

## Supporting Information for

### **Visual artificial tongue for identification of various metal ions in mixtures and real water samples: a colorimetric sensor array using off-the-shelf dyes**

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## 1. Materials and instruments

### 1.1 Materials

Hexadecyl trimethyl ammonium bromide (CTAB), N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid sodium salt (HEPES), sodium hydroxide (NaOH) were from Sangon Biotech Co. (Shanghai, China). Resazurin was purchased from Aladdin Chemical Co. (Shanghai, China). Dithizone were from Innochem Chemical Co. (Beijing, China) and used as received. Metal compounds at analytical reagent or higher grade were purchased from different suppliers and used as received unless otherwise noted. Mercury standard solution (100 ug/ml), lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ), arsenic standard solution (As(III), 1000 ug/ml), silver nitrate ( $\text{AgNO}_3$ ) were purchased from Aladdin Chemical Co. (Shanghai, China). Cadmium nitrate tetrahydrate ( $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ), iron (III) nitrate nonahydrate ( $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ), chromium (III) nitrate nonahydrate ( $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) were obtained from Energy Chemical Co. (Shanghai, China). Nickel (II) chloride ( $\text{NiCl}_2$ ) was from Aldrich Chemical Co. (Shanghai, China). Cupric sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) was from Tianjin Damao Chemical Co. (Tianjin, China). Zinc chloride ( $\text{ZnCl}_2$ ), manganese chloride ( $\text{MnCl}_2$ ), barium chloride ( $\text{BaCl}_2$ ), aluminium chloride ( $\text{AlCl}_3$ ), cobalt chloride ( $\text{CoCl}_2$ ), stannic chloride ( $\text{SnCl}_2$ ) were obtained from our lab as 10 mM stock solution. The water used in all experiments was prepared in a SG water purification system and displayed a resistivity of  $\geq 18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$  unless otherwise stated. The 96 well-plates (Corning) were obtained from BSKC Co. (Chengdu, China). The dyes stock solutions were prepared in DMSO at concentration of 10 mM and diluted as needed in the experiments. The metal ion stock solutions were prepared in water at concentration of 10 mM and diluted as needed in the experiments.

### 1.2 Instruments

The pH measurements were performed using a Mettler Toledo FE28 pH meter. Colour images were acquired with a flatbed scanner (Epson Perfection V33) in 96-well plates

(Corning 3903). UV-vis absorption spectra were recorded in 96-well plates (Corning 3903) using Tecan M1000 Pro plate reader.

## **2. Experimental methods**

### **2.1 Dye solution preparation**

The first dye solution was prepared by mixing 60  $\mu$ l dithizone stock solution (10 mM in DMSO) with 36  $\mu$ l CTAB solution (50 mM in H<sub>2</sub>O), and then mixed with HEPES buffer (25 mM, pH = 7.4) to 10 mL. The second dye solution was prepared by mixing 60  $\mu$ l dithizone stock solution (10 mM in DMSO) with 4  $\mu$ l CTAB solution (50 mM in H<sub>2</sub>O) and 120  $\mu$ l NaOH solution (1 M in H<sub>2</sub>O), and then mixed with HEPES buffer (25 mM, pH = 7.4) to 10 mL. The third dye solution was prepared by mixing 49  $\mu$ l Resazurin solution (10 mM in DMSO) with water to 10 mL.

### **2.2 Discrimination of metal ions solutions**

The optimized dye solutions (100  $\mu$ l) were loaded into a 96-well plate respectively. And then metal ions solutions (100  $\mu$ l each, experimental group) and water (100  $\mu$ l each, control group) were added. After shaking for 2 min in the plate reader, the “before” (from the control group) and “after” (from the experimental group) color images of the plate were recorded on a flatbed scanner (Epson perfection V33) at 1200 dpi. And then, the UV-vis absorbance spectra were recorded using plate reader (Tecan M1000 Pro) from 350 nm-750 nm with a step size of 5 nm. All the analyses of each sample were conducted in quadruple trials.

### **2.3 Discrimination of metal ions in real samples**

Discrimination of real water samples including tap water, deionized water (prepared in our lab), lake water (located in Tsinghua campus, University Town, Shenzhen, China), artificial lake water (located in Peking campus, University Town, Shenzhen, China), Dasha River water (located in Shenzhen, China) and sea water (located in Shenzhen, China) was taken to test the capability of the developed sensor array. All real samples

were directly used after simply filtered by qualitative filter paper. The analysis procedure was the same as the above description, just by using filtered real water samples instead of prepared metal ions solutions.

### **3. Feature extraction and data analysis**

#### **3.1 UV-vis absorbance spectra data extraction and analysis**

The absorbance values of 81 wavelengths (350-750 nm, every 5 nm) were extracted from the spectral data. And the foldchange of absorbance (F) for each dye at all 81 wavelengths were calculated as follows for quantitative analysis:

$$F = \frac{A_0 - A}{A_0}$$

F represents the foldchange of absorbance, A refers to the absorbance value of the experimental group at one wavelength, and that of control group is  $A_0$ . 61 wavelengths (350 nm-650 nm, every 5 nm) and 55 wavelengths (350-620 nm, every 5 nm) were used for resazurin and dithizone dye solution analysis respectively.

#### **3.2 Color image data extraction and analysis**

The red, green and blue (RGB) values of each well were extracted from the center one-fourth of each well to avoid noise at the edges of the spot using home-made python algorithm with the help of OpenCV packages. For each trail, a color difference map was obtained by subtracting the RGB values of “before” image from the RGB values of “after” image.

