SUPPORTING INFORMATION

A Dual-Function Gold–Zinc Ferrite Nanocomposite Sensor for Colorimetric Detection of Phosphates in Environmental Water

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EXPERIMENTAL SECTION

Materials:

Zinc nitrate ($Zn(NO_3)_2$), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH), chloroauric acid, and sodium citrate were purchased from Sigma-Aldrich (St. Louis, MO, USA) without further purification. All solvents (Sigma-Aldrich) used were of HPLC grade. The following reagents were obtained from the specified suppliers: Sodium chloride (extra pure, Thermo Scientific, Waltham, MA, USA), sodium bromide (ACS reagent ≥99.0%, Sigma-Aldrich), sodium iodide (ACS reagent ≥99.5%, Sigma-Aldrich), sodium nitrate (Laboratory grade, Carolina Biological Supply, Burlington, NC, USA), sodium sulfate (powder anhydrous, 99%, Acros Organics, Geel, Belgium), sodium bicarbonate (ACS reagent ≥99.7%, Sigma-Aldrich), sodium thiocyanate (ACS reagent 98+%, Acros Organics), sodium hexafluorophosphate (98%, Sigma-Aldrich), sodium dichromate dihydrate (ACS reagent ≥99.5%, Sigma-Aldrich), sodium hypochlorite solution (reagent grade 4.99%, Sigma-Aldrich), sodium acetate (ACS reagent ≥99.0%, Sigma-Aldrich), sodium pyrophosphate dibasic (practical grade, Sigma-Aldrich), sodium hexametaphosphate (crystalline, 96%, Sigma-Aldrich), malathion (liquid, ≥98.0%, Supelco, Bellefonte, PA, USA), ethion (Liquid, ≥95.0%, Supelco), and dimethoate (powder, ≥98.0%, Sigma-Aldrich).

Characterization:

Transmission electron microscope (TEM) images were obtained using a HR-TEM (JEOL 2100PLUS) equipped with STEM/EDS capabilities to analyze morphologies, crystallinity and selected area electron diffraction (SAED) patterns of all nanoparticles (NPs). Powder X-ray diffraction (XRD) data was collected using a Rigaku SmartLab diffractometer. For XRD measurements, the nanoparticle samples were dried in air on a Si wafer. The elemental compositions were analyzed by X-ray photoelectron spectroscopy (XPS) on an ESCALAB™ Xi$^+$ spectrometer (Thermo Scientific). Drop-cast samples on Si wafers were used to obtain the micro elemental compositions and were matched with the XPS elemental survey analysis. UV-visible absorbance spectra were collected using a UV−Vis−NIR Spectrophotometer (Agilent, Santa Clara, CA, USA).

Synthesis of $ZnFe_2O_4$ NPs

Zinc nitrate [$Zn(NO_3)_2$, 0.1 M] and ferric chloride ($FeCl_3$, 0.2 M) were individually dissolved in 5 mL of deionized water and stirred until fully dissolved. Next, 0.2 M NaOH was added dropwise to the $FeCl_3$ solution under vigorous stirring, and the pH was adjusted to 11. Subsequently, the $Zn(NO_3)_2$ solution was gently introduced into the mixed solution and the temperature was raised to 75 °C and stirred for 3 hours until a dark red-brown precipitate formed. After cooling the solution to room temperature, the mixture was centrifuged and washed three times with deionized water and ethanol. The resulting product was dried in air for 24 hours and then annealed at 500 °C for 3 hours to obtain the final product of $ZnFe_2O_4$ NPs.

Synthesis of $AuZnFe_2O_4$ nanocomposite

A total of 100 µL of 100 mg/mL chloroauric acid ($H[AuCl_4]$) was introduced into 60 mL of deionized water that was preheated to 90 °C. Subsequently, 1 mg of $ZnFe_2O_4$ NPs were sonicated for 5 minutes and then added to the solution under vigorous stirring. Next, 80 mM of sodium citrate (as a stabilizer) was added dropwise to the mixture. The color of the solution changed from pale yellow to reddish upon the addition of $ZnFe_2O_4$ NPs and then transitioned further to pale red, nearly colorless, dark purple, dark red and finally wine-red with slight turbidity. After stirring for 3 hours, the mixture was cooled to room temperature for subsequent use. The observed color transitions confirm the successful synthesis process of the $AuZnFe_2O_4$ nanocomposite.

