Electronic Supplementary Material (ESI) for Analytical Methods.

Detection of Glyphosate with a Copper(II)-Pyrocatechol Violet Based GlyPKit

Prerna Yadav and Felix Zelder*

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Fax: +41 44 635 6803; E-mail: felix.zelder@chem.uzh.ch, www.felix-zelder.net.

Contents

S1. General Information and Instrumentation

Unless otherwise stated, all chemicals were of reagent grade and were commercially purchased from *Sigma–Aldrich-Merck*, *Fluka*, *Apollo Scientific* or *Alfa Aesar*. Solvents for reactions were of p.a. grade. CHROMABOND C18ec cartridges (1 mL, 100 mg) were obtained from *Machery-Nagel AG* Schweiz.

UV-Vis spectra were measured at T = 22 +/- 1 °C with a *Cary* 50 spectrophotometer using quartz cells with a path length of 1 cm. Spectra were recorded between 230 and 800 nm at 1.2 nm resolution and 20 points s^{-1}.

Stock solutions were prepared freshly before use. The desired pH values of the stock solutions of buffers: Acetate (10 mM; pH 5.50), HEPES (10 mM; pH 7.40) and CHES (10 mM; pH 9.00) were adjusted by the addition of either a solution of 2 N NaOH or 1 N HCl. All measurements were performed at a final buffer concentration of 10 mM. The pH values of solutions were measured with a *Metrohm* 827 pH lab.
Selectivity and sensitivity studies were performed with Zn^{II}-zincon and Cu^{II}_2-PV in either a quartz cuvette or a 26-well plate.

S2. Screening Procedure

Indicators used in this study: pyrocatechol violet (PV), xylidyl blue (XB), green B (GB), murexide (MX), xylenol orange (XO), alizarin red S (ARS), pyrogallol red (PR) and zincon (ZCN). Stock solutions of the indicators were freshly prepared in either Milli-Q H_2O or DMSO/ Milli-Q H_2O mixture. *Metal ions* used in this study: $FeCl_3$, $ZnCl_2$, $NiCl_2$, $CuCl_2$ (1 or 2 equiv). *Anions* used in this study: sodium salts except glyphosate (GlyP). *Potential Interfering Ions:* PO_4^{3-}, SO_4^{2-}, NO_3^-, CO_3^{2-}, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, GlyP, Fe^{3+}, Zn^{2+} and Mn^{2+}.[S1] Stock solutions of ions were prepared in Milli-Q H_2O or tap water.

Naked-eye screening of the indicators and the metal ions was performed at pH 5.50, 7.40 and 9.00.

Screenings were performed in a 96-well plate. Changes in color were detected by naked-eye.

First step: Selection criteria for M^{n+}-indicators were: a) a visual color change that is detectable by naked-eye with a high color contrast between metal-free indicator and the respective M^{n+}-indicator complex; b) stability of M^{n+}-indicators, i.e. no change in intensity of colors during the screening experiment (approx. 2h).

Second step: Selection of GlyP sensor candidates were: c) a visual color change of the M^{n+}-indicator complex (selected in the first step) upon additions of GlyP to the color of the metal-free indicator. d) no visual color change of the M^{n+}-indicator complex (selected in the first step) upon additions of PO_4^{3-}.

S3. CSPE Method[S2-S4]

The colorimetric solid phase extraction (CSPE) device consisted of a 5 mL syringe connected to a C18ec cartridge. The procedure is described for immobilized Cu^{II}_2-PV.

1. *Conditioning:* The cartridge was washed with MeOH (2 mL) and distilled H_2O (3 mL).

2. *Adsorption of M^{n+}-Indicator:* An aq. soln. of Cu^{II}_2-PV (2 mL, 5 µM) at pH 6.50 ([HEPES] = 10 mM) was slowly passed through C18ec cartridges (approx. 1 drop per 3 s). The immobilized metal complex was visible as a blue colored ring (height ~1 mm; *detection zone*).
3. *Analysis:* Buffered tap water (pH 6.50; HEPES buffer = [10 mM]) spiked with either GlyP or other potentially interfering ions was slowly passed (approx. 1 drop per 3 s) through the GlyPKit containing immobilized Cu^{II}_2-PV. Detection was indicated by a change of color.

S4. Smartphone Colorimetry

We used an *OPPO F9* with front camera (16 MP) and LED flash. Pictures were taken in the daytime between 2 – 4 p.m. CET in November. All artificial sources of light were switched off and the photos were taken against a white background to get a better picture quality and consistency.

