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## Elucidating the effects of TiO<sub>2</sub> nanoparticles on the toxicity and accumulation of Cu in soybean plants (*Glycine max* L.)

Yinlong Xiao<sup>a,1</sup>, Ying Du<sup>a,1</sup>, Yue Xiao<sup>a</sup>, Xiaohong Zhang<sup>a</sup>, Jun Wu<sup>a</sup>, Gang Yang<sup>a</sup>, Yan He<sup>a</sup>, Yaoyu Zhou<sup>b</sup>, Willie J.G.M. Peijnenburg<sup>c,d</sup>, Ling Luo<sup>a,\*</sup>

<sup>a</sup> College of Environmental Sciences, Sichuan Agricultural University, Chengdu 611130, PR China

<sup>b</sup> Hunan International Scientific and Technological Cooperation Base of Agricultural Typical Pollution Remediation and Wetland Protection, College of Resources and Environment, Hunan Agricultural University, Changsha 410128, China

<sup>c</sup> Institute of Environmental Sciences (CML), Leiden University, P. O. Box 9518, 2300 RA Leiden, The Netherlands

<sup>d</sup> National Institute of Public Health and the Environment, Center for the Safety of Substances and Products, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

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### ABSTRACT

Copper (Cu) pollution is common in the soil. Due to the widespread application of TiO<sub>2</sub> NPs, there is a high propensity for the co-occurrence of TiO<sub>2</sub> nanoparticles (NPs) and Cu in agricultural soils. It is therefore imperative to evaluate the joint effects of TiO<sub>2</sub> NPs and Cu on crops. In this study, the mutual effects of TiO<sub>2</sub> NPs and Cu on their toxicity and accumulation in soybean seedlings and on their fates in a hydroponic system were determined. When Cu was at levels of 1 and 2 mg/L, the co-occurring TiO<sub>2</sub> NPs at a non-toxic concentration (10 mg/L) significantly enhanced the toxicity and accumulation of Cu and Ti in soybeans, and inhibited the translocation of Cu from soybean roots to shoots. However, when the Cu concentration for co-exposure was  $\geq$  5 mg/L, such mutual effects disappeared. The amount of Cu ions adsorbed onto TiO<sub>2</sub> NPs after 48 h of co-exposure gradually increased from 31 to 118 mg/g when the Cu concentration was increased from 1 to 20 mg/L. The aggregation and sedimentation of TiO<sub>2</sub> NPs were significantly increased after 48 h of co-exposure with the Cu at a concentration higher than 5 mg/L, as compared to the single TiO<sub>2</sub> NPs exposure. The increasing aggregation and sedimentation might reduce the bioavailability of TiO<sub>2</sub> NPs associated with the adsorbed Cu to soybeans, and consequently alleviate or even neutralize the enhanced toxicity and accumulation of Cu in soybeans exerted by the co-existing TiO<sub>2</sub> NPs. Our results thus suggest that consideration of the impact of TiO<sub>2</sub> NPs on the phytotoxicity of heavy metals, and specifically Cu, needs to be interpreted with care, and highlight the importance of integrating the interaction and fates of TiO<sub>2</sub> NPs and metals into their risk assessment.

### 1. Introduction

The rapid development of nanotechnology has led to an increasing amount of manufactured nanoparticles (NPs) being unintentionally discharged into the environment. Among the manufactured NPs, TiO<sub>2</sub> NPs undoubtedly are the most widely applied, with an estimation of 2.5 million tons to be produced annually by 2025 (Chavan et al., 2020; Robichaud et al., 2009). Given the large production of TiO<sub>2</sub> NPs, these NPs are inevitably released into the environment, with agricultural soil as a primary sink. It is estimated that there are 89  $\mu$ g of TiO<sub>2</sub> NP every year to be accumulated in per kg of soil (Gottschalk et al., 2009). In the EU region, the total concentration of TiO<sub>2</sub> NPs in the soil can reach as

high as 50 mg/kg (Meesters et al., 2016). Therefore, exposure to TiO<sub>2</sub> NPs is increasingly possible for crops. Several studies have demonstrated that TiO<sub>2</sub> NPs could be accumulated and translocated in edible plants even when the primary sizes of the particles were larger than the root apoplastic barriers (Larue et al., 2012; Kurepa et al., 2010). This raises a growing concern about the threat of TiO<sub>2</sub> NPs to food safety. Furthermore, due to their unique physicochemical characteristics, TiO<sub>2</sub> NPs have a high potential to interact with other co-existing pollutants, such as the so called "heavy metals" (Wang et al., 2019; Tan et al., 2017; Fan et al., 2016a). Subsequently, the bioavailability and toxicity of the co-existing metals to exposed organisms may be altered. For instance, some studies have reported that the co-exposure of NPs significantly

\* Correspondence to: College of environmental sciences, Sichuan Agricultural University.

