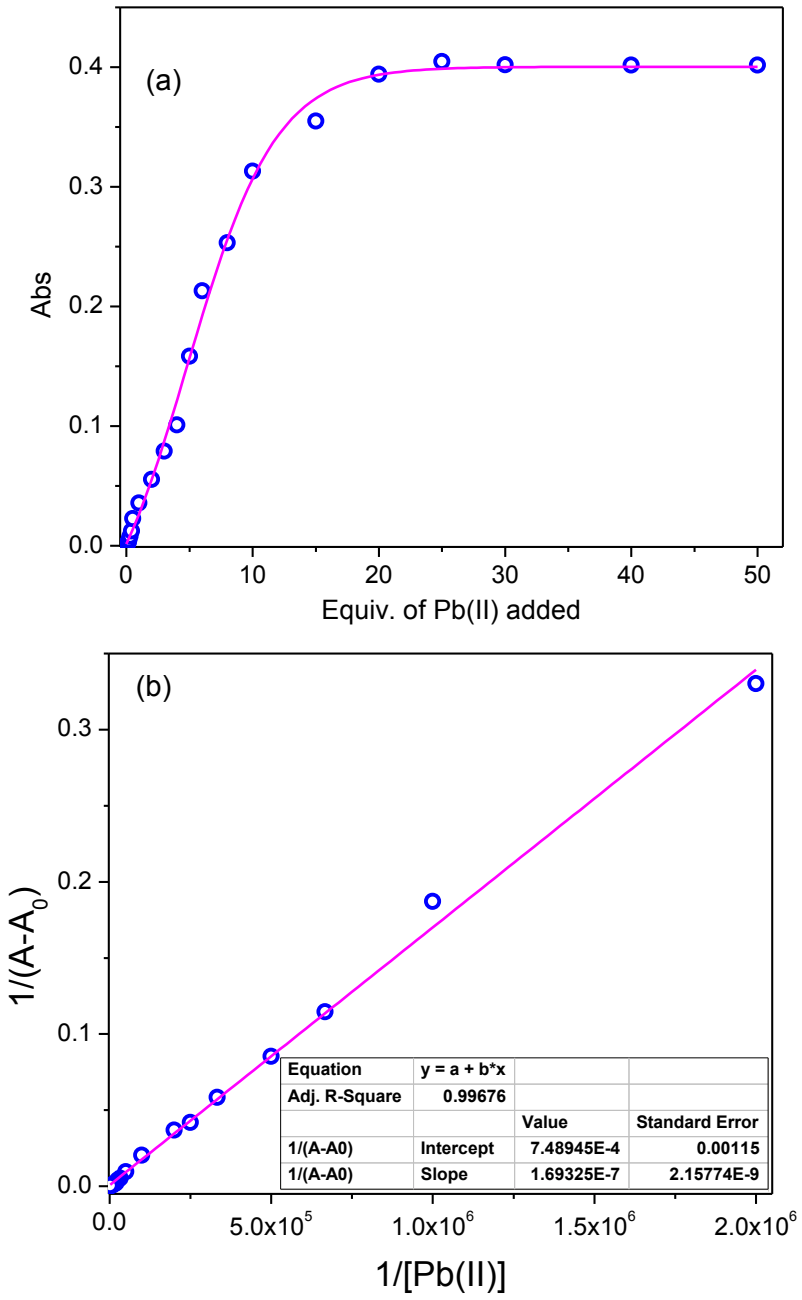

Fig. S7: (a) Absorbance as a function of equivalents of Pb(II) added to $\mathbf{L_1}$(10 μM) in EtOH-H_2O(1:1 v/v) and (b) linear regression plot of $1/(A-A_0)$ vs. 1/[Pb(II)] of the respective titration.