Calibration samples were prepared by slowly passing cartridges separately (approx. 1 drop per 3 s with aq. tap water solns. of GlyP (2 mL; 0, 2, 4, 8, 12, 20, 40 and 200 nmoles) at pH 6.50 (HEPES buffer = [10 mM]) through the GlyPKit. Change in color in the detection zone were observed by naked-eye or analyzed using smartphone colorimetry. In total, 20 photos of each calibration sample were taken and averaged R values from a mix and match of ten pictures were obtained. The average R values were plotted against increasing GlyP concentration.

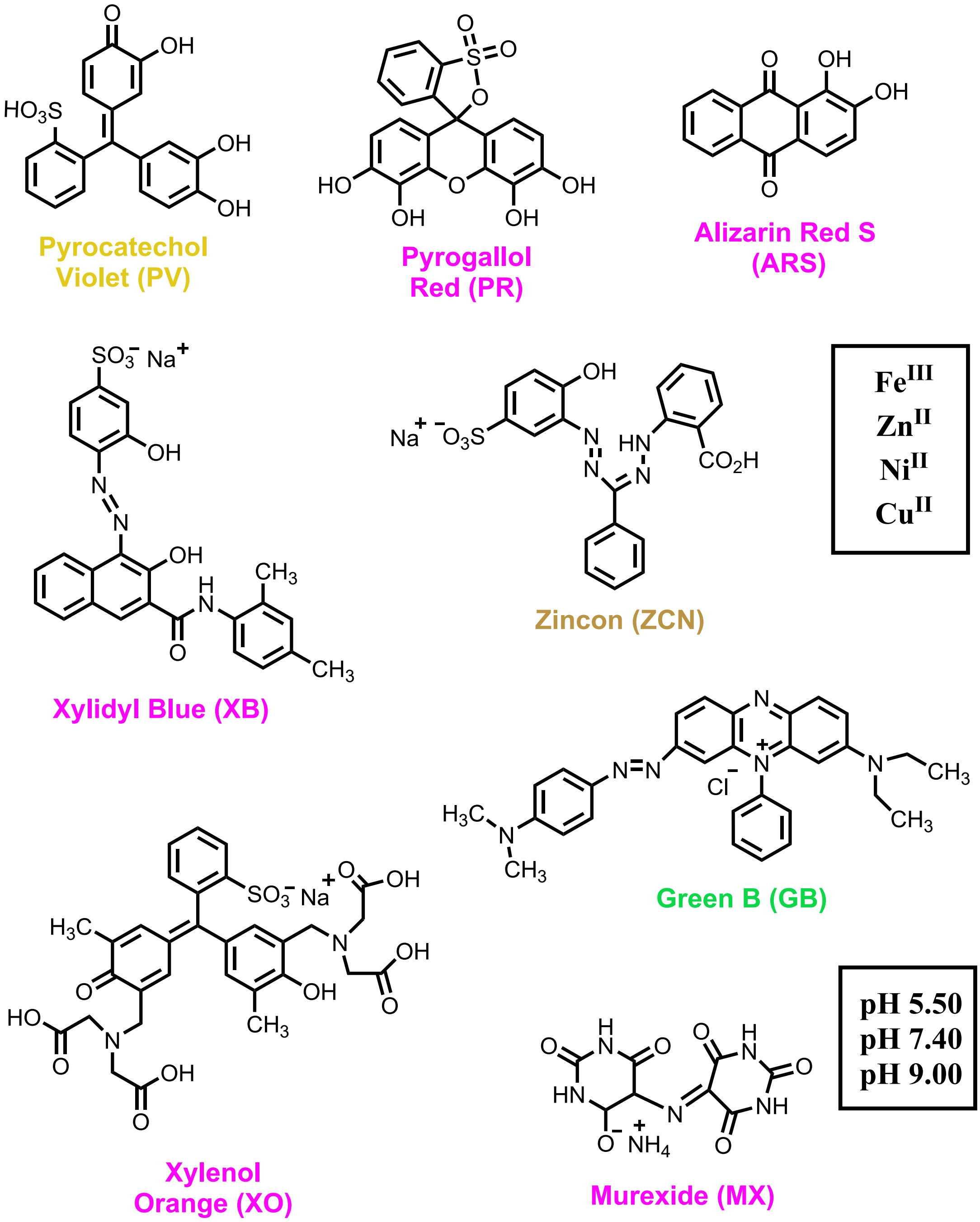
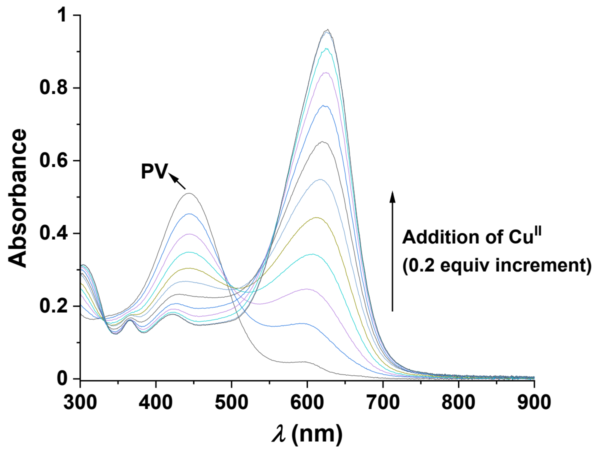
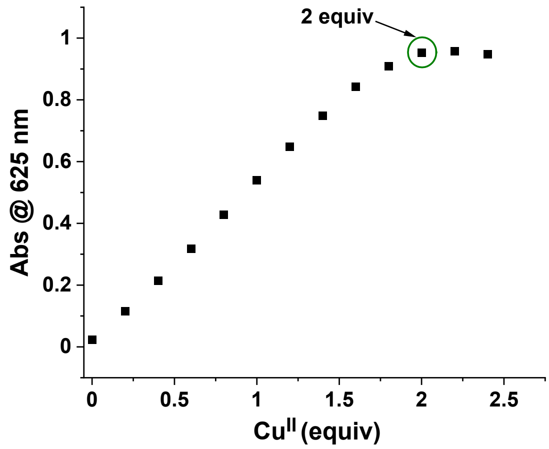


