Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Effects of titanium oxide nanoparticles on tetracycline accumulation and toxicity in *Oryza sativa* (L.)

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Numbers of Pages: **22**

Numbers of Figures: **12**

Numbers of Tables: **6**

S1. Adsorption isothermal experiment

One and a half mg of TiO_2 NPs were weighed into 8 mL glass tubes containing 8 mL 1/2x Hoagland's solution (pH 5.7) amended with different concentrations of TC (0, 0.5, 1.0, 2.5, 5, 7.5, 10.0, 12.5, 15, 20 mg/L). The glass tubes were covered with aluminum-foil lined caps and shaken end-to-end in a rotator at 25 ºC. The suspension was sampled at day 3 (as determined by the kinetic experiment) and filtered with 0.22 µm filter. The TC concentrations were determined using HPLC (Waters 1525) equipped with a UV-detector (Waters 2487) at 355 nm with a C18 column. The mobile phase was a mixture of acetonitrile and oxalic acid (15/8, *v/v*). The mobile phase was adjusted to pH of 2 and flow rate was 1 mL/min. The triplicate was set in the experiment. The adsorption amount was calculated as the followed equation.

$$q = \frac{[(C_0 - C_t) \times V] - q_c}{W}$$

where q (mg/g) is the amount of TC adsorbed onto the NPs; C_0 and C_t is the initial and that at time t (mg/L) concentrations of TC, respectively (calculated based on the standard curve of TC); q_c (mg/g) is the amount of TC in control without sorbents (TiO_2 NPs); V (L) is the initial volume of the solution; W (g) is the weight of the NPs.

S2. Analysis for protein content and antioxidant enzyme activities

For protein content determination, plant fine powder was vigorously mixed with 10 mM Tris-HCl (pH 7.2) at a ratio of 1:10 (*w/v*). The mixture was centrifuged at 4000 rpm, 4 °C for 20 min. One hundred µL of supernatant and 1900 µL of Bradford reagent were reacted in a 2 mL centrifuge tube for 15 min at ambient temperature. The absorbance of each sample was measured at 595 nm. Detailed information for antioxidant enzyme extraction buffer, reaction buffer, reaction time, as well as wavelength, are shown in **Table S3**.

S4. TiO_2 NPs characterization as affected by TC

The hydrodynamic diameter and zeta potential of TiO_2 NPs in single analyte and co-exposure treatments were determined in 1/2x Hoagland's solution (**Figure S5** and **Table S2**). In TiO_2 NPs alone treatments, the hydrodynamic diameters decreased with increasing concentration of NPs in 1/2x Hoagland's solution. One of the possible explanations was that large aggregates might settle down before measurements. The surfaces of TiO_2 NPs were all negatively charged. Interestingly, the addition of different concentrations of TC notably decreased the hydrodynamic diameter of TiO_2 NPs regardless of the doses of TiO_2 NPs. The values of zeta potential also suggested that the presence of TC caused positive charges on the surface of TiO_2 NPs, which could be ascribed to that *pKa* value of TC is positive (3.3 – 9.7) at 25 °C. However, such alteration was only evident in 1000 and 2000 mg/L TiO_2 NPs amended 1/2x Hoagland's solution.

S5. The total protein contents in rice seedlings treated with TiO_2 NPs × TC

As shown in **Figure S11**, the presence of TiO_2 NPs and TC significantly altered the total protein contents in rice shoots and roots. For rice shoots, 2000 mg/L TiO_2 NPs alone and 20 mg/L TC alone significantly increased the total protein content by approximately 30% relative to the control. In the co-exposure scenarios, elevation of the total protein content was evident as compared to 5 mg/L and 10 mg/L TC alone treatment. However, in the 20 mg/L TC treatment, the presence of TiO_2 NPs did not further increase the protein content, which were all decreased by 18.1% relative to its TC alone treatment; these values were still significantly higher than the control. Similar to the shoots, the protein contents in rice roots were significantly increased upon exposure to TiO_2 NPs alone or TC alone, regardless of the dose. It is worth mentioning that co-exposure of TiO_2 NPs and TC resulted in decreases of the root proteins as compared to the control or the single contaminant treatment. For example, in the 10 and 20 mg/L TC treatments with 2000 mg/L TiO_2 NPs, the total protein levels were only 0.5-fold of the control, or 0.2- and 0.12-fold of the respective TC alone treatment.

