

Supporting Information

Reduced Graphene Oxide based ‘Turn-On’ Fluorescence Sensor for Highly Reproducible and Sensitive Detection of Small Organic Pollutants

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There are totally 15 pages, with 10 Figures and 4 Tables in the Supporting Information.

Table S1: Comparison of the existing methods of bisphenol A (BPA) detection.

Pollutant Detected	Materials Used	Methods of Detection	Limit of Detection	Range of Response	Selectivity	Reproducibility and stability	Disadvantages	Reference
BPA	C ₈ and C ₁₈ column	HPLC-ED (Coulometry)	$\sim 10^{-9}$ M	$\sim 10^{-10}$ M to $\sim 10^{-7}$ M	Not shown	RSD $\leq 3\%$ Stability not discussed	Complex technique, long pre-processing time	26
BPA	Au-Pd nanoparticle/graphene composite	ED	$\sim 4 \times 10^{-9}$ M	$\sim 10^{-8}$ M to $\sim 10^{-6}$ M	Selective against ions and few Phenolic compounds tested	RSD = 2.6 % Stable for ~ 7 days with increase in RSD to 3.8 %	Complex fabrications, long pre-processing time	27
BPA	Cyclodextrin modified ionic liquid based carbon paste	ED (Voltammetry and impedance)	$\sim 8 \times 10^{-8}$ M	$\sim 10^{-7}$ M to $\sim 10^{-5}$ M	Selective against ions and few Phenolic compounds tested	RSD = $\sim 5\%$ Stable for one month with 90% activity retained	Poor sensitivity, complex fabrications	28
BPA	Graphene-iron oxide composite	ED	$\sim 10^{-8}$ M	$\sim 6 \times 10^{-8}$ M to $\sim 10^{-5}$ M	Selective against ions and few Phenolic compounds tested. (phenol, naphthol can interfere)	RSD = 2.7 % Stable for two weeks	Poor sensitivity, complex fabrications	29
BPA	Small fluorophore derivitized column	HPLC-fluorescence	$\sim 10^{-10}$ M	$\sim 10^{-10}$ M to $\sim 5 \times 10^{-9}$ M	Not shown	RSD = 8.7 % Stability not discussed	Long processing time, complex fabrications and derivitization process	30
Estriol	Graphene	'Turn-On' Fluorescent Sensor	$\sim 10^{-9}$ M	$\sim 10^{-9}$ M to $\sim 10^{-8}$ M	Selective against few biomolecules	Not discussed	Poor reproducibility	31
BPA and other Phenolic compounds	Polymer materials	HRGC-MS	$\sim 2 \times 10^{-14}$ M	Not shown	Not shown	Not discussed	Long processing time, complex fabrications	32
BPA	Standard HPLC columns	LC-MS	$\sim 10^{-9}$ M	Not shown	Not shown	Not discussed	Long processing time, complex fabrications, poor sensitivity	33
BPA	Graphene-dextran-fluorescein composite	'Turn-On' Fluorescent Sensor	$\sim 8 \times 10^{-12}$ M	$\sim 10^{-10}$ M to $\sim 10^{-6}$ M	Selective against other molecules, ions and few Phenolic compounds tested	RSD $\leq 1\%$ Stable for more than six months with increase in RSD to $\sim 1.5\%$		This Work

HPLC: High Performance Liquid Chromatography**ED:** Electrochemical Detection**LC-MS:** Liquid Chromatography Mass Spectroscopy**HRGC-MS:** High Resolution Gas Chromatography Mass Spectroscopy**RSD:** Relative Standard Deviation

Table S2: Comparison of the existing methods of picric acid (PA) detection.