Absorbance measurements

The synthesized nanocomposite was titrated with phosphate solutions, and absorbance measurements were performed using UV-visible absorbance spectroscopy. To maintain a pH at 7, 0.5 ml of phosphate-buffer saline (PBS) was added to 30 mL of the nanocomposite solution. After the addition of phosphate solutions into the nanocomposite solution, the mixture was stirred for 2 minutes to achieve homogeneity prior to absorbance measurements. In all cases, 700 µL of the resulting mixture was diluted with 300 µL of deionized water to maintain a consistent dilution factor. The color changes were recorded upon phosphate addition, and a TEM grid was immediately prepared to observe any aggregation. For each phosphate analyte, concentration points were selected based on preliminary titrations to identify the working interval that generated a clear and approximately linear plasmonic response suitable for calibration (Table S2), while avoiding response saturation or rapid sedimentation.

Selectivity study

In order to evaluate the selectivity and interference effects of the nanocomposite, the absorbance measurements and colorimetric responses were also investigated in the presence of 0.3 M solutions of Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, HCO_3^-, F_6^-, and CH_3COO^-. A similar absorbance measurement procedure (described above) was performed for these selectivity tests.

Environmental Water Sample Analysis

Following the same procedure, environmental water samples from four different locations (Lake Okeechobee, EREC Canal, EREC groundwater, and sea water from Lake Worth) were evaluated to assess matric effects by spiking ethion and recording UV-Vis spectra and visual color changes.

Sample Collection and Processing

Water samples were collected from four sites in southern Florida: Lake Okeechobee, the Everglades Research and Education Center (EREC) canal, groundwater wells within EREC fields and coastal seawater at Lake Worth Beach. Immediately after collection, samples were transferred into acid-washed high-density polyethylene (HDPE) containers, placed on ice in insulated coolers and maintained at -4 °C during transport and shipment until analysis was performed to minimize changes in phosphorus speciation. Total phosphorus (TP) concentrations were determined following acid digestion and analysis by inductively coupled plasma–optical emission spectroscopy (ICP-OES, Agilent 5110). Soluble reactive phosphorus (SRP) concentrations were quantified colorimetrically using the ascorbic acid method on a UV–Vis spectrophotometer. All data are available upon request from the corresponding author.

Sampling Locations and Times

- Lake Okeechobee – Belle Glade, FL; 26°42′11″ N, 80°42′57″ W; elevation 20 ft; sampled at 09:40 AM, 21 February 2023.
- EREC Canal – Belle Glade, FL; 26°39′45″ N, 80°37′49″ W; elevation 10 ft; sampled at 10:10 AM, 21 February 2023.
- EREC Groundwater Wells – Belle Glade, FL:
    - Well 1: 26°39′31″ N, 80°37′47″ W; elevation 0 ft; sampled at 10:15 AM, 21 February 2023.
    - Well 2: 26°39′31″ N, 80°37′22″ W; elevation 10 ft; sampled at 10:20 AM, 21 February 2023.
    - Well 3: 26°39′36″ N, 80°37′23″ W; elevation 10 ft; sampled at 10:35 AM, 21 February 2023. Groundwater from these three wells was composited into a single representative sample.
- Seawater (Lake Worth Beach) – Lake Worth, FL; 26°36′52″ N, 80°02′12″ W; elevation 10 ft; sampled at 11:40 AM, 17 February 2023.