A

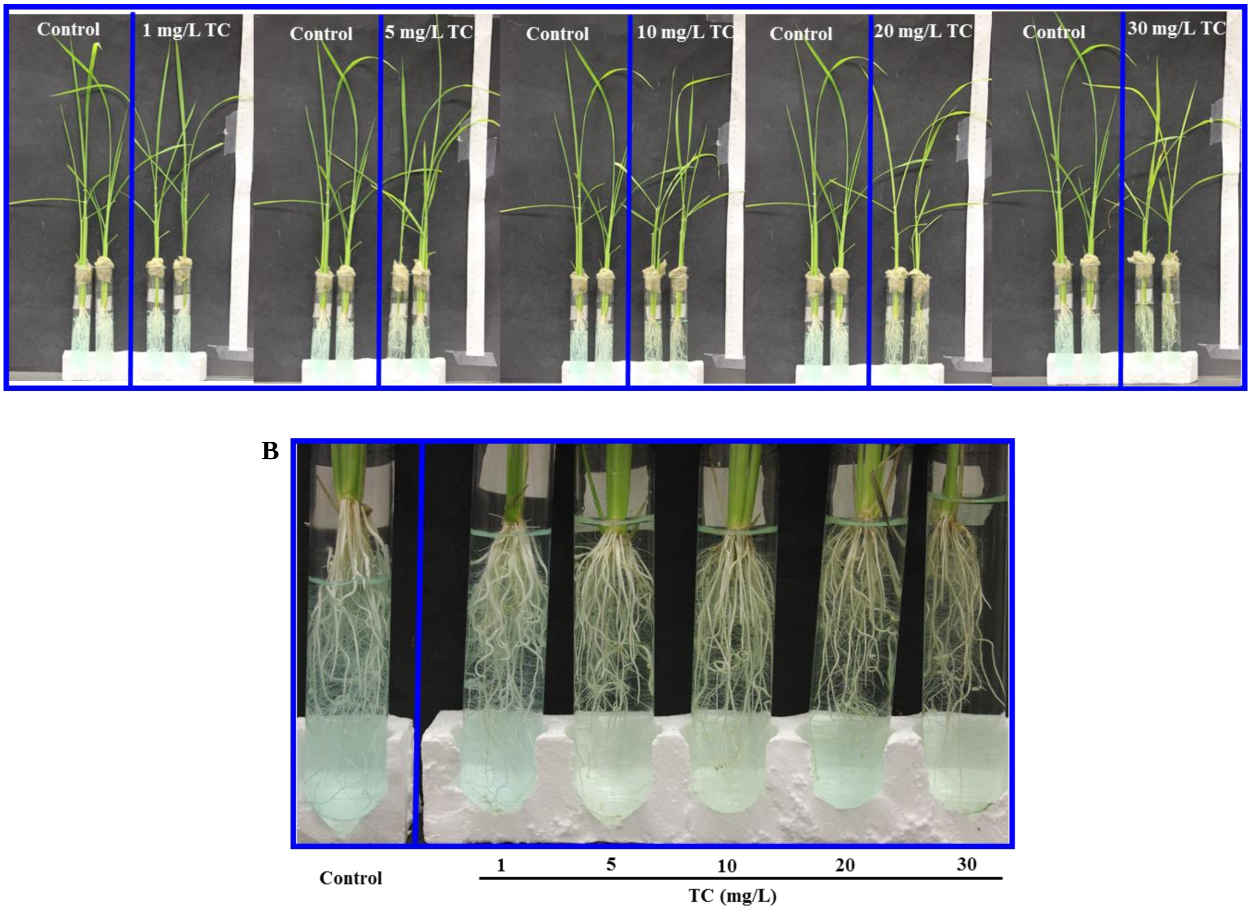


Figure S1. Phenotypic images of rice seedlings treated with different concentrations of TC in 1/2x Hoagland’s solution. (**A**) and (**B**) represent images of whole seedlings and rice roots, respectively, in response to different concentrations of TC.

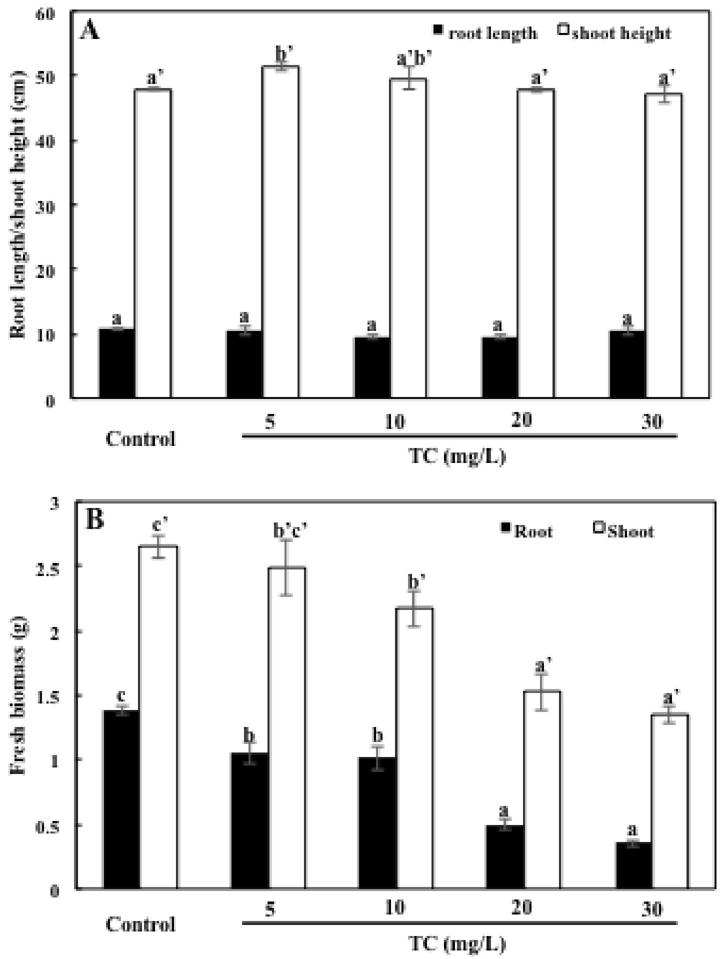


Figure S2. Seedling length and fresh biomass of different concentrations of TC treated rice. (**A**) shows root length and shoot height; (**B**) represents fresh biomass of rice shoots and roots.

