Supporting Information

Rapid and Sensitive Naked Eye Detection of Faecal Pigments Using Their Enhanced Solid-State Green Fluorescence on Zinc Acetate Substrate

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Context

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EXPERIMENTAL SECTION

The steady-state absorbance and fluorescence of FP-Zn(II) crystals were measured using Shimadzu UV-2600, Fluoromax-4, and Aqualog Horiba Jobin-Yvon spectrophotometer with 150 W xenon lamp as the light source. With the integrating sphere attachment of a UV-Visible spectrophotometer, the absorbance of FP-Zn(II) crystals was measured. The fluorescence from the paper strip was measured with the integrating sphere attachment to Aqualog Horiba Jobin-Yvon spectrophotometer. Solid-state emission of FP-Zn(II) crystals was conducted at a 45 ° angle of front face orientation to prevent scattering,. The Horiba Jobin-Yvon FluoroCube TCSPC lifespan device was used to monitor fluorescence decay. Ludox AS40 colloidal silica was used to record the instrument response function (prompt). The deconvolution technique was used to analyse the decay data using IBH (DAS-6) software. All of the lifetime data goodness fit χ^2 values were within the acceptable range ($0.9 \leq \chi^2 \geq 1.4$). The following equation was used to compute the average fluorescence lifetime.

$$\tau_{avg} = \frac{\sum_i B_i \tau_i^2}{\sum_i B_i \tau_i} \quad \text{(Eq. 1)}$$

where 'τ_i' is the fluorescence lifetime and 'B_i' is the pre-exponential factor of the emission of the i^{th} species.

$$\Phi = \frac{k_r}{k_r + k_{nr}} \text{ and } \tau = \frac{1}{k_r + k_{nr}} \quad \text{(Eq. 2)}$$

where 'τ' is the fluorescence lifetime, 'φ' is the quantum yield, 'k_r' is radiative and 'k_{nr}' is the non-radiative decay constant.

The absolute quantum yield was calculated based on the following equation (Eq. 4) with the Horiba software package.

$$\phi = \frac{N_{em}}{N_{ab}} = \frac{\alpha \int \frac{\lambda}{hc} I_{em}\, \lambda(d\lambda)}{\alpha \int \frac{\lambda}{hc} \left[I_{ex}(\lambda) - I_{ex}^{'}(\lambda) \right] d\lambda} \quad \text{(Eq. 3)}$$

Where N_{em} and N_{ab} are the respective numbers of emitted and absorbed photons, the term 'α' is the calibration factor for FP-Zn(II) crystal measurements. The term h, c, I_{em} (λ) are Plank's constant, velocity of light and the intensity of emitted photons at λ (nm). Corresponding I_{ex} (λ), I'_{ex} (λ) are intensities of excited photon beams in the absence and in the presence of the sample, respectively.

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A Nikon Eclipse Ti Fluorescence Microscope was used to photograph the FP-Zn(II) crystals (Nikon, Japan). FTIR spectra were acquired using a JASCO FT/IR-4100 spectrometer. Single-crystal XRD was carried out by Bruker Single-crystal KAPPA APEXII. Powder XRD analysis was performed using Bruker D8 advance powder Xray diffractometer using Cu Kα as the Xray source (λ= 1.54 Å).

MATERIAL AND METHODS

Urobilin hydrochloride and stercobilin hydrochloride were purchased from Genetix biotech, India and Frontier Scientific, USA respectively. Various metal salts used in the experiments were purchased from Sigma Aldrich, Rankem, Fischer Scientific and Alfa aesar India). Commercial humic acid (HA), methylene blue, Protoporphyrin IX, 4- hydroxy-3 nitro coumarin and N, N bis (salicylidene) 1, 2 phenylene diamine (salophen or Schiff base) were purchased from Sigma-Aldrich. Ethylenediaminetetraacetic acid (EDTA) was purchased from Qualigen.

SUPPLEMENTARY FIGURES AND TABLES

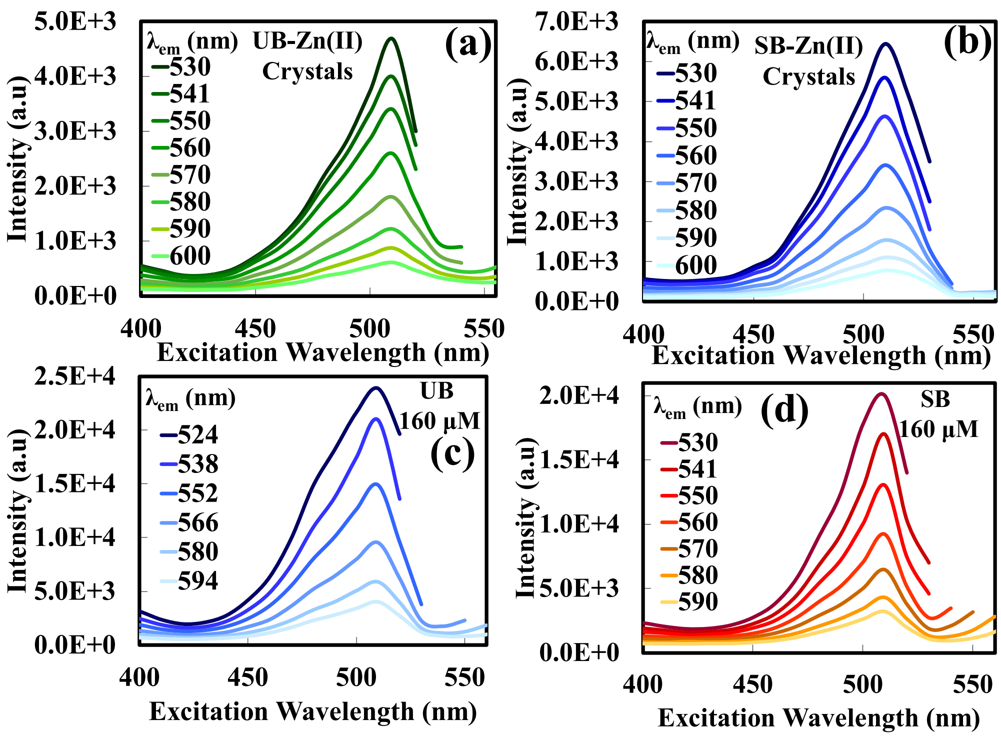

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Figure S1. (a) Excitation spectra of UB-Zn(II) embedded in the surface of zinc acetate crystals with variation of emission wavelength, (b) Excitation spectra of SB-Zn(II) embedded in the surface of zinc acetate crystals with variation of emission wavelength, (c) Excitation spectra of UB drop cast on Zn(II) coated filter paper with variation of emission wavelength and (d) Excitation spectra of SB drop cast on Zn(II) coated filter paper with variation of emission wavelength

