Supplementary Material

Application of a newly developed o-DGT for predicting neonicotinoid insecticide (NNIs) bioavailability in soils

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Text S1. Chemicals and Reagents

This study selected pesticide standards, including Imidacloprid (IMI), Thiamethoxam (TMX), Dinotefuran (DIN), Thiacloprid (THI), and Acetamiprid (ACE). All the standards were procured from J&K Scientific Ltd. (China), with the purities ≥98%, the isotopic standard imidacloprid-D4 was purchased Cambridge Isotope Laboratories, Inc. (USA). High-performance liquid chromatography (HPLC) grade organic solvents, including methanol (MeOH), acetonitrile (ACN), and formic acid were purchased from Tedia Company (USA). All chemicals exhibited purities 98%. Laboratory-purified Milli-Q water (18.24 MΩ·cm) was generated via an in-house purification system (Millipore, Merck, USA).

The solvents used for extracting NNIs from plant tissues included formic acid and ammonia water. Formic acid and ammonia water were purchased from Anpel Laboratory Technology (Shanghai) Inc. (China) and Shanghai Aladdin Biochemical Technology Company Limited (China), respectively. Analytical grade hydrochloric acid was procured from Tianjin Comio Chemical Reagent Company Limited (China).

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Sodium citrate sesquihydrate and disodium citrate dihydrate were supplied by Shandong Keyuan Biochemical Company Limited. Octadecylsilane (C18), Primary Secondary Amine (PSA), anhydrous $MgSO_4$, and Graphitized Carbon Black (GCB, 40–120 μm) were all obtained from Shanghai Xinhu Laboratory Equipment Company Limited (China). NaCl was bought from China National Pharmaceutical Group Company Limited (China).

Text S2. The process of preparing materials for binding and diffusive gel

Preparation of 2% agarose diffusion phase: Dissolve 0.6 g agarose in 30 mL ultrapure water, heat until transparent, pour into preheated glass mold (0.8 mm gap), cool and demold, then cut with 25 mm ring cutter. Prepare AC-bound phase: Dissolve 0.9 g agarose in 30 mL ultrapure water, heat until clear. Add 0.5 g AC, mix well, pour into 0.5 mm mold. The prepared binding phase and diffusive phase were immersed in ultrapure water and maintained in a freezer at 4°C, with the water replaced three times within 24 hours to ensure complete hydration. Subsequently, the gels were preserved in a 0.01 $mol \cdot L^{-1}$ NaCl solution [1, 2].

Text S3. Soil Pretreatment and Physicochemical Properties

Prior to experimentation, all soil samples underwent air-dried and homogenized subsequently via sieving through a 2-mm mesh for the removal of root fragments and gravel, upon which NNIs stock solution and ultrapure water were added to adjust the soil moisture level reaching 25 ~ 30% of the maximum water holding capacity (MWHC). The lower spiked concentrations simulated the ambient environmental levels. To obtain results applicable to a wide range of pesticide pollution levels, a mixture of five pesticides was added to the soil to achieve final concentrations of each chemical at 0.5 $mg \cdot kg^{-1}$, 1 $mg \cdot kg^{-1}$, 3 $mg \cdot kg^{-1}$, 5 $mg \cdot kg^{-1}$ and 8 $mg \cdot kg^{-1}$ dry weight. The study incorporated a control group. Microbial activity was suppressed with 10 mM

NaN_3 prior to month-long dark incubation at controlled temperature (25 ± 1°C).

Text S4. Pot Experiment Samples

A portion of the sample soils were analyzed for bioavailable pesticide fractions, and the rest of soils were used to pot trials. *Bok choy* was selected for the experiment, with seeds purchased from the Guangzhou Vegetable Research Institute, China. Plump seeds were surface-sterilized with 10% H_2O_2 for 30 min, subsequently rinsed thoroughly with deionized water, and subsequently soaked in warm water (40°C) for 2 h until the water cooled to ambient temperature. The treated seeds were sown in the seedling trays filled with culture medium and cultivated in the light incubator. When they grew to the three-leaf stage, the seedlings with consistent growth were selected and transplanted into plastic pots filled with labeled soil. The harvested plants underwent three-times rinsed with ultrapure water to remove surface soil. After measuring their biomass, the plants were separated into shoots and roots, and stored at -20°C for later analysis.

