Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Highly selective simultaneous Cu(II), Co(II), Ni(II), Hg(II), Mn(II) determination in water samples on microfluidic paper-based analytical devices

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Table S1. Reagents.

Chemicals	Supplier
1. Complexing agent	
Dimethylglyoxime (DMG)	Merck
Dithizone (DTZ)	Fluka
4-(2-Pyridylazo) resorcinol (PAR)	Sigma-Aldrich
Bathocuproine (Bc)	Aldrich
2. Masking agents	
Hydroxylamine hydrochloride	Merck
Sodium fluoride	Merck
Ethylenediamine(en)	Fluka
trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (DCTA)	Sigma-Aldrich
Potassium cyanide (KCN)	Merck
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich

Table S1. Reagents (Cont.).

Chemicals	**Supplier**
Thiourea	Sigma-Aldrich
Triethylenetetramine	Aldrich
3. Others	
Polyethylene glycol (PEG 400)	Sigma-Aldrich
Chloroform	RCI Labscan Limited
Methanol	Merck
Ammonium hydroxide	Sigma-Aldrich
Acetic acid	Merck
Poly (diallyldimethylammonium chloride) (PDDA)	Sigma-Aldrich
Sodium hydroxide (NaOH)	Merck
Sodium acetate	Merck
Sodium tetraborate decahydrate	Sigma-Aldrich
Sodium chloride	Sigma-Aldrich
Sodium phosphate dibasic	Sigma-Aldrich

Table S2. Reagents preparation.

Reagents	Concentration	Solvent
Dimethylglyoxime (DMG)	60 mM	Methanol
Dithizone (DTZ)	2 mM	Chloroform
4-(2-Pyridylazo) resorcinol (PAR)	5 mM	Borate buffer (0.1 M, pH 9.3)
Bathocuproine (Bc)	10 mM	Chloroform
Hydroxylamine hydrochloride	0.5 g/mL	Milli-Q water
Sodium fluoride	0.5 M	Milli-Q water
Ethylenediamine (en)	4, 8, 10 M	Acetate buffer (2 M, pH 5)
trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (DCTA)	0.1 M	NaOH (0.4 M)
Potassium cyanide	0.1 M	Milli-Q water
Ethylenediaminetetraacetic acid (EDTA)	0.2 M	NaOH (0.4 M)
Triethylenetetramine (TETA)	0.4 M	Phosphate buffer (0.1 M, pH 10)
Thiourea	1 M	Milli-Q water

S1. ImageJ Processing for Quantitative Analysis

1. An image was scanned and saved as JPEG format after optimum reaction time.
2. The scanned image was imported into ImageJ software.
3. The image was split into channels by using “image tool”, “color”, “split channels”, resulting in 3 windows (red, green, blue), or using “image tool”, “type”, “8-bit”, respectively for gray channel.
4. The optimum channel was inverted by using “edit tool” and then “invert”.
5. An analysis region (circle, width = 69, height = 69) was specified to measure the color intensity of the detection regions.
6. The color intensity of each detection zone was determined by “analysis” and then “measure” (or “control + M”).
7. All processes are illustrated in **Fig. S1.**

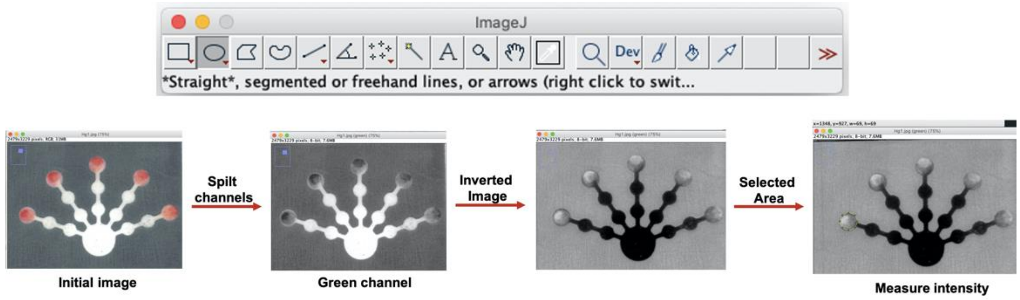


Fig. S1 ImageJ processes for image analysis.

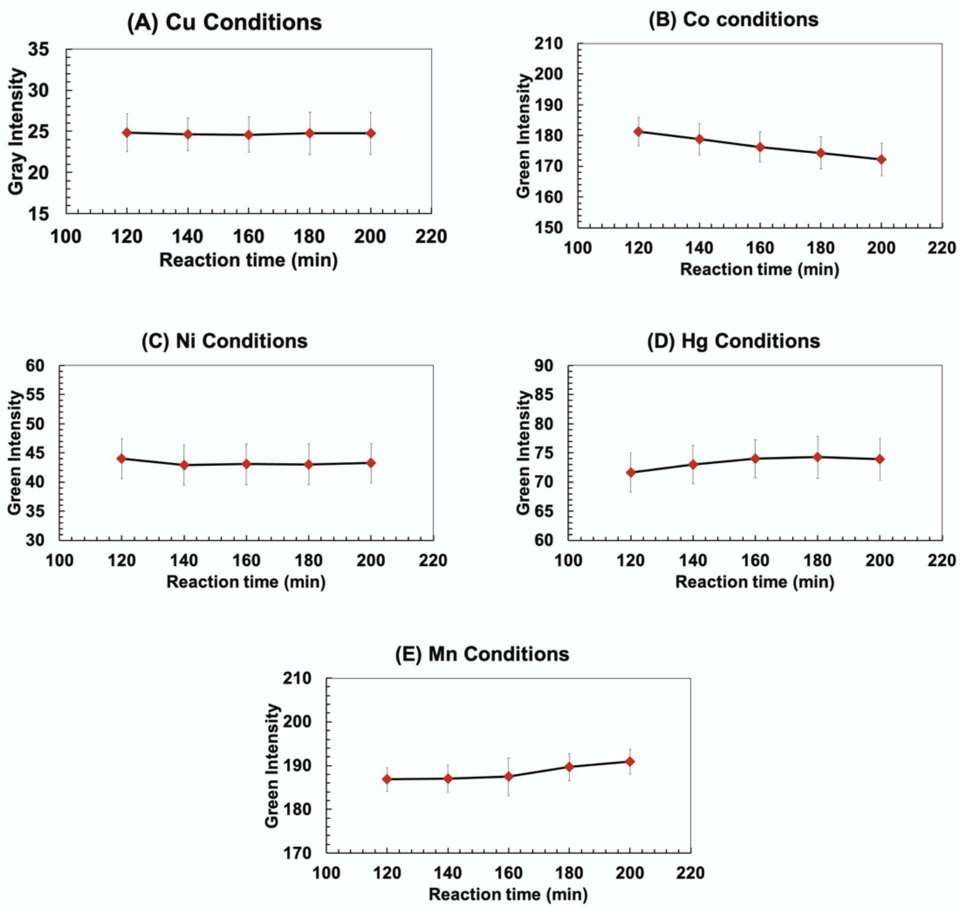


Fig. S2 Effect of reaction time (120-200 min) on the color intensity response for (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), and (E) Mn(II) analysis using a mixture solution of five metal ions.