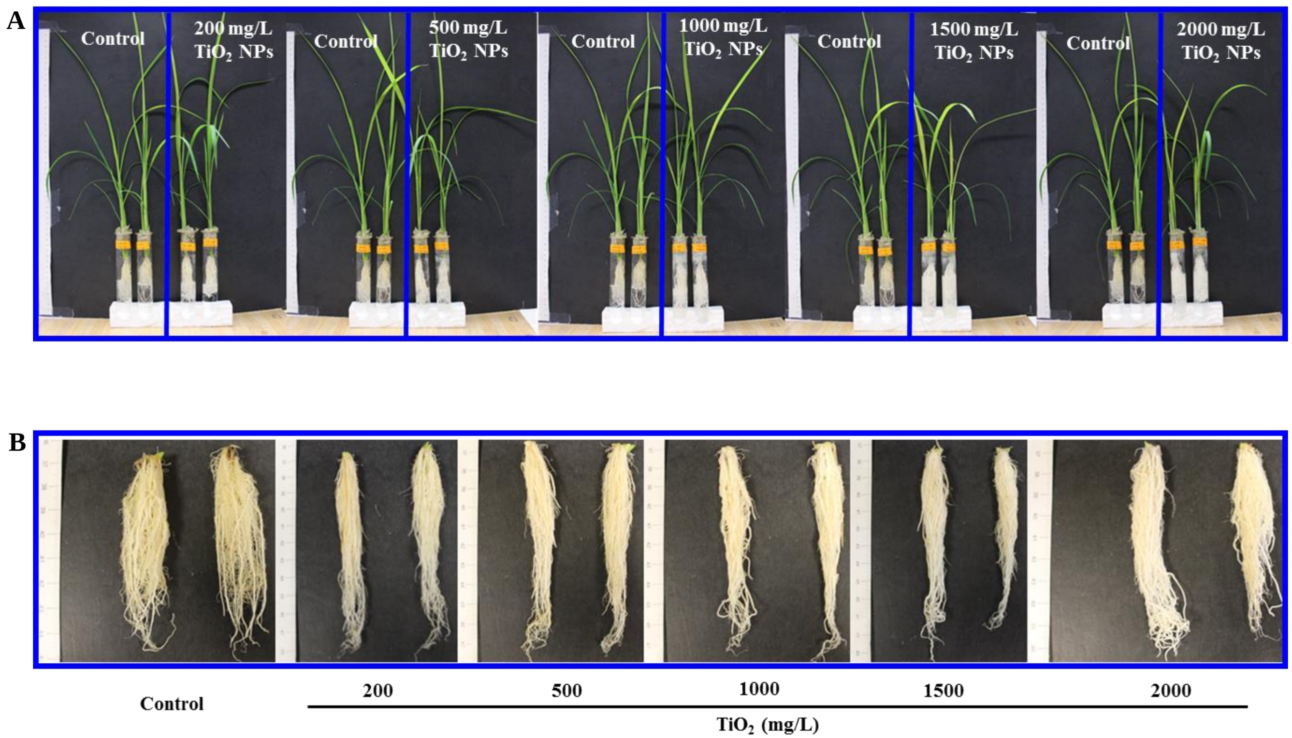


Figure S3. Phenotypic images of rice seedlings treated with different concentrations of TiO_2 NPs in 1/2x Hoagland's solution. (**A**) and (**B**) represent images of whole seedlings and rice roots, respectively, in response to different concentrations of TiO_2 NPs.

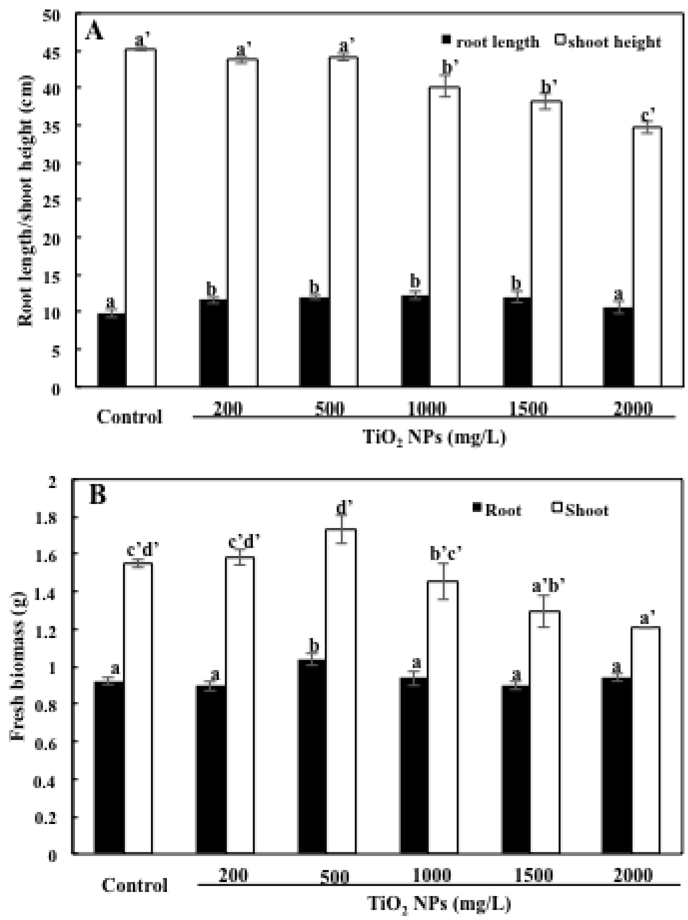


Figure S4. Seedling length and fresh biomass of different concentrations of TiO_2 NPs treated rice. (**A**) shows root length and shoot height; (**B**) represents fresh biomass of rice shoots and roots in response to TiO_2 NPs exposure.

