

*Electronic Supplementary Information (ESI)*

## **Controlled Release of Agrochemicals and Heavy Metal Ions Capture Dual-Functional Redox-responsive Hydrogel for Soil Remediation**

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## **Materials**

Sodium carboxymethyl cellulose (CMC, medium viscosity), N-Hydroxysuccinimide (NHS), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), naphthylacetic acid (NA), 6-benzyladenine (6-BA), dithiothreitol (DTT), cysteine, 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), Tris base, copper dichloride and mercuric chloride were purchased from Sigma-Aldrich, Inc. Nitric acid, hydrogen peroxide (30%), cystamine dihydrochloride (CYS·2HCl) and phosphate buffer saline (PBS, pH=6.0 and 8.0) were purchased from Alfa Aesar.

## **Preparation of CMC Hydrogel and Agrochemicals Loading**

To prepare the cellulose-based redox responsive hydrogel as carrier and stabilizer, 1 g of CMC was dissolved in 35 mL of PBS solution (pH=6) with vigorous stirring and 287 mg EDC (1.5 mmol) and 173 mg NHS (1.5 mmol) were added into the solution. Then, 5 mL of PBS solution (pH=6) containing 0.9 g of CYS·2HCl crosslinker was mixed with abovementioned solution. After mixing for 2 minutes, the mixture was transferred into a sealed vial with moisture circumstance for 1 h at room temperature. Finally, the resulting transparent hydrogel was dialyzed ( $M_w$  cutoff 8000) with distilled and deionized water which was changed every 8 h for 3 days.

The loading of agrochemicals was carried on the hydrogel which was freeze-dried first. Dehydrated gel was soaked in 150 mg/L solution of agrochemicals and the agrochemicals could be accommodated in the networks of cellulose hydrogel along with swelling process. This post-loading method was aimed at avoiding the deterioration of active auxins in complex reaction system. For the agrochemicals which do not bear amino groups and carboxyl groups (e.g. 6-BA), the agents could be added with cystamine dihydrochloride simultaneously before gelation i.e. the pre-loading method. Finally, two types of hydrogel were obtained i.e. CMC hydrogel loaded with NA (GNA) and CMC hydrogel loaded with 6-BA (GBA).

## **Entrapment efficiency and loading capacity**

Entrapment efficiency (EN%) of GNA was measured by the following process: 0.5 g of dehydrated hydrogel was soaked in the solution of NA, after the hydrogel was swelling completely, the concentration of NA in solution was measured by UV-visible spectrometer (Genesys 10-s, Thermo Electron Corporation). The entrapment efficiency of GBA was determined by the cumulative release in release experiment directly. The entrapment efficiency was calculated by Eq 1:

$$EN\% = \frac{C_o - C_t}{C_o} \times 100\% \quad (1)$$

where  $C_o$  and  $C_t$  are the initial and equilibrium concentrations of agrochemicals in solution. The loading capacity ( $q$ ) was calculated by Eq 2:

$$q = \frac{C_e \times V}{m} \quad (2)$$

where  $C_e$  is the equilibrium concentration of agrochemicals after release;  $V$  and  $m$  are the solution volume and weight of xerogel in release experiment, respectively.

### ***Characterization***

Scanning electron microscopy analysis (JEOL JSM-6400 SEM instrument, Japan) was conducted on freeze-dried, gold-coated samples to reveal the microstructures of hydrogel networks. The chemical structures bonds of redox-responsive hydrogel were characterized with Fourier transform infrared spectroscopy (FT-IR NEXUS 470 spectrophotometer, Nicolet Thermo Instruments, Canada) after being grinded with KBr. <sup>1</sup>H Nuclear Magnetic Resonance was recorded on Varian Unity INOVA 400 spectrometer (400 MHz) with the samples dissolved by deuterium peroxide.

### ***Swelling Test***

Gravimetric analysis was used to measure the swelling properties of the hydrogels<sup>1</sup>. Specifically, the freeze-dried hydrogels were immersed in deionized water first; after adequate swelling of the stabilizers, excess water on the surface was wiped away by the filter paper. The weights of dried and swollen gel were measured by analytical balance and the swelling ratio was calculated by Eq. 3:

$$\text{Swelling Ratio} = \frac{W_s - W_d}{W_d} \times 100\% \quad (3)$$

where  $W_d$  and  $W_s$  are the weights of dried and swollen gel, respectively.