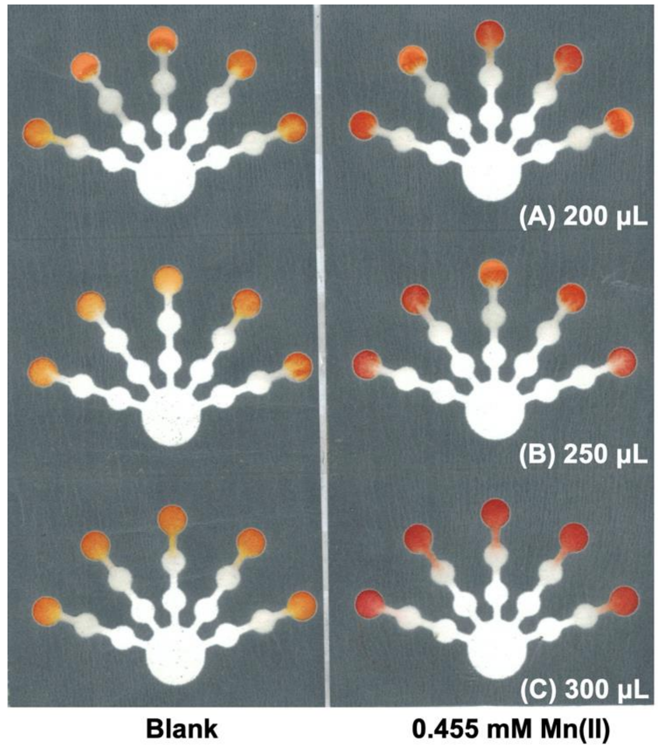


Fig. S3 Effect of metal ion volumes (A) 200 µL, (B) 250 µL, (C) 300 µL Mn(II) (0.455 mM) on the color change for Mn(II) analysis.

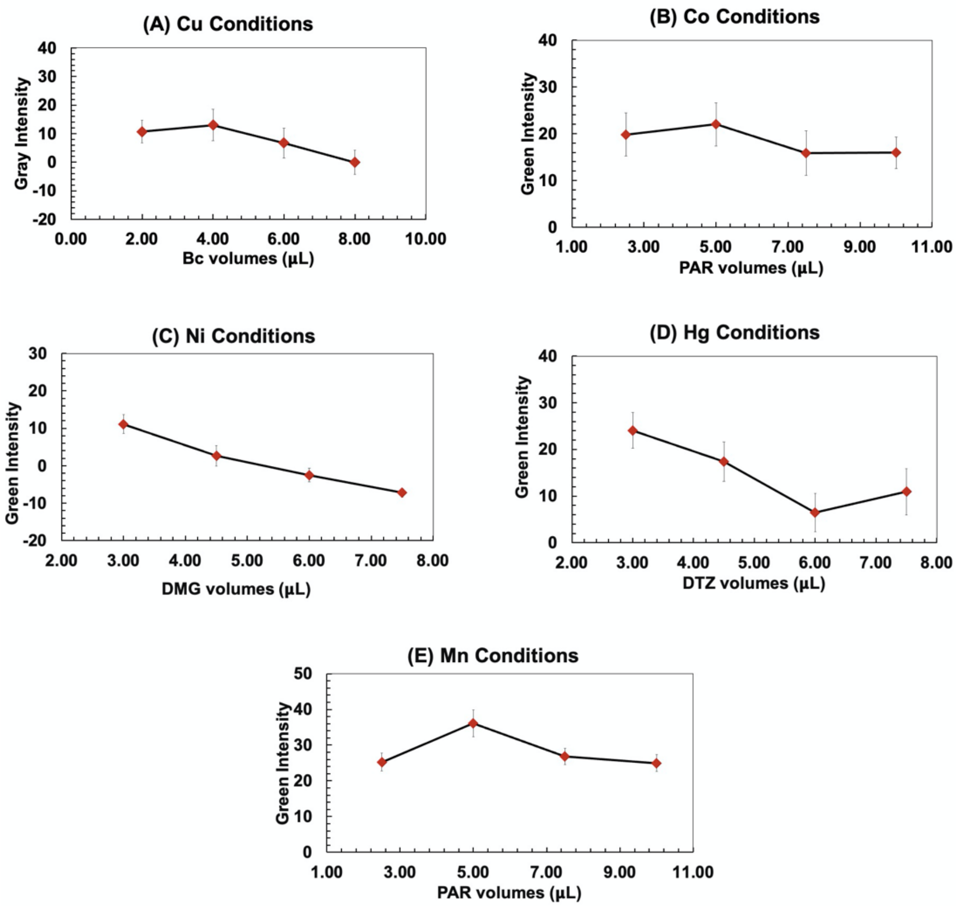


Fig. S4 Effect of complexing agent volumes on the color intensity response for (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), and (E) Mn(II) analysis, respectively.

