Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Pyrene-Based Ratiometric Probe for Nanomolar Level Detection of Glyphosate in Food and Environmental Samples and its Application for Live-Cell Imaging

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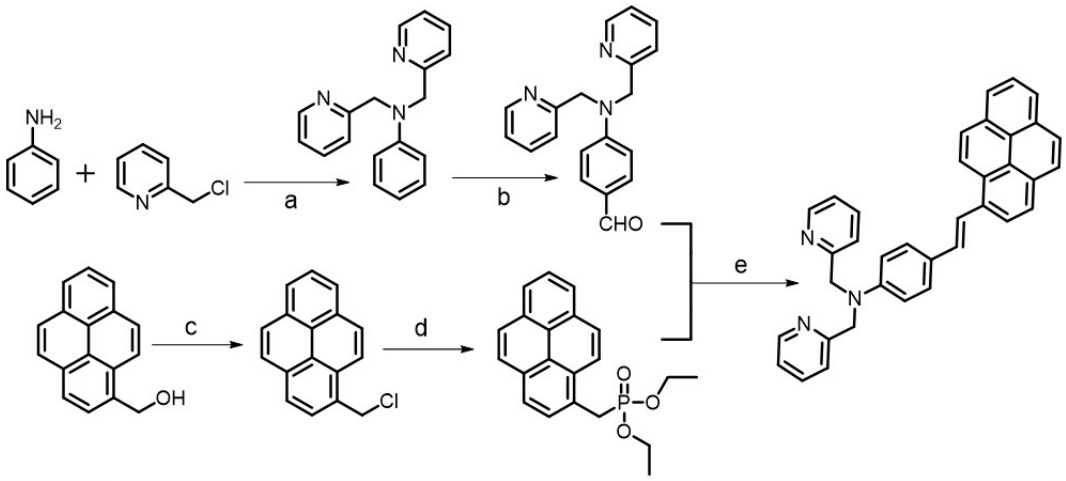
Synthesis of Probe molecule

The probe molecule was synthesized following the procedure reported in the literature [1]



Reagents and conditions: (a) K_2HPO4, CH_3CN, reflux, 24 h, 82 % (b) $POCl_3$/ DMF, 90 °C, 2 h, 56 % (c) $SOCl_2$, toluene, 90 % (d) $P(OCH_2CH_3)_3$ (e) NaH, THF, 42 %

Characterization of compound **1**. Yield 0.5 g, yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): d= 4.95 (s, 4 H), 6.72 (d, J=9.3 Hz, 2 H), 7.28–7.30 (m, 2 H), 7.32–7.40 (m, 3 H), 7.60 (d, J=8 Hz, 2 H), 7.75–7.79 (m, 2H), 8.03 (s, 1 H), 8.05–8.12 (m, 3 H), 8.20 (d, J=8 Hz, 1H), 8.24–8.28 (m, 3 H), 8.42 (d, J=8 Hz, 1 H), 8.57–8.59 (m, 2 H), 8.71 ppm (d, J=8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): d=57.2, 120.8, 121.6, 122.5, 123.2, 124.4, 125.3, 125.7, 126.2, 126.7, 127.0, 127.3, 127.9, 129.3, 130.2, 131.3, 132.2, 133.7, 135.4, 136.3, 147.7, 149.5, 149.8, 158.2 ppm; IR (KBr): $\tilde{\upsilon}$= 3030.2, 2938.2, 1735.6, 1592.5, 1518.3, 1434.7, 1347.8, 1223.4, 1177.5 cm^{-1}; MS (ESI): m/z: 502 $[M+H]^+$; HRMS: m/z calcd for $C_{36}H_{27}N_3$ $[M+H]^+$: 502.2283; found: 502.2288; elemental analysis calcd (%) for $C_{36}H_{27}N_3$: C 86.2, H 5.43, N 8.38; found: C 86.6, H 5.44, N 8.35.

Reference: Bhattacharya et al., ChemPlusChem 2014, 79, 1059 – 1064

Materials and Methods

Materials and Methods All necessary reagents including starting materials, precursor molecules, solvents and silica gel (for TLC and column chromatography) were obtained from the best-known local supplier and were used in present study without further purification. Solvents were distilled and dried prior to use. FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). ^{1}H-NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 MHz. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane (TMS). Mass spectra were recorded on Micro mass Q-TOF Micro TM spectrometer.