Pollutant Detected	Materials Used	Methods of Detection	Limit of Detection	Range of Response	Selectivity	Reproducibility and stability	Disadvantages	Reference
Picric Acid	Fluorescent polymer and metalloles	'Turn-Off' Fluorescent Sensor	$\sim 10^{-5}$ M	Not shown	Not shown	Not discussed	Poor sensitivity, complex synthesis	40
Picric Acid	Fluorescent polymer	'Turn-Off' Fluorescent Sensor	$\sim 10^{-5}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity, complex synthesis	42
Picric Acid	Fluorescent polymer	'Turn-Off' Fluorescent Sensor	$\sim 10^{-5}$ M	Not shown	Not shown	Not discussed	Poor sensitivity, complex synthesis	43
Picric Acid	Fluorescent polymer	'Turn-Off' Fluorescent Sensor	$\sim 10^{-5}$ M ($\sim 10^{-12}$ M in TLC strip)	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity, complex synthesis	44
Picric Acid	Eu (III) based metal organic framework (MOF)	'Turn-Off' Fluorescent Sensor	$\sim 10^{-7}$ M	$\sim 10^{-5}$ M to $\sim 10^{-2}$ M	Selective towards other nitro aromatics	Not discussed	Poor sensitivity and reproducibility, complex chemistry	45
Picric Acid	Metallogel based MOF	'Turn-On' Fluorescent and colorimetric sensing	$\sim 10^{-5}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity, complex synthesis	46
Picric Acid	Chitosan	'Turn-Off' Fluorescent Sensor	$\sim 10^{-8}$ M	Not shown	Selective towards other nitro aromatics	Stable for six months. Reproducibility not discussed	Poor sensitivity and reproducibility, complex synthesis	47
Picric Acid	Fluorescent polysiloles	'Turn-Off' Fluorescent Sensor	$\sim 10^{-7}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity and reproducibility	48
Picric Acid	Small fluorophores	'Turn-Off' Fluorescent Sensor	$\sim 10^{-6}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity and reproducibility	49
Picric Acid	Small fluorophores	'Turn-Off' Fluorescent Sensor	$\sim 10^{-14}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor reproducibility, complex chemistry	50
Picric Acid	Small fluorophores	'Turn-Off' Fluorescent Sensor	$\sim 10^{-7}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity and reproducibility	51
Picric Acid	Graphene oxide-methyl cellulose hybrid	'Turn-Off' Fluorescent Sensor	$\sim 10^{-6}$ M	Not shown	Poor Selectivity as tested with other nitro aromatics	Not discussed	Poor sensitivity	52
Picric Acid	Graphene oxide-pyridine hybrid	'Turn-Off' Fluorescent Sensor	$\sim 10^{-6}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity	53
Picric Acid	Carbon nanoparticle	'Turn-Off' Fluorescent Sensor	$\sim 10^{-6}$ M	Not shown	Selective towards other nitro aromatics	Not discussed	Poor sensitivity	55
Picric Acid	Graphene-dextran-fluorescein composite	'Turn-On' Fluorescent Sensor	$\sim 5 \times 10^{-12}$ M	$\sim 10^{-11}$ M to $\sim 10^{-6}$ M	Selective against other molecules, ions and few Phenolic compounds tested	RSD $\leq 1\%$ Stable for more than six months with increase in RSD to $\sim 1.5\%$		This Work

Table S3: Comparison of the existing methods of 1-naphthol (NP) and phenol (PH) detection.

Pollutant Detected	Materials Used	Methods of Detection	Limit of Detection	Range of Response	Selectivity	Reproducibility and stability	Disadvantages	Reference
1-naphthol	Beta-Cyclodextrin	HPLC-fluorescence	$\sim 10^{-9}$ M	Not shown	Not shown although in report it stated as selective	Not discussed	Long processing time, complex fabrication	17
1-naphthol	C ₁₈ column	LC-fluorescence	$\sim 10^{-9}$ M	$\sim 7 \times 10^{-8}$ M to $\sim 3 \times 10^{-6}$ M	Not shown	RSD $\leq 5\%$ Stability not discussed	Long processing time, complex fabrication	24
1-naphthol	Graphene-dextran-fluorescein composite	'Turn-On' Fluorescent Sensor	$\sim 10^{-11}$ M	$\sim 10^{-10}$ M to $\sim 10^{-6}$ M	Selective against other molecules, ions and few Phenolic compounds tested	RSD $\leq 1\%$ Stable for more than six months with increase in RSD to $\sim 1.5\%$		This Work
Phenol	Filter paper with MBTH	Colorimetric detection via enzymatic reaction	$\sim 10^{-6}$ M	$\sim 10^{-6}$ M to $\sim 10^{-4}$ M	Not shown	RSD $\leq 6\%$ Stable for three weeks with decrease in activity to 37%	Poor sensitivity and costly	34
Phenol	Copper phosphate-enzyme hybrid	Colorimetric detection via enzymatic reaction	$\sim 10^{-6}$ M	$\sim 10^{-6}$ M to $\sim 10^{-4}$ M	Selective against other organic molecules tested	RSD $\leq 6\%$ Stable for two weeks as tested for 10 cycles.	Poor sensitivity, complex derivitization process and costly	35
Phenol	Carbon paste electrode	Voltammetric detection	$\sim 10^{-6}$ M	$\sim 2.5 \times 10^{-6}$ M to $\sim 5 \times 10^{-5}$ M	Selective against other phenolic compounds	Not discussed	Poor sensitivity, complex fabrication process	36
Phenol	BPX-5 column	GC-MS and HPLC fluorescence	Order of microgram	Not shown	Not shown although in report it stated as selective	RSD $\leq 5\%$ Poor robustness of the system	Poor sensitivity, long processing time, complex fabrication	37
Phenol	Graphene-dextran-fluorescein composite	'Turn-On' Fluorescent Sensor	$\sim 7 \times 10^{-12}$ M	$\sim 10^{-11}$ M to $\sim 10^{-6}$ M	Selective against other molecules, ions and few Phenolic compounds tested	RSD $\leq 1\%$ Stable for more than six months with increase in RSD to $\sim 1.5\%$		This Work

HPLC: High Performance Liquid Chromatography

LC: Liquid Chromatography

GC-MS: Gas Chromatography Mass Spectroscopy

Table S4: List of conventional analytical techniques for quantitative detection of organic pollutants under study.