Text. S5 Quality Control/Quality Assurance

All experimental materials were thoroughly cleaned to prevent the introduction of any NNIs that could interfere with the experimental results. All new plasticware used in the experiments, including DGT molds and water sample containers, were pre-soaked in methanol overnight and thoroughly rinsed with ultrapure water before utilization. All DGT filter membranes were washed three times with methanol followed by three rinses with ultrapure water before use. All experiments were conducted under cool conditions, and all containers were wrapped in aluminum foil to prevent light exposure, thereby avoiding the photolysis of NNIs during the experimental process. Three replicates were set up for each experiment and sample. To account for potential contamination or changes during the experimental process, blank and control tests were conducted for each experimental group. No target analytes were detected in the blanks,

and the loss of target compounds in the control groups remained within 5% (e.g., due to degradation or adsorption onto testing materials, container walls, or DGT devices). Additionally, the recovery rate was calculated. These steps collectively ensured the reliability of the experimental results.

Text. S6 solid-phase extraction (SPE)

The solid-phase extraction (SPE) was applied to clean up the extracts from soil. The HLB cartridge was conditioned sequentially with 12 mL of methanol and 12 mL of ultrapure water at a flow rate of 1 ~ 2 drops per second. When approximately 1 mL of ultrapure water remained above the sorbent bed, the sample was loaded onto the cartridge at a flow rate of 3 ~ 5 mL/min. The cartridge was then rinsed with 6 mL of ultrapure water and dried under vacuum for 20 minutes. Finally, the analytes were eluted with 12 mL of pure methanol at a flow rate of 1 ~ 2 drops per second, and the eluate was collected. The eluate was concentrated to near dryness under a gentle stream of nitrogen, reconstituted with pure methanol to a final volume of 1.0 mL, passed through a 0.22 μm aqueous-phase filter membrane into an autosampler vial, and then ready for analysis.

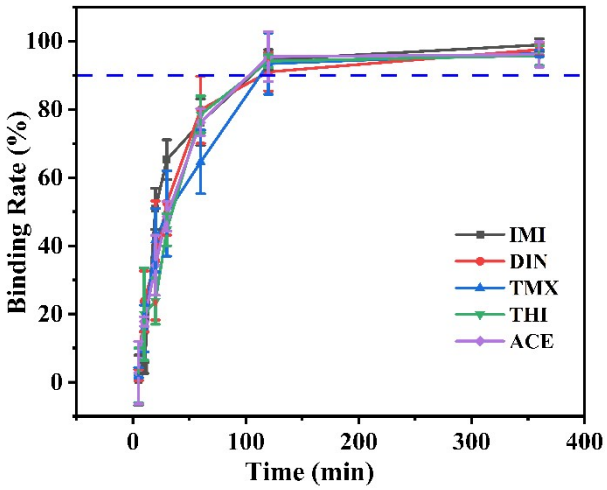

Fig. S1. Binding kinetics of five pesticides (IMI, DIN, TMX, THI and ACE) on AC binding gel in aqueous solutions. Error bars were derived from the standard deviation (SD) of three replicates.

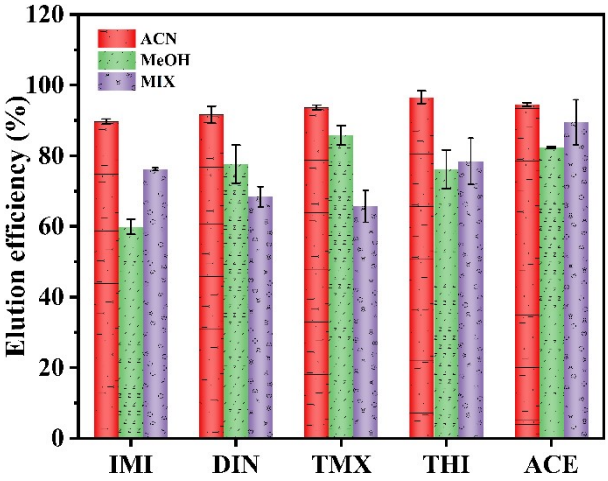

Fig. S2. The ultrasonic extraction recoveries (%) for different extraction solvents (ACN, MeOH and MIX). Error bars: SD.

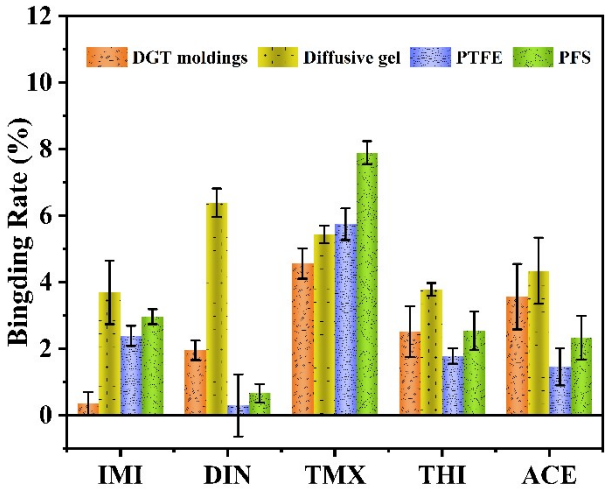

Fig. S3. Adsorption of IMI, DIN, TMX, THI, and ACE on two different filter membranes (PTFE and PES), AG (agarose diffusive gel) and DGT moldings. Error bars = SD (n=3)