UV-visible and fluorescence Experiment The UV−vis and fluorescence spectra were recorded on a Shimadzu model 2100 UV-vis spectrometer and Cary Eclipse spectrofluorimeter respectively. In the emission experiments, the slit widths (for both the excitation and emission channel) were fixed at 5 nm and the excitation wavelength was chosen 350 nm.

Sampling Procedure of Sensing. Sensing of the herbicides/ pesticides in pure water was carried out by adding 10 μL DMSO solution of **1**.Cu^{2+} from a stock ([**1**] = [Cu^{2+}] = 1 × 10^{-3} M) in pure water to make the final volume of 1 mL ([**1.**Cu^{2+}] = 1 × 10^{-5} M) followed by addition of DMSO solution of the analytes (1 equiv). In case of sensing in the micellar medium, 10 μL of the DMSO stock solution ([**1**] = [Cu^{2+}] = 1 × 10^{-3} M) into the Brij-58 (1 mM), SDS (8 mM) or CTAB (1 mM) micellar solution to make the final volume of 1 mL. This was followed by addition of an aliquot of DMSO solution of the analytes (1 equiv). Similar procedure has been followed for the sensing in buffered media of different pH (HCO_2Na/HCl buffer for pH 2, Tris/HCl for pH 7 and $Na_2B_4O_7 \cdot 10H_2O$/NaOH for pH 12). The final concentration of DMSO in the solution did not exceed 1%.

Detection limit determination. The method used for the calculation of the detection limit is known as the blank variability method. In this method, the calibration curve was prepared by recording fluorescence spectra of **1**.Cu^{2+} with different amounts of glyphosate.

From the equation obtained from the calibration plot, the added glyphosate concentrations were calculated. Then another calibration curve was drawn between the C_{real} (added glyphosate) vs. $C_{calc.}$ (Calculated amount of glyphosate). This afforded a value of the slope (b).

The signal of the ligand in absence of the added analyte was taken as blank reading. 10 replicates of the blank were measured. The standard deviation from the blank readings was calculated by fitting the fluorescence reading into the equation obtained from the first calibration curve (titration spectra). Using this standard deviation value, we calculated decision limit by this following equation.

$L_C = t_C \times s \times (1 + 1/N)^{1/2}$..... (1)

where, N = the number of blank replicates taken; the value of t_c for 10 blank readings is 1.833; and s = the standard deviation value.

The detection limit (LD) was calculated as the double of the decision limit obtained,

$L_D = 2\ L_c$ ..... (2)

In concentration term, the detection limit appeared as,

$x_D = 2 \times C = 2\ L_C / b$ ..... (3)

where, b = slope of the second calibration curve (Creal vs. Ccalc.).

Determination of Fluorescence Quantum Yield: The fluorescence quantum yield was calculated by using rhodamine 6G (Φ = 0.94 in EtOH) as a reference. And the quantum yield is calculated using the equation

$\Phi_{unk} = \Phi_{std}[(I_{unk}/A_{unk})/(I_{std}/A_{std})](\eta_{unk}/\eta_{std})^2$

where, Φ_{unk} and Φ_{std} are the radiative quantum yields of the sample and standard, Iunk and Istd are the integrated emission intensities of the corrected spectra for the sample and standard, A_{unk} and A_{std} are the absorbances of the sample and standard at the excitation wavelength, and η_{unk} and η_{std} are the indices of refraction of the sample and standard solutions, respectively.

Fluorescence Decay Experiment

Fluorescence lifetime values were measured by using a time-correlated single photon counting fluorimeter (Horiba Jobin Yvon). The system was excited with nano LED of Horiba - Jobin Yvon with pulse duration of 1.2 ns. Average fluorescence lifetimes (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