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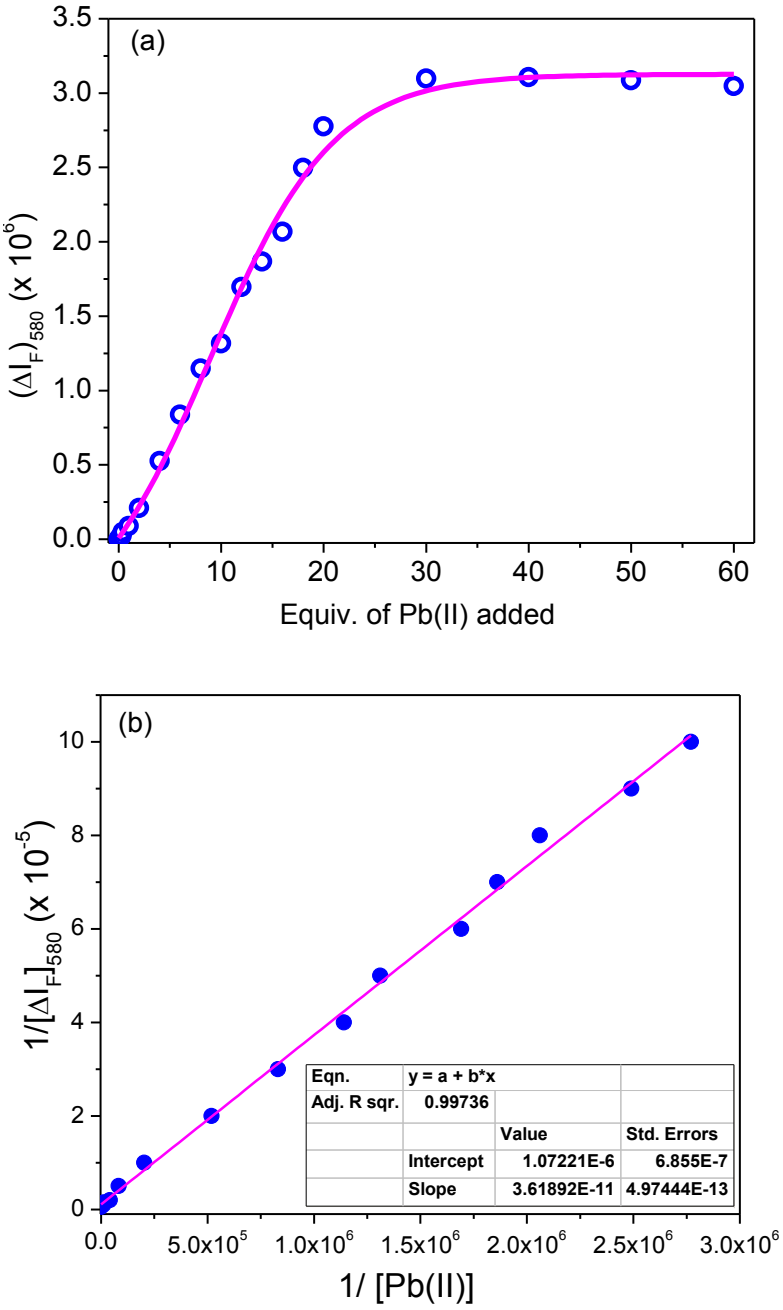

Fig. S8: (a) Change in fluorescence intensity as a function of equivalents of Pb(II) added to $\mathbf{L_1}$(1 μM) in EtOH-H_2O(1:1 v/v) and (b) linear regression plot of $1/(F-F_0)$ vs. 1/[Pb(II)] of the same titration depicting association constant(K_a) determination.

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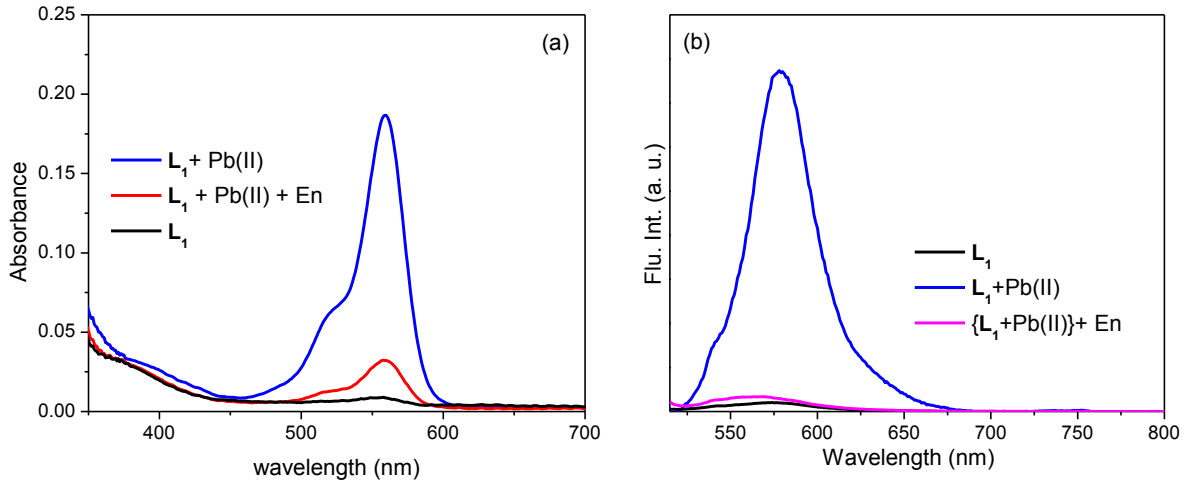