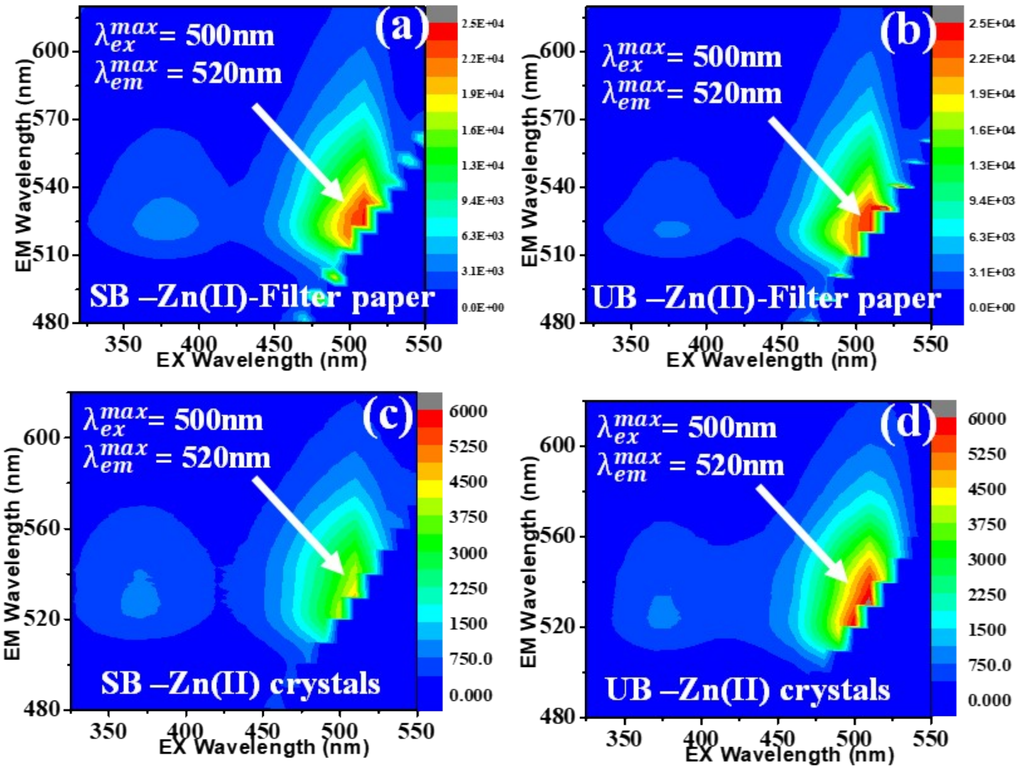

Figure S2. (a)EEMF of SB-Zn(II) filter paper, (b) EEMF of UB-Zn(II) filter paper, (c) EEMF of SB-Zn(II) embedded in the surface of zinc acetate crystals and (d) EEMF of UB-Zn(II) embedded in the surface of zinc acetate crystals

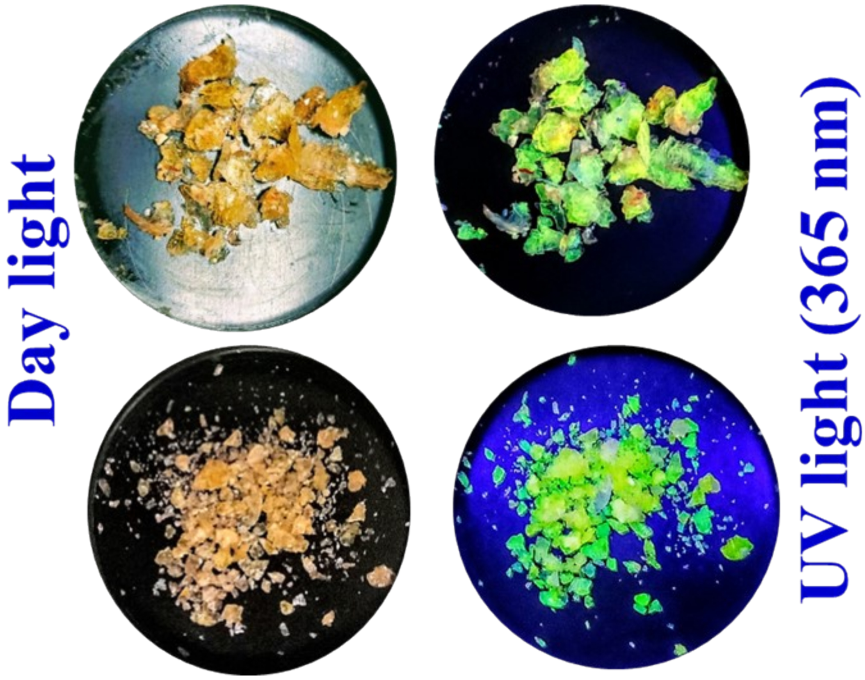

Figure S3. Digital images of FP-Zn(II) embedded in the surface of zinc acetate crystals in day light and in UV 365 nm