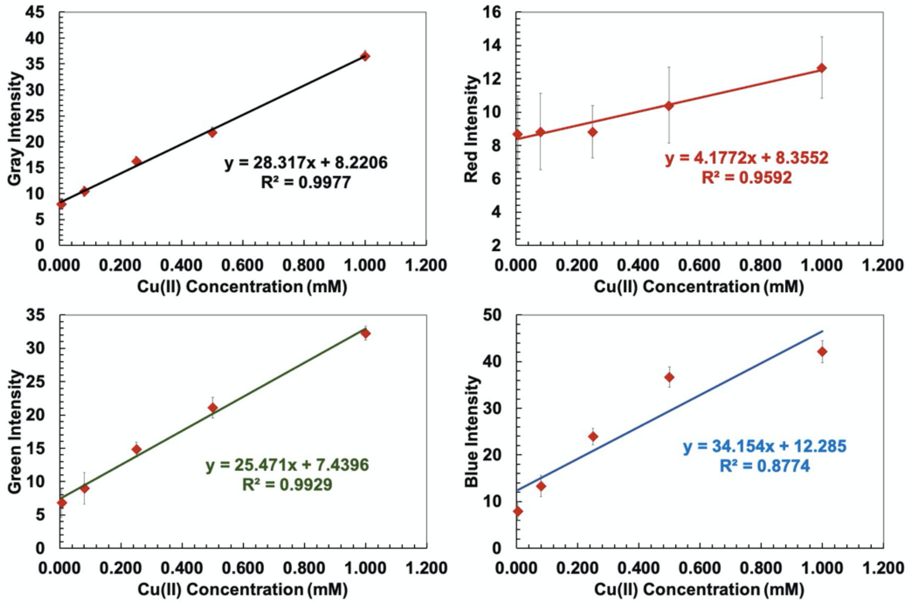


Fig. S5a Optimization of the color intensity (gray, red, green, blue channel) by using ImageJ processes for Cu(II) calibration.

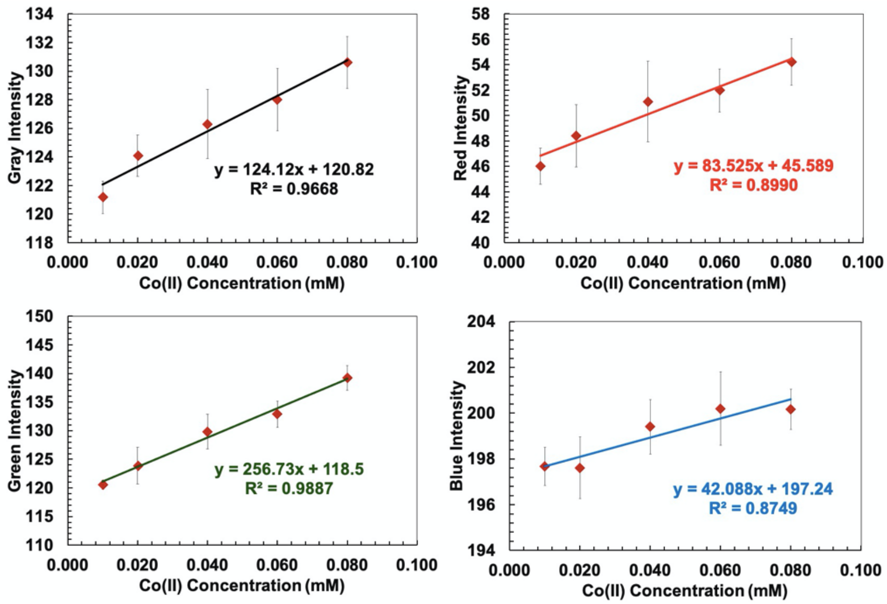


Fig. S5b Optimization of the color intensity (gray, red, green, blue channel) by using ImageJ processes for Co(II) calibration.

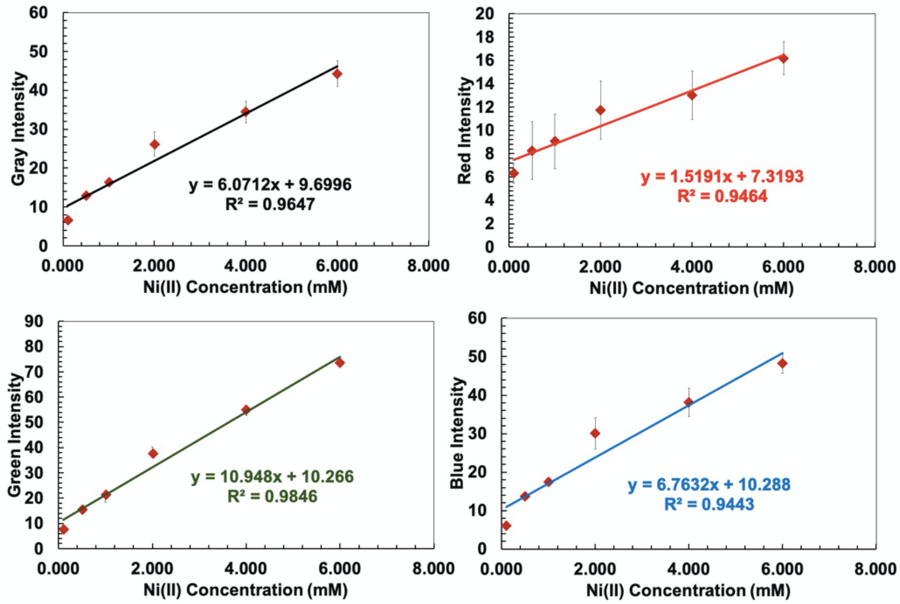


Fig. S5c Optimization of the color intensity (gray, red, green, blue channel) by using ImageJ processes for Ni(II) calibration.

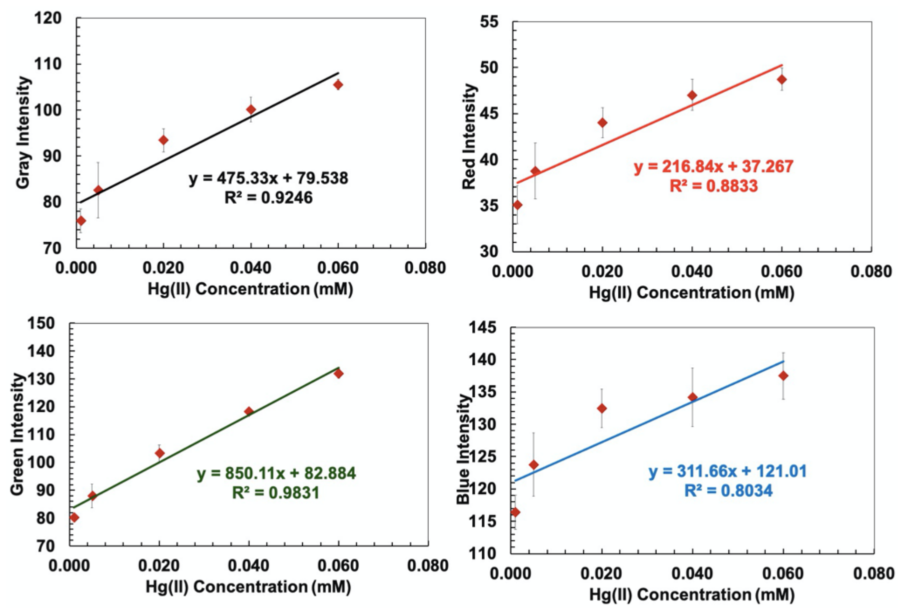


Fig. S5d Optimization of the color intensity (gray, red, green, blue channel) by using ImageJ processes for Hg(II) calibration.