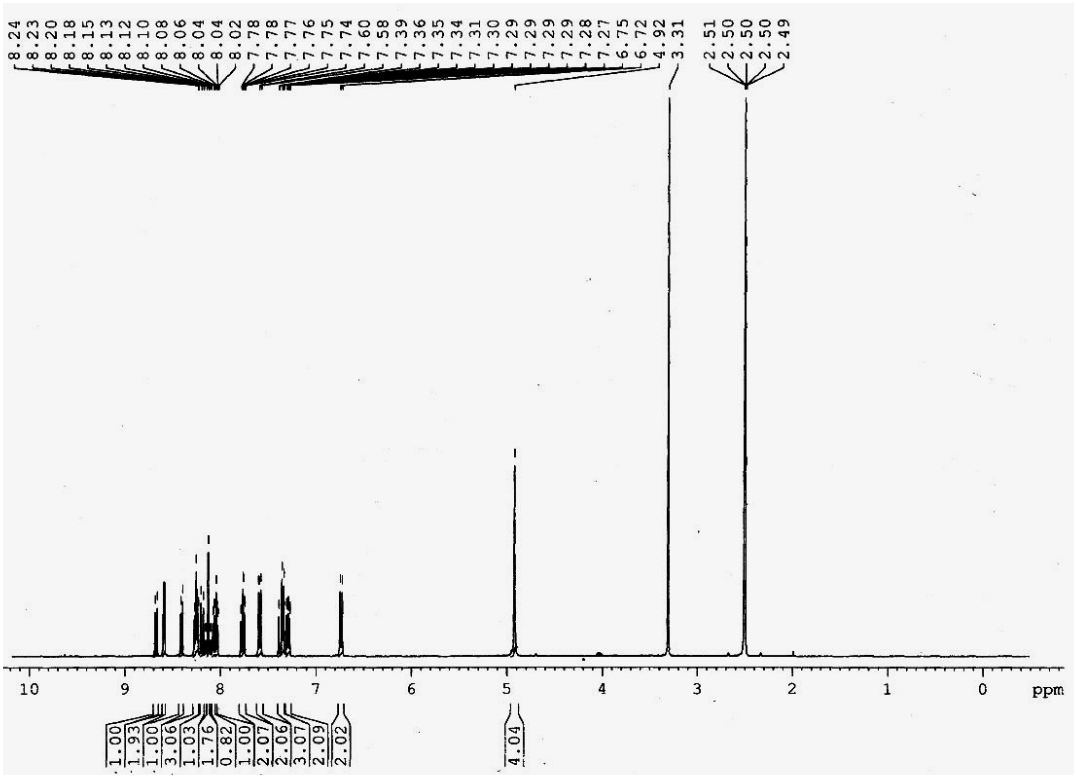
$\tau_{av} = (a_1\tau_1^2+a_2\tau_2^2 +a_3\tau_3^2)/(a_1\tau_1+a_2\tau_2+a_3\tau_3)$

Where a_1, a_2 and a_3 are the relative amplitudes and τ_1, τ_2, and τ_3 are the lifetime values, respectively. For data fitting, a DAS6 analysis software version 6.2 was used.

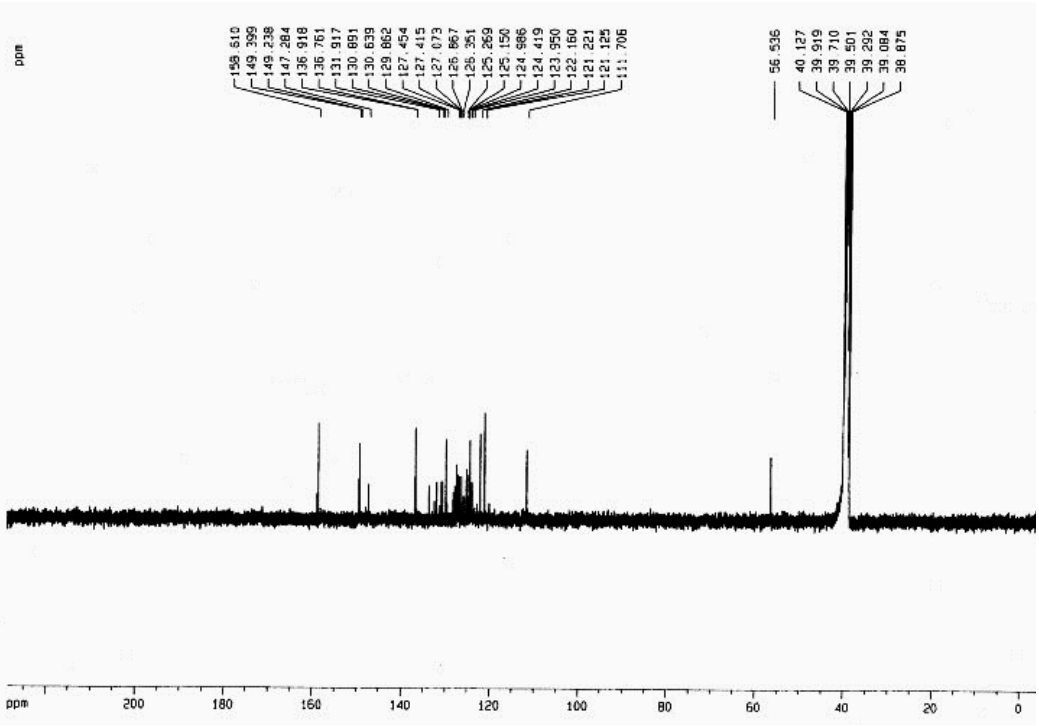
Analysis of Food Samples: The agricultal crops collected from local organic farm, were thoroughly washed with distilled water to remove insoluble dirts. Then the cereals or grain samples were powdered using mortar-pestle. About 500 mg of powdered samples were added to water (5 mL) and sonicated at 50 °C for 1 h. Then, the mixtures were subjected to centrifugation at 5000 rpm for 15 min and the clear supernatants were collected for spectroscopic analysis. For the fluorescence analysis, each supernatant (0.5 mL) was mixed with Brij-58 (1.5 mL), mixed with **1**.Cu^{2+}, and incubated for 1 h at RT. The emission spectra were recorded upon spiking with different amounts of glyphosate. The detection limit in each case was calculated by using the blank variation method.