Name of the pollutant	Methods and materials used	Source	Limit of Detection	Applications
BPA	UPLC with PDA or UV detector. C18 column	Methods Development Team, Industrial Hygiene Chemistry Division, OSHA Salt Lake Technical Centre Sandy UT 84070-6406 (United States Department of Labor, Method No. 1018)	0.041 nanogram (ng)	Applied in the sample of workplace air from which BPA is extracted
BPA	LC/Tandem Mass Spectrometry. C8 or C18 column	ASTM COMPASS Designation: D7574 – 16	40 ng /L	Applied in environmental waters
BPA	HPLC with UV or FL detector. C18 column	Sigma Aldrich Food and Beverage analysis	~ 1 µg/mL	Applied in drinking waters
Phenol	GLC with hydrogen flame ionization detector. carbowax 20-M column	ASTM COMPASS Designation: D2580 – 06	~ 120 mg/L	Applied in water samples
Phenol	GC/MS Fused silica capillary column	National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268 Method: 528	~ 0.025 g/L	Applied in drinking waters
Phenol	GC with flame ionization detector	United States Environment Protection Agency (EPA) Method: 604	~ 0.14 µg/L	Applied in industrial waste water
Picric acid and other nitro phenols	HPLC	The National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention Method: S228	~ 0.036 µg/L	Applied in particulate picric acid
Picric acid	HPLC	U.S. Geological Survey, Open File Report No. 79-207, Menlo Park, CA, 1979	NA	Applied in water samples
1-naphthol	HPLC-FL detector with post column derivitization	United States Environment Protection Agency (EPA) Method: 531.2	~ 0.063 µg/L	Applied in water samples

GLC: Gas Liquid Chromatography

UPLC: Ultra Performance Liquid Chromatography

PDA: Photo Diode Array

FL: Fluorescence

Derivation of Binding constant:

One site Binding model Equation:

$$F - F_0 = (F_{\max} - F_0) \times L_0 / (K_D + L_0) \dots\dots\dots(1)$$

Where,

F = Recovered Fluorescence intensity in presence of ligands (organic pollutant)

F₀ = Fluorescence intensity in the absence of ligands (organic pollutant)

L₀ = Equilibrium concentration of the ligand (organic pollutant)

F_{max} = Maximum recovered fluorescence intensity at equilibrium

K_D = Dissociation constant

K_B = Binding constant (= 1/K_D)

Rearranging equation (1),

$$(F_{\max} - F_0)/(F - F_0) = K_D/L_0 + 1$$

$$\rightarrow \{(F_{\max} - F_0)/(F - F_0)\} - 1 = K_D/L_0$$

$$\rightarrow \text{Log} [\{(F_{\max} - F_0)/(F - F_0)\} - 1] = -\text{Log } L_0 + \text{Log } K_D \quad (y = mx + c)$$

F_{max} is obtained by plotting Log L₀ against ΔF (F-F₀)

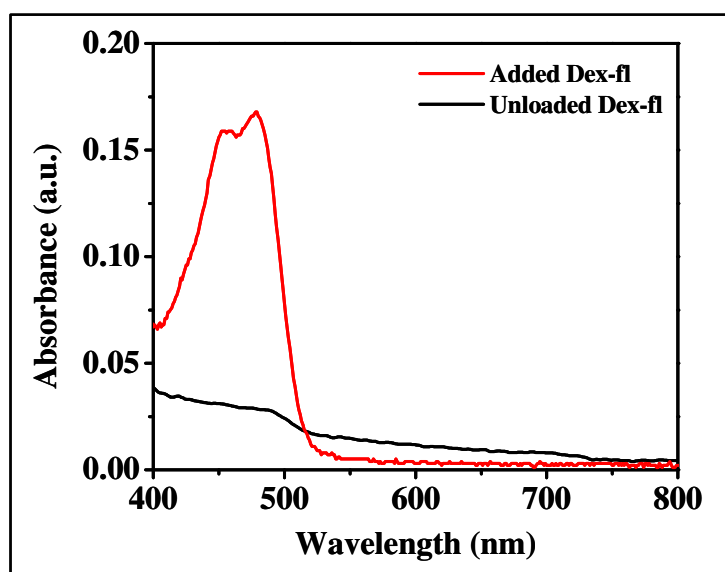


Figure S1: UV-Visible spectroscopy has been performed to calculate the loading of dextran-fluorescein. Here, absorbance of the added dextran-fluorescein and unloaded dextran-fluorescein has been measured after appropriate dilution. From the difference in absorbance at 490 nm of the above absorbances loaded dye amount has been calculated.