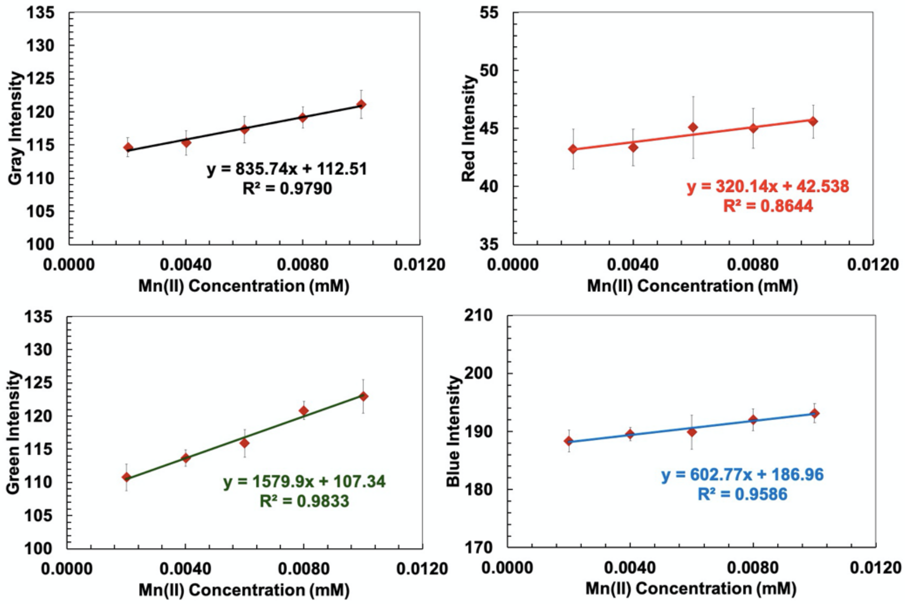
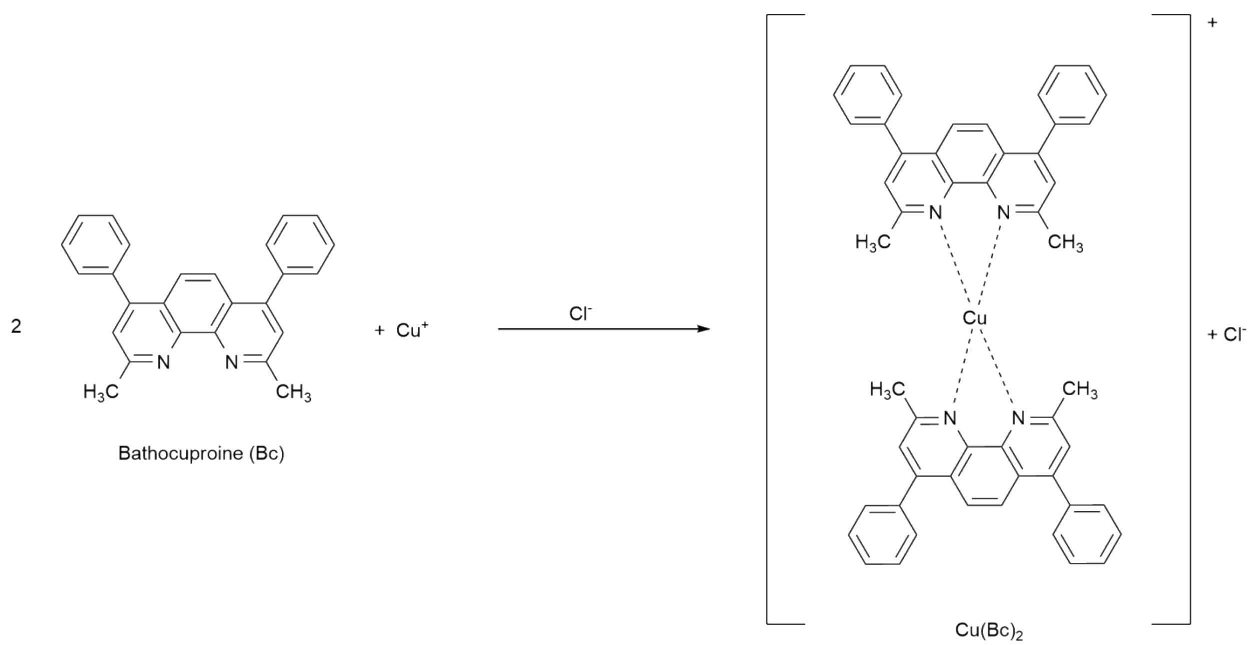
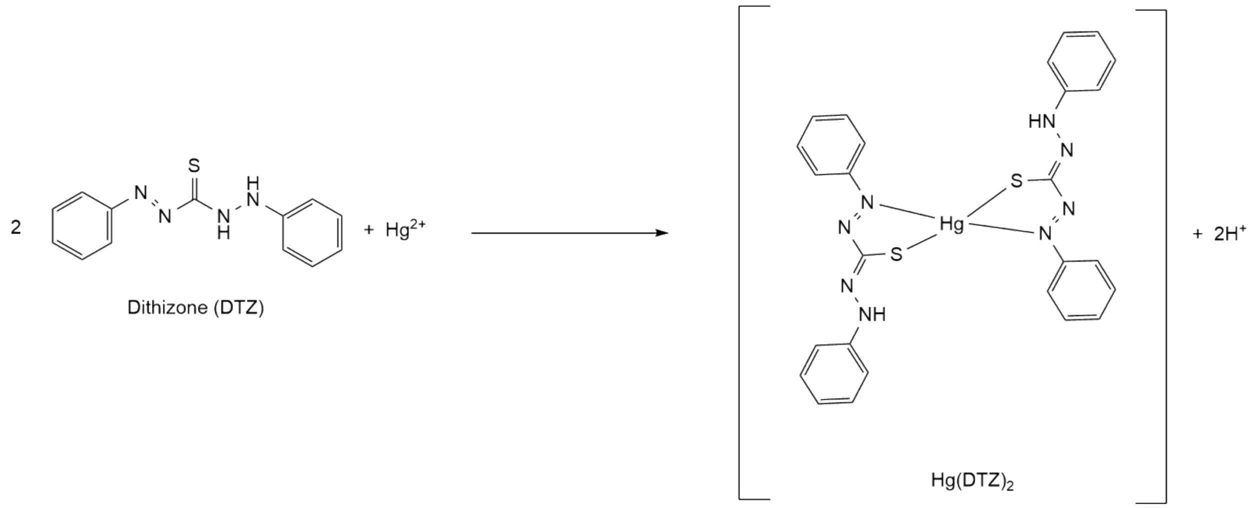