Design of Paper Strips: To prepare the compound-coated paper strips, 40 µL of CH_2Cl_2 solution of **1** (0.02 mM) was drop-cast onto the filter paper strips (rectangular in shape, 1 x 1.5 cm) using a micropipette in two intervals (2 x 20 µL). The concentration of **1** in the solution as well as immersion time were varied systematically to obtained optical brightness and stability. The solution was completely absorbed in filter paper within 5-10 min and then the filter papers were kept overnight to airdry. The stability of paper strips was verified over a couple days under ambient condition. Finally, the paper strips were ready for sensing studies.

Characterization Data



^{1}H-NMR spectrum of **1** in DMSO-d_6 medium



^{13}C-NMR spectrum of **1** in DMSO-d_6 medium

Additional Spectral Data

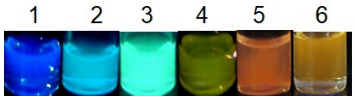


Figure S1. Fluorescence images of **1** in different organic solvents, 1: in THF, 2: in $CHCl_3$, 3: in acetone, 4: DMSO, 5: acetonitrile 6: MeOH

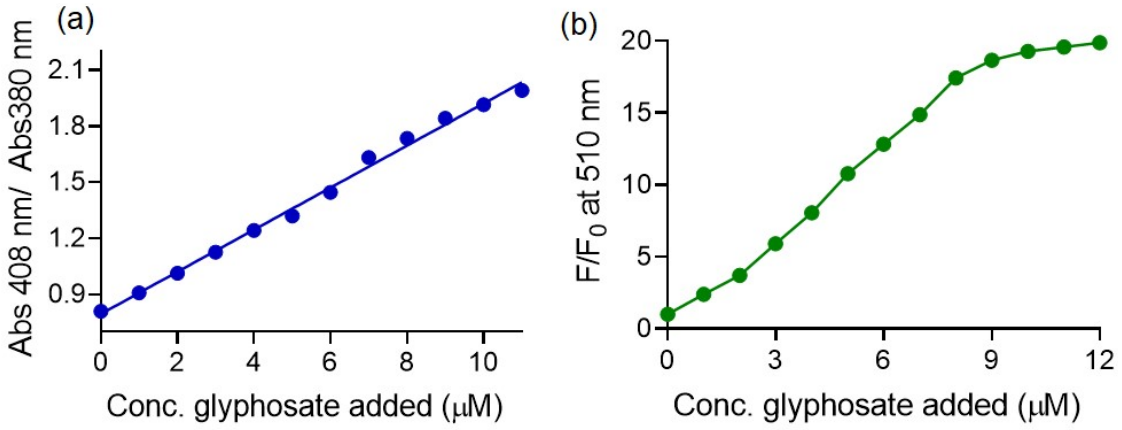


Figure S2. (a) Plot of absorbance ratio at 408 nm and 380 nm of **1**.Cu^{2+} (10 μM, 1:1) vs. concentration of glyphosate added (0 – 12 μM) in Brij-58 micelle. (b) Change in fluorescence intensity of **1**.Cu^{2+} (10 μM, 1:1, λex = 350 nm) upon addition of glyphosate (0 – 12 μM) in Brij-58 micelle.