### ***Characterization of Redox Responsive Behaviors***

The redox responsive behaviors were characterized with the unloaded hydrogels as-prepared above. Firstly, 1.0 mL of the mixed reactant was transferred to a vial after the addition of CYS. After the solution was no longer flowing, the hydrogel was treated with reductive DTT solution (100  $\mu$ L, 50 mM, dissolved in pH=8 PBS solution) at room temperature and kept dark for 1 h. For the regeneration of hydrogel, the solution of last step was treated with an oxidizing agent H<sub>2</sub>O<sub>2</sub> (50  $\mu$ L, 30 wt%). During those periods, the transition time and experimental phenomena were recorded.

### ***Determination of the thiol group content***

The thiol group content of hydrogels was quantified using an Ellman's test<sup>2</sup>. Briefly, 0.1 g of disjunct hydrogel (pre-treated by dialysis and lyophilization) were dispersed in 10 mL of Tris-HCl solution (0.25 mol/L of Tris base with pH adjusted to 8.3 by HCl) and then mixed with 10 mL of Ellman's reagent (0.04 g of DTNB dissolved in 1 L of Tris-HCl solution). The UV adsorption was recorded at 412 nm after 10 mins reaction. The standard curve was established by recording the UV adsorption of a series of standard solutions of cysteine; and the unmodified CMC was served as a control.

### ***Preparation of simulated soil leachate***

As an important condition for redox responsiveness, the redox potential of the flooded paddy field is from 100 ~ -300 mV<sup>3</sup>. Maximum of 50 mM DTT solution, also the frequently-used concentration in the research of drug release, was prepared for

simulating the reduce environment of flooded soil leachate. The redox potential of simulated soil leachate was -190 mV which is similar to the natural environment.

### ***Agrochemicals Releasing Experiments***

To demonstrate the agrochemicals release, the hydrogels loaded with 6-BA and NA by pre-loading and post-loading were immersed in 50 mL of simulated soil leachate with various concentrations of DDT (i.e., blank, 10 mM, 30 mM, 50 mM DTT) in PBS solution at pH=8 in dark. The concentrations of agrochemicals were dynamically measured using an UV-visible spectrometer at a wavelength of 272 nm (6-BA) and 281 nm (NA). The releasing data were fitted to Zero Order model, First Order model<sup>4</sup>, Higuchi model<sup>5</sup>, Korsmeyer-Peppas model<sup>6</sup>, Kopcha model<sup>7</sup> and Makoid-Banakar model<sup>8</sup>, their equations are listed in Table S1:

Table S1 Kinetic models for the release of agrochemicals

Model	Equation
Zero Order	$Q_t = Q_0 + K_0t$
First Order	$\ln Q_t = \ln Q_0 - K_1t$
Higuchi	$Q_t = Q_0 + K_Ht^{0.5}$
Korsmeyer-Peppas	$Q_t = K_{K-P}t^n$
Kopcha	$Q_t = At^{0.5} + Bt$
Makoid-Banakar	$Q_t = K_{M-B}t^n e^{(-ct)}$

where  $Q_0$  and  $Q_t$  are the release capacity at initial and time  $t$ ;  $K_0$ ,  $K_1$ ,  $K_H$ ,  $K_{K-P}$  and  $K_{M-B}$  are the release constants of each model;  $A$  and  $B$  are Kopcha constant and  $t$  is time;  $k$  and  $n$  are empirical parameters.