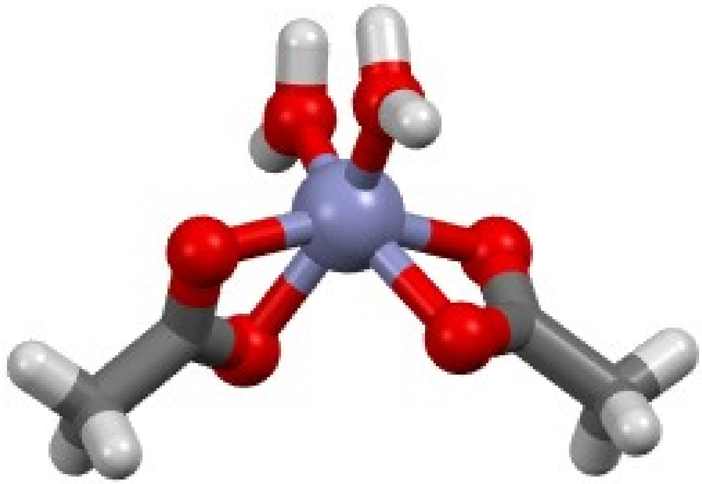

Figure S4. Single-crystal XRD of FP-Zn(II) embedded in the surface of zinc acetate crystals indicates the presence of only zinc acetate dihydrate salt crystal

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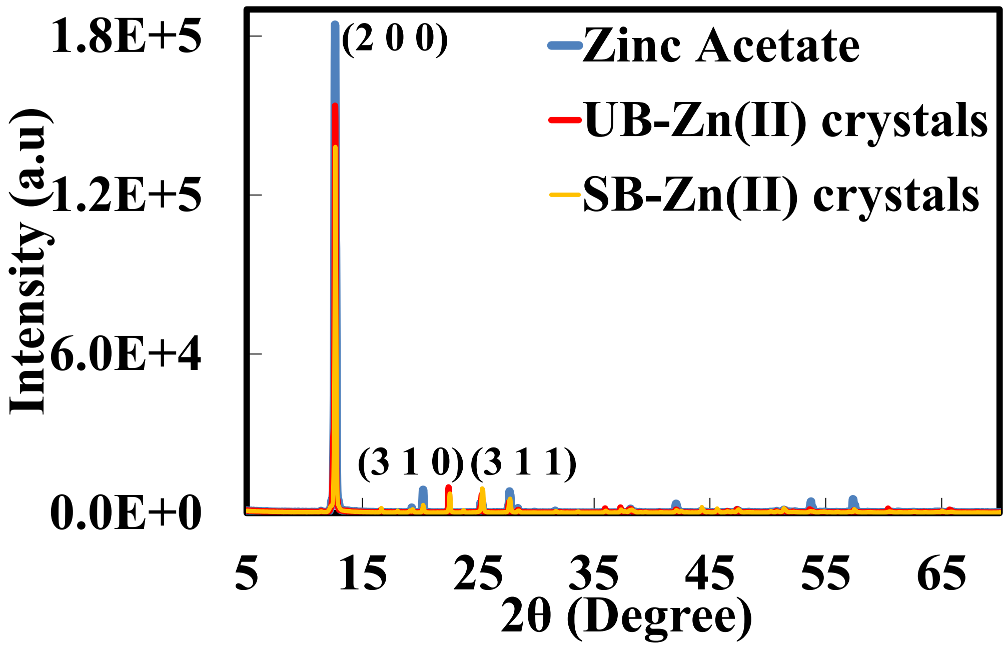

Figure S5 Powder XRD of FP-Zn(II) embedded in the surface of zinc acetate crystals and comparison with zinc acetate dihydrate salt crystals

Table S1. Fluorescence lifetime (τ, ns) of FP-Zn(II) complexes in crystal form and in aqueous media at emission maximum wavelengths. The relative amplitude is given in parentheses. λ_{ex} = 450 nm. The error in the measurement = ±5%. The lifetime data for FP-Zn(II) complexes were taken from *S. Prakash, S. K. Panigrahi, R. P. Dorner, M. Wagner, W. Schmidt and A. K. Mishra, **Chemosphere**, 2021, 265, 129189* for comparison.

Samples	**λ_{ex}= 450 nm**	**Lifetime (ns)**		**τ_{avg} (ns)**	**χ^2**	**k_r(ns^{-1})**	**k_{nr}(ns^{-1})**
	λ_{em} (nm)	**τ_1(β)**	**τ_2(β)**				
SB-Zn(II)Water	511	0.80 (65)	1.82 (35)	1.36	1.20	0.02	0.64
UB-Zn(II)Water	511	1.03 (55)	2.03 (45)	1.64	1.21	0.04	0.62
SB-Zn(II) embedded crystal	520	0.57(27)	3.16 (73)	3.01	1.25	0.03	0.30
UB-Zn(II) embedded crystal	520	1.33 (21)	3.65 (79)	3.45	1.18	0.05	0.21

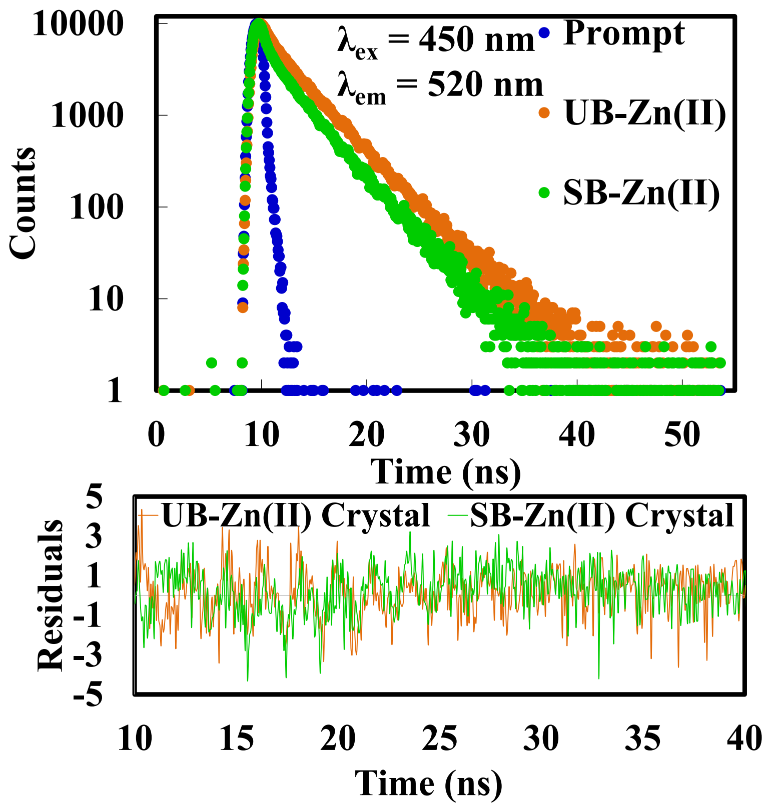

Figure S6. Fluorescence lifetime decay of FP-Zn(II) complexes embedded in the surface of zinc acetate crystals