#### **3.3 Pattern recognition data analysis**

Pattern recognition methods were performed both on the UV-vis absorbance spectra foldchange values and the obtained  $\Delta R$ ,  $\Delta G$  and  $\Delta B$  values respectively using home-made python algorithm with the help of Scikit-learn scientific analysis packages. Data analyses were performed by Linear discriminant analysis (LDA), and hierarchical clustering analysis (“average” linkage method was used in HCA). The “leave-one-out” cross-validation was used to evaluate the LDA prediction capacity.

## 4. Supplementary figures

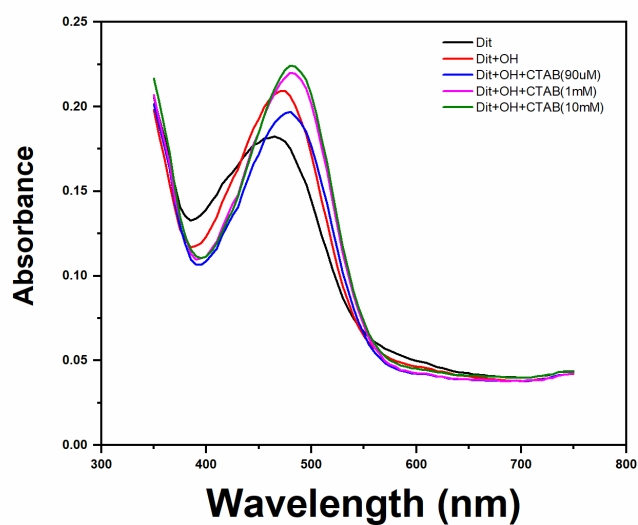


Fig. S1 UV-visible absorption spectra of dithizone (30  $\mu\text{M}$ ), dithizone (30  $\mu\text{M}$ ) with NaOH, and different concentrations of CTAB (0, 90  $\mu\text{M}$ , 1 mM and 10 mM).

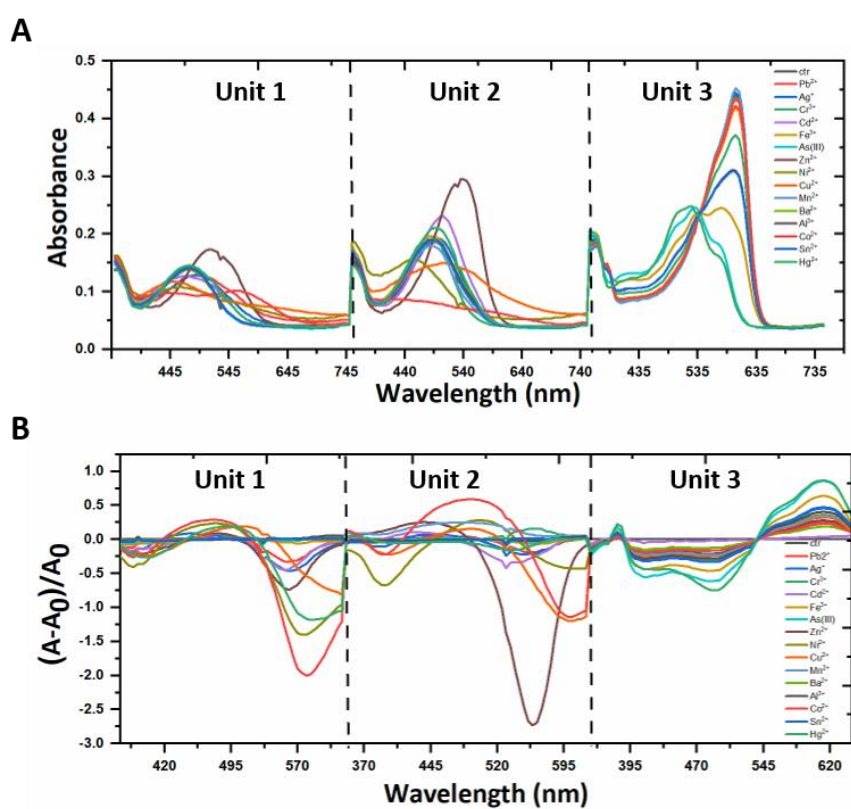


Fig. S2 (A) UV-vis absorption spectra of three units upon addition of 15 different single metal ions at 5  $\mu\text{M}$ . (B) fold change of absorption spectra used for further pattern recognition data analysis.