E-mail address: [luoling@sicau.edu.cn](mailto:luoling@sicau.edu.cn) (L. Luo).

<sup>1</sup> The first two authors have the equivalent contributions to the work.

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enhanced the accumulation and phytotoxicity of heavy metals via acting as metal carriers (Lian et al., 2020; Wang et al., 2018a). On the other hand, NPs have also shown to mitigate the uptake and toxicity of co-existing metals to plants due to adsorb metals on NPs (Rossi et al., 2018; Ji et al., 2017; Wang et al., 2015). The contradicting effects indicate that more comprehensive studies involved in the joint effects of NPs and heavy metals on edible plants are needed to better elucidate the potential risk of the simultaneous presence of NPs and heavy metals to food safety.

Copper (Cu) is a naturally occurring metal and Cu pollution is common in the environment due to both geogenic and anthropogenic activities (Cao et al., 2018; Thounaojam et al., 2012). Recently, there have been some studies finding that TiO<sub>2</sub> NPs could modulate the adverse effects of excessive Cu to a series of aquatic organisms, such as *Daphnia magna* (Fan et al., 2011; Rosenfeldt et al., 2015a, 2016), *Mytilus galloprovincialis* (Torre et al., 2015) and *Gammarus fossarum* (Rosenfeldt et al., 2015b). However, the current knowledge in terms of the joint effects of TiO<sub>2</sub> NPs and Cu to edible terrestrial plants is rather limited. To the best of our knowledge, there is only one study investigating the combined effects of TiO<sub>2</sub> NPs and Cu on edible plant, in which a mitigation of toxicity of Cu to rice by the co-exposure of TiO<sub>2</sub> NPs was reported (Wang et al., 2015).

In addition, mechanistic insights into the interactions between TiO<sub>2</sub> NPs and Cu are lacking. Adsorption of heavy metals on NPs has been proposed as a key interaction to regulate the bioavailability and subsequent toxicity of heavy metals in organisms (Sebastian et al., 2019; Sharifan et al., 2018; Ji et al., 2017; Wang et al., 2015). On the one hand, heavy metals have a strong propensity to adsorb on co-existing NPs (Rossi et al., 2017; Fan et al., 2016b). On the other hand, adsorbed metals have the potential to alter the physicochemical characteristics of NPs (e.g., hydrodynamic diameter and zeta-potential) (Sharifan et al., 2018; Wang et al., 2018b). Consequently, the fate and bioavailability of the NPs together with the adsorbed metals may be altered. However, there is a lack of studies integrating the reciprocal effects of NPs and heavy metals on their fates in the environment, whilst specifically considering the joint toxicity of NPs and heavy metals to crops. This undoubtedly hinders to uncover the possible mechanisms behind the toxicological interactions of NPs and heavy metals in the environment.

In view of that, one of the objective of this study was to explore the particular impact of the co-presence of TiO<sub>2</sub> NPs and Cu ions on the toxic effects and accumulation of this heavy metal in soybeans. Soybean was selected as it is the fifth most produced crop in the world and accounts for 30% of the global production of vegetable oil (Rossi et al., 2018). Moreover, the reciprocal effects of TiO<sub>2</sub> NPs and Cu on their fates were monitored in a hydroponic system, including assessment of the adsorption of Cu onto TiO<sub>2</sub> NPs, TiO<sub>2</sub> NPs characterization, and quantification of the residual amounts of TiO<sub>2</sub> NPs and Cu in the exposure suspensions within the incubation period, with an attempt to better understand the joint toxicity and accumulation of TiO<sub>2</sub> NPs and Cu in soybean seedlings.

