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Supporting Information

Response of wheat (*Triticum aestivum* L. cv.) seedlings to combined effects of polystyrene nanoparticles and tetracycline

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2. Materials and methods

2.2 Interactive mechanism of PS and TC

Briefly, 0.1 g PS NPs was mixed with TC solution in a brown glass bottle, and the concentrations of TC were 5, 10, 20, 30, 40 and 50 mg L^{-1}, respectively. Each group was replicated three times. Subsequently, the isothermal experiment was performed at 298 K and 200 rpm at a reciprocating oscillator. After equilibrium, samplings were filtered by a 0.22 μm membrane, and TC concentrations were determined using high-performance liquid chromatography equipped with ultraviolet detection (Shimadzu, Japan). The temperature of the C18 column (4.6 mm × 150 mm, 4 μm, USA) was 25 °C. Potassium dihydrogen phosphate solution (0.02 mol L^{-1}) and methanol (phosphoric acid to adjust the pH value to 2.0) (80: 20) was used as the mobile phase, with a total flow rate of 1 mL min^{-1}. The standard curves were determined to calculate the concentrations of TC according to peak area response, and the correlation coefficient (R^2) ≥ 0.99. A procedural blank was routinely analyzed every 10 samples. A spiked blank was detected every 15 samples to correct the deviation in the operation of the instrument during testing. The detection limit of the instrument was 5 μg kg^{-1}.

The content of TC in supernatant and solid phase were fitted using the Langmuir and Freundlich model:

Langmuir model: $$Q_e = \frac{Q_{max} C_e K_L}{1 + C_e K_L} \quad (1)$$

Freundlich model: $$Q_e = K_f C^{1/n} \quad (2)$$

Where Q_{max} (mg g^{-1}) and Q_e (mg g^{-1}) are maximum and equilibrium uptake capacities of TC, respectively. K_L (L mg^{-1}) is an equilibrium constant and C_e (mg g^{-1})

is the equilibrium TC concentration. n (dimensionless) represents the bond distribution, which is the heterogeneity factor and K_f (mL g^{-1}) is the Freundlich constant and is related to the adsorption ability.

2.6 Physiological and biochemical analyses

2.6.1 Biomass

The Shoots and roots of wheat were washed with deionized water, dried at 105 ° C for 30 min to be fixed. Subsequently, the samples were dried at 70 ° C until constant weight. The biomass of wheat roots and Shoots was measured using a balance (JA2003N, YoKo Instrument Co. Ltd., China).

2.6.2 Determination of *Chl* content

Fresh and clean wheat Shoots (0.1 g) were ground in 1 mL distilled water, a small amount of calcium carbonate ($CaCl_2$), and silicon dioxide (SiO_2). The homogenate was extracted with 10 mL 60% acetone in dark conditions until the samples completely turned white. Subsequently, the absorbance values of the supernatant were measured at 663 nm and 645 nm using an ultraviolet spectrophotometer (Jinghua, 723PC, China), respectively.

2.6.3 Enzyme assays

The content of $O_2^{\bullet -}$ and MDA were measured by the sulfonamide colorimetric method and the thiobarbituric acid colorimetric method, respectively. The root or Shoot samples (0.1 g) were treated with phosphate buffer solution (PBS) and grounded in an ice bath under liquid nitrogen protection. The homogenate was centrifuged at 10,000 g for 20 min at 4 °C and 8,000 g for 10 min at 4 °C, respectively. For $O_2^{\bullet -}$, 500 μL

supernatant was mixed evenly with 500 μL hydroxylamine hydrochloride solution at 37℃ for 20 min in a water bath. Subsequently, the mixture was mixed evenly with 300 μL p-aminobenzene sulfonic acid solution and α- naphthylamine solution, respectively, and watered bath at 37 ℃ for 20 min again. Chloroform 500 μL was added and centrifuged at 8,000 g for 5 min at 25 °C. The 1 mL upper aqueous phase was sucked and determined the absorbance at 530 nm. For MDA, 200 μL supernatant was well-mixed with 600 μL PBS and watered bath at 95℃ for 30 min. Subsequently, the mixture cooled on the ice and centrifuged at 10,000 g for 10 min at 25 °C to determine the absorbance at 532 and 600 nm.