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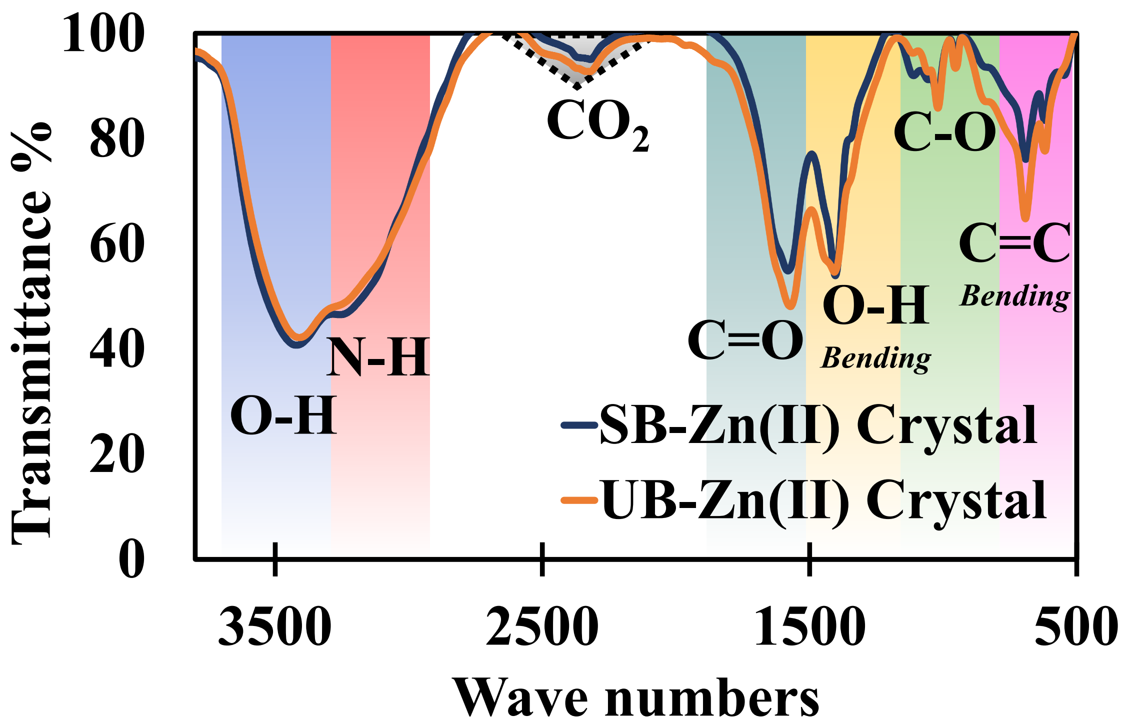

Figure S7. FTIR of FP-Zn(II) embedded in the surface of zinc acetate crystals

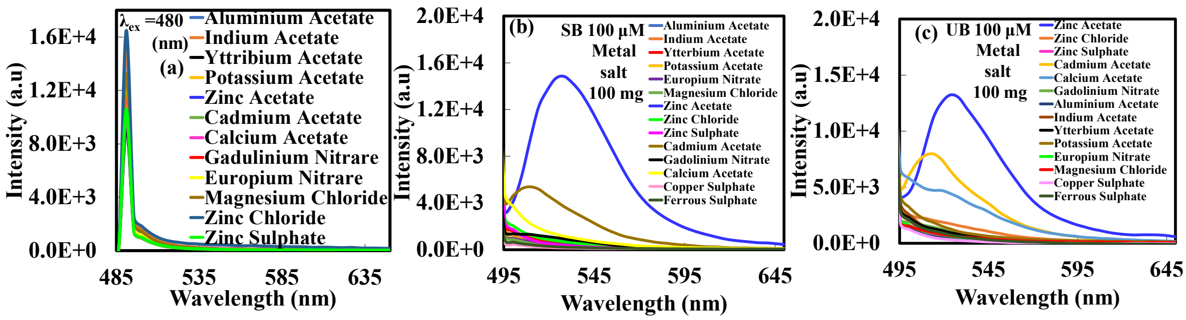

Figure S8. (a) Steady-state emission spectral behaviour of metal salts with excitation 480 nm, (b) Steady-state emission spectral behaviour of various other metal salts drop cast of SB with excitation 480 nm and Steady-state emission spectral behaviour of various other metal salts drop cast of UB with excitation 480 nm

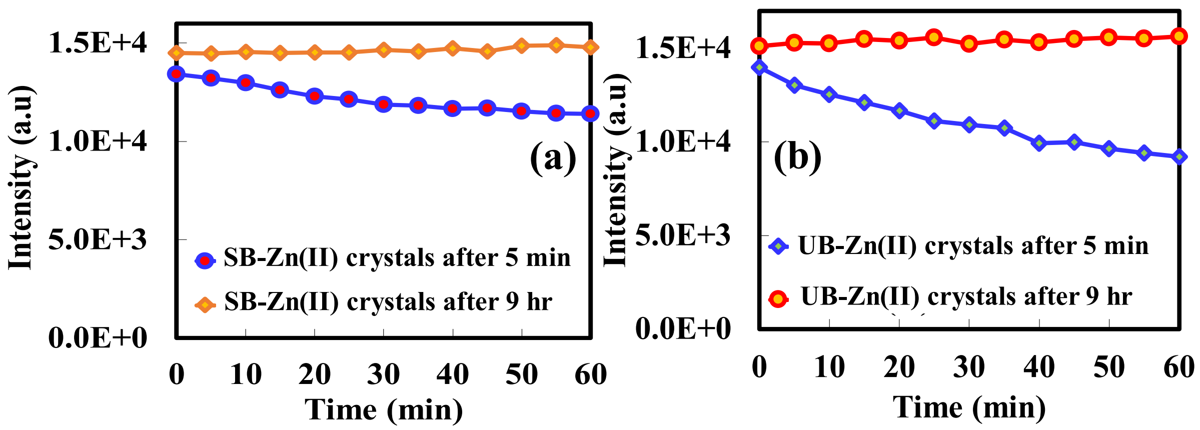

Figure S9. (a) Time-dependent change of solid-state fluorescence of SB-Zn(II) embedded in the surface of zinc acetate crystals and (b) Time-dependent change of solid state fluorescence UB-Zn(II) embedded in the surface of zinc acetate crystals