## 2. Materials and methods

### 2.1. Growth analysis of soybean plants

Soybean seeds (*Glycine max* L. cv. Nandou 12) were provided by Sichuan Engineering Research Center for Crop Strip Intercropping System (China). The seeds were disinfected with 0.5% (w/v) sodium hypochlorite solution for 20 min and then rinsed thoroughly with distilled water three times. The sterilized seeds were germinated on moistened filter paper in Petri dishes. The Petri dishes were covered with tinfoil for light blocking and cultured in a growth chamber at 25 °C for 5 d. The germinated seedlings with similar growth status were transplanted into 50 mL glass beakers filled with 10% strength Hoagland solution (pH 6.8) (Hoagland and Arnon, 1950). The TiO<sub>2</sub> NPs (anatase, purity ≥ 99%) were purchased from XFNANO Materials Technology Co., Ltd (Nanjing,

China) with advertised particle size and surface area equaling 20–40 nm and 77.37 m<sup>2</sup>/g, respectively. CuSO<sub>4</sub>·5H<sub>2</sub>O was provided by the Sino-pharm Chemical Reagent Co., China. Before transplanting the seedlings, the TiO<sub>2</sub> NP and Cu stocks were introduced into the 10% strength Hoagland solutions to achieve the target exposure concentrations, as follows: individually 10 mg/L TiO<sub>2</sub> NPs and Cu at 1, 2, 5, and 20 mg/L; combinations of 10 mg/L TiO<sub>2</sub> NPs + 1 mg Cu/L, 10 mg/L TiO<sub>2</sub> NPs + 2 mg Cu/L, 10 mg/L TiO<sub>2</sub> NPs + 5 mg Cu/L and 10 mg/L TiO<sub>2</sub> NPs + 20 mg Cu/L. Soybean seedlings were transplanted into the 10% strength Hoagland solutions without the addition of TiO<sub>2</sub> NPs and Cu were regarded as the control samples in the experiment. The concentration of 10 mg/L of TiO<sub>2</sub> NPs as selected in this study did not lead to growth differences of soybean seedlings, as compared to the control treatment. There were two seedlings planted in each beaker filled with 25 mL of the amended Hoagland solution. All the seedlings were cultured in the growth chamber at 25 °C under a 6000 lux light intensity for 6 d (16: 8 h light/dark photoperiod). The beakers were sealed with perforated parafilm to avoid high evaporation while maintaining air circulation. The culture media were refreshed every 2 days. After harvesting, the roots and shoots were rinsed with distilled water three times and their lengths were measured with a ruler. After being dried for 48 h at 70 °C, the dry weights of roots and shoots were determined.

### 2.2. Accumulation of Cu and Ti in soybean seedlings

After determining the dry weight of each seedling as described above, the two seedlings in each beaker were mixed to analyze the Cu and Ti contents associated with roots and shoots. The dry roots and shoots were digested at room temperature with 4 mL of 69% (v/v) HNO<sub>3</sub> and 2 mL of 30% (w/v) H<sub>2</sub>O<sub>2</sub> overnight, and then the digests were heated at 180 °C on an electric hot plate until the digests turned achromatic or pale yellow. After cooling down to room temperature and dilution of the digests with 1% HNO<sub>3</sub> to 15 mL, the Cu and Ti contents in the digestion solution were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6300 DUO, Thermo, U.S. A.). To evaluate the reliability of the digestion method, four replicates of pure TiO<sub>2</sub> NPs were digested in the same method as mentioned above and determined by ICP-OES. Consistent results with the concentration of pure TiO<sub>2</sub> NPs were obtained.

To clarify in this study, the measured Cu and Ti contents associated with the soybean roots were defined as the sum of the metals internalized into the roots and adsorbed on the surface of roots. After rinsing the roots with distilled water, the TiO<sub>2</sub> NPs associated with adsorbed Cu might not be completely removed from the surface of roots. It has been reported that the surface adsorbed NPs can still exert adverse effects on the growth of plants by inhibiting the transportation of water and nutrients (Margenot et al., 2018; Servin and White, 2016; Dietz and Herth, 2011; Asli and Neumann, 2009). Therefore, both the internalized and adsorbed fractions of NPs were considered as being accumulated by the soybean roots in this study.