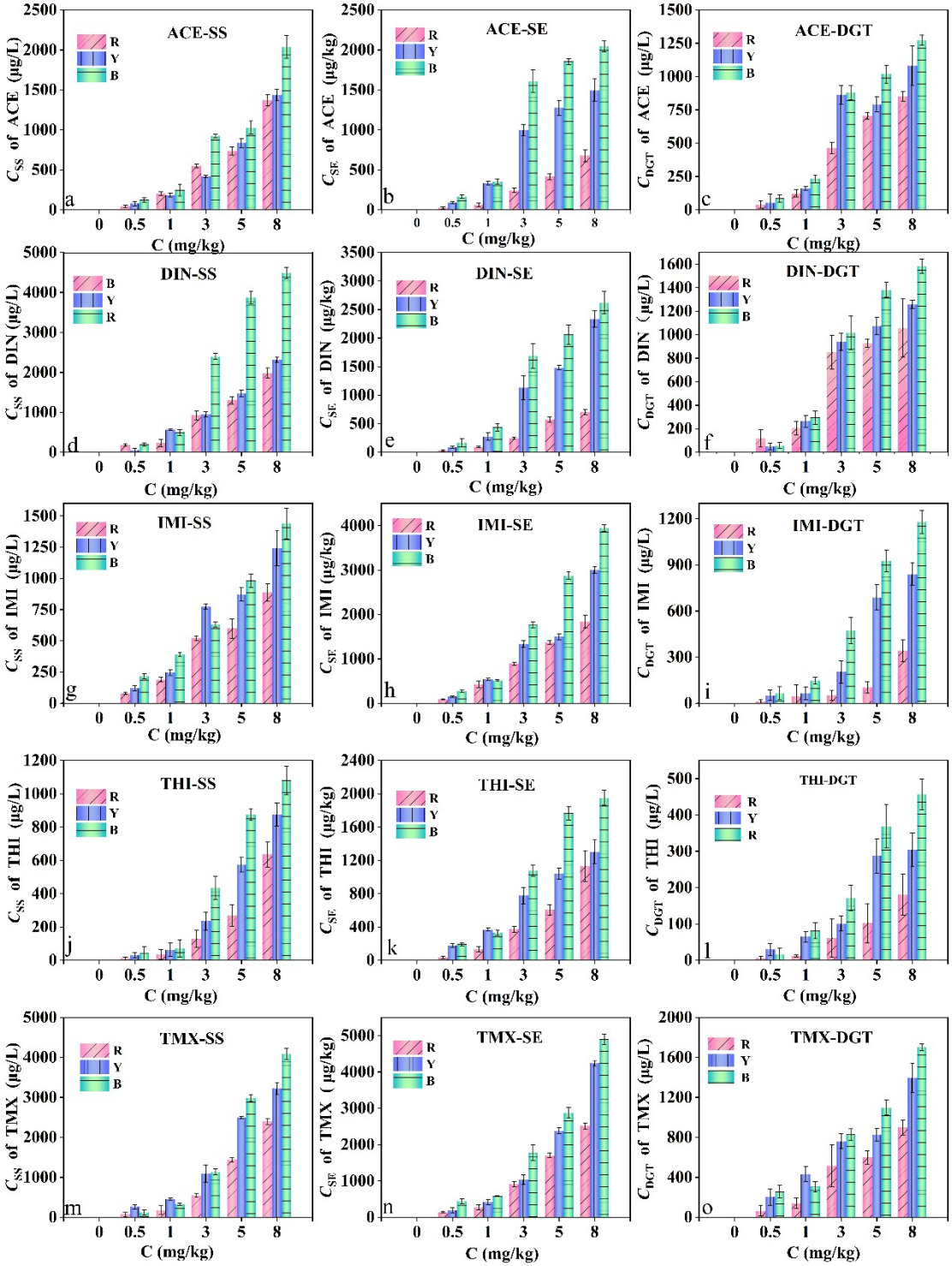

Fig. S4. The pesticide concentrations in soils. C_{DGT}, C_{SS} and C_{SE} presented the concentrations derived from AC-DGTs determination, soil solution method and solvent extraction method, respectively.