Computational methodology

First principal quantum calculations were performed with the periodic spin-polarized density functional theory (DFT) using the Perdew–Burke–Ernzerhof (PBE) exchange-correlation functional, as implemented in the Vienna *ab initio* Simulation Package (VASP).[42–44] The projector augmented wave (PAW) potential and the generalized gradient approximation (GGA) were used to describe the interactions between the valence electrons and ions and the electronic exchange−correlation interaction, respectively.[44–47] The cut-off energy and k-point grids (Monkhorst−Pack) were chosen as 500 eV and 1×1×1 for the structural relaxations of these systems. The convergence criteria for full geometry optimizations included an energy change of less than 10^{-6} eV, and a maximum force of less than 0.02 eV $Å^{-1}$. The unit cell of $ZnFe_2O_4$, including 56 atoms, was obtained from the Materials Project (https://next-gen.materialsproject.org/). Based on this unit cell, a 2×2×1 supercell with 224 atoms was constructed. To prevent interactions between repeated supercells, 20 Å of vacuum space was added along the z-direction. The lattice parameters of the optimized $ZnFe_2O_4$ surface (001) were 16.871×16.871×28.435 Å, with the angles between the two vectors being 90°. Subsequently, eight Au atoms were randomly embedded on the optimized $ZnFe_2O_4$ surface ($AuZnFe_2O_4$) and the entire structure was reoptimized. Finally, phosphates were adsorbed on the $AuZnFe_2O_4$ surface, and the adsorption energy (ΔE_{Ads}) was calculated as:

$$\Delta E_{Ads} = E_{Total} - E_{Surface} - E_{Adsorbate}$$

where E_{Total} is the total energy of adsorbed species on $AuZnFe_2O_4$, and $E_{Surface}$ and $E_{Adsorbate}$ are the corresponding energies of the Au-embedded nanomaterial $ZnFe_2O_4$, and free adsorbates (phosphate molecules), respectively.