### 2.3. Characterization of TiO<sub>2</sub> NPs

Stock solutions of TiO<sub>2</sub> NPs (1000 mg/L) were freshly prepared in MilliQ water after 30 min of sonication in a water bath sonicator. Stock solutions of TiO<sub>2</sub> NPs and Cu were added into the 10% strength Hoagland medium in glass bottles to prepare the target exposure concentrations used for the growth analysis of soybeans, as depicted above. The particle size and shape of TiO<sub>2</sub> NPs in the 10% strength Hoagland medium were characterized using transmission electron microscopy (TEM, JEOL 1010, JEOL Ltd, Japan). The prepared TiO<sub>2</sub> NP suspensions were maintained in the growth chamber at 25 °C under a 6000 lux light intensity for 48 h (16 h light-8 h dark photoperiod). Within the 48 h of incubation, the hydrodynamic diameters of the TiO<sub>2</sub> NPs were determined in triplicate in the prepared culture medium at 1, 12, 24, and 48 h, respectively, by dynamic light scattering (DLS) analysis (Malvern,

Instruments Ltd., UK). At the same time points, the zeta-potential of each TiO<sub>2</sub> NP suspension was measured by the same instrument via electrophoretic light scattering. At each sampling timepoint (namely 1, 12, 24, and 48 h after preparation), a 5 mL sample of the suspension was collected carefully from the position approximately 2 cm below the surface of each suspension, and the sample was then digested by concentrated nitric acid for at least 1 d, before being subjected to ICP-OES analysis. Standard TiO<sub>2</sub> NPs solution was used to validate the digestion method. In this way, the contents of Ti remaining in the water column with varying concentrations of Cu during the 48 h incubation were monitored.

#### 2.4. Adsorption of Cu on TiO<sub>2</sub> NPs

At the sampling time point of 48 h, after drawing the 5 mL of suspension used to analyze the residual TiO<sub>2</sub> NPs in each suspension as depicted above, a 10 mL sample was drawn from the water column of each TiO<sub>2</sub> NP suspension. The samples were then centrifuged at 16,089 g for 30 min, followed by filtering through a syringe filter with a 0.02 μm pore diameter (Anotop 25, Whatman, UK). Cu contents in the filtrates were determined by means of ICP-OES. The Cu concentrations determined in the filtrates were considered as being non-adsorbed. The amount of adsorption of Cu on TiO<sub>2</sub> NPs was determined by analysis of the mass difference between the initial Cu content and the non-adsorbed Cu content. It has been demonstrated that the adsorption of heavy metals on TiO<sub>2</sub> NPs could be fitted well by the Freundlich model (Chen et al., 2012). The adsorption isotherm of Cu on TiO<sub>2</sub> NPs was therefore fitted with the Freundlich equation:

$$q_e = KC_e^{1/n}$$

In this equation,  $q_e$  and  $C_e$  are the adsorbed and non-adsorbed amounts of Cu (mg/L) after 48 h of equilibration, respectively.  $K$  indicates the adsorption coefficient (mg/g) (L/g)<sup>n</sup>, and  $1/n$  refers to the Freundlich coefficient.

#### 2.5. Statistical analysis

Statistical analysis was conducted with the SPSS 16.0 package for Windows. Two-way analysis of variance (ANOVA) was used to analyze the significance of the interaction effects between TiO<sub>2</sub> NPs and Cu on the growth characters of soybean plants, the Ti and Cu contents in soybean seedlings, as well as the translocation factors of Ti and Cu in soybean seedlings. One-way ANOVA and the  $t$ -test were applied to analyze the significance of the differences of the growth characters of soybean seedlings, the contents of Ti or Cu in soybean seedlings, the adsorption of Cu onto TiO<sub>2</sub> NPs, the hydrodynamic-diameters of TiO<sub>2</sub> NPs, and the residual of TiO<sub>2</sub> NPs remaining in the water column among different treatments. The significance level in all analysis was set at  $\alpha = 0.05$ . Except for the data of the amount of Cu adsorbed onto TiO<sub>2</sub> NPs after 48 h of equilibration, all data were expressed as mean values  $\pm$  standard deviation (SD).