FP-Zn(II) crystal fluorescence intensity slightly decreased in the inertial period because FPs drop cast was not dried properly. But after complete dry of the sample, the fluorescence intensity of FP-Zn(II) crystals was stable with respect to time.

Stepwise drop casting procedure of FPs on the zinc acetate coated substrate

1) The stock solution of zinc acetate was made by taking 1g dissolved in 2 ml of water and sonicating.

2) The zinc acetate salt-coated filter papers and TLC plates were prepared by taking a square shaped paper and dipping it into zinc acetate stock solution in water. Then the zinc acetate coated substrate was dried properly as shown in Figure S10.

3) The µM concentration range was chosen for FP and the volume of solution taken was 30 µL. The volume of 30 µL was added by micropipette in three steps of 10 µL each, to enable concentrate the sample within a small area on the zinc acetate coated substrate, due to water evaporation.

4) The sample illuminated using a UV torch of 365 nm to check green fluorescence of the FP-Zn(II) complexes on the zinc acetate coated substrate as shown in Figure S10.

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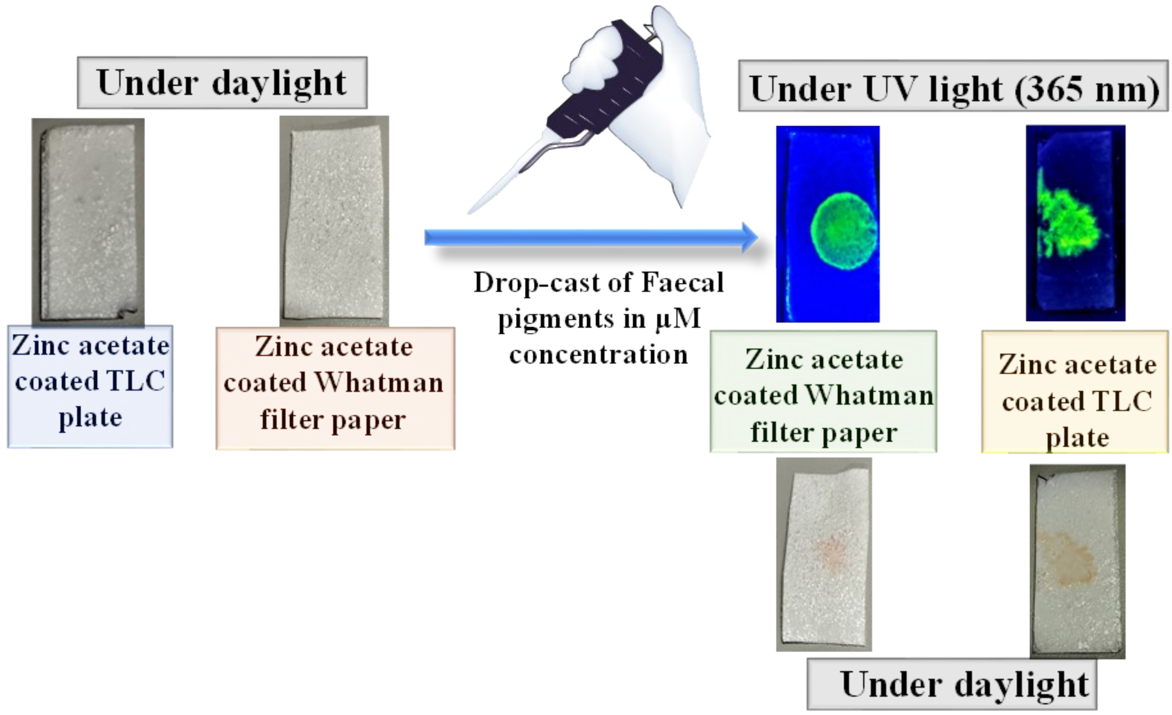

Figure S10. Digital photograph presentation of zinc acetate coated over various substrate (TLC plate and cellulose paper). The bright green fluorescence of FP-Zn(II) can be detected with naked eye under UV 365 nm.