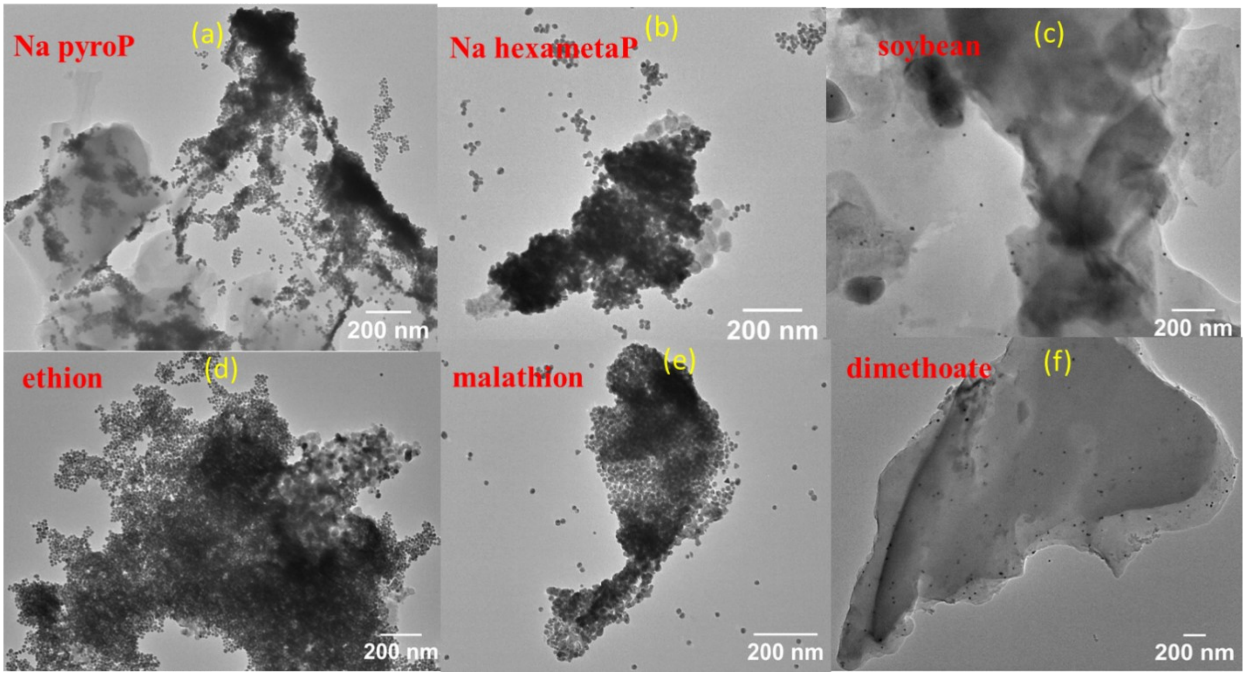

Figure S2. TEM images of AuZnFe$_2$O$_4$ nanocomposite after exposure to (a) sodium pyrophosphate, (b) sodium hexametaphosphate, (c) soybean phospholipids, (d) ethion, (e) malathion, and (f) dimethoate. TEM grids were prepared immediately upon the first visible color change. The corresponding analyte concentrations when the TEM samples were prepared are: Na pyrophosphate 15.84 mM; Na hexametaphosphate 12.26 mM; soybean phospholipid 0.348 µM; ethion 3.44 nM; malathion 0.2051 µM; and dimethoate 9.44 µM (see Table S2 for the corresponding titration point).

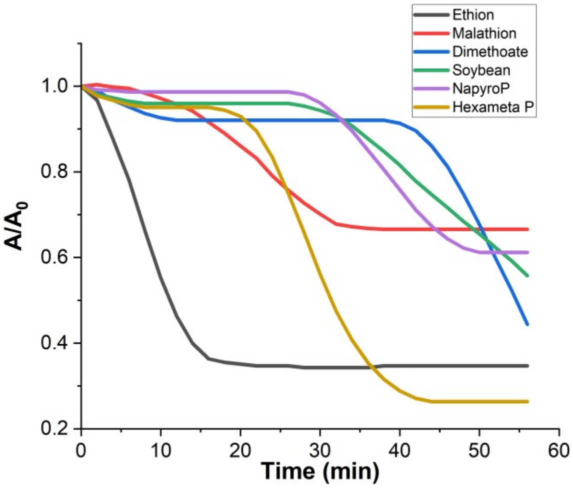

Figure S3. Sedimentation plots of AuZnFe$_2$O$_4$ nanocomposite during the titration of sodium pyrophosphate, sodium hexametaphosphate, soybean phospholipids, ethion, malathion and dimethoate. Calibration titration aliquot ranges for each phosphate are summarized in Tables S2 and S3.

Phosphate Type	**Total Solution Volume (µL) Added to Produce the First Visible Color Change**[‡]	**Equation**	**R^2**	**Linearity Range (Added Volume; µL)**	**Linearity Range (µM)**	**LOD (µM)** [†]
Sodium pyrophosphate	1,700	y = 0.0411x + 0.8738	0.9994	700 – 1,400	6,731 - 13,166	6.29
Sodium hexametaphosphate	1,300	y = 0.1847x + 0.43	0.9422	1000 – 1,400	9,524 – 13,166	1.40
Soybean phospholipid	4,000	y = 0.6887x - 1.8536	0.9900	1000 – 6,000	0.0952 – 0.493	0.37
Ethion	3.5	y = 1.6071x +0.5783	0.9825	0.5 - 2.5	0.000492 - 0.002465	0.16

System	ΔE_{Ads}	R(Au-S)	R(Au-O)	R(Fe-O)
Na pyroP	-2.54	/	2.050/2.066/2.344	1.975/1.919
Na hexametaP	-1.11	/	2.057/ 2.025/ 2.013/ 2.033	2.003/1.938
soybean	-3.69	/	2.066/2.059	/
ethion	-4.04	2.266	/	/
malathion	-2.56	2.263	2.075	/
dimethoate	-2.22	2.278	/	/

Table S1. Adsorption (ΔE_{Ads}, eV) and bond distances (R, in Å) of phosphates on $AuZnFe_2O_4$.

Table S2. Summary of calibration curves of LOD calculations for different phosphates.

Malathion	210	y = 0.5628x - 0.6067	0.9958	35 - 500	0.0344 – 0.484	0.46
Dimethoate	14,000	y = 0.2619x - 0.7639	0.9490	6,000 – 12,500	4.93 – 8.72	0.99

‡: The reported volume is the cumulative aliquot volume (µL) of the specified analyte standard solution (phosphate type) added during titration to a fixed volume of $AuZnFe_2O_4$ nanocomposite dispersion that produced the first visually observable color change.