Fig. S5e Optimization of the color intensity (gray, red, green, blue channel) by using ImageJ processes for Mn(II) calibration.

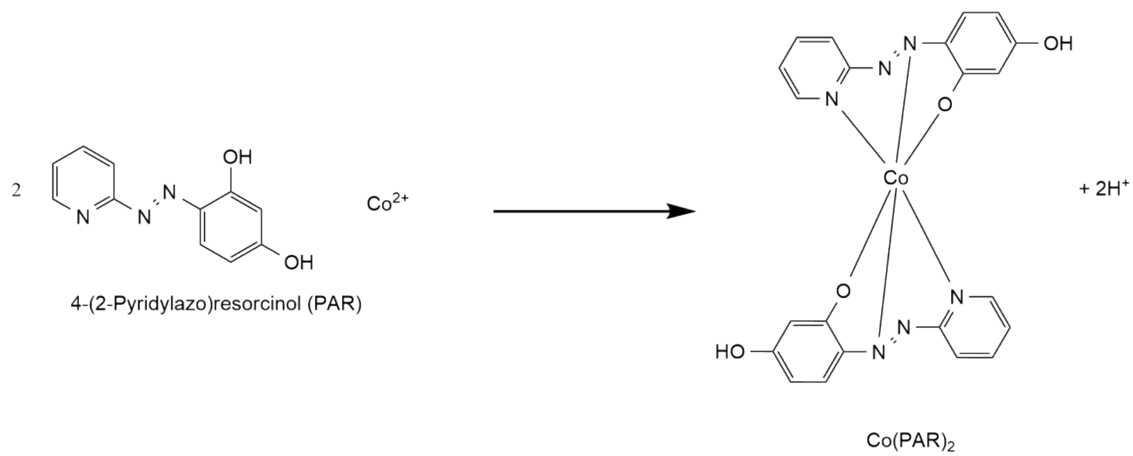
S2. Cu-Bathocuproine (Bc) reaction



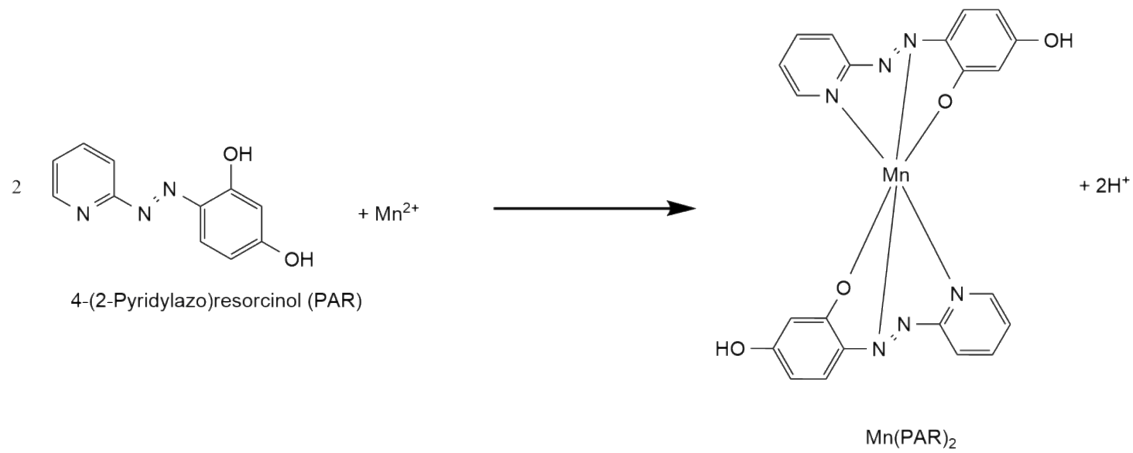
S3. Hg-Dithizone (DTZ) reaction



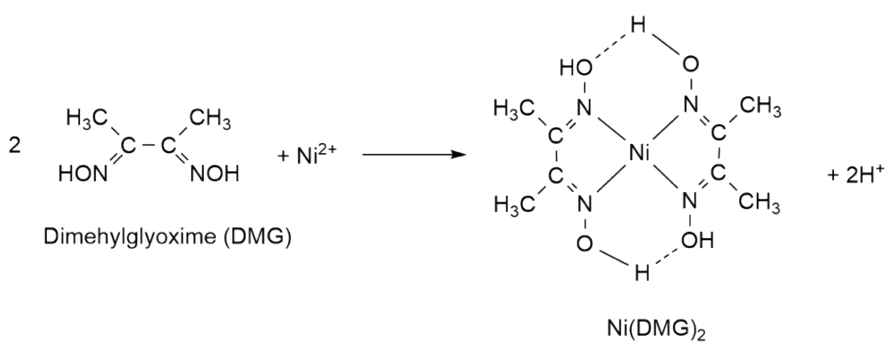
S4. Co-4-(2-Pyridylazo) resorcinol (PAR) reaction