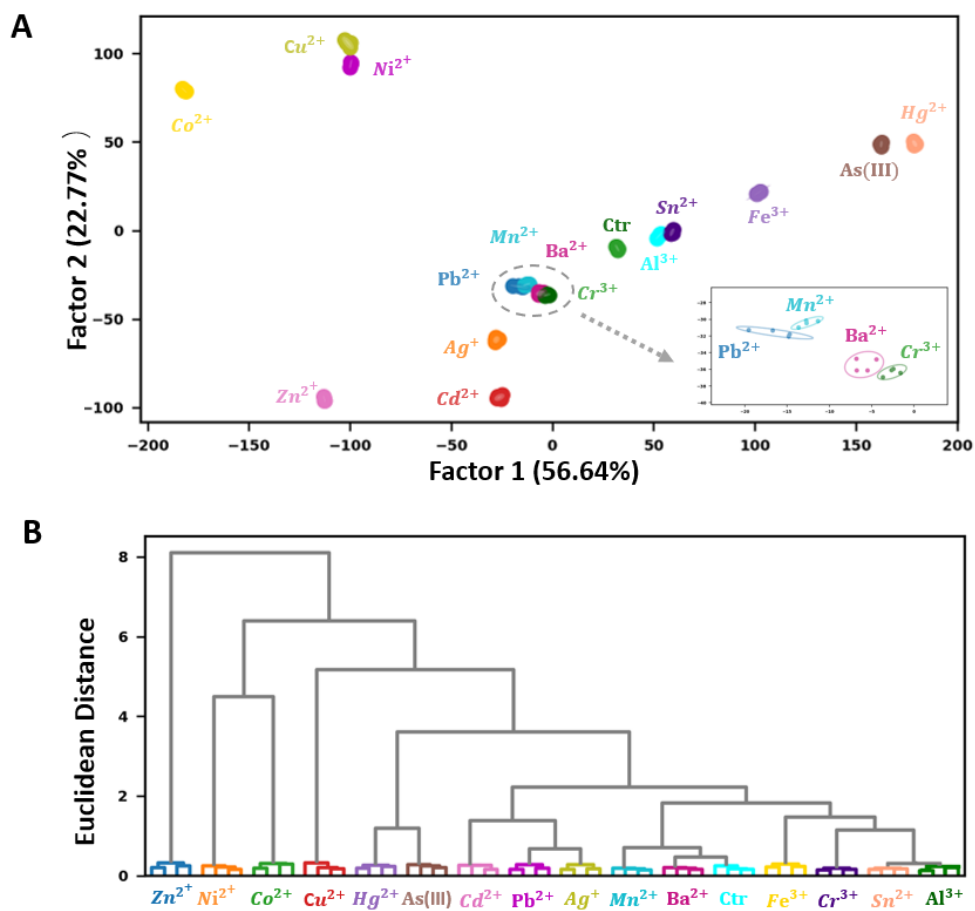


Fig. S3 Performance of sensor array to 15 metal ions single solutions based on UV-vis absorbance spectra changed pattern. (A) LDA 2D plot and (B) HCA dendrogram derived from the UV-vis absorbance spectra change pattern of sensor array upon addition of 15 different single metal ions at 5  $\mu$ M .

Table S1. Identification of unknown metal ion samples at 5  $\mu\text{M}$  using the proposed sensor array.

Samples	Identification	Verification	Samples	Identification	Verification
1	$\text{Pb}^{2+}$	$\text{Ag}^{2+}$	16	$\text{Ni}^{2+}$	$\text{Ni}^{2+}$
2	$\text{Pb}^{2+}$	$\text{Cd}^{2+}$	17	$\text{Cu}^{2+}$	$\text{Cu}^{2+}$
3	$\text{Ag}^{2+}$	$\text{Ag}^{2+}$	18	$\text{Cu}^{2+}$	$\text{Cu}^{2+}$
4	$\text{Ag}^{2+}$	$\text{Ag}^{2+}$	19	$\text{Mn}^{2+}$	$\text{Mn}^{2+}$
5	$\text{Cr}^{3+}$	$\text{Cr}^{3+}$	20	$\text{Mn}^{2+}$	$\text{Mn}^{2+}$
6	$\text{Cr}^{3+}$	$\text{Cr}^{3+}$	21	$\text{Ba}^{2+}$	$\text{Ba}^{2+}$
7	$\text{Cd}^{2+}$	$\text{Cd}^{2+}$	22	$\text{Ba}^{2+}$	$\text{Ba}^{2+}$
8	$\text{Cd}^{2+}$	$\text{Cd}^{2+}$	23	$\text{Al}^{3+}$	$\text{Al}^{3+}$
9	$\text{Fe}^{3+}$	$\text{Fe}^{3+}$	24	$\text{Al}^{3+}$	$\text{Al}^{3+}$
10	$\text{Fe}^{3+}$	$\text{Fe}^{3+}$	25	$\text{Co}^{2+}$	$\text{Co}^{2+}$
11	$\text{As(III)}$	$\text{As(III)}$	26	$\text{Co}^{2+}$	$\text{Co}^{2+}$
12	$\text{As(III)}$	$\text{As(III)}$	27	$\text{Sn}^{2+}$	$\text{Sn}^{2+}$
13	$\text{Zn}^{2+}$	$\text{Zn}^{2+}$	28	$\text{Sn}^{2+}$	$\text{Sn}^{2+}$
14	$\text{Zn}^{2+}$	$\text{Zn}^{2+}$	29	$\text{Hg}^{2+}$	$\text{Hg}^{2+}$
15	$\text{Ni}^{2+}$	$\text{Ni}^{2+}$	30	$\text{Hg}^{2+}$	$\text{Hg}^{2+}$