†: LOD was calculated using the 3σ method (LOD = kσ/S), where k is a numerical factor chosen according to the level of confidence (k=3), σ is the standard deviation of the nanocomposite solution and S is the slope of the calibration curve (σ value was calculated at 0.08614).

Table S3. Cumulative aliquot volume added versus calculated final phosphate concentration in the reaction mixture for each titration series.

Sodium pyrophosphate

Volume (µL)	Final concentration (µM)
200	1,954.40
700	6,730.77
850	8,133.97
925	8,830.55
1,000	9,523.81
1,100	10,443.04
1,200	11,356.47
1,300	12,264.15

Ethion

Volume (µL)	Final concentration (µM)
0.5	0.000492
1.0	0.000984
1.5	0.00148
2.5	0.00246
3.5	0.00344
4.5	0.00442

Sodium hexametaphosphate

Volume (µL)	Final concentration (µM)
200	1,954.40
350	3,403.57
375	3,643.72
400	3,883.50
430	4,170.71

Malathion

Volume (µL)	Final concentration (µM)
35	0.03439
50	0.04910
150	0.14682
350	0.34036
400	0.38835

500	4,838.71
550	5,314.01
600	5,787.78
650	6,260.03
700	6,730.77
750	7,200.00

500	0.48387
650	0.62600
700	0.67308
750	0.72000
800	0.76677
850	0.81340
950	0.90620

Soybean phospholipid

Volume (µL)	Final concentration (µM)
400	0.0388
600	0.0579
800	0.0767
1,000	0.0952
1,200	0.114
2,000	0.185
2,300	0.210
2,600	0.236
3,000	0.267
3,500	0.309
4,000	0.348
4,500	0.386
5,000	0.422
6,000	0.493
8,000	0.623

Dimethoate

Volume (µL)	Final concentration (µM)
1,000	0.952
1,500	1.41
3,000	2.59
4,000	3.44
6,000	4.93
7,500	5.92
10,000	7.41
12,500	8.72
15,000	9.89

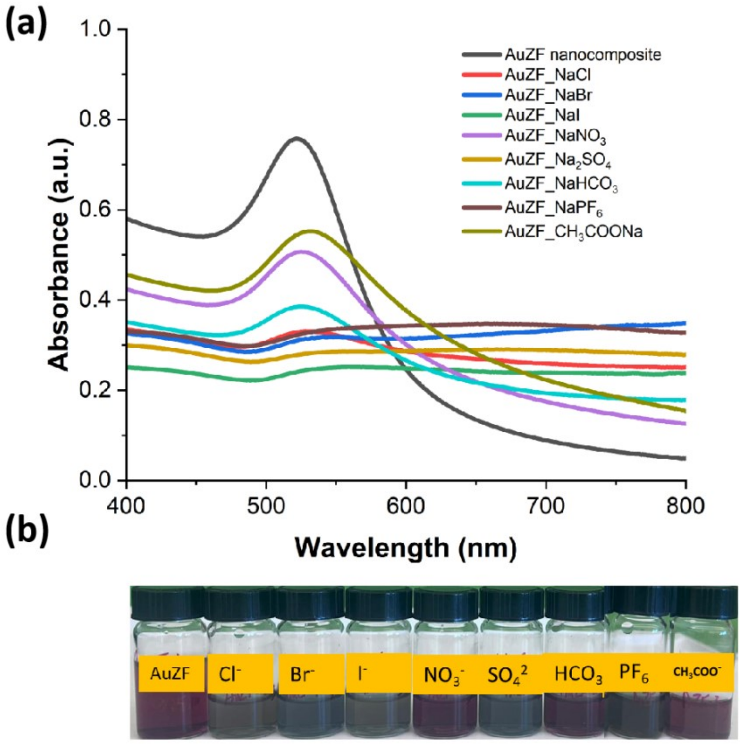

Figure S6. (a) Absorbance measurements of the AuZnFe$_2$O$_4$ nanocomposite in the presence of different interferences and (b) the corresponding color changes. Interferents were tested at 0.30 M (Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, SO$_4^{2-}$, HCO$_3^-$, F$_6^-$, and CH$_3$COO$^-$).