Fig. S9: Change in (a) absorption and (b) fluorescence intensity upon addition of a competitive chelators (10 equiv.) such as ethylenediamine to the solution containing $\mathbf{L_1}$ (1 equiv.) and Pb(II) (5 equiv.) showing reversibility in signaling pattern. [$\mathbf{L_1}$] = 5.0×10^{-6} M (for absorption) and 1.0×10^{-7} M (for emission). λ_{ex} = 500nm, RT, *ex* and *em* b. p. = 5nm.

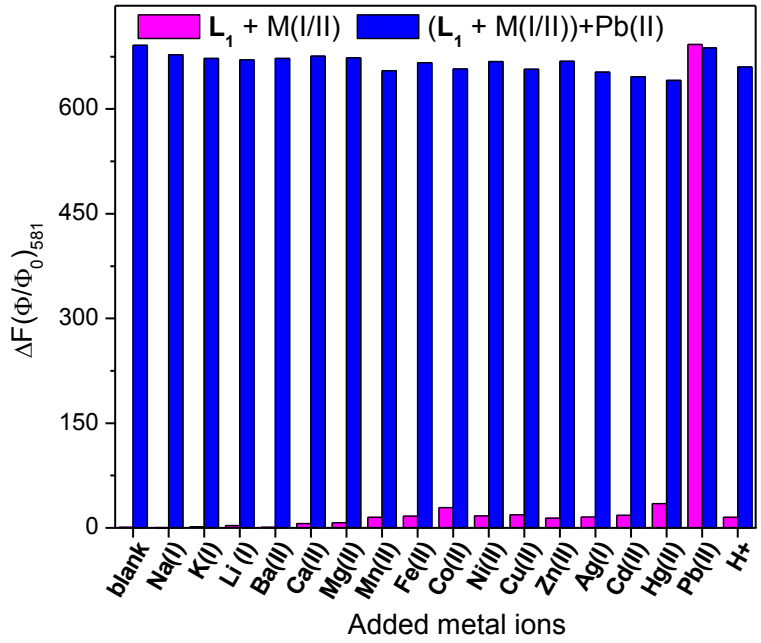

Fig. S10: Extent of fluorescence enhancement in $\mathbf{L_1}$ with various metal ions in absence and presence of Pb(II) in EtOH-H_2O(1:1 v/v). Conditions: [$\mathbf{L_1}$]=1 μM, λ_{ex} = 500nm, RT, *ex* and *em* b. p. = 5nm. The error in ϕ_F is within 5 %.

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Detailed Procedure for fluorescence imaging of lead accumulation in root tissues of A. lanata.

Plant material

One month old plants of *Aerva lanata*, maintained in the green house was used for experimental purpose. The experiments were carried out at the CSIR-Institute of Minerals & Materials Technology, Bhubaneswar (200 17' 45" N latitude and 850 49' 15" E longitude), India.

Lead ion treatment

Plants with well developed root system were hydroponically grown in test tubes, secured by wrapping a cotton plug at the stem-root junction, thus ensuring immersion of the whole root system (Fig S11) in nutrient solution (1/4 Murashige Skoog salts) and maintained in an incubation room with mean temperature of 25 ± 2 ºC. Lead ion have been supplied with aqueous $Pb(NO_3)_2$ solution and the pH was adjusted to 6.6 5 using 1 M KOH or 1 N HCl. Lead concentrations used in the present experiments were 0, 25, 50, 75, and 100ppm. In each concentration 5 uniform plants were cultured and allowed for 7 days growth period.

Treatment with $\boldsymbol{L_1}$ *solution*

After 7 days of growth period, the plants were removed from the respective hydroponic solution and the root portions were rinsed in distilled water for 2-3 minutes, then were cut into 2-3 cm long pieces from the root tips. Root segments from each plants were treated in equimolar aqueous-ethanolic solution of $\mathbf{L_1}$ for 0, 15, 30 minutes.

Binding studies by fluorescence microscope

The Pb(II)-$\mathbf{L_1}$ interaction in root segment were probed using a fluorescence microscope (Nikon 80i, Nikon Inc., Japan).