Table S1. Interactions between TiO_2 NPs and TC in the hydroponic system

TiO_2 NPs / TC (mg/L)	A1 (500)	A2 (1000)	A3 (2000)
B1 (5)	A1B1	A2B1	A3B1
B2 (10)	A1B2	A2B2	A3B2
B3 (20)	A1B3	A2B3	A3B3

Table S2. Hydrodynamic diameter and zeta potential of TiO_2 NPs as affected by TC

TC (mg/L)	TiO_2 NPs (mg/L)	Hydrodynamic diameter (nm)	Zeta potential (mV)
0	500	521.4 ± 22.1	-15.10 ± 0.97
	1000	268.0 ± 10.9	-2.75 ± 9.23
	2000	64.5 ± 12.7	-16.08 ± 5.92
5	500	235.0 ± 17.7	-11.41 ±2.07
	1000	144.6 ± 11.3	1.87 ± 11.70
	2000	186.5 ± 21.4	-1.28 ± 4.59
10	500	264.5 ± 13.7	-10.49 ± 3.65
	1000	168.2 ± 25.8	8.73 ± 6.83
	2000	65.6 ± 7.2	19.45 ± 6.64
20	500	325.3 ± 23.5	-12.86 ± 1.35
	1000	174.1 ± 11.8	1.68 ± 7.67
	2000	99.3 ± 16.8	5.71 ± 7.85

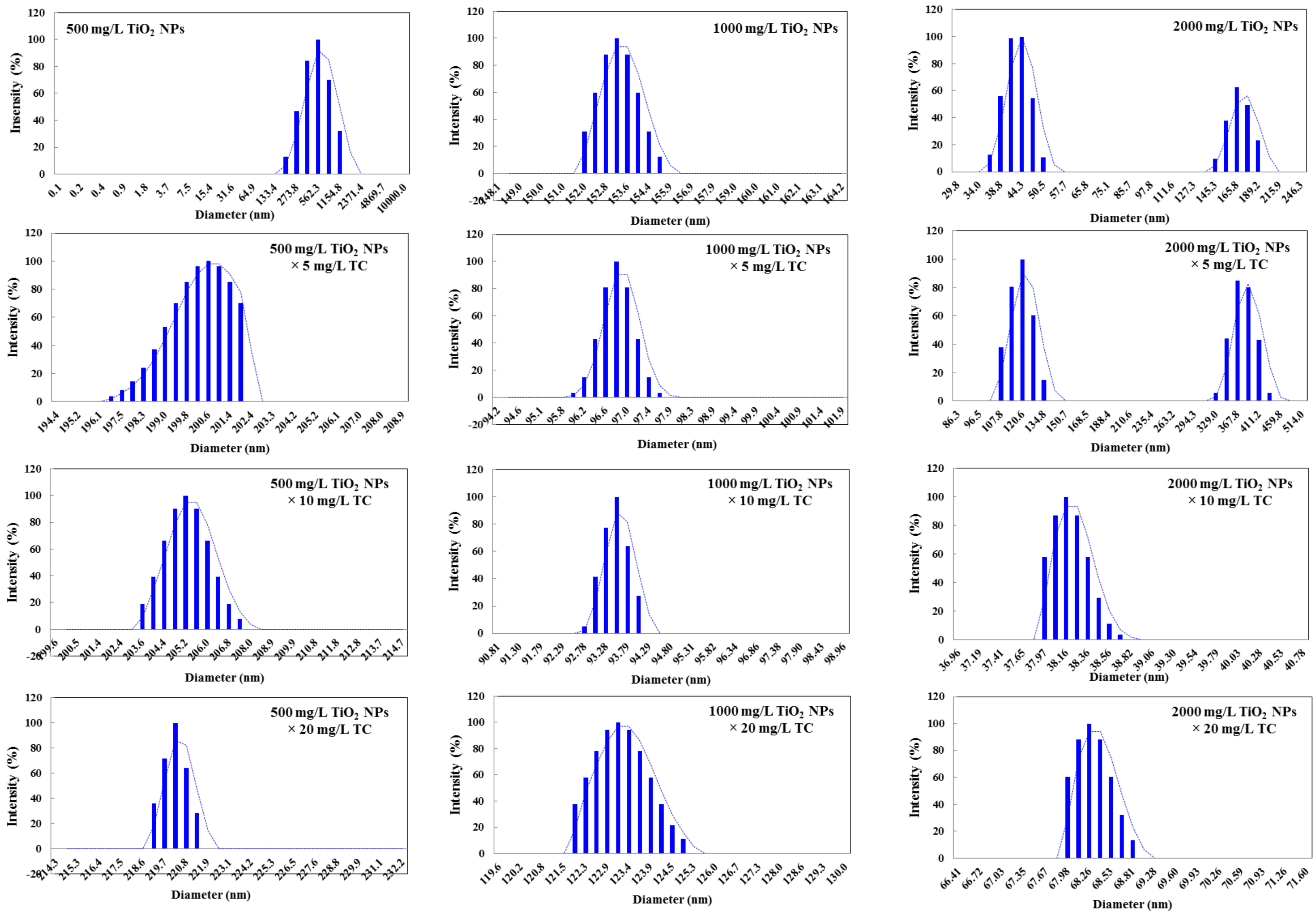


Figure S5. Particle distribution of TiO_2 NPs in different concentrations of TiO_2 NPs amended 1/2x Hoagland's solution as affected by TC.