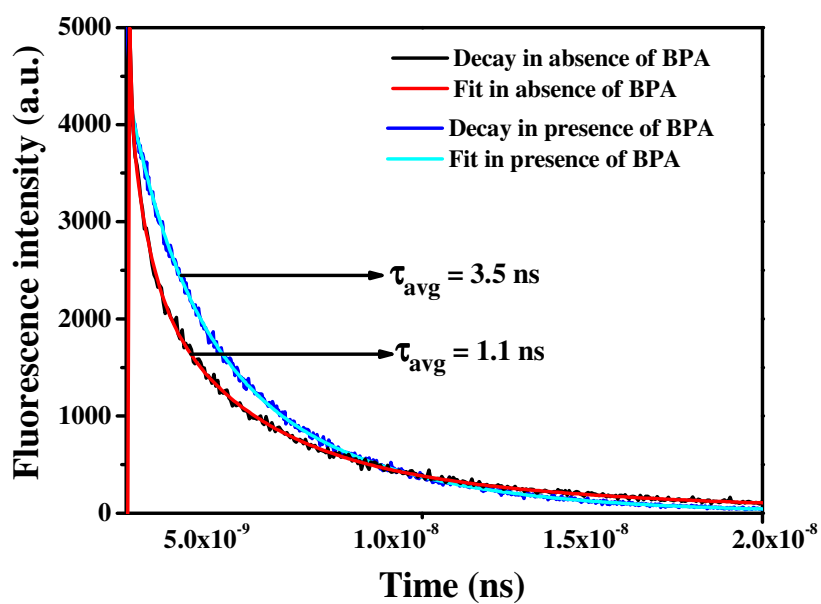


Figure S2: Fluorescence lifetime measurement of the composite, before and after addition of organic pollutant bisphenol A (BPA). Excitation wavelength is 405 nm. in the presence of BPA average lifetime increases due to decrease in non-radiative energy transfer.