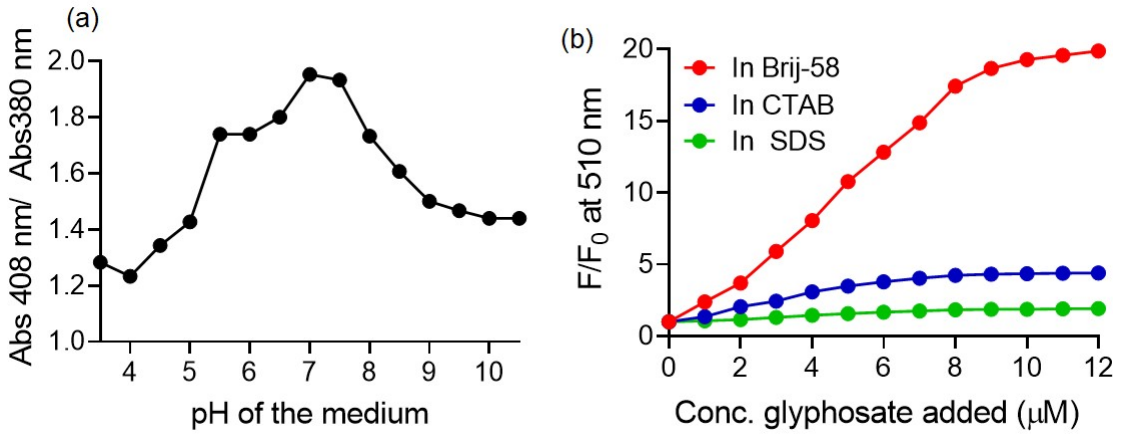


Figure S3. (a) Effect of pH on absorbance ratio at 408 nm and 380 nm of **1**.Cu^{2+} (10 μM, 1:1) upon addition of glyphosate (15 μM) in Brij-58 micelle. (b) Change in fluorescence intensity of **1**.Cu^{2+} (10 μM, 1:1, λex = 350 nm) upon addition of glyphosate (0 – 12 μM) in different micelle medium.

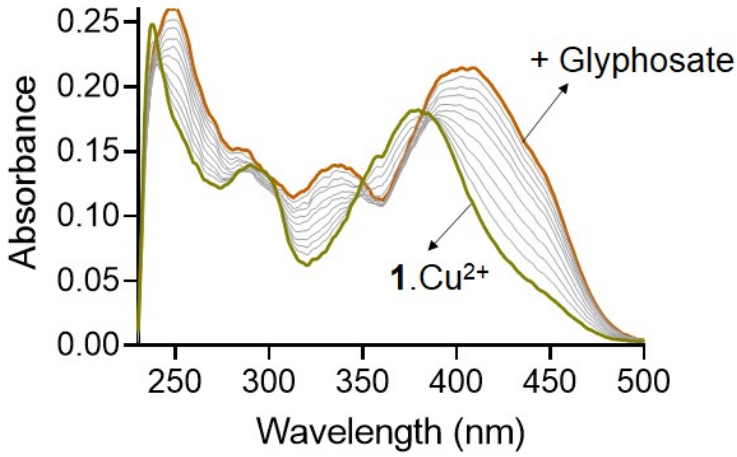


Figure S4. UV-visible titration of **1**.Cu^{2+} (10 μM, 1:1) with glyphosate (0 – 15 μM) in aqueous medium.