Table S3. Extraction and reaction buffer of antioxidant enzymes

Antioxidant enzyme	Extraction buffer	Reaction buffer	Reaction time (min)	Wavelength (nm)
SOD	50 mM phosphate (pH 7.8) containing 0.1% (*w/v*) ascorbate, 0.1 % (*w/v*) bovine serum albumin (BSA), and 0.05% (*w/v*) β-mercaptoethanol	100 μL of enzyme extract + 1900 μL of 50 mM phosphate buffer (pH 7.8) containing 9.9 mM L-methionine, 57 μM NBT, 0.0044% (*w/v*) riboflavin and 0.025% (*w/v*) Triton X-100	20	560
POD	50 mM phosphate (pH 7.0) containing 1% (*w/v*) polyvinylpyrrolidone	50 μL of enzyme extract + 1.75 mL of 50 mM sodium phosphate buffer (pH 7.0) +0.1 mL of 4% guaiacol in cuvette + 0.1 mL of 1% (*v/v*) H_2O_2 to initiate the reaction	2	470
CAT	25 mM KH_2PO_4 (pH 7.4)	100 μL of enzyme extract + 1900 μL of 10 mM H_2O_2	3	240

Table S4. Methods for joint toxicity evaluation of TC and TiO_2 NPs to rice seedlings

Method	Equation	Description	Assessment
Toxicity Unit (TU)	$TUi = Ci/IC_{50}i$ $TU = \Sigma TUi$ $TU_0 = TU/max(TUi)$	Where *TUi* is the toxicity unit of the contaminant *i*; *Ci* represents the contaminant *i* caused 50% reduction of fresh biomass in the scenario of single exposure; $IC_{50}i$ represents the concentration of contaminant *i* that caused 50% reduction of fresh biomass in the scenario of co-exposure;	$TU = 1$, additive; $TU > TU_0$, antagonistic; $TU < 1$, synergistic; $TU = TU_0$, independent; $TU_0 > TU > 1$, partially additive
Additional Index (AI)	When $TU \leq 1$, $AI = (1/TU)-1$; When $TU \geq 1$, $AI = TU(-1)+1$		$AI > 0$, synergistic; $AI = 0$, additive; $AI < 0$, antagonistic
Mixture Toxicity Index (MTI)	$MTI = 1\text{-lg}TU/\text{lg}TU_0$		$MTI = 0$, independent; $0 < MTI < 1$, partially additive; $MTI = 1$, additive; $MTI > 1$, synergistic; $MTI < 0$, antagonistic

Table S5. Freely dissolved concentration of TC in the co-exposure treatments

TC (mg/L) \ TiO_2 NPs	A1 (500)	A2 (1000)	A3 (2000)
B1 (5)	4.013	3.157	1.903
B2 (10)	8.859	7.759	5.736
B3 (20)	18.775	17.561	15.174

Note: The freely dissolved concentration of TC was calculated based on the difference between the addition amount of TC and the portion adsorbed onto TiO_2 NPs, which was calculated by the fitting result of Langmuir model.

Table S6. Bioaccumulation factor of TC in rice shoots and root

Treatment	BAF in shoot	BAF in root
5 mg/L TC	9.618	119.318
10 mg/L TC	10.397	214.879
20 mg/L TC	10.860	663.690
5 mg/L TC×500 mg/L TiO_2 NPs	4.030	4.780
5 mg/L TC×1000 mg/L TiO_2 NPs	3.266	2.570
5 mg/L TC×2000 mg/L TiO_2 NPs	3.424	1.770
10 mg/L TC×500 mg/L TiO_2 NPs	2.289	7.709
10 mg/L TC×1000 mg/L TiO_2 NPs	1.485	3.042
10 mg/L TC×2000 mg/L TiO_2 NPs	1.524	1.712
20 mg/L TC×500 mg/L TiO_2 NPs	2.059	47.667
20 mg/L TC×1000 mg/L TiO_2 NPs	2.007	21.302
20 mg/L TC×2000 mg/L TiO_2 NPs	0.915	3.377

Note: BAF= $C_{\textit{TC in plant tissues}}/C_{\textit{TC in Hoagland's solution}}$

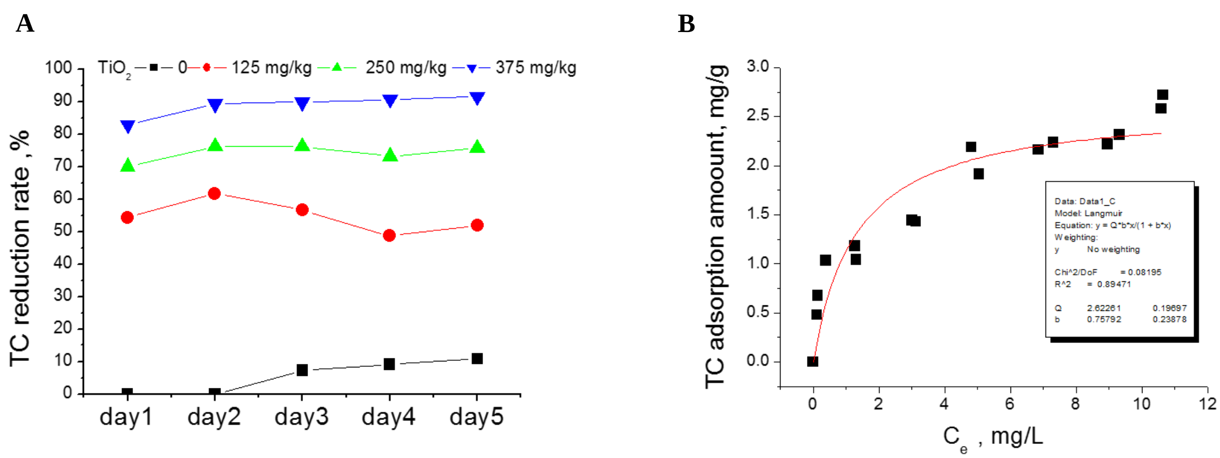


Figure S6. Interactions between TC and TiO_2 NPs in 1/2x Hoagland's solution in the absence of rice. (**A**) is TC reduction rate at ambient temperature over 5 day; (**B**) is TC adsorption amount on TiO_2 NPs.