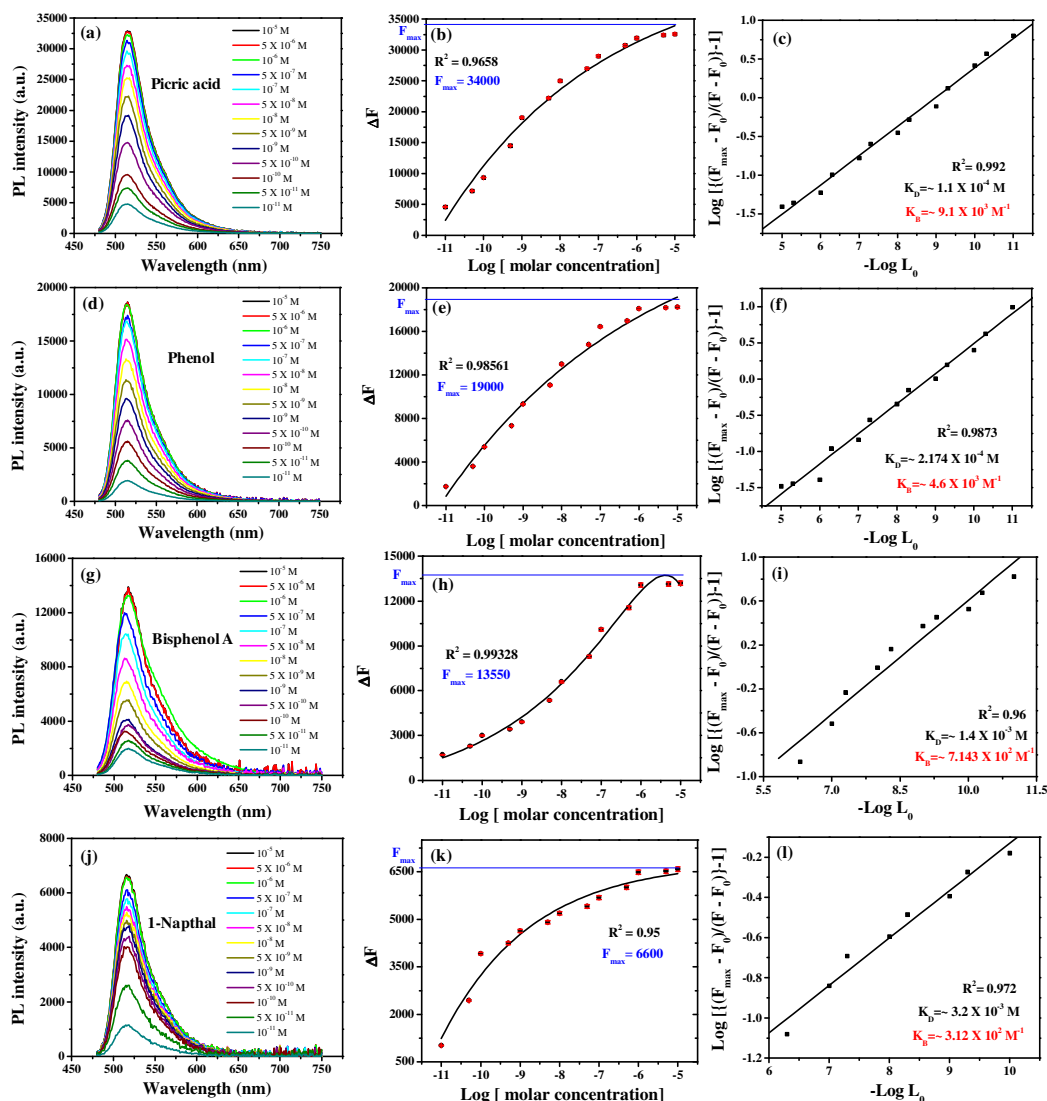


Figure S3: Binding constant measurement of picric acid (a-c), phenol (d-f), bisphenol A (g-i) and 1-naphthal (j-l). Recovered fluorescence is first plotted for varying concentration of pollutant (a, d, g, j), then difference in fluorescence (ΔF) is plotted against Logarithm of molar concentration of pollutant (b, e, h, k) to obtain F_{\max} , finally K_D is obtained from the graph according the above mentioned equation (c, f, i, l). Binding constant K_B is obtained from the value of K_D . From the measurement it has been observed that picric acid has the highest binding constant, while 1-naphthal has the lowest. Error bars are indicated in each data point.

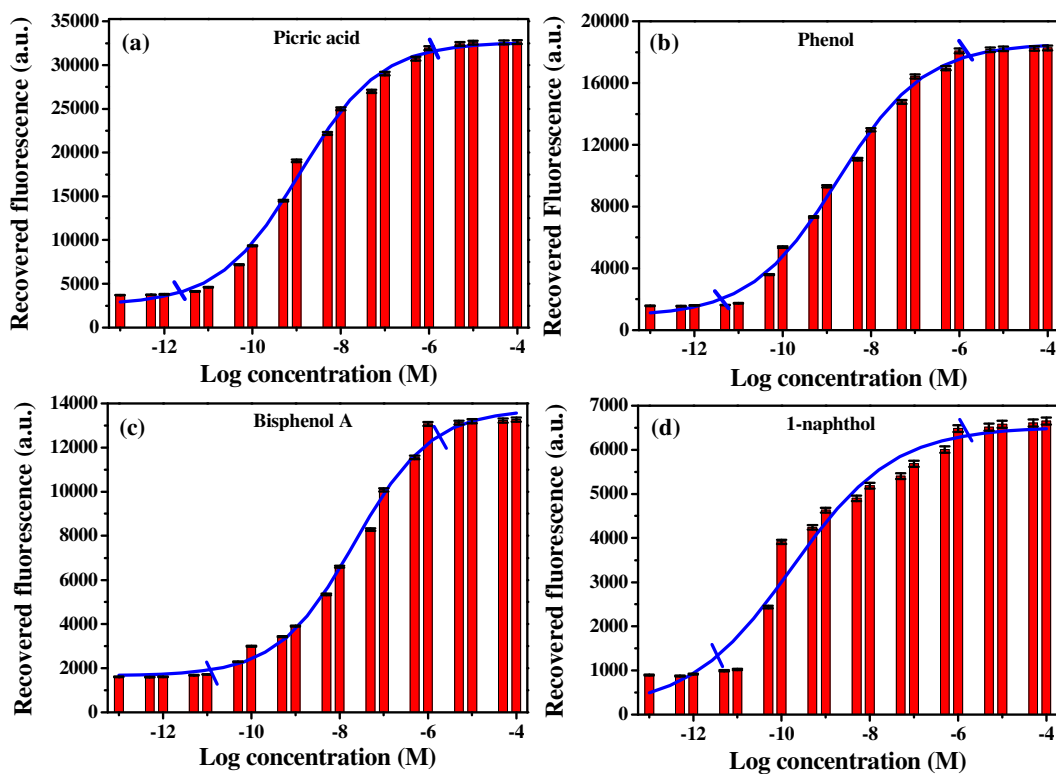


Figure S4: To confirm the range of detection and linearity of response all the organic pollutants are tested between 10^{-4} M to 10^{-13} M. The results obtained clearly indicates that practical detection of the pollutants are only possible in the range of detection. The sigmoidal plot shows that above and below the range of linearity there is no significant change in fluorescence.