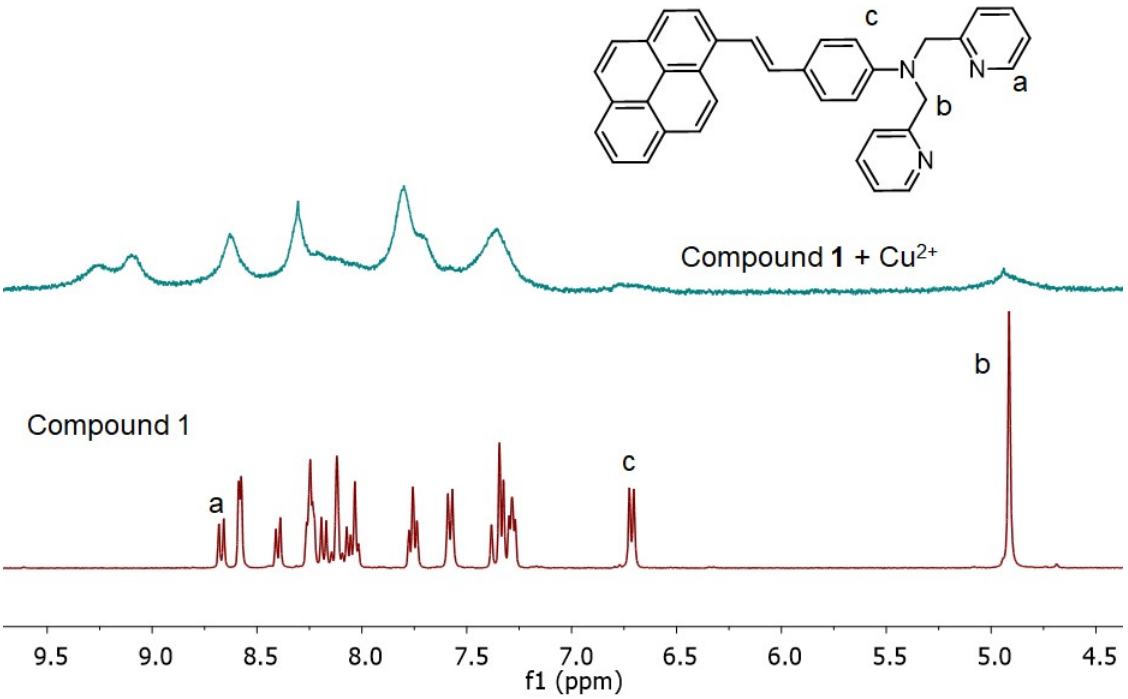


Figure S5. Partial ^{1}H-NMR spectra of **1** (5 mM) upon addition of Cu^{2+} (1:1) in DMSO-d_6 medium (the relevant proton peaks are assigned).

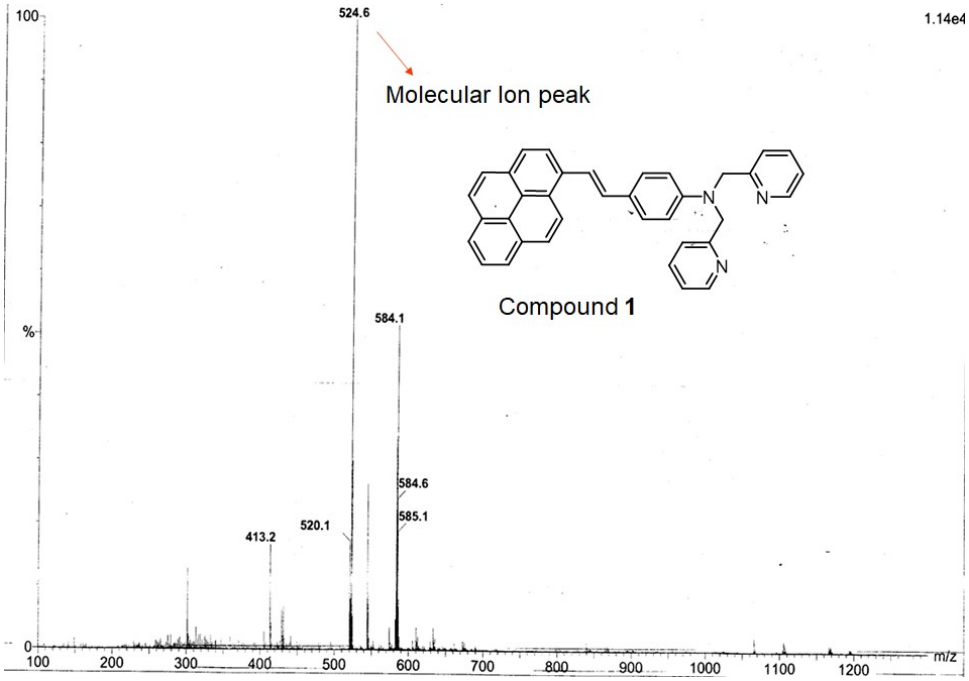
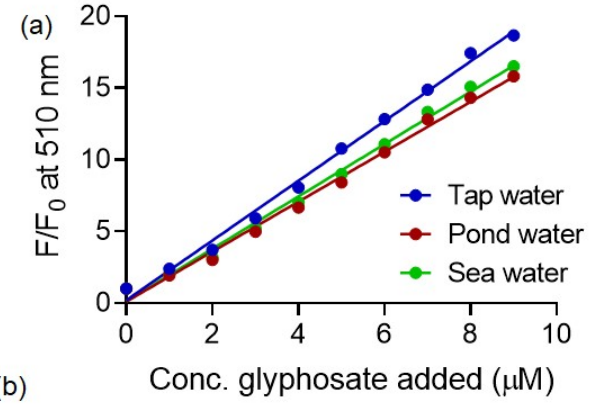
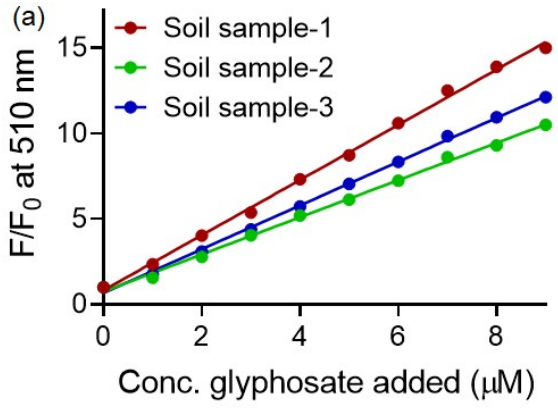