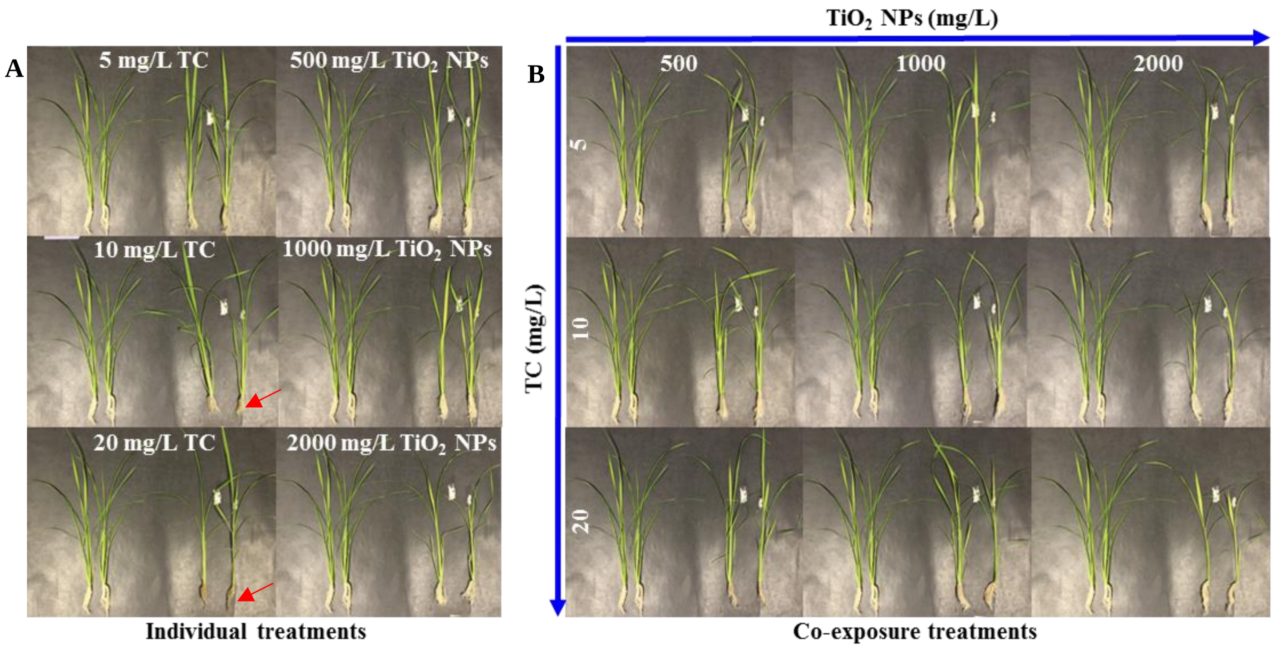


Figure S7. Phenotypic images of rice seedlings co-exposed to different concentrations of TC and TiO_2 NPs in 1/2x Hoagland's solution. (**A**) shows the images of TC alone and NPs alone treatments; (**B**) shows the images of co-contaminated treatments. In each image, two seedlings on the left-hand side are the control plants grown in 1/2x Hoagland's solution, and the ones on the right-hand side are the treated plants.

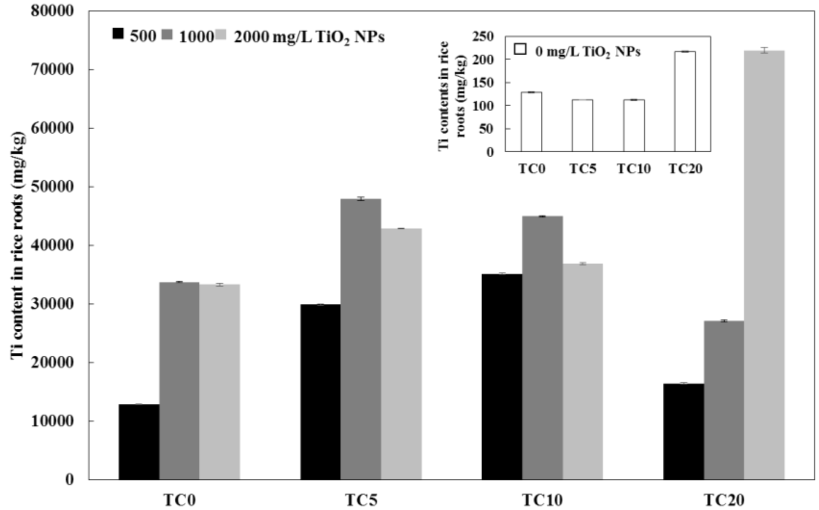


Figure S8. The contents of Ti in rice roots treated with different concentrations of TiO_2 NPs and TC.