Histological study

At the end of the exposure period, plants were rinsed thoroughly in three changes of distilled water. Root samples were taken for fluorescence microscopy and microanalysis from control plants and plants that had been exposed to the different concentrations of Pb(II) ions. In

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order to prepare thinner (<20 micron) sections, a simple technique of hand-sectioning of samples using sharp razor was followed.

Visible effect of lead treatment

Lead ion exposure has shown an observable toxic effect (Fig. S11B) on root growth as well as shoot growth on *A. lanata* seedlings. In comparison to control (Fig. S11A)plants without any treatment, plants exposed to Pb(II) ions for 3 days have showed no adverse effects such as wilting or necrosis irrespective of concentration. However, the adverse effect of phyto-toxicity on plant growth was well pronounced after three days in plants cultured in solution with 100ppm Pb(II) concentration, after five days in those with 50ppm, where as those with 25ppm have shown lesser impact. No adverse effect of phyto-toxicity on root growth was observed for at least 7 days upon exposure of the control plants to the $\mathbf{L_1}$ solution.

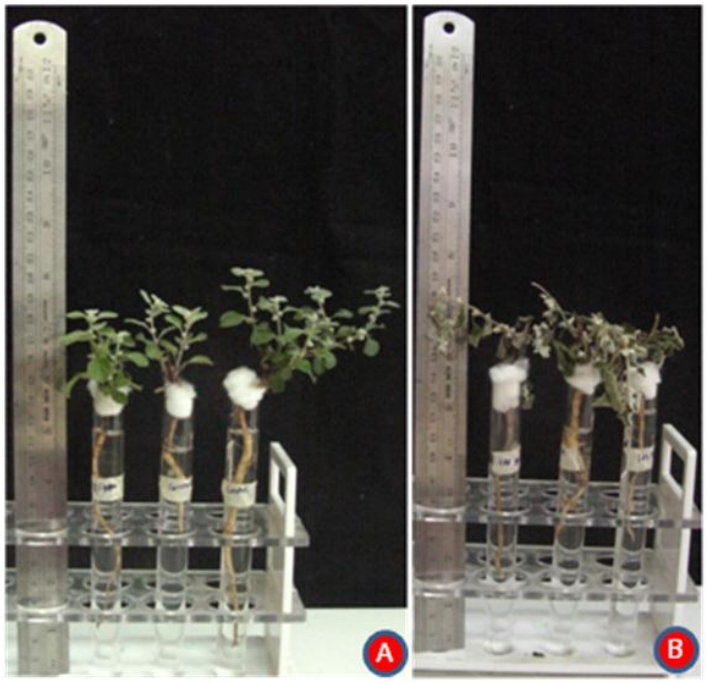

Fig. S11: Effect of Pb(II) treatment on *Aerva lanata* seedlings without lead treatment(A) and with 100ppm Pb(II) treatment(B), both in a 7 day time frame showing Pb(II) induced phyto-toxicity on plant growth.

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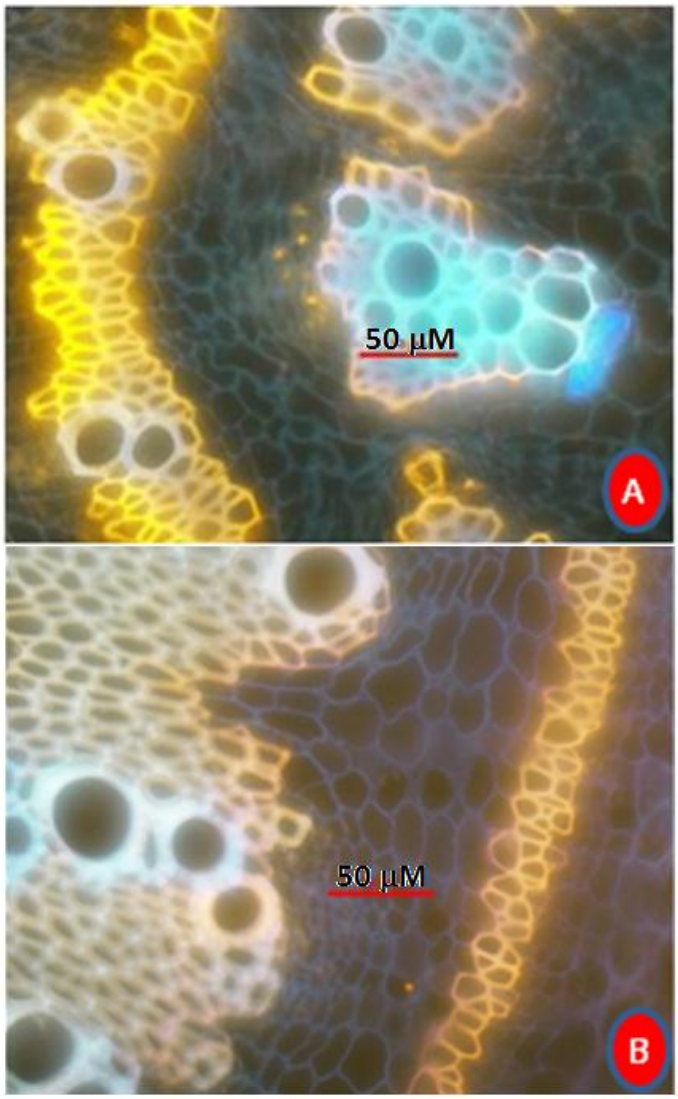