S5. Mn-4-(2-Pyridylazo) resorcinol (PAR) reaction



S6. Ni-Dimethylglyoxime (DMG) reaction



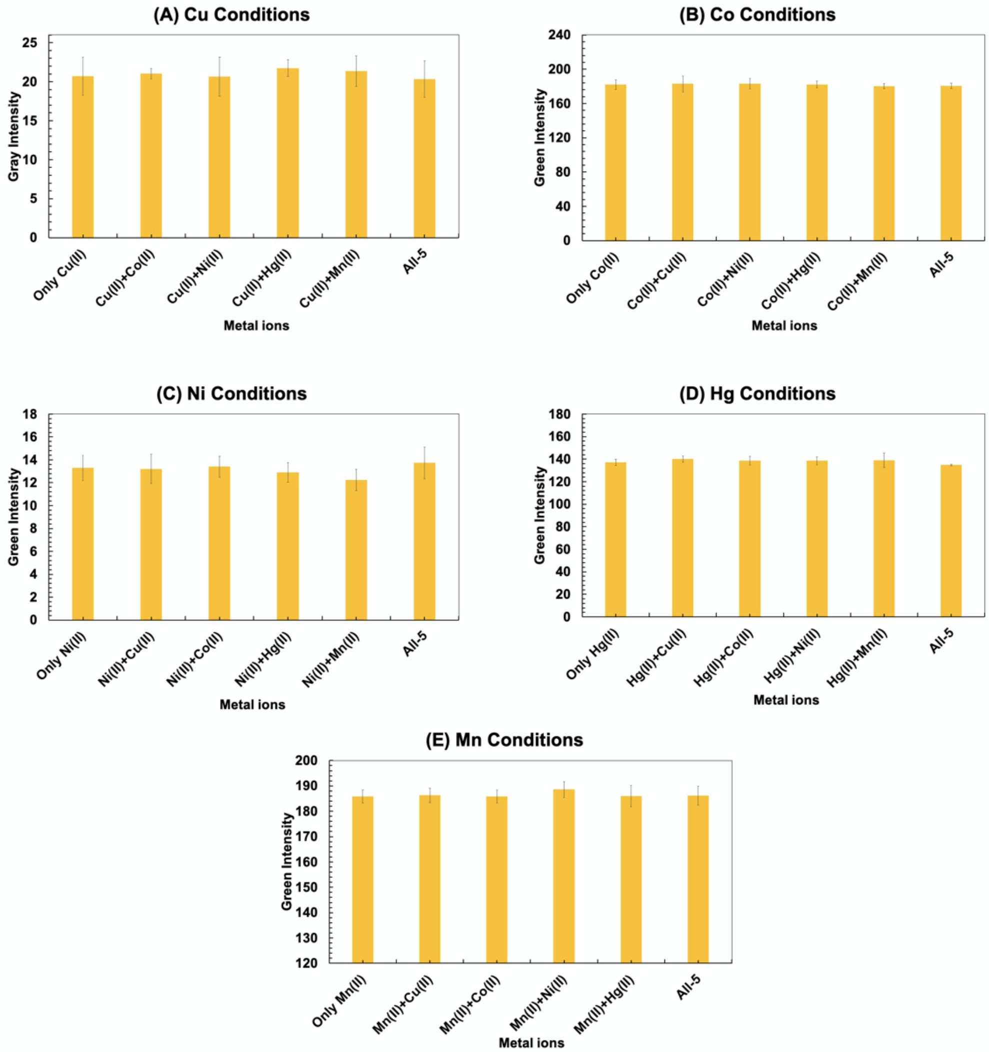


Fig. S6 The color intensities of 0.455 mM (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), (E) Mn(II) in the presence of 0.455 mM Cu(II), Co(II), Ni(II), Hg(II), Mn(II), and All-5 for Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) analysis, respectively (1:1 ratio). The error bars were obtained from the standard deviation (n=5) on one device.