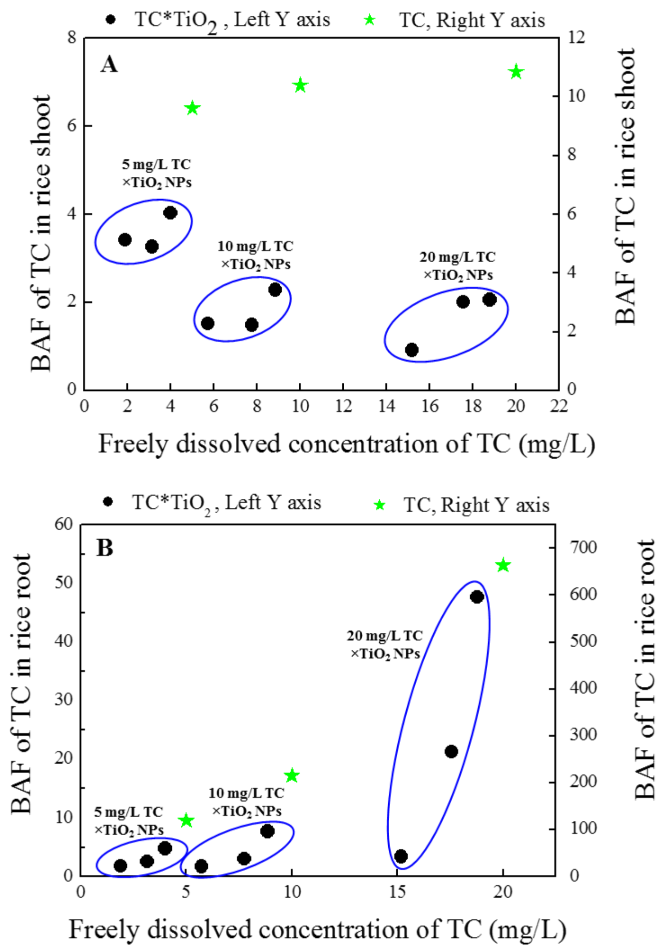


Figure S9. Bioaccumulation factor of tetracycline in rice shoots (**A**) and roots (**B**) *versus* freely dissolved concentration of TC in 1/2X Hoagland's solution.

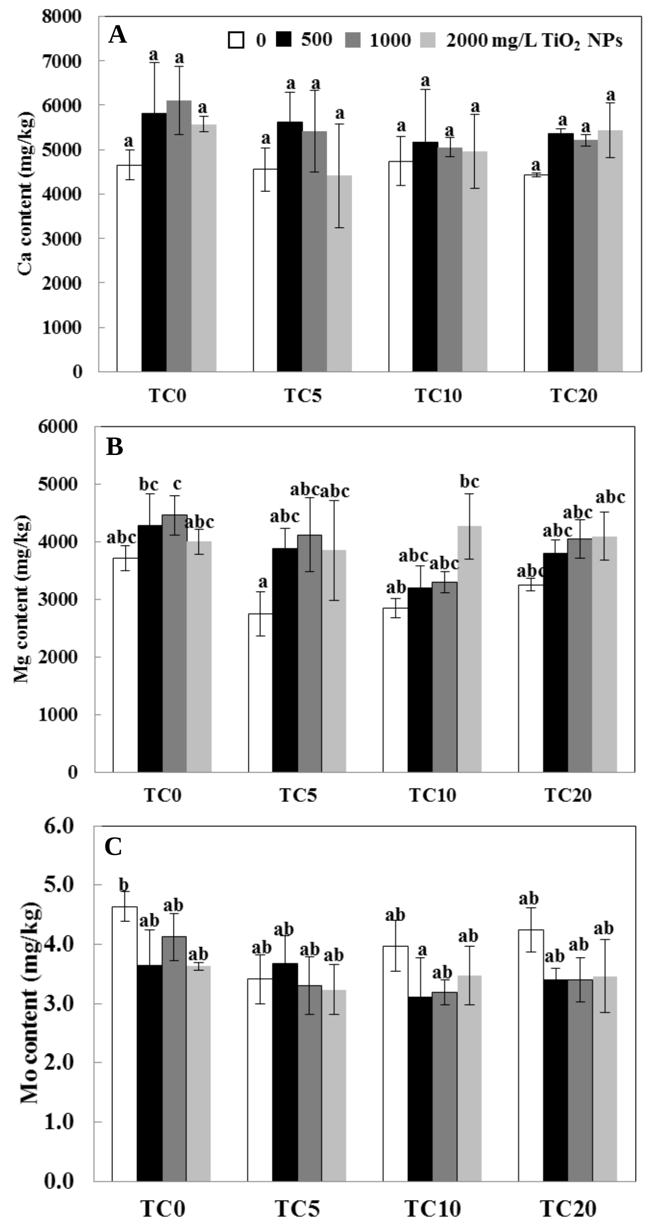


Figure S10. The contents of other nutrient elements in rice shoots co-treated with TC and TiO_2 NPs. (**A**) – (**C**) represent the contents of Ca, Mg, and Mo, respectively.