Figure S1. Overview of the eight indicators, four metal ions and three different pH used in the screening process.



Absorbance
PV
Addition of Cu^{II}
(0.2 equiv increment)
λ (nm)



2 equiv
Abs @ 625 nm
Cu^{II} (equiv)

Figure S2. *Left:* Changes of absorbance of PV (30 μM; λ_{max} = 625 nm) upon addition of Cu^{II} (0 – 2.4 equiv) at pH 6.50, [HEPES buffer] = 10 mM. *Right:* Change of absorbance of PV (30 μM; λ_{max} = 625 nm) at 625 nm upon addition of Cu^{II} (0 – 2.4 equiv) at pH 6.50 ([HEPES buffer] = 10 mM) depicting saturation after 2 equiv of Cu^{II}.

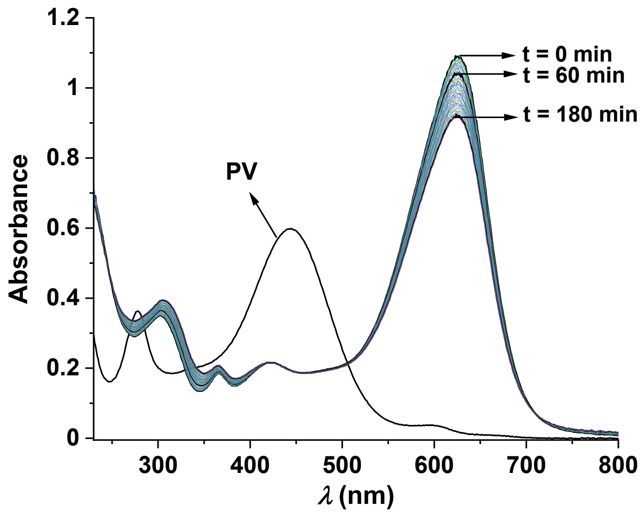


Figure S3. Changes of absorbance of Cu^{II}_2-PV (30 μM) over a period of 180 min in the absence of any analyte at pH 6.50 ([HEPES buffer] = 10 mM).

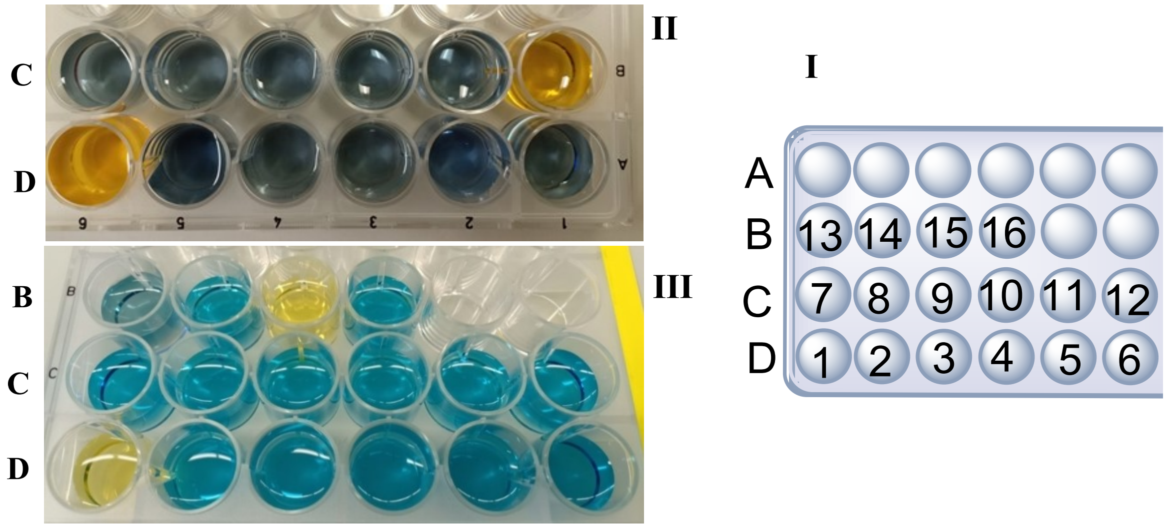
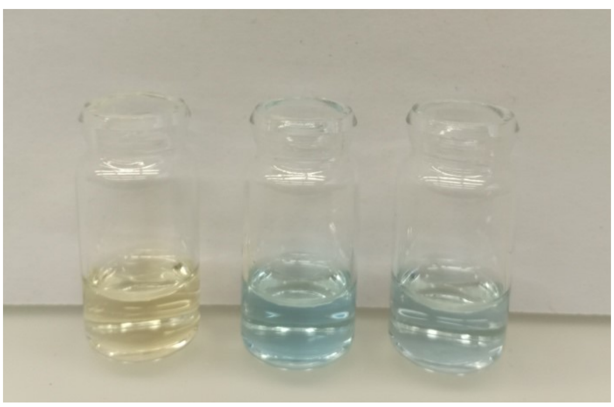