Figure S6. ESI-MS mass spectra of **1**.Cu^{2+} upon addition of glyphosate.



Systems	LOD (in nM)	% Recovery values	% RSD values
Tap water	1.5	98.5 – 102.1	1.8 – 2.5
Pond water	2.1	97.4 – 102.9	2.1 – 3.2
Sea water	3.2	97.2 – 104.3	1.9 – 3.5

Figure S7. (a) Change in fluorescence intensity of **1.**Cu^{2+} (10 μM, 1:1, λex = 350 nm) upon addition of glyphosate (0 – 12 μM) in different natural water samples. (b) Detection limit, percentage recovery and RSD values for glyphosate estimation in different natural water samples.



(b)

Systems	LOD (in nM)	% Recovery values	% RSD values
Soil-1	1.8	97.7 – 103.1	2.1 – 3.3
Soil-2	3.5	96.1 – 102.6	1.7 – 2.7
Soil-3	2.8	98.3 – 103.8	1.5 – 2.8

Figure S8. (a) Change in fluorescence intensity of **1.**Cu^{2+} (10 μM, 1:1, λex = 350 nm) upon addition of glyphosate (0 – 12 μM) in different soil samples. (b) Detection limit, percentage recovery and RSD values for glyphosate estimation in different soil samples.

Systems	LOD (in nM)	% Recovery values	% RSD values
Peanut	1.9	96.3 – 103.2	2.2 – 3.8
Coriander	2.6	97.5 – 104.6	2.5 – 4.2
Rice	2.2	95.2 – 102.5	2.0 – 3.8
Mung Bean	3.4	97.8 – 103.1	1.8 – 2.6
Soy Bean	3.0	96.0 – 102.3	1.7 – 2.8
Maize	4.8	95.5 – 103.8	2.3 – 3.6
Cumin	3.5	94.8 – 102.2	2.6 – 3.8
Mustard	4.5	95.2 – 103.4	2.5 – 3.7
Cloves	5.8	97.6 – 102.5	2.8 – 3.9

Table S1. Detection limit, percentage recovery and RSD values for glyphosate estimation in aqueous extracts of different agricultural crop samples.

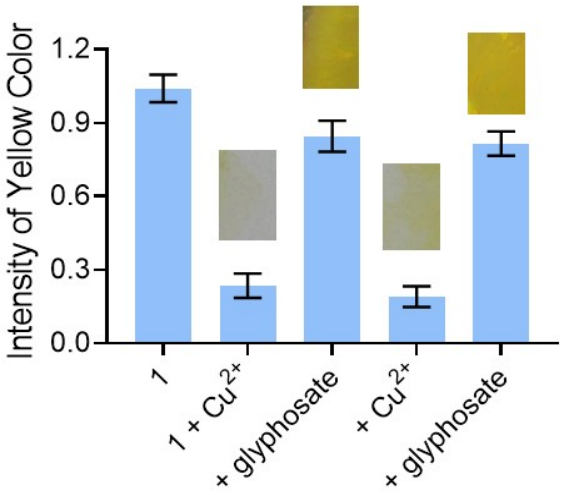


Figure S9. Change color of the paper strips coated by **1** (0.5 mM) upon sequential addition of Cu^{2+} and glyphosate.