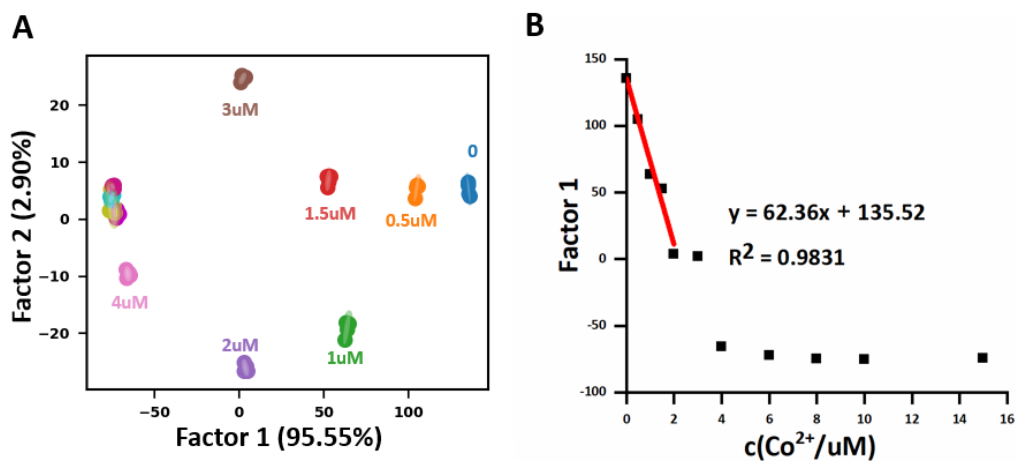


Fig. S4 Discrimination of  $\text{Co}^{2+}$  at different concentrations. (A) LDA plots for the detection of metal ions at different concentrations. (B) The relationship between Factor1 and different concentrations of metal ions.



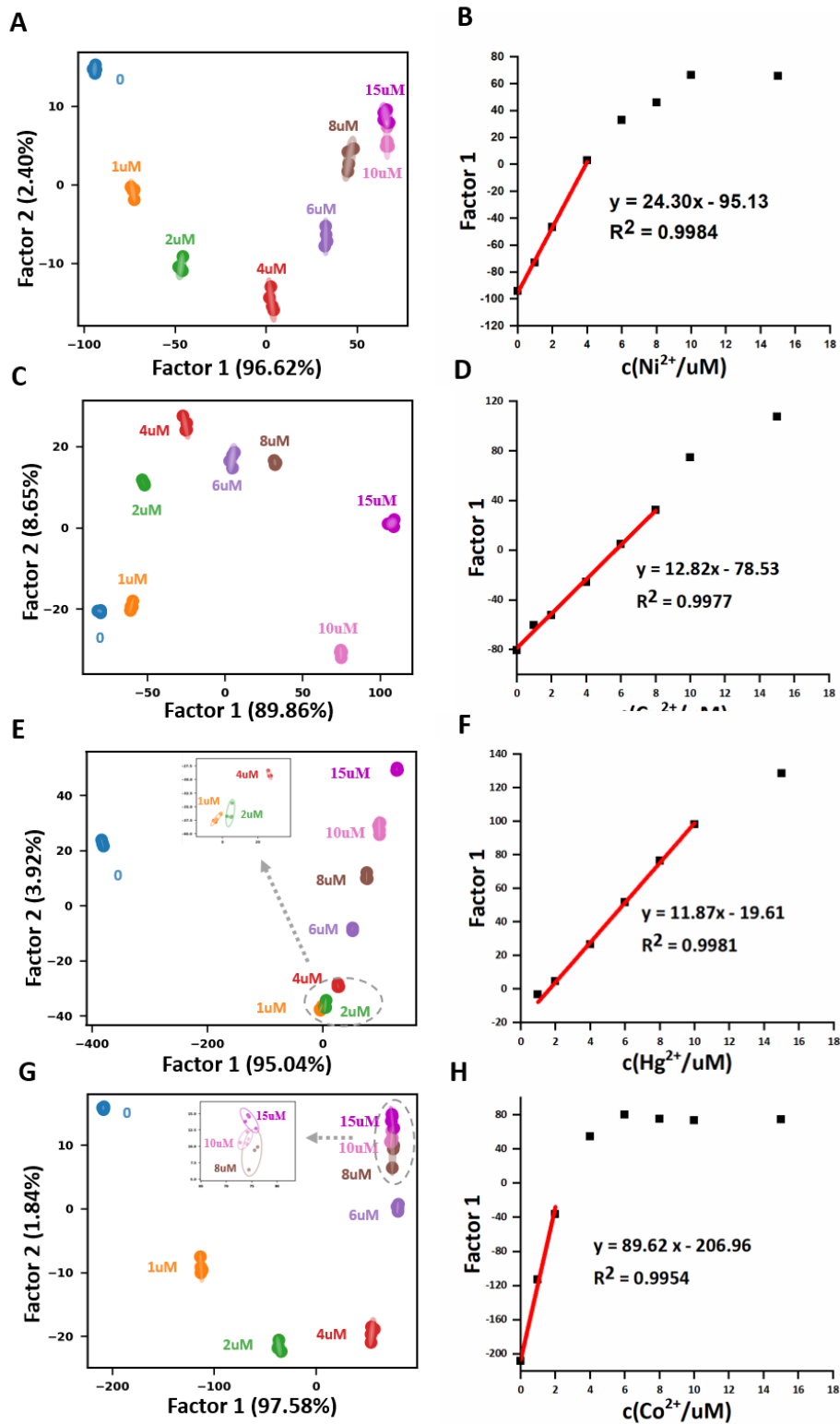


Fig. S5 Discrimination of Ni<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup> and Co<sup>2+</sup> at different concentrations based on the UV-vis absorbance spectra change pattern. (A, C, E, G) LDA plots for the detection of metal ions at different concentrations. (B, D, F, H) The relationship between Factor1 and different concentrations of metal ions.