Figure S4. I. A depiction of a portion of a 26-well plate alongwith the rows (A, B, C, D) and numbers of the well (from 1 to 16). Note that only a portion of wells are shown which were used in experiments (I and II). **II**: Naked-eye selectivity test in a 26-well plate at pH 7.20 ([HEPES buffer] = 10 mM) of Zn^{II}-zincon (30 µM) with different ions (10 equiv; 0.3 mM). Row D: ZCN (*1*), Zn^{II}-zincon (*2*), Zn^{II}-zincon + [PO_4^{3-} (*3*), NO_3^- (*4*), CO_3^{2-} (*5*), Cl^- (*6*), Row C: SO_4^{2-} (*7*), NH_4^+ (*8*), Ca^{2+} (*9*), Mg^{2+} (*10*), K^+ (*11*), GlyP (*12*)], **III**: Naked-eye selectivity test in a 26-well plate (at pH 6.50, HEPES buffer] = 10 mM) of Cu^{II}_2-PV (30 µM) with different anions (0.5 mM). Row D: PV (*1*), Cu^{II}_2-PV (*2*), Cu^{II}_2-PV + [PO_4^{3-} (*3*), NO_3^- (*4*), CO_3^{2-} (*5*), Cl^- (*6*), Row C: SO_4^{2-} (*7*), NH_4^+ (*8*), Ca^{2+} (*9*), Mg^{2+} (*10*), K^+ (*11*), Mn^{2+} (*12*), Row B: Fe^{3+} (*13*), Zn^{2+} (*14*), GlyP (*15*), Na^+ (*16*)].

(I)



(II)

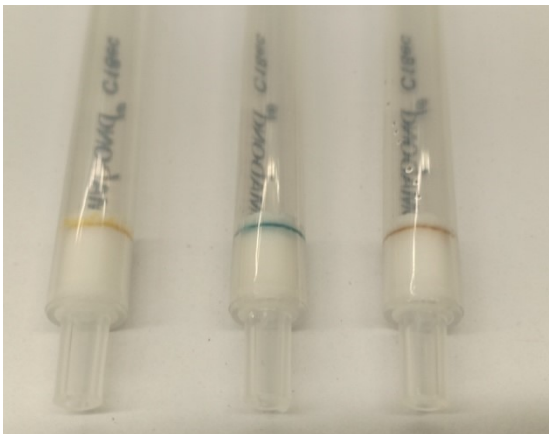
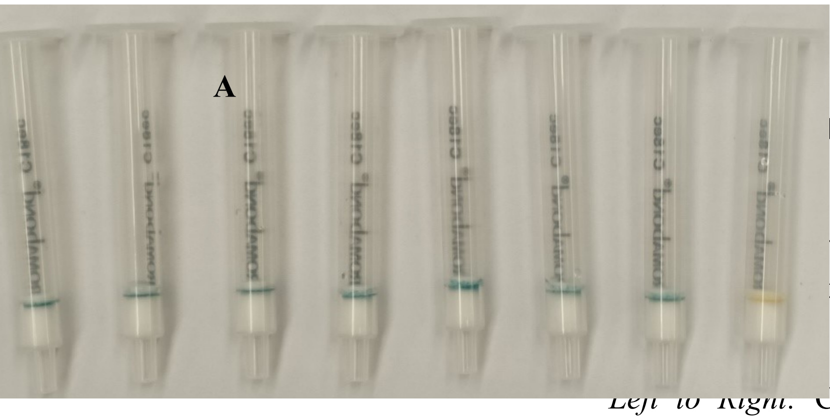