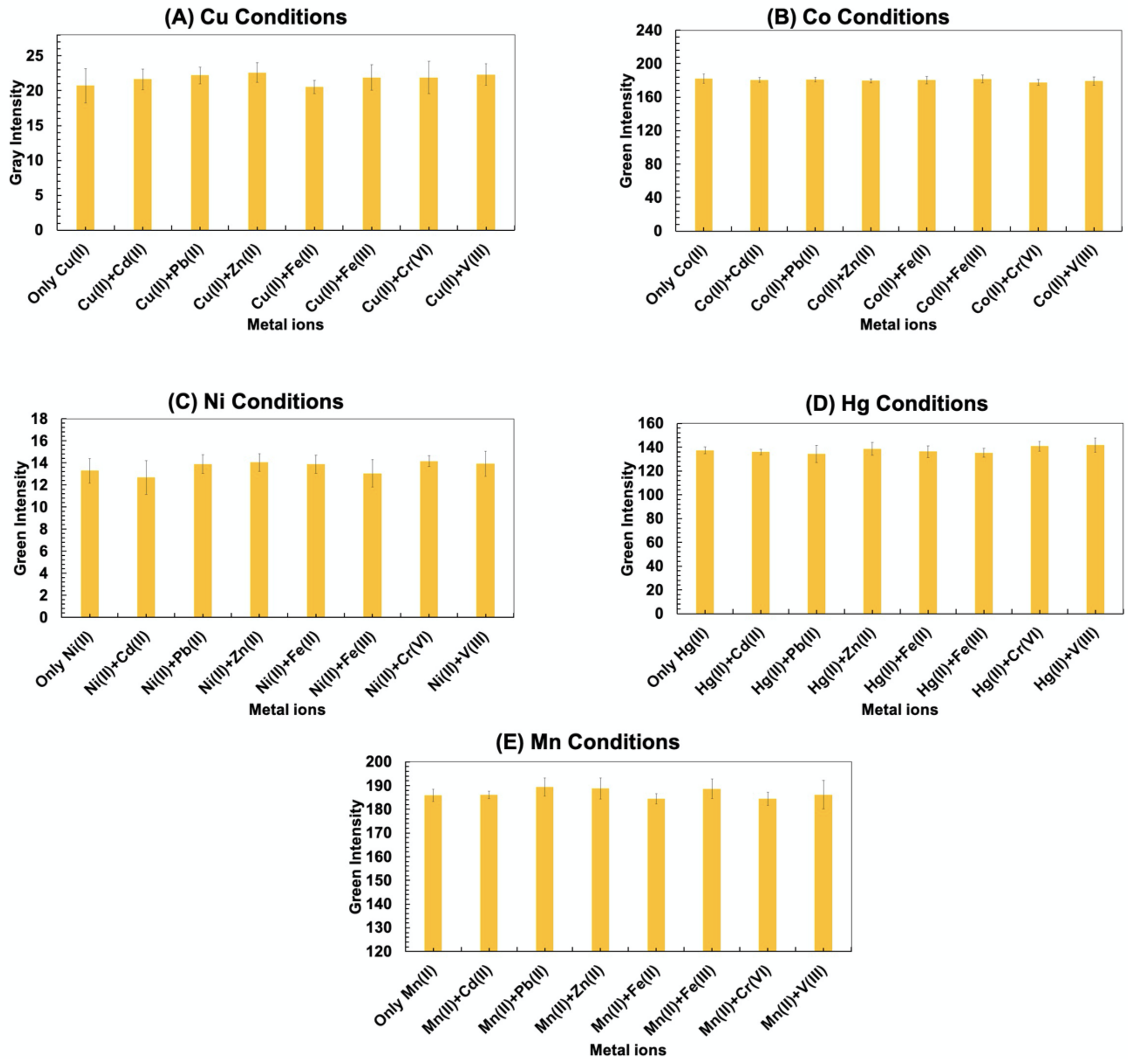


Fig. S7 The color intensities of 0.455 mM (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), (E) Mn(II) in the presence of 0.455 mM Cd(II), Zn(II), Pb(II), Fe(II), Fe(III), Cr(VI), and V(III) for Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) analysis, respectively (1:1 ratio). The error bars were obtained from standard deviation (n=5) on one device.

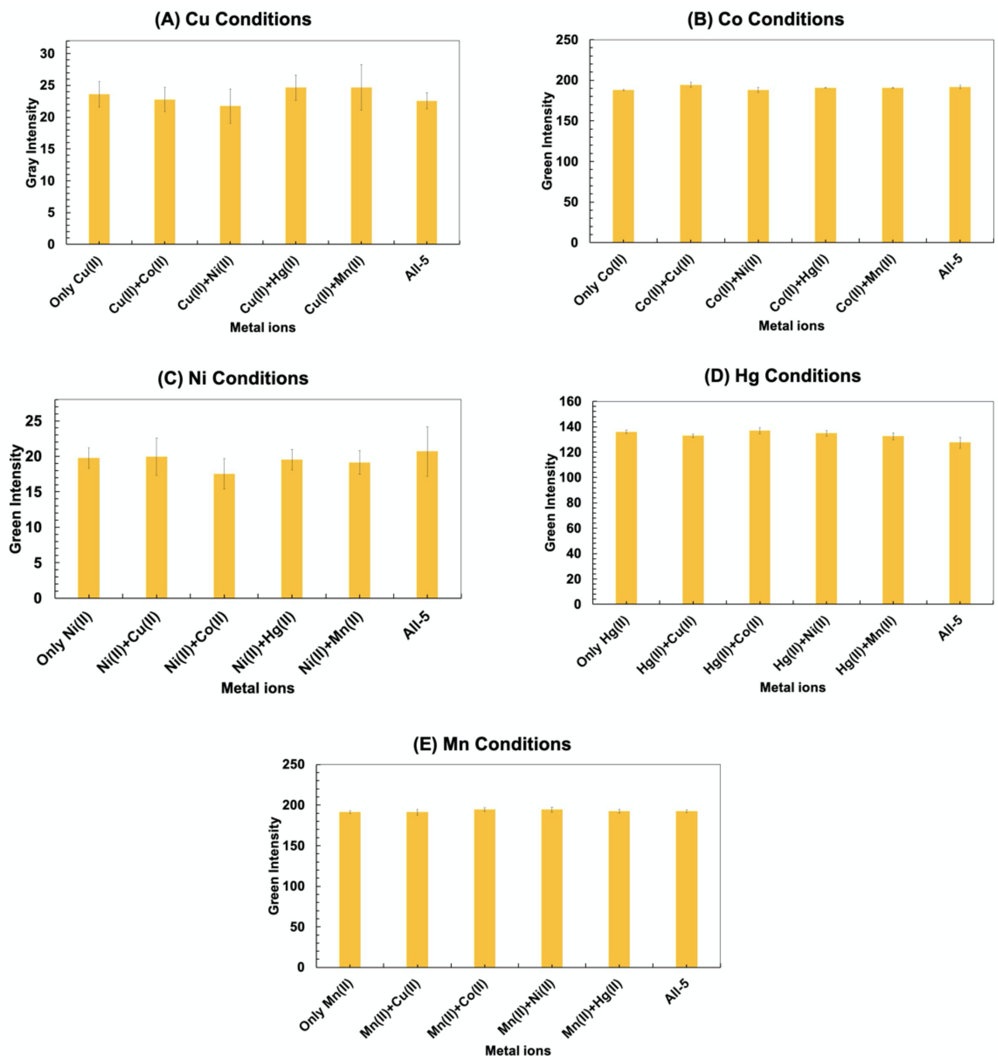


Fig. S8 The color intensities of 0.455 mM (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), (E) Mn(II) in the presence of 0.910 mM Cu(II), Co(II), Ni(II), Hg(II), Mn(II), and All-5 for Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) analysis, respectively (1:2 ratio). The error bars were obtained from the standard deviation (n=5) on one device.