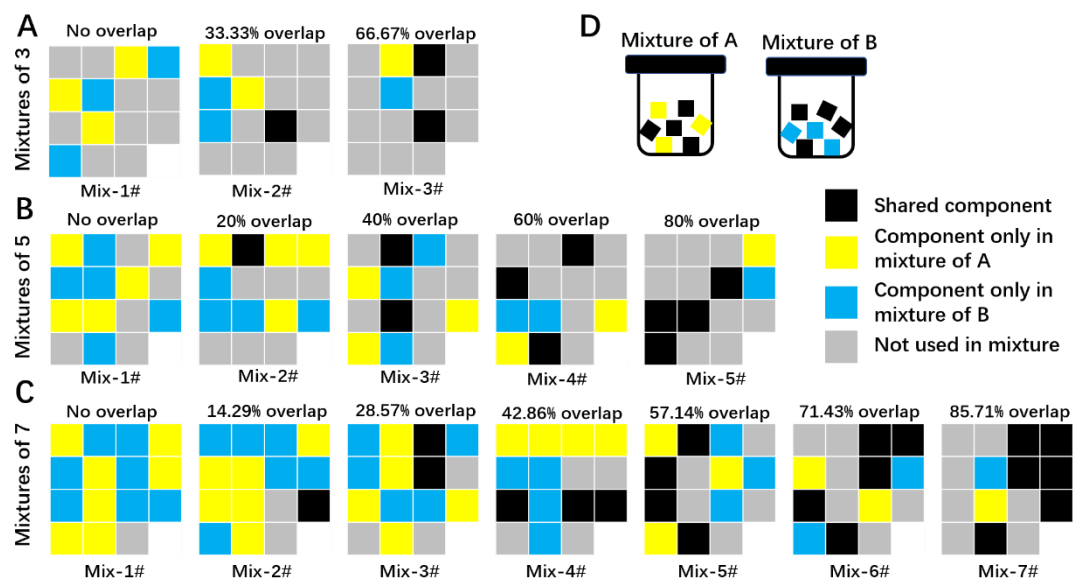


Fig. S6 Illustration of 15 metal ions mixtures pairs used in mixture discrimination. Metal ions mixture pairs consists of exactly (A) 3, (B) 5 and (C) 7 components selected from 15 metal ions with varying overlap rate. (D) Example of one mixture pair.

Table. S2 Details about 15 metal ions mixtures pairs used in mixture discrimination