Figure S5. I. Naked-eye test in solution phase (pH 6.50, [HEPES] = 10 mm). *Left:* PV (5 µM, 2 mL, light yellow); *Middle:* Cu^{II}_2-PV (5 µM, 2 mL, light blue); *Right:* Cu^{II}_2-PV + GlyP (GlyP conc. = 4 µM, 2 mL, blue color of complex fades). **II.** Immobilizations of PV and Cu^{II}_2-PV on the top of C18ec cartridges at pH 6.50 ([HEPES] = 10 mM). *Left:* PV (2 mL, 5 µM; 10 nmol; dark yellow), *Middle:* Cu^{II}_2-PV (2 mL, 5 µM; 10 nmol; *detection zone*: blue), *Right:* Cu^{II}_2-PV + GlyP (2 mL, 4 µM, 8 nmol; *detection zone*: dark yellow).



photograph of the immobilized with 5 µM; 10 nmol) in he presence of the ons in tap water; *Left to Right:* Cu^{II}_2-PV, Cu^{II}_2-PV with either Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, CO_3^{2-}, Cl^{-} or GlyP (2 mL, 50 µM; 100 nmol). **II.** A photograph of the C18ec cartridges immobilized with Cu^{II}_2-PV (2 mL, 5 µM; 10 nmol) in the absence and the presence of other potential polydentate interferents; *Left to Right:* Cu^{II}_2-PV, Cu^{II}_2-PV with $C_2O_4^{2-}$ and PPi (2 mL, 50 µM; 100 nmol).*

*Note that $C_2O_4^{2-}$ or PPi are not abundantly found in tap water and thus should not interfere with testing GlyP in tap water.

Table S1: A documentation of respective change in colors of six indicators (30 µM) upon addition of four metal ions (1 or 2 equiv) at pH 5.50, 7.40 and 9.00 as a part of the 35 selected M^{n+}-indicators after first step in the screening process. *

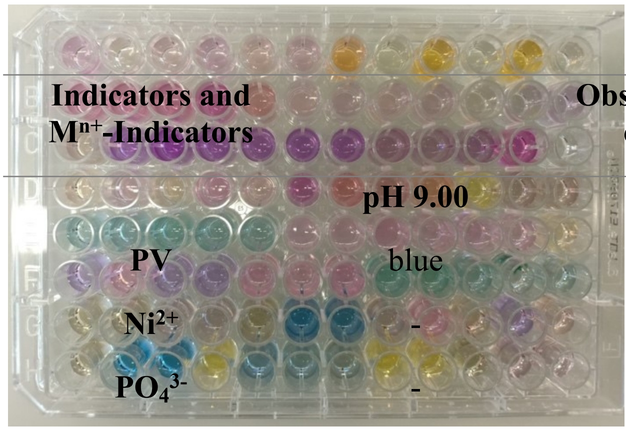
Indicators and M^{n+}-Indicators		**Observed Colors at different pH**	
	pH 9.00	**pH 7.40**	**pH 5.50**
PV	blue	light green	yellow
Fe^{3+}	-	blue green	blue
Ni^{2+}	-	blue	-

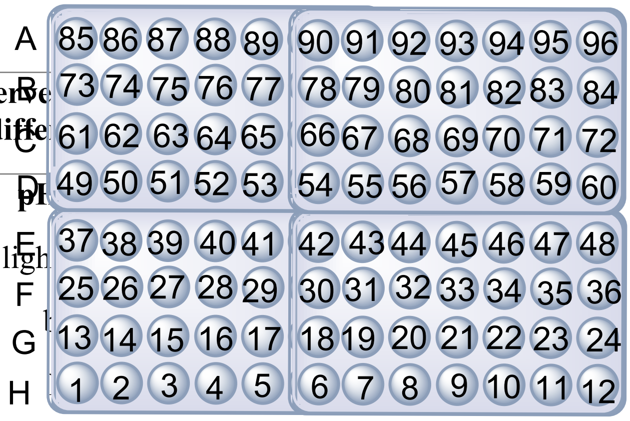
Zn^{2+}	violet	blue	-
Cu^{2+}	-	-	blue
XB	purple	pink	pink
Ni^{2+}	pink	-	-
Zn^{2+}	pink	-	-
MX	pink	pink	pink
Zn^{2+}	yellow	yellow	-
Ni^{2+}	yellow	yellow	-
Cu^{2+}	yellow	yellow	-
XO	pink	magenta	yellow
Fe^{3+}	-	-	light pink
Ni^{2+}	purple	purple	light purple
Zn^{2+}	-	pink	pink
Cu^{2+}	-	pink	pink
PR	pink	pink	pink
Fe^{3+}	-	-	blue purple
Ni^{2+}	purple	purple	-
Cu^{2+}	purple	purple	purple
ZCN	orange	yellow orange	yellow
Fe^{3+}	-	-	light pink
Ni^{2+}	grey-blue	grey-blue	-
Zn^{2+}	dark blue	dark blue	-
Cu^{2+}	blue	blue	-

The documented change in colors suggests formation of M^{n+}-indicators. Only 35 combinations of the selected M^{n+}-indicators are shown.