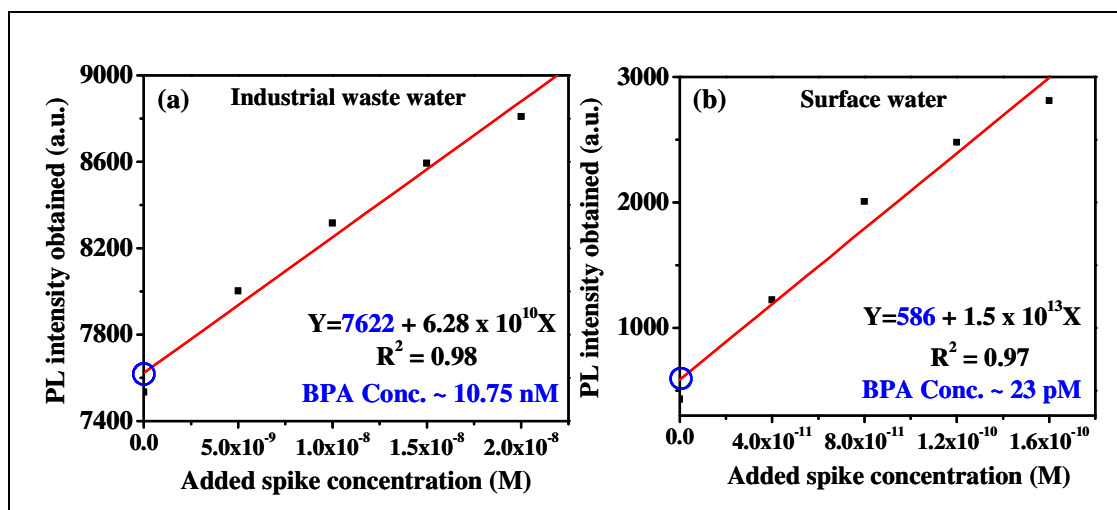


Figure S5: Quantitative estimation of bisphenol A in industrial waste water and surface water by standard addition method. In this method, we have used our detection approach and have found ~10.75 nM bisphenol A in industrial waste water and ~23 pM bisphenol A in surface water.

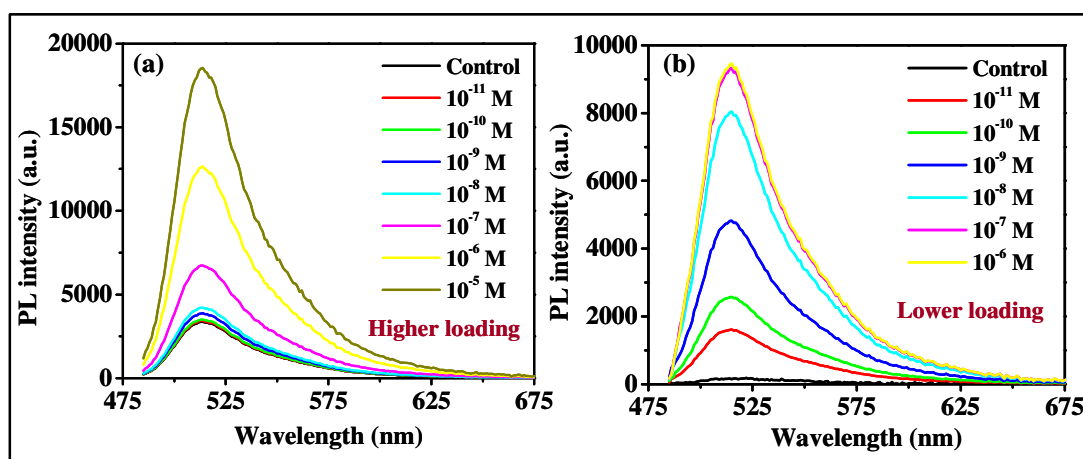


Figure S6: To optimize the loading concentration we have tried both higher (~60 μM / 5 mg composite) and lower loading conditions (~5 μM / 5 mg composite). Bisphenol A has been studied as organic pollutant. In case of higher loading (a) high background signal diminishes its performance especially in the lower range of detection. Lower loading (b) limits its detection sensitivity especially in the upper range of detection.

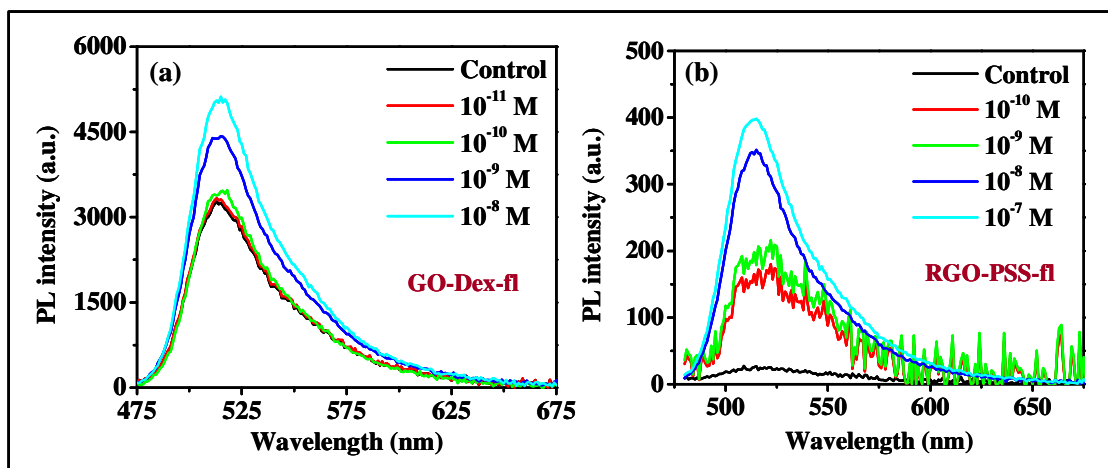


Figure S7: Two control composites have been tested for bisphenol A detection. In graphene oxide-dextran fluorescein composite (GO-Dex-fl) diminished sensitivity has been observed due to high background signal results from inefficient fluorescence quenching of the fluorophore (a). In the other composite (RGO-PSS-fl) also poor sensitivity observed (b). Here the fluorophore strongly binds with the composite surface results in inefficient fluorescence recovery.