Mixture	Overlap	Mixture A	Mixture B
3	0	3-0-A (Mix1)	3-0-B (Mix1)
		(Cr <sup>3+</sup> , Fe <sup>3+</sup> , Mn <sup>2+</sup> )	(Cd <sup>2+</sup> , As, Co <sup>2+</sup> )
	33.33%	3-1/3-A (Mix2)	3-1/3-B (Mix2)
		(Pb <sup>2+</sup> , As, Ba <sup>2+</sup> )	(Fe <sup>3+</sup> , Cu <sup>2+</sup> , Ba <sup>2+</sup> )
	66.67%	3-2/3-A (Mix3)	3-2/3-B (Mix3)
		(Ag <sup>+</sup> , Cr <sup>3+</sup> , Ba <sup>2+</sup> )	(Cr <sup>3+</sup> , As, Ba <sup>2+</sup> )
5	0	5-0-A (Mix1)	5-0-B (Mix1)
		(Pb <sup>2+</sup> , Cd <sup>2+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> )	(Ag <sup>+</sup> , Fe <sup>3+</sup> , As, Al <sup>3+</sup> , Sn <sup>2+</sup> )
	20.00%	5-1/5-A (Mix2)	5-1/5-B (Mix3)
		(Pb <sup>2+</sup> , Ag <sup>+</sup> , Cr <sup>3+</sup> , Cd <sup>2+</sup> , Ba <sup>2+</sup> )	(Ag <sup>+</sup> , Fe <sup>3+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> , Al <sup>3+</sup> )
	40.00%	5-2/5-A (Mix3)	5-2/5-B (Mix3)
		(Ag <sup>+</sup> , Fe <sup>3+</sup> , Mn <sup>2+</sup> , Al <sup>3+</sup> , Co <sup>2+</sup> )	(Ag <sup>+</sup> , Cr <sup>3+</sup> , As, Mn <sup>2+</sup> , Sn <sup>2+</sup> )
60.00%	5-3/5-A (Mix4)	5-3/5-B (Mix4)	
	(Cr <sup>3+</sup> , Fe <sup>3+</sup> , Al <sup>3+</sup> , Co <sup>2+</sup> , Sn <sup>2+</sup> )	(Cr <sup>3+</sup> , Fe <sup>3+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> , Sn <sup>2+</sup> )	
80.00%	5-4/5-A (Mix5)	5-4/5-B (Mix5)	
	(Cd <sup>2+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> , Co <sup>2+</sup> )	(Zn <sup>2+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> , Co <sup>2+</sup> )	
7	0	7-0-A (Mix1)	7-0-B (Mix1)
		(Pb <sup>2+</sup> , Cd <sup>2+</sup> , As, Ni <sup>2+</sup> , Mn <sup>2+</sup> , Co <sup>2+</sup> , Sn <sup>2+</sup> )	(Ag <sup>+</sup> , Cr <sup>3+</sup> , Fe <sup>3+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Ba <sup>2+</sup> , Al <sup>3+</sup> )
	14.29%	7-1/7-A (Mix2)	7-1/7-B (Mix2)
		(Cd <sup>2+</sup> , Fe <sup>3+</sup> , As, Cu <sup>2+</sup> , Mn <sup>2+</sup> , Al <sup>3+</sup> , Sn <sup>2+</sup> )	(Pb <sup>2+</sup> , Ag <sup>+</sup> , Cr <sup>3+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> , Al <sup>3+</sup> , Co <sup>2+</sup> )
	28.57%	7-2/7-A (Mix3)	7-2/7-B (Mix3)
		(Ag <sup>+</sup> , Cr <sup>3+</sup> , As, Zn <sup>2+</sup> , Cu <sup>2+</sup> , Al <sup>3+</sup> , Sn <sup>2+</sup> )	(Pb <sup>2+</sup> , Cr <sup>3+</sup> , Cd <sup>2+</sup> , Fe <sup>3+</sup> , Zn <sup>2+</sup> , Mn <sup>2+</sup> , Ba <sup>2+</sup> )
	42.86%	7-3/7-A (Mix4)	7-3/7-B (Mix4)
		(Pb <sup>2+</sup> , Ag <sup>+</sup> , Cr <sup>3+</sup> , Cd <sup>2+</sup> , Cu <sup>2+</sup> , Ba <sup>2+</sup> , Al <sup>3+</sup> )	(Fe <sup>3+</sup> , As, Cu <sup>2+</sup> , Mn <sup>2+</sup> , Ba <sup>2+</sup> , Al <sup>3+</sup> , Sn <sup>2+</sup> )
57.14%	7-4/7-A (Mix5)	7-4/7-B (Mix5)	
	(Pb <sup>2+</sup> , Ag <sup>+</sup> , Fe <sup>3+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Co <sup>2+</sup> , Sn <sup>2+</sup> )	(Ag <sup>+</sup> , Cr <sup>3+</sup> , Fe <sup>3+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Ba <sup>2+</sup> , Sn <sup>2+</sup> )	
71.43%	7-5/7-A (Mix6)	7-5/7-B (Mix6)	
	(Cr <sup>3+</sup> , Cd <sup>2+</sup> , Fe <sup>3+</sup> , Zn <sup>2+</sup> , Cu <sup>2+</sup> , Ba <sup>2+</sup> , Sn <sup>2+</sup> )	(Cr <sup>3+</sup> , Cd <sup>2+</sup> , Cu <sup>2+</sup> , Mn <sup>2+</sup> , Ba <sup>2+</sup> , Co <sup>2+</sup> , Sn <sup>2+</sup> )	
85.71%	7-6/7-A (Mix7)	7-6/7-B (Mix7)	
	(Cr <sup>3+</sup> , Cd <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> , Mn <sup>2+</sup> , Al <sup>3+</sup> , Sn <sup>2+</sup> )	(Cr <sup>3+</sup> , Cd <sup>2+</sup> , As, Zn <sup>2+</sup> , Ni <sup>2+</sup> , Al <sup>3+</sup> , Sn <sup>2+</sup> )	

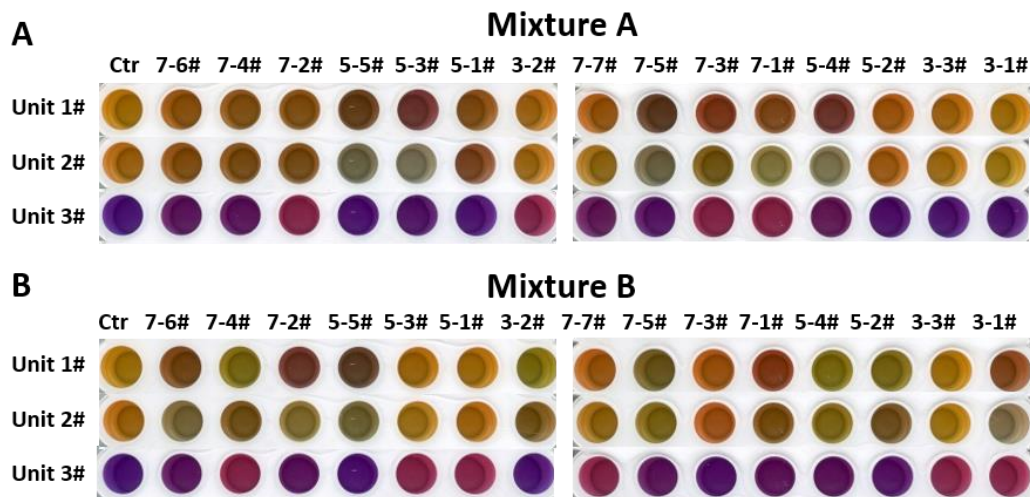


Fig. S7 Colour image of sensor array recorded by flatbed scanner upon addition of 15 different pairs of metal ions mixtures.