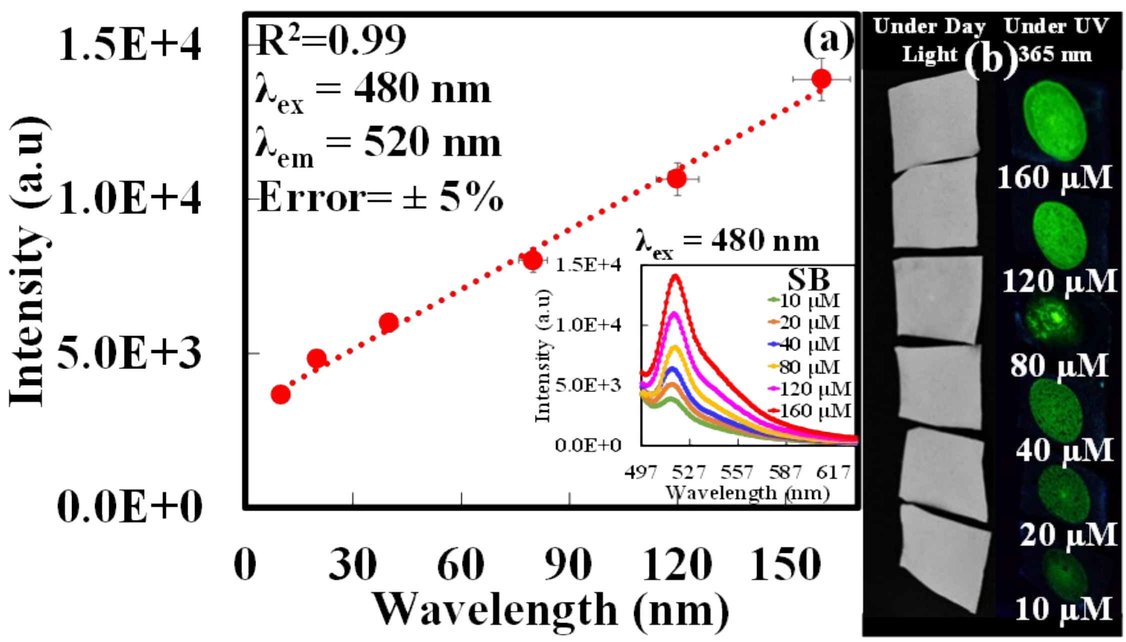

Figure S11. (a) Calibration plot of SB-Zn(II) after drop cast of SB on cellulose paper coated with zinc acetate salt at excitation wavelength 480 nm and emission wavelength 520 nm. The concentration range of SB was taken as 160-10 μM of volume 30 μL.

Table S2. The comparison between the suggested method and previous methods for FPs detection.

Methods for detection of faecal pigments, urobilin (UB) and stercobilin (SB)	**Novelty**	**Detection of faecal pigments in solution state**	**Detection of faecal pigments in solid-state**
Complexation of Zn(II) with urinary UB, significantly enhances the weak fluorescence in absolute alcohol.[1]	Estimation of UB	✓	✗
Detection of UB and SB by high-performance liquid chromatography with fluorimetric detection using Zn(II) complexation.[2]	Estimation of UB and SB up to detection limit (0.2 μg L^{-1})	✓	✗
Solid-phase extraction coupled with high-performance liquid chromatography (HPLC) method.[3]	Estimation of UB 0.1 mg L^{-1}	✓	✗
Hydrophilic-lipophilic balance (HLB) cartridges and combing it with high-performance liquid chromatography-electrospray mass spectrometry (HPLC–ES-MS).[4]	Detection limit of UB 300 ng L^{-1}	✓	✗
UB contained in river sediment was extracted with alkaline buffer solution.[5]	Established UB as an faecal indicator in river water analysis	✓	✗
Integrating cavity to detect UB using Zn(II) complexation method in ethanol medium.[6]	Femtomolar concentrations of UB detection	✓	✗
Extraction of UB, SB with 1-hexanol and complexation with Zn(II).[7]	Picomolar concentrations of UB and SB detection	✓	✗
Rapid and sensitive detection of faecal pigments using their enhanced solid-state green fluorescence on zinc acetate solid substrate (current study).	**Rapid, nonhazardous and naked eye detection of UB and SB. Detection limit up to 10 μM under UV 365 nm.**	✗	✓

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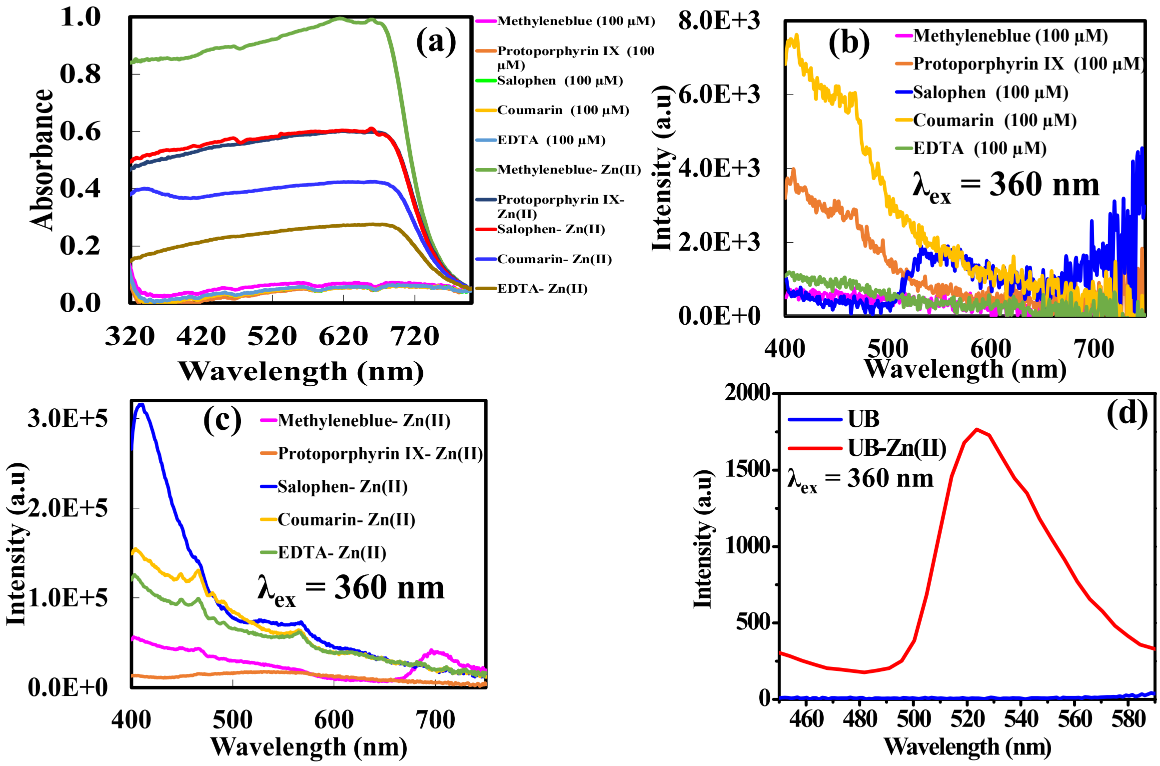

Figure S12. (a) Steady-state absorbance spectra of different organic dyes of 100 µM concentration and their zinc complexes in the solid state, (b) Steady-state emission spectral behaviour of various organic dyes drop cast of 50 µL of 100 µM concentration on quartz slide, (c) Steady-state emission spectral behaviour of various organic dyes drop cast of 50 µL of 100 µM concentration on 100 mg of zinc acetate salt and (d) emission spectra of UB drop cast of 50 µL of 100 µM concentration on 100 mg of zinc acetate salt. Absorbance and emission spectra were recorded after complete drying of the samples in the solid state.