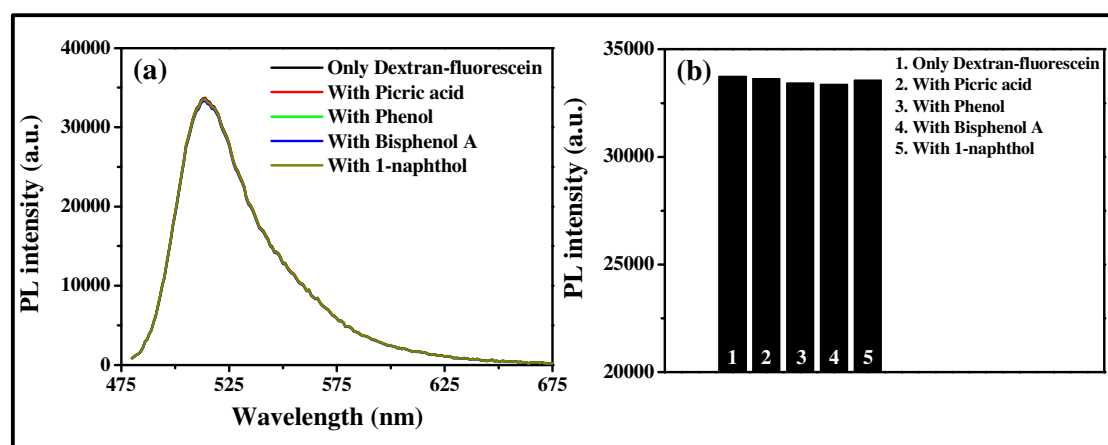


Figure S8: Control experiments have been performed to test the role of graphene oxide (GO) in the detection approach. The experiments were performed in the absence of GO, keeping all other parameters same. All the organic pollutants tested have 10^{-8} M concentration. Without GO, there is no significant quenching of dex-fl was recorded in presence of organic pollutants. PSS also have found negligible effect on the fluorescence of dex-fl. (a) The results are shown in bar diagram for better understanding of the effect of GO in the detection approach.

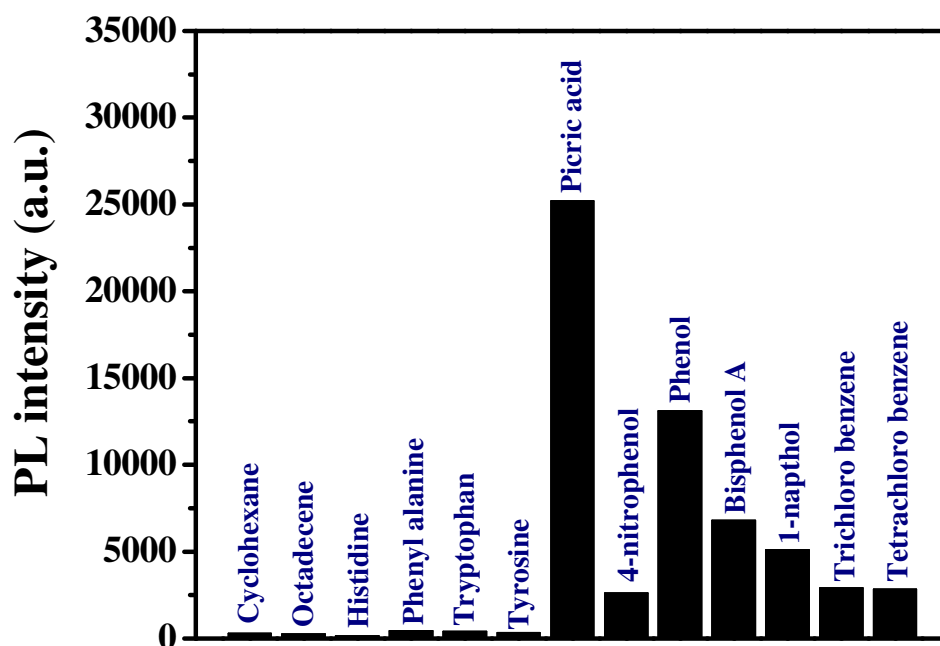


Figure S9: Control experiments have been performed to test the sensitivity of the detection approach. It has been found that common organic solvents or lipophilic amino acids do not respond in this approach. Only the organic pollutants tested respond in this approach. This data also demonstrate different sensitivity of different organic pollutants towards this approach due to varying extent of interaction with the detection probe. Polychloro benzenes (PCBs) and 4-nitrophenol were also tested for interference by this approach since these molecules present in water samples as common organic pollutants. All the samples tested have 10^{-8} M concentration.