Table S2: A documentation of respective change in colors of selected nine M^{n+}-indicators (30 μM) upon addition of PO_4^{3-} and GlyP (10 equiv) at pH 5.50, 7.40 and 9.00 as a part of the second step in the screening process. *

NR: No reaction (i.e. no visual color change); color of the M^{n+}-indicator remains unchanged. 'Reacts' indicates formation of metal free indicator. Only 9 combinations of the selected M^{n+}-indicators are shown.





Indicators and M^{n+}-Indicators		**Observe[illegible] diffe[illegible]**	
	pH 9.00	**pH [illegible]**	[illegible]
PV	blue	ligh[illegible]	[illegible]
Ni^{2+}	-	[illegible]	[illegible]
PO_4^{3-}	-	[illegible]	[illegible]
GlyP	-	reacts	-
Zn^{2+}	violet	blue	-
PO_4^{3-}	-	NR	-
GlyP	-	reacts	-
Cu^{2+}	-	-	blue
PO_4^{3-}	-	-	NR
GlyP	-	-	reacts
MX	pink	pink	pink
Ni^{2+}	yellow	yellow	-
PO_4^{3-}	NR	NR	-
GlyP	reacts	reacts	-
PR	pink	pink	pink
Ni^{2+}	purple	purple	-
PO_4^{3-}	NR	NR	-
GlyP	reacts	reacts	-
ZCN	orange	yellow orange	yellow
Zn^{2+}	dark blue	dark blue	-
PO_4^{3-}	NR	NR	-
GlyP	reacts	reacts	-

Figure S7. A picture of the whole screening process of plate 1 including the first and second step of the screening process. It includes change in colors of eight indicators (30 µM) in presence of four metal ions at pH 5.50, 7.40 and 9.00 and change in color of the selected M^{n+}-indicators (30 µM) upon addition of PO_4^{3-} and/or GlyP (10 equiv). Numbering of well plates is depicted in right.

	PLATE 1		
Plate hole number	**Constituent**	**Plate hole number**	**Constituent**
1	**PV (light green, 7.40)**	**49**	MU + **Zn^{2+}**+ PO_4^{3-} (reacts overtime)
2	PV + **Fe^{3+}** (blue-green)	**50**	MU + **Zn^{2+}**+ GlyP (reacts)

Table S3: A documentation of changes in colors observed in plate 1 (Figure S7). *

3	PV + **Fe^{3+}** + PO_4^{3-} (reacts)	**51**	MU + **Zn^{2+}** (yellow-pink, 7.40)
4	PV (yellow, **5.50**)	**52**	MU + **Zn^{2+}**+ GlyP (reacts)
5	PV + **Fe^{3+}** (light blue)	**53**	MU + **Zn^{2+}**+ PO_4^{3-} (reacts overtime)
6	PV + **Fe^{3+}** + PO_4^{3-} (NR)	**54**	**XO (pink, 9.00)**
7	PV + **Fe^{3+}** + GlyP (reacts but fading)	**55**	XO + **Fe^{3+}** (NR)
8	PV (yellow, **5.50**)	**56**	XO (light red/magenta, **7.40**)
9	PV + **Ni^{2+}** (NR)	**57**	XO + **Fe^{3+}** (NR)
10	PV (light green, **7.40**)	**58**	XO (yellow, **5.50**)
11	PV + **Ni^{2+}** (reacts but fading)	**59**	XO + **Fe^{3+}** (light pink)
12	PV + **Ni^{2+}** + PO_4^{3-} (NR, fading)	**60**	XO + **Fe^{3+}**+ PO_4^{3-} (NR)
13	PV + **Ni^{2+}** + GlyP (reacts)	**61**	XO + **Fe^{3+}**+ GlyP (NR)
14	PV (light green, **7.40**)	**62**	XO + **Ni^{2+}** (purple, **9.00**)
15	PV +**Zn^{2+}** (blue color)	**63**	XO + **Ni^{2+}**+ PO_4^{3-} (NR)
16	PV +**Zn^{2+}**+ PO_4^{3-} (NR)	**64**	XO + **Ni^{2+}**+ GlyP (NR)
17	PV +**Zn^{2+}**+ GLY (reacts)	**65**	XO + **Ni^{2+}** (purple, **7.40**)
18	PV +**Zn^{2+}**+ Fe^{3+} (initial blue color deepens)	**66**	XO + **Ni^{2+}**+ PO_4^{3-} (NR)
19	PV + **Ni^{2+}**+ Zn^{2+} (sky blue color)	**67**	XO + **Ni^{2+}**+ GlyP (NR)
20	PV + **Ni^{2+}**+ Fe^{3+} (slight color change in blue)	**68**	XO + **Ni^{2+}** (light purple, **5.50**)
21	**XB (pink, 5.50)**	**69**	XO + **Ni^{2+}**+ PO_4^{3-} (NR)
22	XB + **Fe^{3+}** (NR)	**70**	XO + **Ni^{2+}**+ GlyP (NR)
23	XB (purple, **9.00**)	**71**	XO + **Zn^{2+}** (**9.00**, NR)
24	XB + **Fe^{3+}** (NR)	**72**	blank
25	XB (purple, **9.00**)	**73**	XO + **Zn^{2+}**(**5.50**, pink)
26	XB + **Ni^{2+}** (pink)	**74**	XO + **Zn^{2+}**+ PO_4^{3-} (NR)