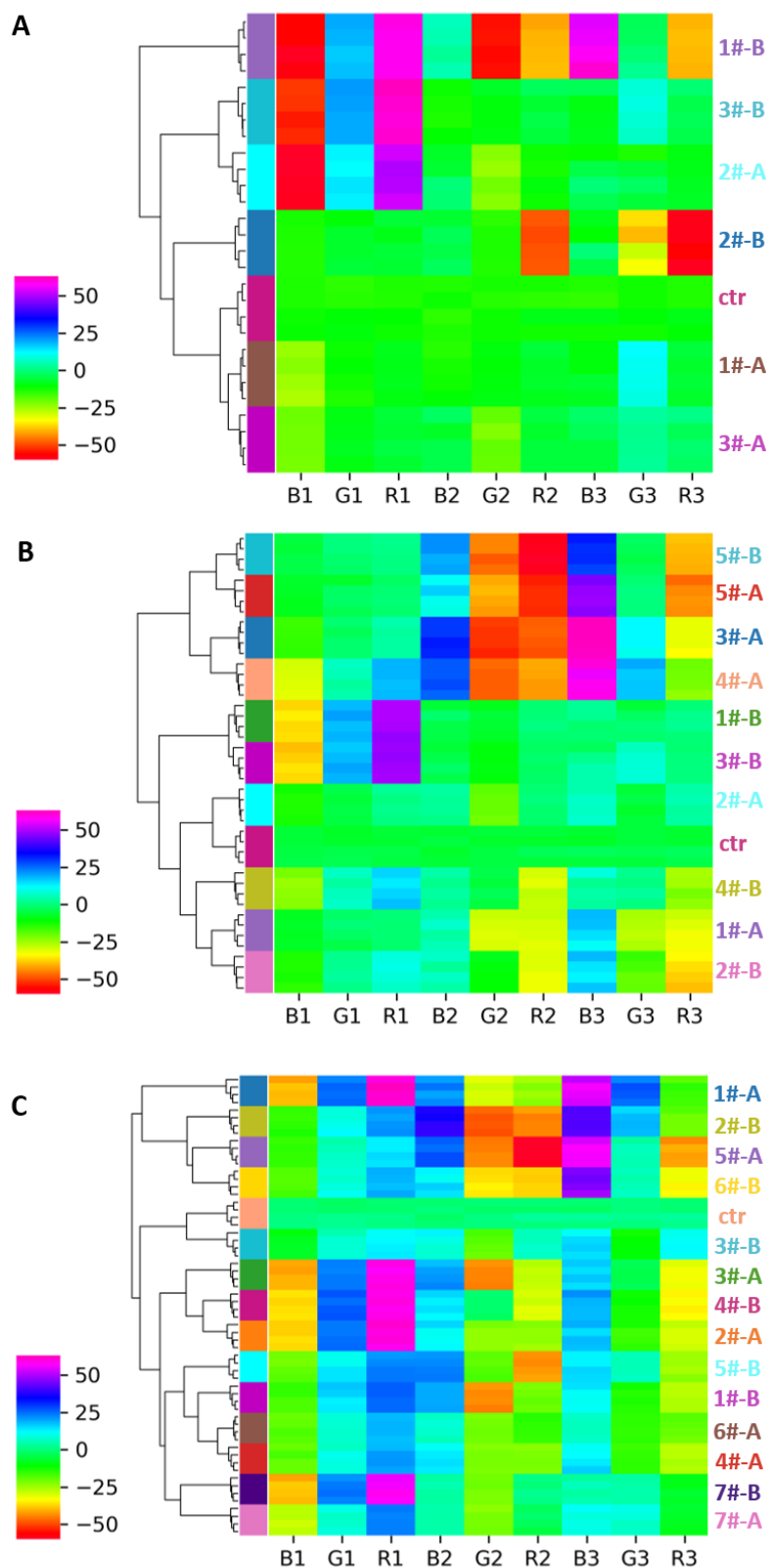


Fig. S8 Heatmap and HCA dendrogram derived from the colour response pattern of sensor array upon addition of metal-ions mixtures of (A) 3, (B) 5 and (C) 7 components selected from detested 15 metal ions.