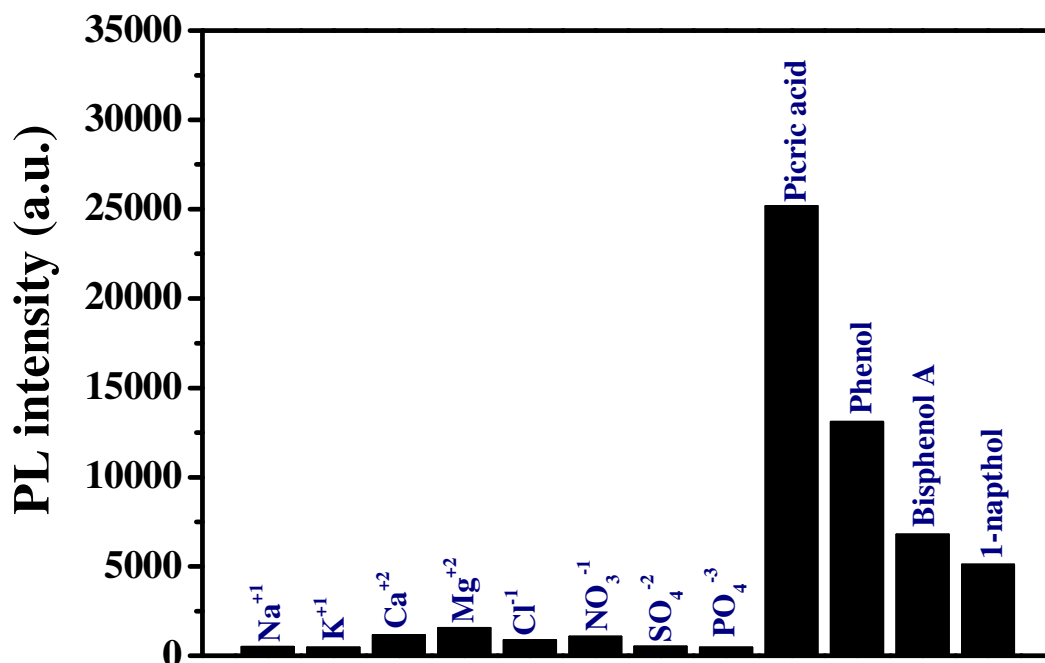


Figure S10: Control experiments have been performed with common ions generally present in water samples. It has been found that these ions do not have any significant interference in the detection approach. All the ions tested have 10^{-7} M concentration.

Calculation for the estimation of dye loading:

5 mg composite sample (RGPD-fl) first dissolved in water (pH ~9.0 with carbonate-bicarbonate buffer). Precipitation redispersion method followed to completely precipitate the composite sample. The supernatant containing unloaded dye was collected and absorbance measured by appropriate dilution (B). Known concentration of dye has been added to RGO-PSS composite (A).

Absorbance of total added dye (A): 0.167 (10 times dilute)

Absorbance of unloaded dye (B): 0.028 (10 times dilute)

Thus, absorbance of loaded dye: 0.139

Molar extinction coefficient of FITC-Dex: $\sim 75,000 \text{ M}^{-1}\text{cm}^{-1}$

(Assuming same as fluorescein dye)

$$A = \epsilon cl$$

$$c = A/\epsilon l$$

$$A = 0.139$$

$$l = 1 \text{ cm}$$

$$\epsilon = 75,000 \text{ M}^{-1}\text{cm}^{-1}$$

$$c = 1.85 \times 10^{-6} \text{ Molar}$$

Since, the loading experiment has been done by diluting 10 times of all the solution, the final concentration of loaded dye is $\sim 20 \mu\text{M}$.

Standard addition method for quantitative bisphenol A detection:

This method is well known and commonly employed in quantitative detections routinely. We have collected industrial waste water, surface water and packaged drinking water from different sources. The sample waters were first centrifuged at

10000 rpm (5 minutes) and the supernatant collected followed by filtering with Whatman 40 filter paper to remove dust and agglomerated impurities. Next, the filtrate was concentrated 5 times the original volume by boiling. Next five different batches with 1 ml sample each was prepared followed by addition of spike solution by varying volumes in those batches. Final spike concentration has been calculated since added spike concentration is known. Next the batches were treated with the composite probe (RGPD-fl) and recovered fluorescence was measured. Obtained fluorescence was then plotted with the final spike concentration. From the intercept in the y axis, we calculate the exact concentration of the bisphenol A in the given sample (Figure S2). Packaged drinking water estimation could not be performed in this approach since it is beyond the linearity range of this approach.