### 3. Results

#### 3.1. Adsorption of Cu on TiO<sub>2</sub> NPs

The adsorption isotherm of Cu onto TiO<sub>2</sub> NPs is shown in Fig. 1. The amounts of adsorbed Cu onto TiO<sub>2</sub> NPs were gradually increased with an increase of the initial Cu concentration applied in the solutions (Fig. 1). Specifically, the amounts of adsorbed Cu onto TiO<sub>2</sub> NPs were around 31, 47, 79, and 118 mg/g at dissolved Cu concentrations of 1, 2, 5, and 20 mg/L, respectively, after 48 h of equilibration. However, the percentage of non-adsorbed Cu relative to the initial amount of Cu applied for exposure increased with increasing initial Cu concentrations (Table S1). In particular, there was around 67%, 76%, 84%, and 94% of

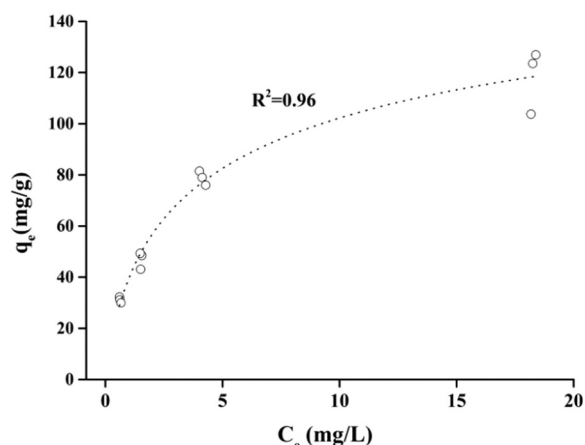


Fig. 1. The adsorption isotherm of Cu onto TiO<sub>2</sub> NPs.  $q_e$  (mg/g) is the amount of Cu adsorbed per unit weight of TiO<sub>2</sub> NPs after 48 h of equilibration, and  $C_e$  (mg/L) is the non-adsorbate Cu concentration in solution at 48 h.

Cu remaining in the supernatants after 48 h of incubation with the co-existing TiO<sub>2</sub> NPs when Cu was applied at concentrations of 1, 2, 5, and 20 mg/L, respectively.

#### 3.2. Characterization of TiO<sub>2</sub> NPs

A TEM image of TiO<sub>2</sub> NPs in the 10% strength Hoagland solution after 1 h of incubation is presented in the Supplementary information (Fig. S1). The shape and primary size of the TiO<sub>2</sub> NPs were spherical and around 23 nm, respectively. As the incubation medium of the soybean seedlings was refreshed every 48 h, the aggregation status of TiO<sub>2</sub> NPs was monitored for 48 h. The DLS results showed that the hydrodynamic diameter of TiO<sub>2</sub> NPs without the addition of Cu shifted from 374 nm after 1 h of incubation to around 1064 nm after 48 h of incubation in the culture medium (Table 1). In the presence of 5 and 20 mg Cu/L, the hydrodynamic diameters of TiO<sub>2</sub> NPs increased to about 2000 nm after 48 h of incubation, and they were significantly higher than the hydrodynamic diameter in case of no addition of Cu to the suspension ( $P < 0.05$ ) (Table 1). Additionally, the absolute value of the zeta-potential of TiO<sub>2</sub> NPs was reduced from 10 in the absence of Cu to 4 in the presence of 20 mg Cu/L after 48 h of incubation (Table 1). The absolute zeta-potential values of TiO<sub>2</sub> NPs when Cu was added at concentrations of 5 and 20 mg/L were significantly lower than the value without the addition of Cu ( $P < 0.05$ ) (Table 1).