Table S2 Parameters of release models

Concentration of DTT (mM)	GBA				GNA			
	Blank	10	30	50	Blank	10	30	50
Zero Order								
$K_0=(\times 10^{-2})$	0.08	0.48	0.90	1.44	0.13	0.26	0.38	0.51
Adj. $R^2=$	0.989	0.997	0.997	0.998	0.653	0.959	0.948	0.972
First Order								
$K_1=(\times 10^{-2})$	0.38	0.48	0.83	1.23	0.32	0.65	0.89	1.16
Adj. $R^2=$	0.865	0.833	0.838	0.902	0.606	0.854	0.861	0.914
Higuchi								
$K_H=(\times 10^{-2})$	2.24	12.6	16.3	20.7	2.71	4.72	5.58	6.43
Adj. $R^2=$	0.973	0.952	0.917	0.853	0.904	0.989	0.989	0.981
Korsmeyer-Peppas								
$K_{K-P}=(\times 10^{-2})$	0.43	0.91	1.44	1.48	8.88	2.34	3.02	2.74
$n=$	0.75	0.90	0.92	0.99	0.30	0.62	0.62	0.67
Adj. $R^2=$	0.998	0.998	0.999	0.997	0.969	0.998	0.998	0.999
Kopcha								
$A=(\times 10^{-2})$	0.82	1.52	1.74	0.20	5.12	3.11	3.85	3.62

B=( $\times 10^{-2}$ )	0.13	0.42	0.81	1.41	-0.15	0.09	0.13	0.24
Adj. R <sup>2</sup> =	0.998	0.998	0.999	0.998	0.996	0.999	0.998	0.999
<b>Makoid-Banakar</b>								
K <sub>M-B</sub> =( $\times 10^{-2}$ )	0.56	0.56	0.11	1.13	3.85	2.46	2.77	3.79
n=	0.69	1.01	0.97	0.98	0.54	0.61	0.64	0.57
c=( $\times 10^{-4}$ )	-2.15	3.58	3.54	-1.99	20.8	-1.06	2.95	-15
Adj. R <sup>2</sup> =	0.998	0.997	0.998	0.998	0.998	0.998	0.998	0.997

### ***Capture of Heavy Metal Ions***

The reduced hydrogels in last section was used to test the performance on capturing heavy metal ions. 0.5 mL of the solution (reduced from 25 mg xerogel) was dropped into 10 mL of simulated soil leachate of CuCl<sub>2</sub> and HgCl<sub>2</sub> at different concentrations (from 50 to 1000 ppb) individually; and then the mixtures were stirred for 3 h to ensure the reaching of the equilibrium adsorption. The precipitations were separated by centrifugation (10000 r/min, 3 min) and the solutions were diluted by 5% HNO<sub>3</sub> for the analysis of Inductive Coupled Plasma Emission Spectrometer (ICP-ES, VISTA-MPX CCD, USA). The removal efficiency and complex capacity were calculated by Eqs. 4 and 5:

$$Removal\ Efficiency = \frac{C_e - C_0}{C_0} \times 100\% \quad (4)$$

$$Complex\ Capacity = \frac{(C_e - C_0) \times V}{M} \quad (5)$$

where  $C_0$  and  $C_e$  are the initial and equilibrium concentrations of heavy metal ion solution,  $V$  is the volume of solution and  $M$  is weight of xerogel.

### ***Adsorption isotherm***

The assumption of this Langmuir adsorption isotherm model is monolayer adsorption; adsorption can only occur in a limited number of cases; there is no lateral interaction and steric hindrance between adsorption. Langmuir isotherm model is illustrated by Eq 6:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} \cdot K_L} \quad (6)$$

where  $q_{max}$  and  $K_L$  are the Langmuir constants related to maximum adsorption capacity and equilibrium constant or energy of adsorption. The type of the Langmuir isotherm was examined by a constant  $R_L$  which is dimensionless.

$$R_L = \frac{1}{1 + C_0 K_L} \quad (7)$$

The lower the  $R_L$  value indicates that the adsorption is more favorable. Specifically, the type of isotherm to be linear ( $R_L=1$ ), irreversible ( $R_L=0$ ), favorable ( $0 < R_L < 1$ ) or unfavorable ( $R_L > 1$ ).

Freundlich isotherm is used to describe reversible adsorption. This empirical model

can be applied to multi-layer adsorption and heterogeneous adsorption on heterogeneous surfaces. Freundlich isotherm model is illustrated by Eq 8:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (8)$$

where  $K_F$  is the adsorption capacity at unit concentration and  $1/n$  is the adsorption intensity. The values of  $1/n$  indicate the type of isotherm to be unfavorable ( $1/n > 1$ ), irreversible ( $1/n = 0$ ) and favorable ( $0 < 1/n < 1$ ). Table S3 shows the parameters of Langmuir and Freundlich models for  $\text{Cu}^{2+}$  and  $\text{Hg}^{2+}$  removal.