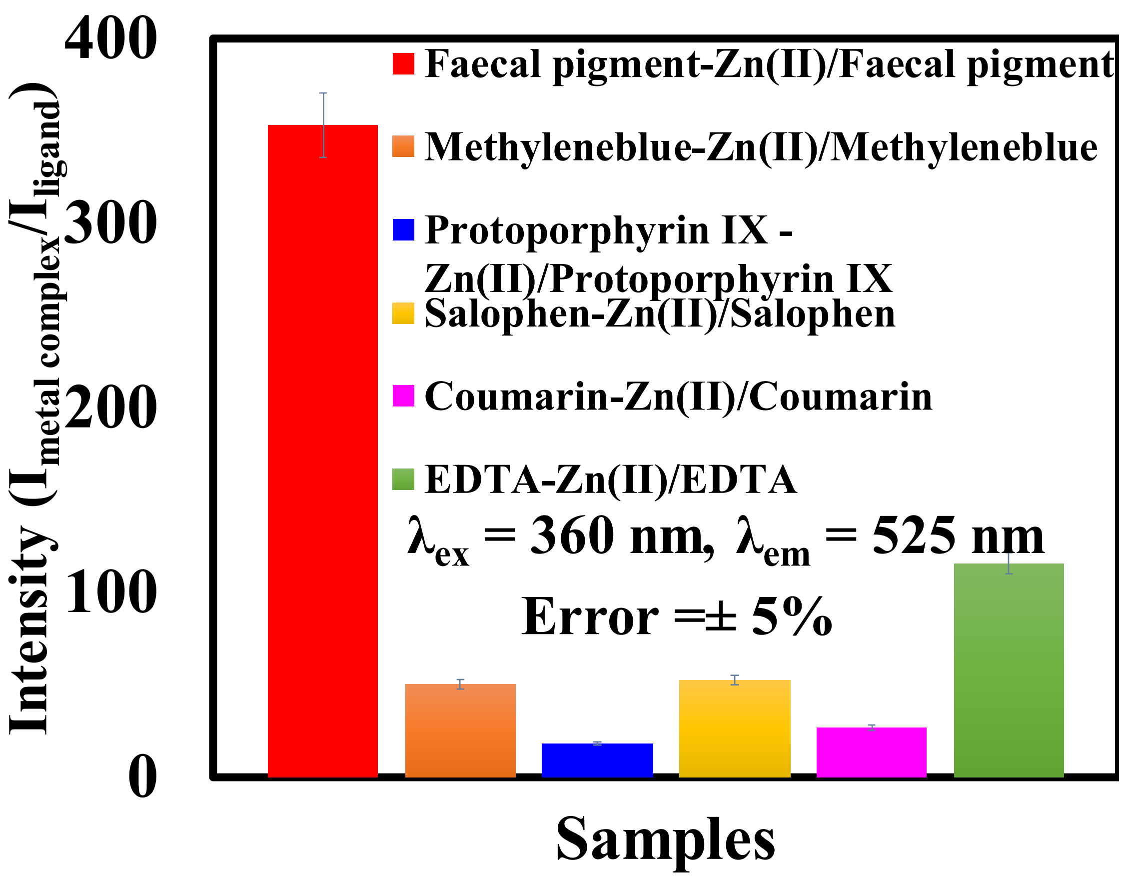

Figure S13. Comparison of fluorescence enhancement intensity at excitation of 360 nm and emission 525 nm.

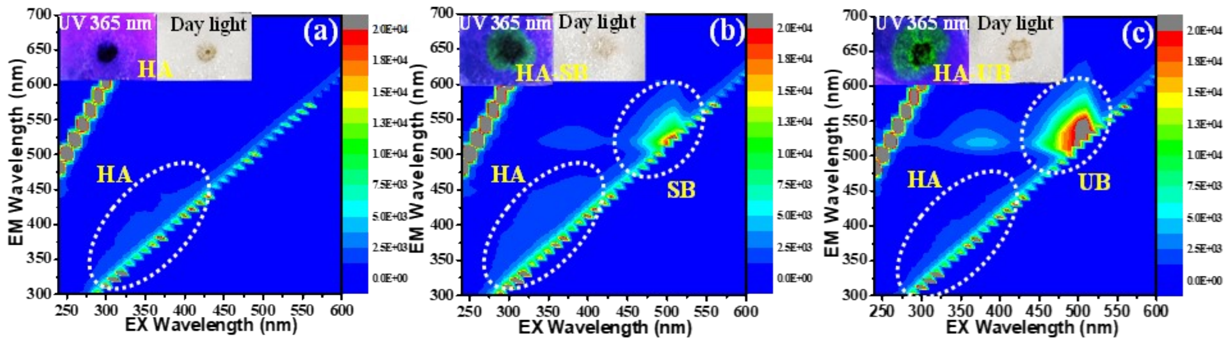

Figure S14. (a) EEMF of humic acid (1 mM) drop cast of 30 µL volume on zinc coated filter paper, (b) EEMF of humic acid (1 mM)-SB (80 µM) drop cast of 30 µL volume on zinc coated filter paper and (c) EEMF of humic acid (1 mM)-UB (80 µM) drop cast of 30 µL volume on zinc coated filter paper