27	XB + + **Ni^{2+}** + GlyP (NR)	**75**	XO + **Zn^{2+}**+ GlyP (NR)
28	XB + + **Ni^{2+}** + PO_4^{3-} (NR)	**76**	XO + **Cu^{2+}** (**9.00**, NR)
29	XB (pink, **7.40**)	**77**	XO + **Cu^{2+}** (**5.50**, pink) + GlyP (NR)
30	XB + **Ni^{2+}** (NR)	**78**	**ARS (light pink, 9.00)**
31	XB + **Zn^{2+}** (pink)+ PO_4^{3-} (NR)+GlyP (NR)	**79**	ARS + **Fe^{3+}** (NR)
32	**GB (green, 9.00)**	**80**	ARS (light pink, **7.40**)
33	GB + **Fe^{3+}** (NR)	**81**	ARS + **Fe^{3+}** (NR)
34	GB (green, **5.50**)	**82**	ARS (v. light pink, **5.50**)
35	GB + **Fe^{3+}** (NR, green aggregate)	**83**	ARS + **Fe^{3+}** (pink color intensified)
36	GB + **Ni^{2+}** (**9.00**, NR)	**84**	ARS + **Ni^{2+}** (**9.00**, pink color intensified)
37	GB + **Ni^{2+}** (**5.50**, NR)	**85**	ARS + **Ni^{2+}** (**7.40**, pink color intensified)
38	GB + **Ni^{2+}** (**7.40**, NR)	**86**	ARS + **Ni^{2+}** (**5.50**, NR)
39	GB + **Zn^{2+}** (**9.00**, NR)	**87**	ARS + **Cu^{2+}** (**9.00**, pink color intensified)
40	GB + **Zn^{2+}** (**7.40**, NR)	**88**	ARS + **Cu^{2+}** (**7.40**, pink color intensified)
41	GB + **Zn^{2+}** (**5.50**, NR)	**89**	ARS + **Cu^{2+}** (**5.50**, pink color intensified)
42	**MU (pink, 9.00)**	**90**	ARS + **Zn^{2+}** (**9.00**, pink color intensified)
43	MU + **Fe^{3+}** (NR)	**91**	**ZCN (orange, 9.00)**
44	MU (pink, **7.40**)	**92**	ZCN + **Fe^{3+}** (NR)
45	MU + **Fe^{3+}** (NR)	**93**	ZCN (yellow-orange, **7.40**)
46	MU (pink, **5.50**)	**94**	ZCN + **Fe^{3+}** (NR)
47	MU + **Fe^{3+}** (NR)	**95**	ZCN (yellow, **5.50**)

48	MU + $\mathbf{Zn^{2+}}$ (**9.00**, yellow)	**96**	ZCN + $\mathbf{Fe^{3+}}$ (light pink-fades overtime)

NR: No reaction (i.e.no visual color change). 'Reacts' indicates either formation of M^{n+}-indicator or metal free indicator in presence of analyte.