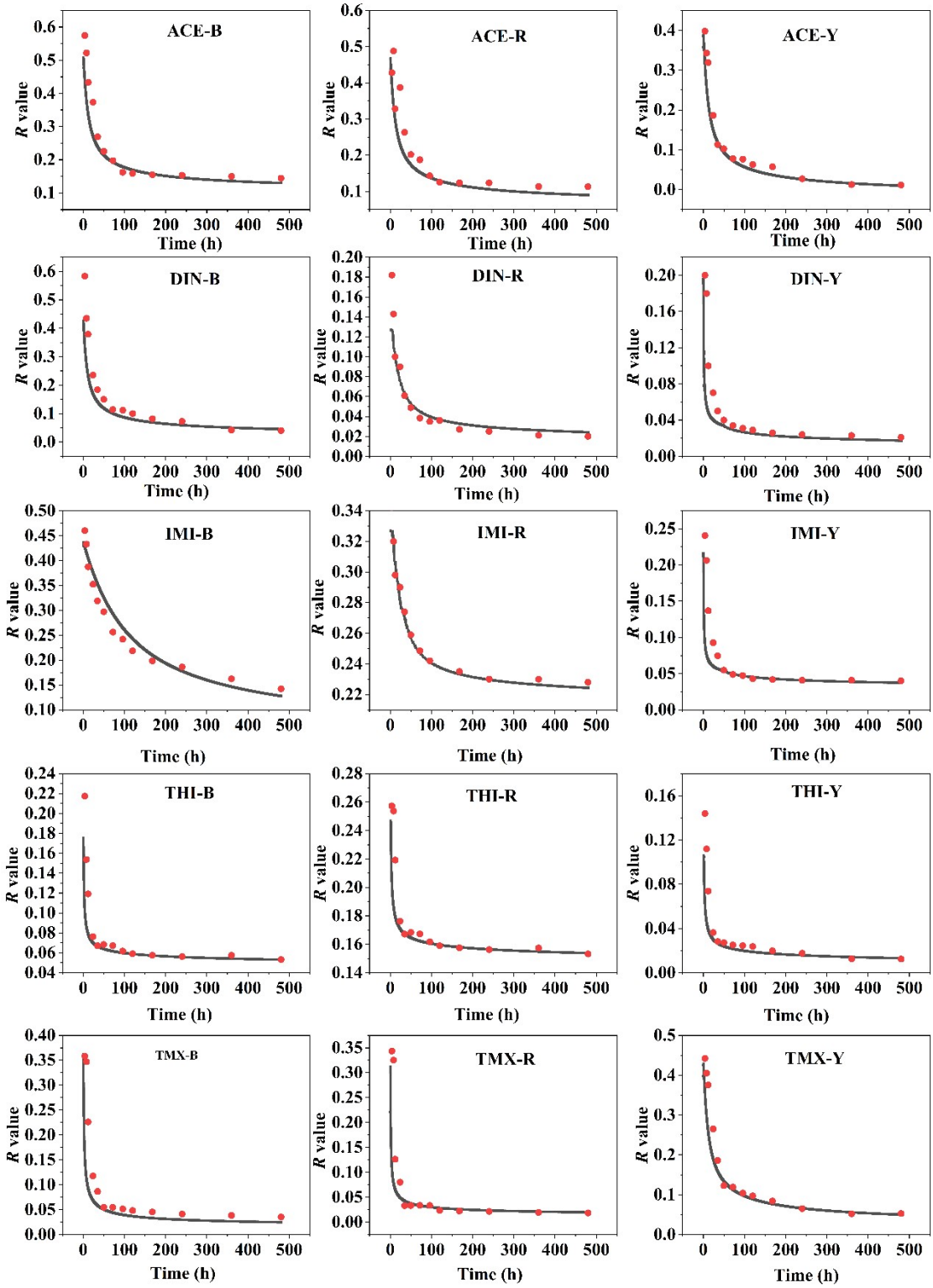

Fig. S5 The variation of measured *R* value of 5 NNIs with time and optimal fit curves using DIFS model

Table S1. Physicochemical properties of different soils

properties	Yunnan Red soil Abbreviation. R	Northeast Black soil Abbreviation. B	Shaanxi Yellow soil Abbreviation. Y
Maximum water hold capacity, MWHC (%)	41.19	35.05	33.07
Dissolved organic matter, DOM ($mg \cdot kg^{-1}$)	10.87	58.36	17.78
Cation exchange capacity, CEC	3.39	31.21	18.13
pH	5.41	7.25	7.95
Clay (%) (< 2 μm)	5.60	1.30	0.80
Silt (%) (2 ~ 50 μm)	62.10	36.40	28.00
Sand (%) (> 50 μm)	32.30	62.30	71.20