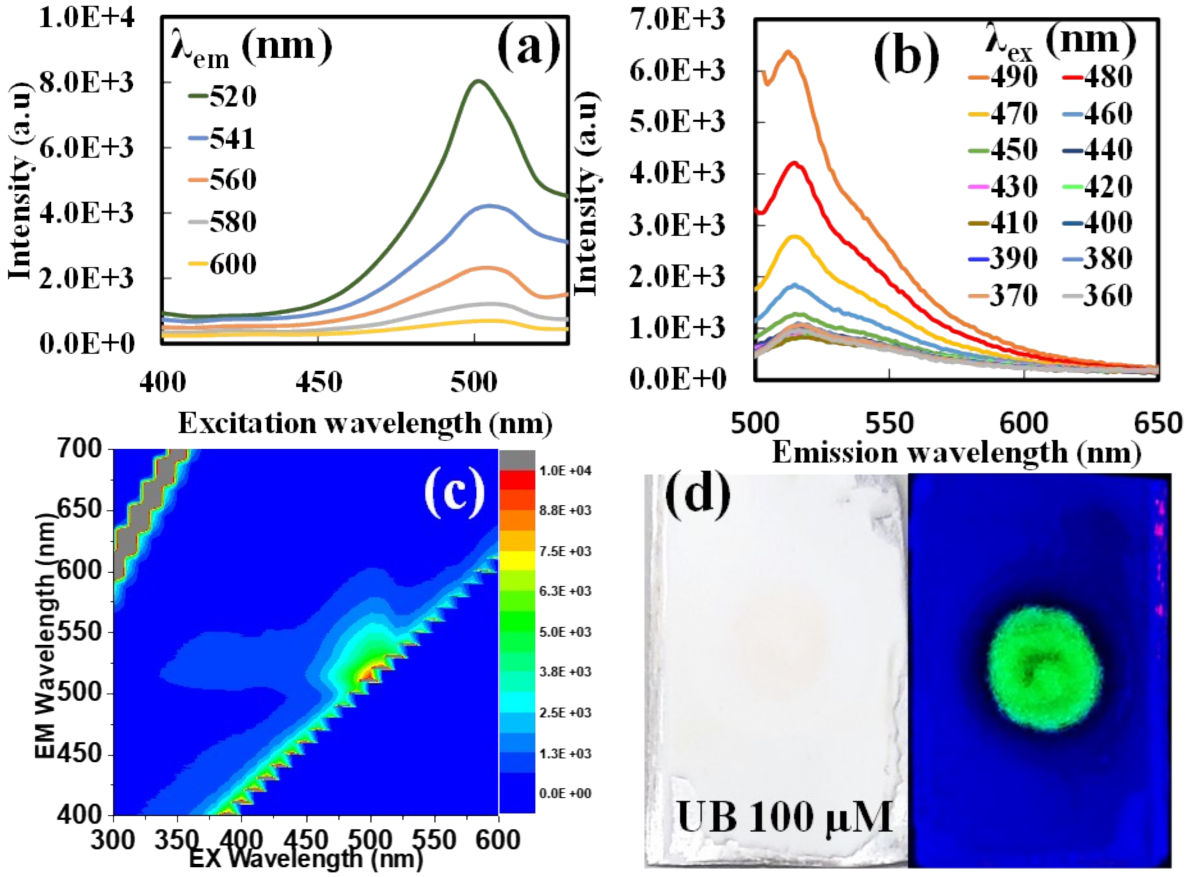

Figure S15. (a) Excitation spectra of UB-Zn(II) drop cast on TLC plate, (b) Emission spectra of UB-Zn(II) drop cast on TLC plate (c) EEMF of UB-Zn(II) drop cast of 30 μL volume on TLC plate and (d) digital photograph of UB-Zn(II) drop cast of 30 μL volume on TLC plate.

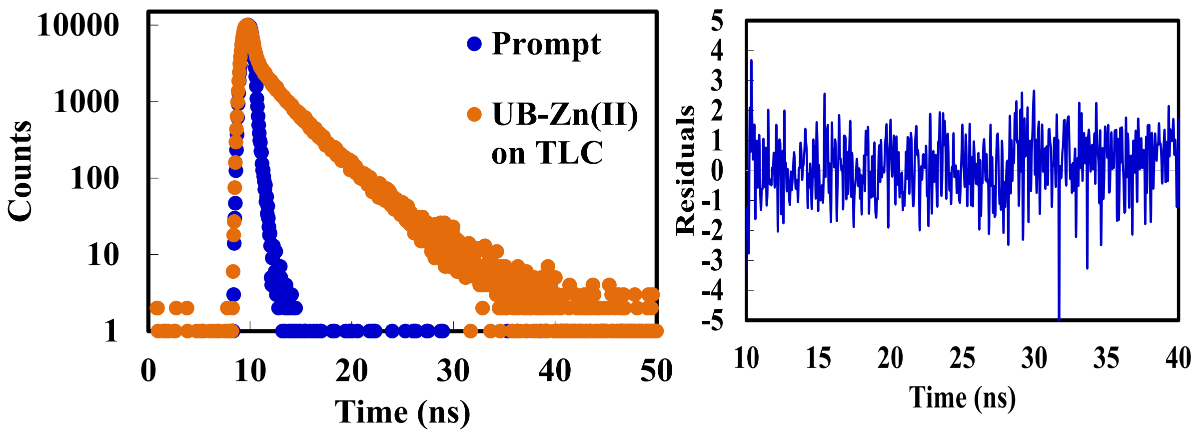

Figure S16. Fluorescence lifetime decay of UB-Zn(II) complexes on the silica coated TLC plate

Table S3. Fluorescence lifetime (τ, ns) of UB-Zn(II) complexes on TLC at emission maximum 520 nm wavelength. The relative amplitude is given in parentheses. λ_{ex} = 450 nm. The error in the measurement = ±5%.

Samples	λ_{ex}= 450 nm λ_{em} (nm)	Lifetime (ns) $\tau_1(\beta)$	$\tau_2(\beta)$	$\tau_3(\beta)$	χ^2
UB-Zn(II) on TLC	520	1.82 (26)	0.27 (38)	3.85 (36)	1.12

Bi- exponential decay fitting was not achieved due to very high scattering on the surface of silica coated TLC plate. Hence the fitting for lifetime was carried out by tri- exponential decay fitting. Lifetime component τ_1, τ_3 and their relative amplitude reasonable match with UB-Zn(II) crystals as shown in Table S1. τ_2 was assigned as a scattering component.

References

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