The content of H_2O_2 was measured by the titanium sulfate colorimetric method. The root or Shoot samples (0.1 g) were treated with acetone and grounded in an ice bath under liquid nitrogen protection. The homogenate was diluted to 1 mL with acetone and centrifuged at 8,000 g for 10 min at 4 °C. The supernatant was mixed evenly with 1000 μL acetone, 100 μL titanium sulfate and 200 μL concentrated ammonia. The mixtures were centrifuged at 4,000 g for 10 min at 25 °C. The precipitate was dissolved with 1000 μL sulfuric acid. The absorbance of the mixture was determined at 415 nm.

The activity of RuBisCO was measured by the ultraviolet absorption method. The Shoot tissue samples (0.1 g) were treated with Tris-HCl, $MgCl_2$ and ethylene diamine tetraacetic acid mixed liquid and grounded in an ice bath under liquid nitrogen protection. The homogenate was crushed by ultrasonic and centrifuged at 8,000 g for 10 min at 4 °C. 50 μL supernatant mixed evenly with 50 μL ribose-1,5-diphosphate, 450 μL triphosphate glycerate kinase and 450 μL the mixture of $NaHCO_3$ and

adenosine triphosphate, and nicotinamide adenine dinucleotide and creatine phosphate. The absorbances were determined at 20 s and after 5 min at 340 nm, respectively.

The activity of SOD and CAT was measured by the nitroblue tetrazole method and the ultraviolet absorption method, respectively. The root or Shoot tissue samples (0.1 g) were treated with PBS and grounded in an ice bath under liquid nitrogen protection. The homogenate was centrifuged at 8,000 g for 10 min at 4 °C. For SOD, 50 μL supernatant was well-mixed with 50 μL nitroblue tetrazole solution, 800 μL xanthine oxidase solution and 100 μL hypoxanthine. The mixture was strewed for 30 min at 25℃ and determined the absorbance at 450 nm. For CAT, 35 μL supernatant well-mixed with 1000 μL the mixture of H_2O_2 and PBS, and determined the absorbance at 240 nm.

Table S1 The formula of modified Hoagland nutrient solution

	Compound	Mass (g)	Concentration ($mg \cdot L^{-1}$)
A	$Ca(NO_3)_2 \cdot 4H_2O$	94.5	945
	KNO_3	60.7	607
	$NH_4 \cdot H_2PO_4$	11.5	115
	$MgSO_4 \cdot 7H_2O$	49.3	493
B	H_3BO_3	2.86	2.86
	$MnCl_2 \cdot 4H_2O$	1.81	1.81
	$ZnSO_4 \cdot 7H_2O$	0.22	0.22
	$CuSO_4 \cdot 5H_2O$	0.08	0.08
	$(NH_4)_2 \cdot 4H_2OMo_7O_{24}$	0.02	0.02
C	$FeSO_4 \cdot 7H_2O$	5.57	27.85
	Na_2-EDTA	7.45	37.25

Table S2 The isothermal model parameter of tetracycline adsorbed on polystyrene

Langmuir			Freundlich		
a	b	R^2	K_f	n	R^2
5.28	0.23	0.99	2.05	4.44	0.89

Table S3 Two-way ANOVA analysis of polystyrene (PS) and tetracycline (TC) on the physiological and biochemical indexes in wheat seedlings

Treatment		Dry weight		Malondialdehyde		$O_2^{\bullet-}$		H_2O_2		Superoxide dismutase		Catalase	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
PS	*p*	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001
TC	*p*	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001
PS*TC	*p*	0.949	0.810	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	＜0.0001	0.090	0.282	0.110	＜0.0001

Table S4 The transfer factor of TC in the wheat plant tissues affected by PS NPs

Treatment	None-PS	PS5	PS10	PS20
TC1	0.24±0.03d	0.33±0.01c	0.20±0.02d	0.36±0.03bc
TC10	0.17±0.03de	0.22±0.05d	0.22±0.02d	0.37±0.01bc
TC50	0.23±0.01d	0.40±0.04b	0.61±0.03a	0.62±0.01a

Note: Transfer factor = $\frac{TC\ content\ in\ shoot}{TC\ content\ in\ root}(dry\ weight)$.