Table S3 the parameters of Langmuir and Freundlich models.

	Langmuir		Freundlich	
$\text{Cu}^{2+}$	$K_L$	0.948	$K_F$	17.9
	$R^2$	0.999	$R^2$	0.938
	$R_L$ ( $C_0=10$ ppm)	0.091	$1/n$	0.169
$\text{Hg}^{2+}$	$K_L$	0.840	$K_F$	15.7
	$R^2$	0.999	$R^2$	0.940
	$R_L$ ( $C_0=10$ ppm)	0.106	$1/n$	0.204

### Soil remediation test

1 kg of mercury contaminated paddy mud (collected from Kunming, China) was placed in a wide-mouth glass container and immersed by 1 L of deionized water. Then the container was covered with plastic membrane to block oxygen thoroughly and was placed beside window for 7 days. The soil leachate was obtained after filtration and the amount of  $\text{Hg}^{2+}$  detected was 3.1 mg/L. In addition, the pH value and redox potential of this leachate were 6.4 and -102 mV, respectively. To determine the release behaviors and remediation effects in a complex soil environment, 1 g of xerogel was added into the container and the values of  $\text{Hg}^{2+}$  concentration, redox potential (Eh) and cumulative release of agrochemicals were measured at set interval.

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**Figures**

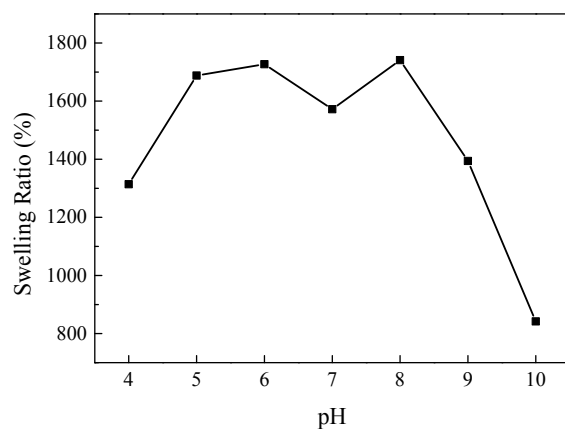


Fig S1 Effects of pH on swelling ratio of CMC hydrogel

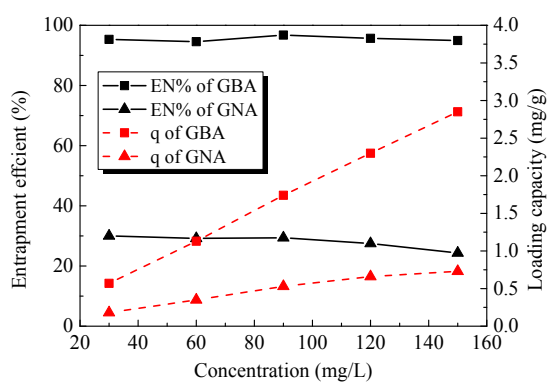


Fig S2 Entrapment efficiency and loading capacity of GBA and GNA

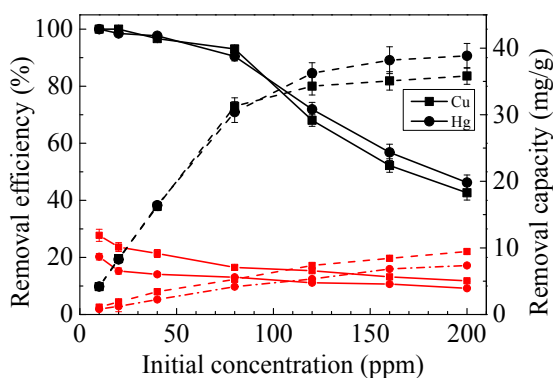


Fig S3 Removal efficiency (solid lines) and complex capacity (dash lines) of the disjunct (black) and original (red) hydrogel towards  $\text{Cu}^{2+}$  and  $\text{Hg}^{2+}$  as simulated soil leachate