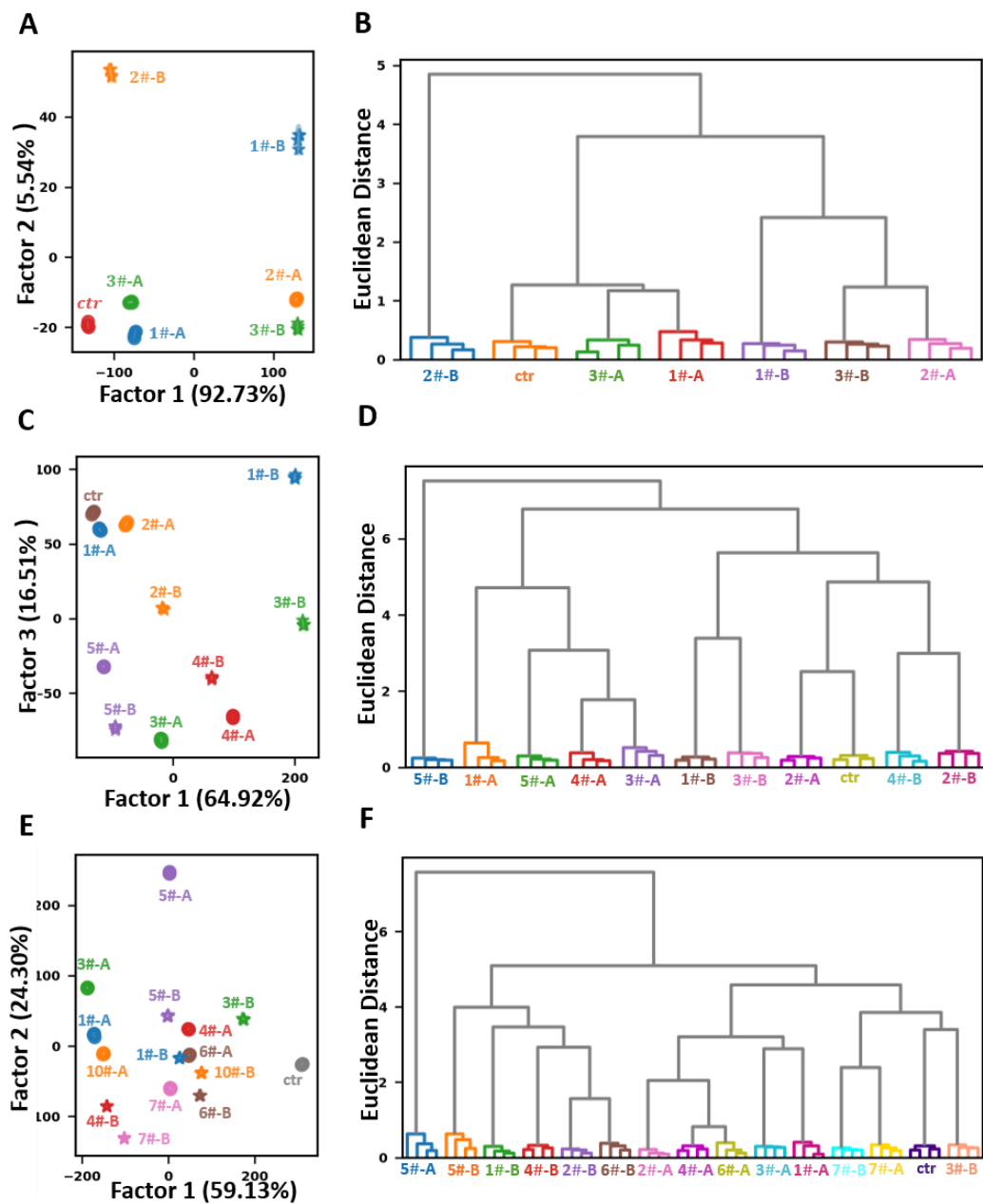


Fig. S9 (A, C, E) LDA plot and (B, D, F) HCA dendrogram of sensor array against metal ions mixture pairs consists of exactly (A, B) 3 components, (C, D) 5 components and (E, F) 7 components using UV-vis absorbance spectra change pattern.

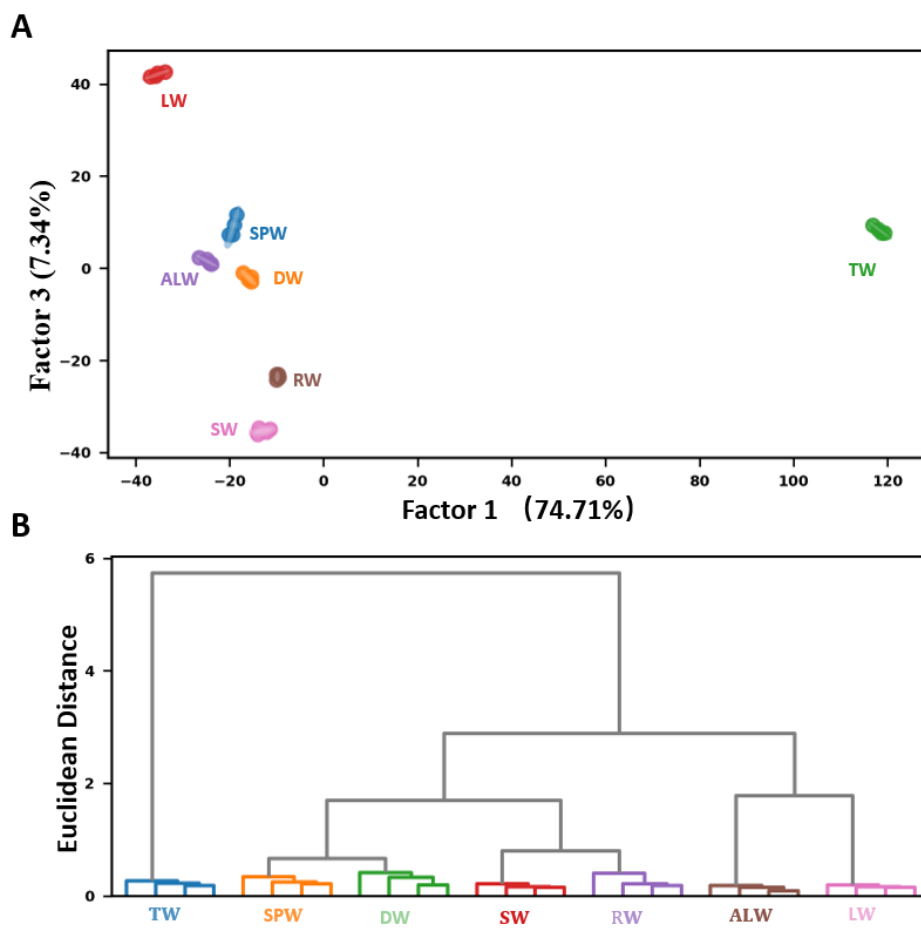


Fig. S10 Performance of the sensor array on distinguishing real water samples. (A) 2D LDA plot and (B) HCA dendrogram using UV-vis absorbance spectra change pattern.