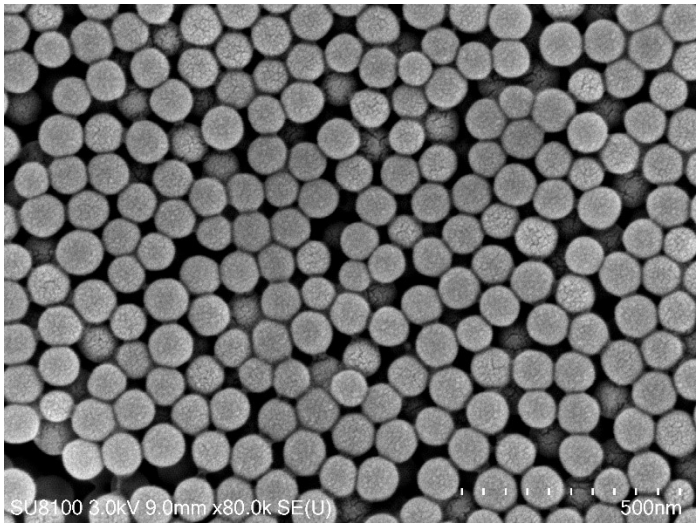

Fig. S1. Scanning electron microscope image of polystyrene nanoplastics

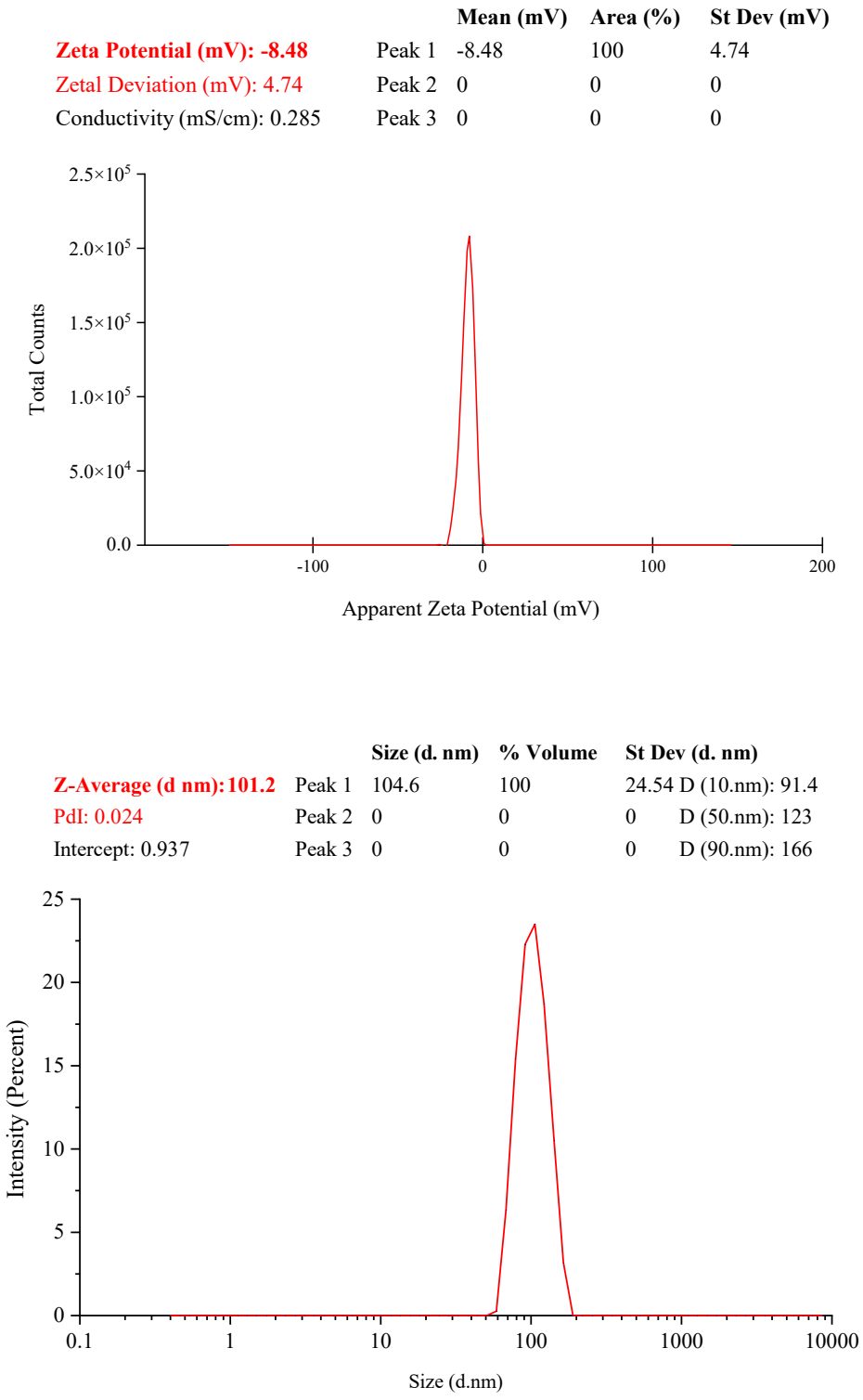

Fig. S2 Zeta potential analysis