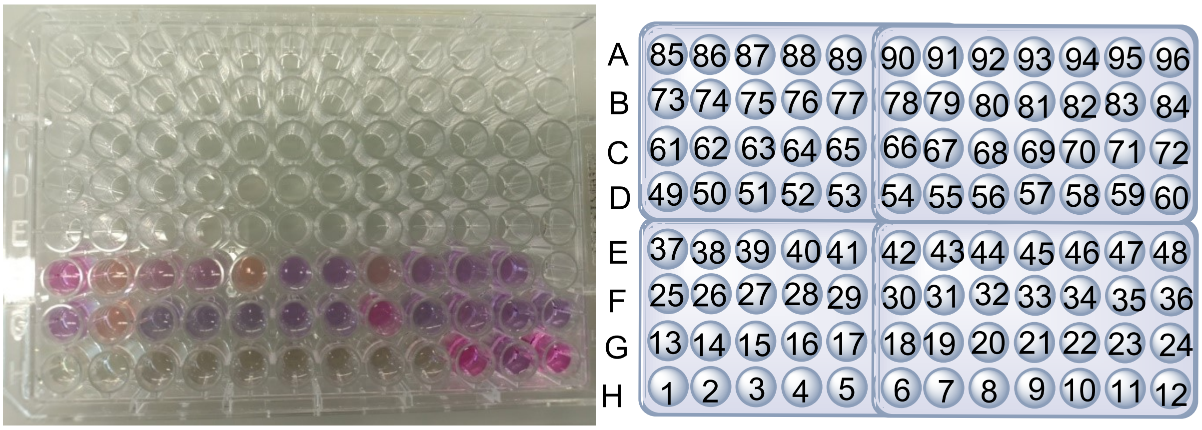


Figure S8. A picture of the whole screening process of plate 2 including the first and second step of the screening process. It includes change in colors of eight indicators (30 µM) in presence of four metal ions at pH 5.50, 7.40 and 9.00 and change in color of the selected M^{n+}-indicators (30 µM) upon addition of PO_4^{3-} and/or GlyP (10 equiv). Numbering of well plates is depicted in right.

Table S4: A documentation of changes in colors observed in plate 2 (Figure S8). *

Plate hole number	Constituent	Plate hole number	Constituent
1	ZCN + $\mathbf{Fe^{3+}}$ + PO_4^{3-} (**5.50**, NR)	**18**	PR + $\mathbf{Ni^{2+}}$ (**9.00**, purple)
2	ZCN + $\mathbf{Fe^{3+}}$ + GlyP (**5.50,** NR)	**19**	PR + $\mathbf{Ni^{2+}}$ + PO_4^{3-} (NR)
3	ZCN + $\mathbf{Ni^{2+}}$ (**9.00**, grey-blue)	**20**	PR + $\mathbf{Ni^{2+}}$ + GlyP (reacts)
4	ZCN + $\mathbf{Ni^{2+}}$ + PO_4^{3-} (NR)	**21**	PR + $\mathbf{Ni^{2+}}$ + Fe^{3+} (NR)

5	ZCN + **Ni^{2+}** + GlyP (NR)	**22**	PR + **Ni^{2+}** + Zn^{2+} (slight color change to pinkish violet)
6	ZCN + **Ni^{2+}** (**7.40**, grey-blue)	**23**	PR + **Ni^{2+}** (**7.40**, purple-pink)
7	ZCN + **Ni^{2+}** + PO_4^{3-} (NR)	**24**	PR + **Ni^{2+}** + PO_4^{3-} (NR)
8	ZCN + **Ni^{2+}** + GlyP (NR)	**25**	PR + **Ni^{2+}** + GlyP (reacts)
9	ZCN + **Ni^{2+}** (**5.50**, NR)	**26**	PR + **Ni^{2+}** (**5.50**, NR)
10	**PR (pink, 9.00)**	**27**	PR + **Zn^{2+}** (**9.00**, NR)
11	PR + **Fe^{3+}** (NR)	**28**	PR + **Zn^{2+}** (**7.40**, NR)
12	PR (pink, **7.40**)	**29**	PR + **Zn^{2+}** (**5.50**, NR)
13	PR + **Fe^{3+}** (NR)	**30**	PR + **Cu^{2+}** (**5.50**, purple)
14	PR (pink-red, **5.50**)	**31**	PR + **Cu^{2+}**+ PO_4^{3-} (NR)
15	PR + **Fe^{3+}** (blue-purple)	**32**	PR + **Cu^{2+}**+ GlyP (slow, lightens purple color)
16	PR + **Fe^{3+}** + PO_4^{3-} (NR)	**33**	PR + **Cu^{2+}** (**9.00**, purple)
17	PR + **Fe^{3+}** + GlyP (NR)	**34**	PR + **Cu^{2+}**+ PO_4^{3-} (NR)
		35	PR + **Cu^{2+}**+ GlyP (NR)

**NR: No reaction (i.e.no visual color change). 'Reacts' indicates either formation of M^{n+}-indicator or metal free indicator in presence of analyte.*

S5. References

[S1]. *Hydrology Project, Training Module, Major Ions in Water. (http://nhp.mowr.gov.in/docs/HP2/MANUALS/Water%20Quality/5014/-download-manuals-WaterQuality-WQManuals-28MajorIonsinWater.pdf).*

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[S4]. D. B. Gazda, J. S. Fritz, M. D. Porter, *Anal. Chim. Acta* **2004**, *508*, 53-59.