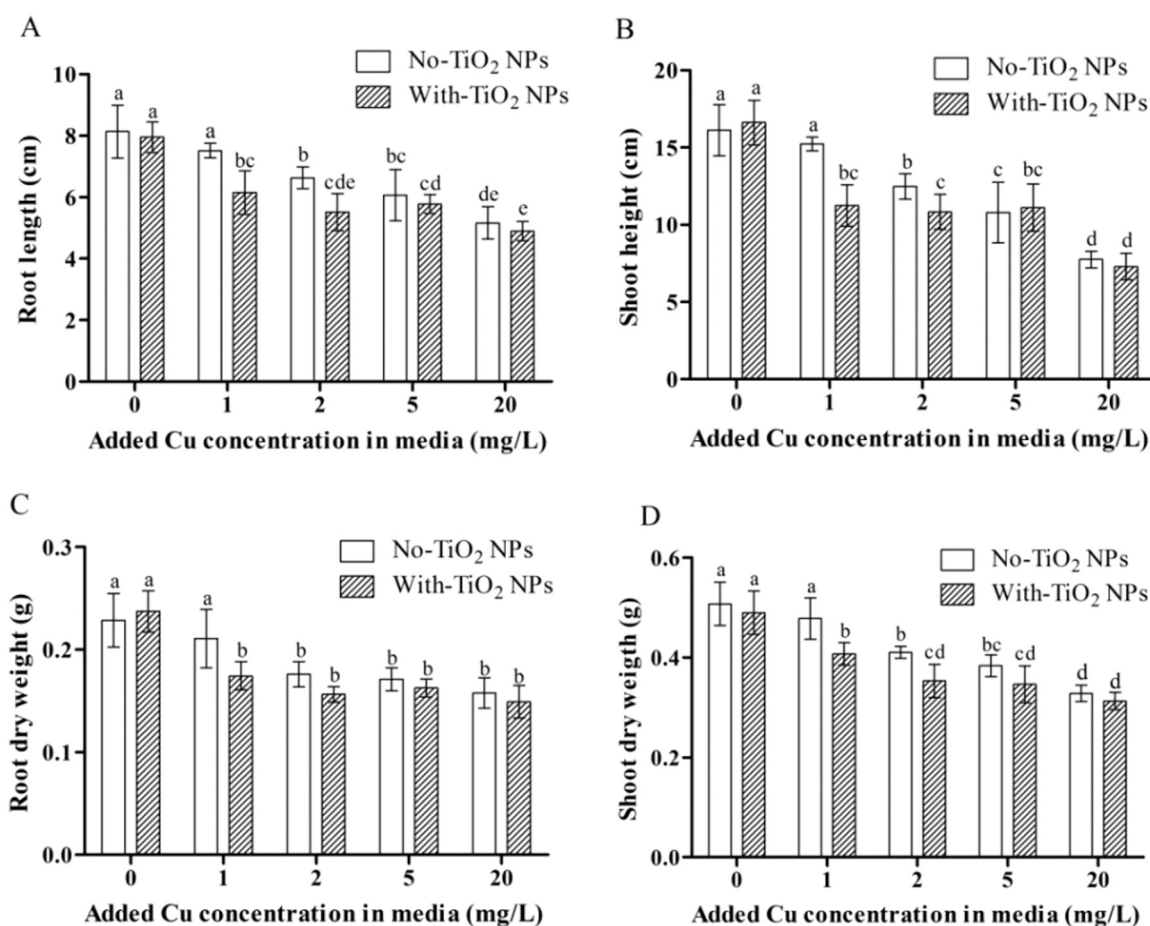
#### 3.3. Growth analysis of soybean plants

The effects of Cu on the growth characteristics of soybean plants in the presence and absence of TiO<sub>2</sub> NPs are presented in Fig. 2. Upon the co-exposure of TiO<sub>2</sub> NPs and Cu, the changes of the root length and shoot height were significantly contributed by Cu, TiO<sub>2</sub> NPs and their interactions ( $P < 0.05$ ) (Table 2). In the absence of TiO<sub>2</sub> NPs, the root length and shoot height were gradually decreased with an increase of the Cu concentration, with reductions of the root length and shoot height from 8.1 and 16.1 cm, at 0 mg Cu/L, to 5.3 and 7.7 cm at 20 mg Cu/L, respectively, after 6 days of incubation (Fig. 2A). Although the joint toxicity of TiO<sub>2</sub> NPs and Cu was mainly attributed to the impact of the heavy metal Cu (Table 2), the co-presence of TiO<sub>2</sub> NPs modulated the phytotoxicity of Cu. The specific effects of TiO<sub>2</sub> NPs on the phytotoxicity of Cu were dependent on the amount of Cu. When Cu was at levels of 1 and 2 mg/L, the presence of TiO<sub>2</sub> NPs significantly aggravated the phytotoxicity of Cu ( $P < 0.05$ ), as reflected by the 15% and 12% more reduction of the soybean root lengths and by the 27% and 12% more decrease of the shoot heights as compared to the exposure to Cu alone at 1 and 2 mg/L, respectively (Fig. 2A and B). Additionally, in the