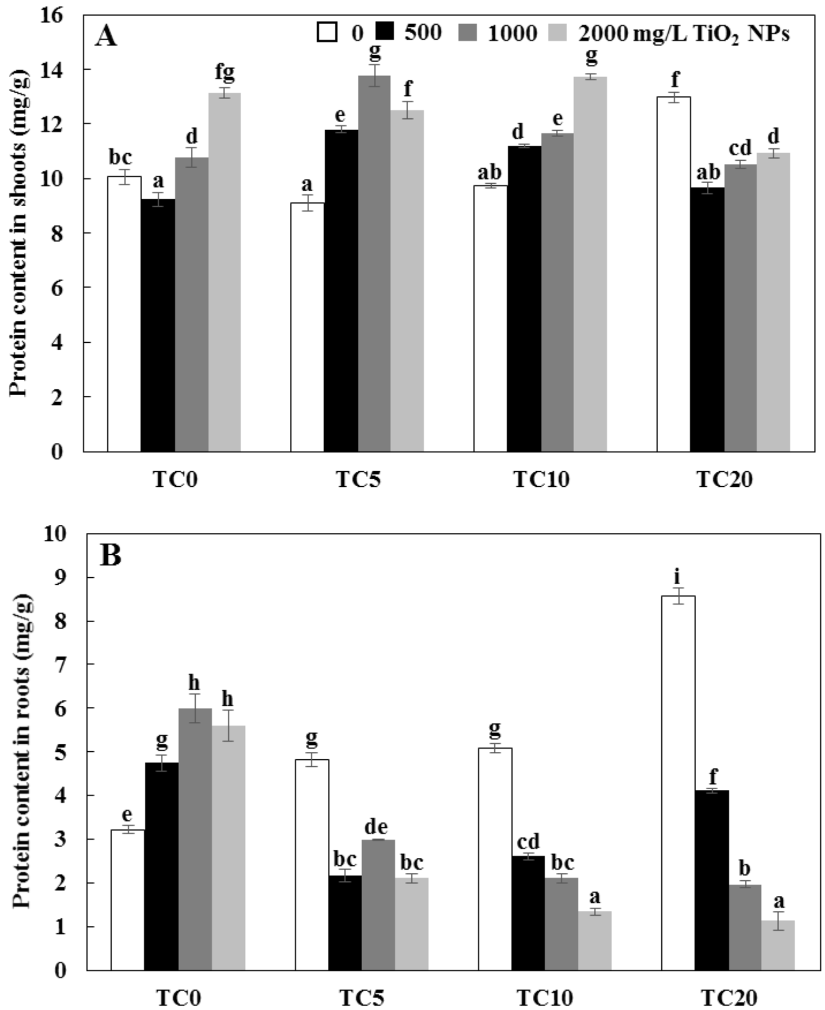
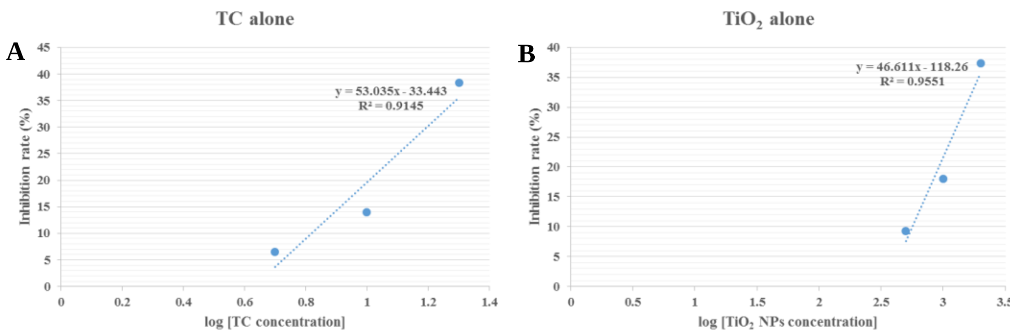


Figure S11. The total protein contents in rice shoots (**A**) and roots (**B**) upon exposure to TC and TiO_2 NPs.



Treatment	IC 50 (mg/L)
TC alone	37.41
TiO_2 NPs alone	3981
5mg/L TC×TiO_2 NPs	5211.947
10mg/L TC×TiO_2 NPs	3258.367
20mg/L TC×TiO_2 NPs	41304.75

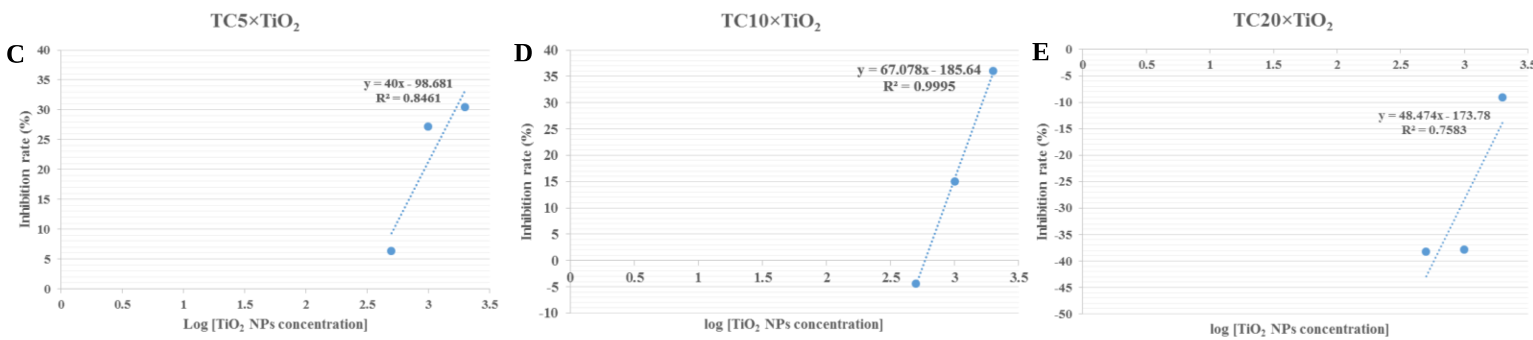


Figure S12. Inhibition rate of rice biomass among all the treatments. (**A**) and (**B**) represent biomass inhibition rate in the TC alone and TiO_2 NPs alone treatment, respectively; (**C**) – (**E**) represent biomass inhibition rate in the co-treatments when the TC concentration is at 5, 10, and 20 mg/L, respectively.