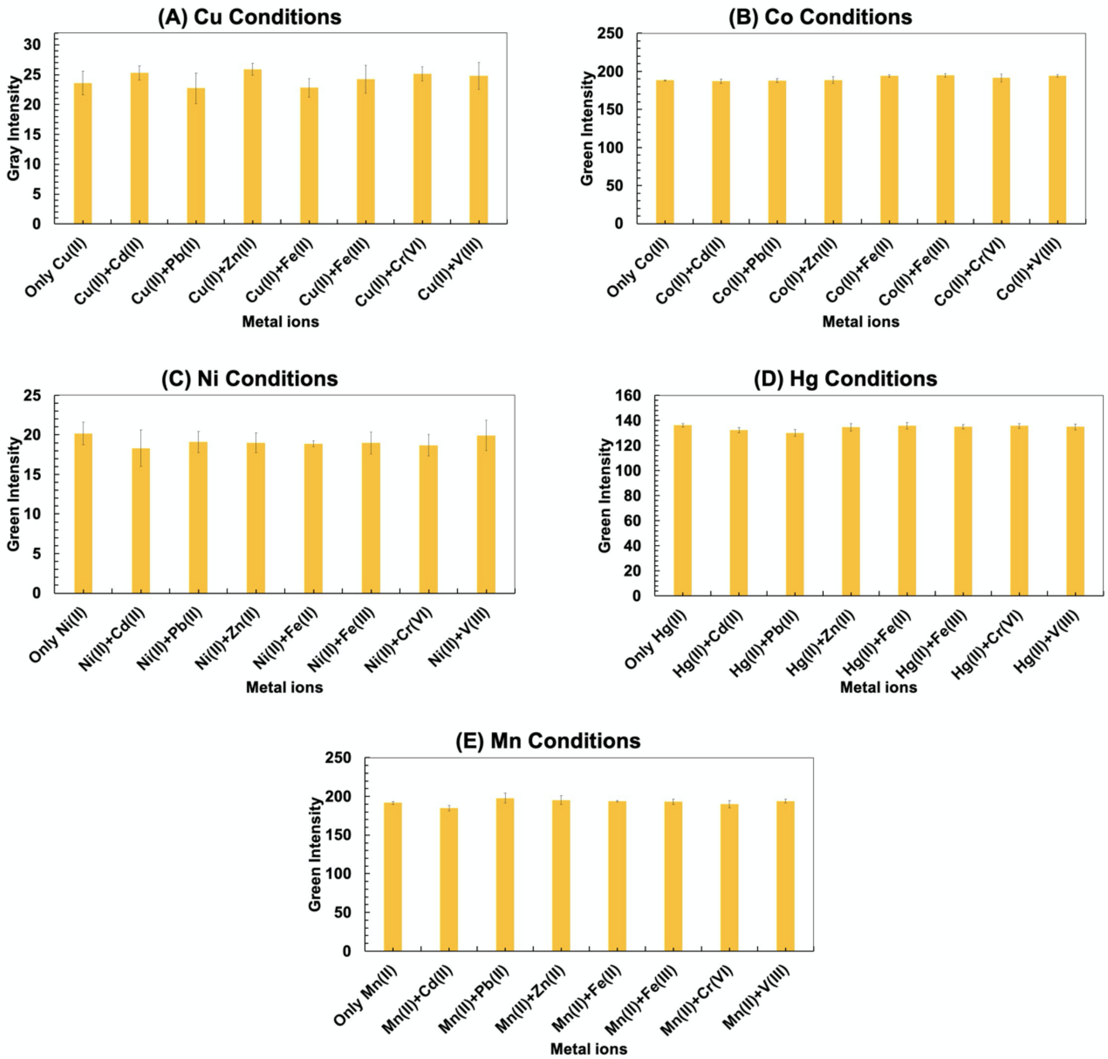


Fig. S9 The color intensities of 0.455 mM (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), (E) Mn(II) in the presence of 0.910 mM Cd(II), Zn(II), Pb(II), Fe(II), Fe(III), Cr(VI), and V(III) for Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) analysis, respectively (1:2 ratio). The error bars were obtained from standard deviation (n=5) on one device.

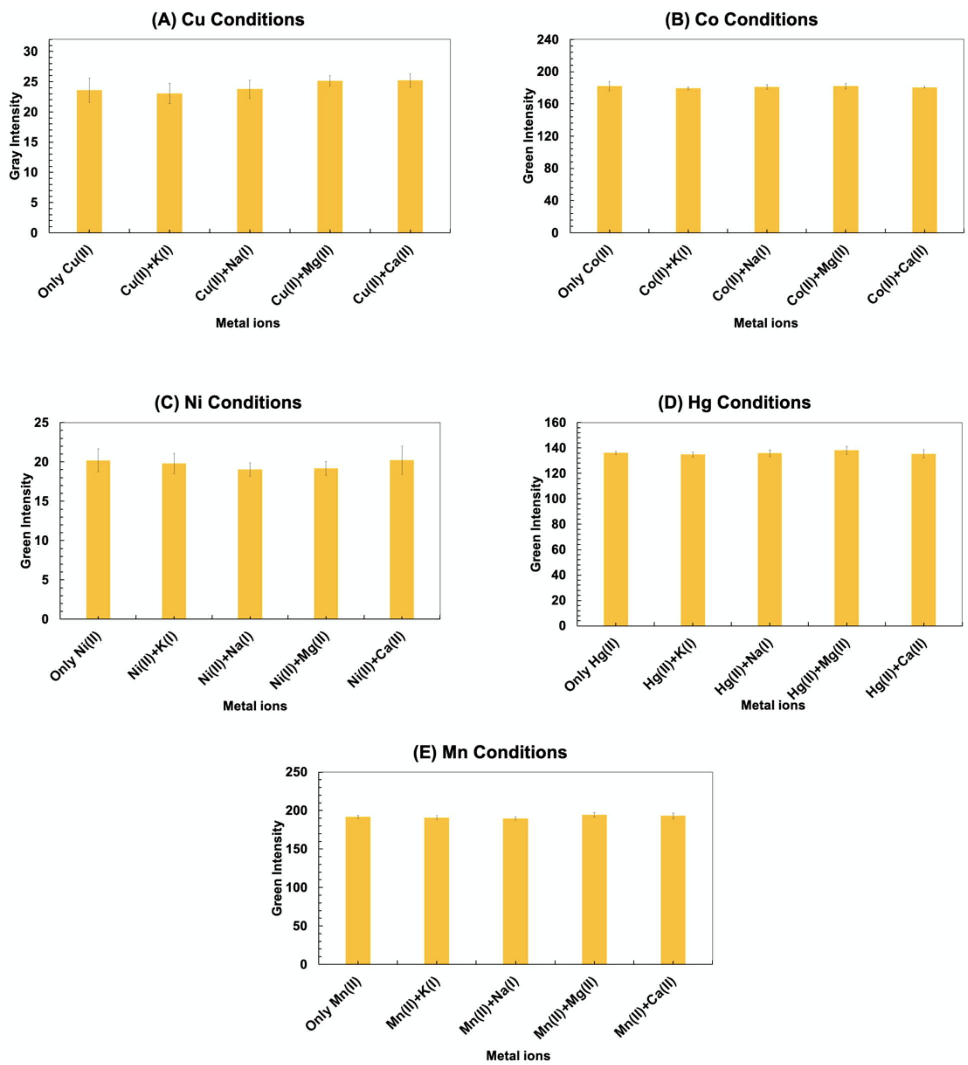


Fig. S10 The color intensities of 0.455 mM (A) Cu(II), (B) Co(II), (C) Ni(II), (D) Hg(II), (E) Mn(II) in the presence of 45.5 mM (1:100) Na(I), K(I), Mg(II), Ca(II) for Cu(II), Ni(II), Hg(II), Mn(II) analysis and 455 mM (1:1000) Na(I), K(I), Mg(II), Ca(II) for Co(II) analysis. The error bars were obtained from standard deviation (n=5) on one device.