and particle size distribution of polystyrene in 1/4 Hoagland nutrition solution

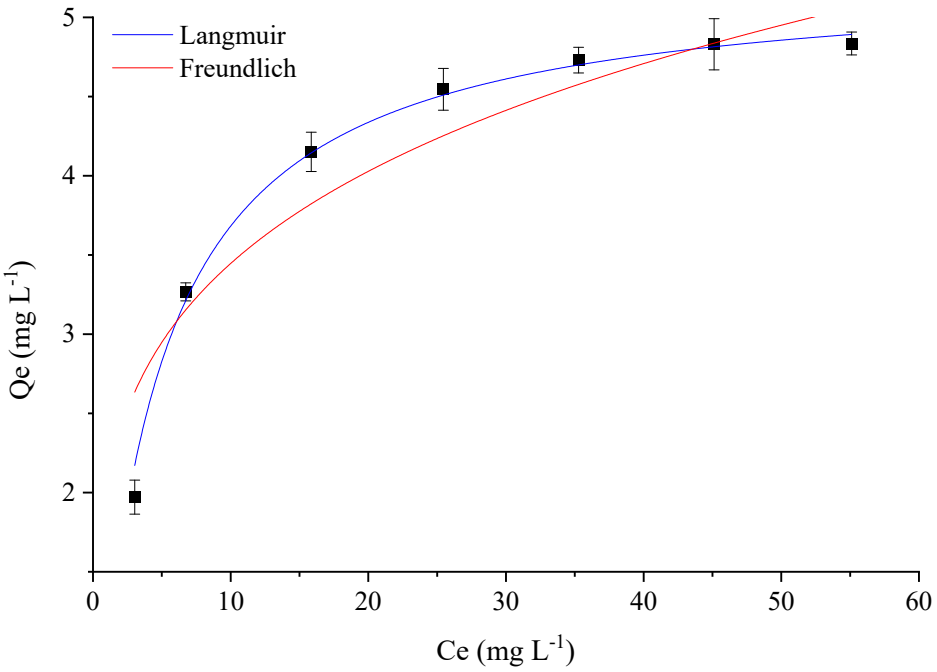

Fig. S3 The adsorption isotherms of tetracycline onto polystyrene