Fig. S12: Fluorescence microscopic images of cross section of root of *Aerva lanata* seedlings grown in hydroponic solution, which (a) first received Pb(II) treatment (100 ppm), then soaked in $\mathbf{L_1}$ solution (for at least 15 mins), and (b) only in $\mathbf{L_1}$ solution (2×10^{-4} M), exited at 543nm, observed after 12 days. The bright emission (as shown in Fig. 5B in the manuscript) due to interaction of $\mathbf{L_1}$ with Pb(II) accumulation located primarily in pericycle fibers and xylem vessels in its root slowly diminishes after 10 days (as observed in Fig. S12A).

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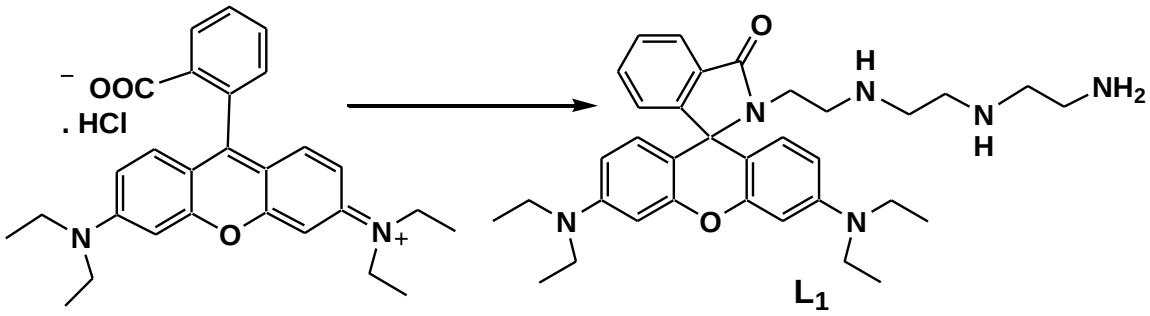
Synthesis of ***L₁***

The compound was synthesized and characterized following the procedure reported from our laboratory (ref. 12a).



Reaction conditions: triethylene tetramine, Et_3N, EtOH, reflux, 15h.

Scheme S1: Synthetic route to $\mathbf{L_1}$

Yield: 0.43 g (76 %); mixed mp: 112-114 °C; ESI-MS, m/z$^+$ (%): 571 $[\mathbf{L_1}]^+$ (45%); ^{1}H-NMR (400MHz, $CDCl_3$, 25 °C, TMS, δ): 7.80 (br s, 1H), 7.35 (t, *J* = 3.99 Hz, 1H), 7.22 (br s, 1H), 7.0 (br s, 1H), 6.34 (d, *J* = 7.99 Hz, 2H), 6.30 (s, 2H), 6.20 (d, *J* = 3.99 Hz, 2H), 3.25 (d, *J* = 3.99 Hz, 8H), 2.75 (br s, 2H), 2.61 (br s, 2H), 2.51 (br s, 8H), 2.30 (br s, 4H), 1.08 (t, *J* = 7.99 Hz, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$, 25 ^{o}C, TMS, δ):168.51, 153.13(d), 148.64, 132.27(d), 130.98(t), 128.55(d), 127.94, 123.65, 122.57, 107.95, 105.36(t), 97.56, 64.77(d), 50.94, 48.47, 48.05, 47.47, 44.20, 40.73, 39.85, 12.44; Anal. Calcd. for $C_{34}H_{46}N_6O_2$: C, 71.55, H, 8.12, N, 14.72. Found: C, 71.46, H, 8.21, N 14.63 %.