Table S2 Gradient elution procedure of the mobile phases of NNIs

Time/min	**Mobile phase A (%)**	**Mobile phase B (%)**
0	30	70
7	65	35
9	80	20
11	90	10
16	50	50
18	30	70

Table S3 Mass Analyzer Parameters

Compounds	**t_R (min)**	**Precursor Ion (m/z)**	**Product Ion (m/z)**	**Fragmentor Voltage (V)**	**Collision Energy (eV)**
Imidacloprid	6.270	256.7	209.1	105	29
			175.1		17
Acetamiprid	6.934	223.7	126	125	25
			56		17
Dinotefuran	2.997	203.2	73	65	21
			43.1		60
Thiamethoxam	4.429	292.7	211.1	90	9
			181.1		21
Thiacloprid	8.807	253.7	126	110	25
			90.1		49

Table S4 Information on the chemical properties of the NNIs, as well as the LOD and LOQ of NNIs

Compounds	Abbreviation	CAS. No.	MW	Molecular formula	soil		pore water		AC-binding gel	
					LOD (ng/g)	LOQ (ng/g)	LOD (μg/L)	LOQ (μg/L)	LOD (ng/g)	LOQ (ng/g)
Imidacloprid	IMI	138261-41-3	255.66	$C_9H_{10}ClN_5O_2$	0.133	0.439	0.443	1.463	0.032	0.156
Acetamiprid	ACE	135410-20-7	222.67	$C_{10}H_{11}ClN_4$	0.034	0.112	0.113	0.373	0.020	0.022
Dinotefuran	DIN	165252-70-0	202.21	$C_7H_{14}N_4O_3$	0.029	0.095	0.097	0.317	0.007	0.028
Thiamethoxam	TMX	153719-23-4	291.71	$C_8H_{10}ClN_5O_3S$	0.056	0.184	0.187	0.613	0.013	0.055
Thiacloprid	THI	111988-49-9	252.72	$C_{10}H_9ClN_4S$	0.071	0.234	0.237	0.780	0.021	0.069

Table S5 The dynamic adsorption/desorption parameters of NNIs in soils derived from DIFS

Soil types	Parameters	NNIs				
		IMI	THI	ACE	TMX	DIN
Black soil	K_{de}/(mL·g^{-1})	4.84	5.60	6.50	4.90	67.87
	K_d/(mL·g^{-1})	3.21	1.00	3.50	4.50	35.0
	T_c (×10^3)/s	1.77	0.99	1.30	10.95	2.07
	K_a (× 10^{-5})/s^{-1}	48.6	60.7	65.6	7.20	47.2
	K_b (× 10^{-6})/s^{-1}	75.9	394	104	18.9	8.16
Red soil	K_{de}/(mL·g^{-1})	1.76	0.61	4.67	2.63	5.97
	K_d/(mL·g^{-1})	0.86	0.50	3.50	1.80	4.50
	T_c (×10^3)/s	1.01	1.04	1.10	13.72	7.40
	K_a (× 10^{-5})/s^{-1}	43.4	17.7	53.2	5.50	8.40
	K_b (× 10^{-6})/s^{-1}	547	777	376	27.9	50.0
Yellow soil	K_{de}/(mL·g^{-1})	2.90	0.64	4.52	53.64	1.86
	K_d/(mL·g^{-1})	1.47	0.10	2.30	44.97	1.20
	T_c (×10^3)/s	3.721	1.45	1.01	0.52	8.23
	K_a (× 10^{-5})/s^{-1}	18.6	63.3	69.3	97.7	6.70
	K_b (× 10^{-6})/s^{-1}	82.1	624.8	296	13157	5.30

Note. K_{de}, soil-water partition coefficient; K_d, distribution coefficient of labile analyte; T_c, response times; K_a, adsorption rate constant; K_b, desorption rate constant.

1. G. T. Chavan, H. Kim, K. Y. Shim, Y. K. Mishra, H. Lee, J. An and K. J. J. o. H. M. A. Nam, Innovative binding gels in diffusive gradients in thin film to detect hazardous contaminants: A critical review, 2025, **17**, 100530.
2. X. H. Li, G. J. Meng, Z. B. Chang, X. X. Lian, J. H. Ma, R. C. Guo and Y. L. Wang, Development of organic-diffusive gradients in thin films technique for measuring freely dissolved concentrations of tetracyclines using a commercial SPE packing, *Ecotoxicol*, 2022, **234**, 113359.