**Table 1**  
Hydrodynamic diameter and zeta-potential of TiO<sub>2</sub> NPs upon varying Cu concentrations.

Treatment	Hydrodynamic diameter (nm) <sup>a</sup>			Zeta-potential (mV) <sup>a</sup>		
	1 h	24 h	48 h	1 h	24 h	48 h
TiO <sub>2</sub> NPs	374 ± 126	898 ± 105	1064 ± 206	-15 ± 1	-12 ± 2	-10 ± 1
TiO <sub>2</sub> NPs+ 1 mg Cu/L	410 ± 60	915 ± 84	1175 ± 274	-14 ± 1	-11 ± 1	-9 ± 1
TiO <sub>2</sub> NPs+ 2 mg Cu/L	391 ± 82	1203 ± 249	1459 ± 186	-14 ± 1	-10 ± 1	-8 ± 2
TiO <sub>2</sub> NPs+ 5 mg Cu/L	627 ± 103 *	1473 ± 151 *	1963 ± 388 *	-9 ± 2 *	-6 ± 2 *	-5 ± 1 *
TiO <sub>2</sub> NPs+ 20 mg Cu/L	848 ± 111 *	1850 ± 247 *	2275 ± 333 *	-11 ± 1 *	-6 ± 1 *	-4 ± 1 *

<sup>a</sup>Hydrodynamic diameter and zeta-potential are expressed as mean ± SD ( $n = 3$ ). (\*) Statistical differences of hydrodynamic diameter and zeta-potential were determined by one-way ANOVA, as compared to the treatment without the addition of Cu at the same incubation time ( $P < 0.05$ ).



**Fig. 2.** Root length (A) and shoot height (B) of soybean plants after exposure to different concentrations of Cu and TiO<sub>2</sub> NPs. (C) and (D) represent the dry weights of roots and shoots determined after incubation, respectively. Data are presented as mean ± SD ( $n = 6$ ). Different letters indicate significant differences among different treatments tested by one-way ANOVA and  $t$ -test ( $P < 0.05$ ).

co-presence of TiO<sub>2</sub> NPs, the shoot biomass (dry weight) was significantly reduced when Cu was at concentrations of 1 and 2 mg/L ( $P < 0.05$ ), compared to the shoot biomass of soybeans upon the treatments with Cu exposure alone. Similarly, the co-presence of TiO<sub>2</sub> NPs and Cu at a concentration of 1 mg/L significantly reduced the root biomass (dry weight) ( $P < 0.05$ ), relative to the root biomass with Cu exposure alone (Fig. 2C and D). However, when Cu was present at concentrations of 5 and 20 mg/L, there were no significant differences in growth (including the lengths and dry weights of roots and shoots) between the treatments upon the co-presence of TiO<sub>2</sub> NPs and upon the Cu exposure alone ( $P > 0.05$ ) (Fig. 2).

### 3.4. Uptake of Cu and Ti in soybean plants

Both the Cu and the Ti contents were determined in soybean roots

and shoots (Fig. 3). The two-way ANOVA results showed that the Cu content in the soybean roots was only significantly affected by the Cu concentration upon the co-exposure of TiO<sub>2</sub> NPs and Cu ( $P < 0.05$ ), while both the Cu concentration and the co-presence of TiO<sub>2</sub> NPs significantly affected the Cu content in the shoots ( $P < 0.05$ ) (Table 2). The Cu content in the roots was gradually enhanced upon increasing Cu concentration in the exposure medium (Fig. 3A). When Cu was at a concentration of 2 mg/L in the exposure medium, the Cu content in roots was significantly increased from 77.8 mg Cu/kg without the addition of TiO<sub>2</sub> NPs to 113.1 mg Cu/kg upon the addition of TiO<sub>2</sub> NPs ( $P < 0.05$ ) (Fig. 3A). However, there were no significant differences of Cu contents in roots between the treatments with and without the addition of TiO<sub>2</sub> NPs at other Cu concentrations employed in this study ( $P > 0.05$ ) (Fig. 3A). In addition, when the Cu concentrations were 1 and 2 mg/L, the Cu contents in the shoots were significantly decreased from



**Table 2**

Significance (*F*-values) of the impact of TiO<sub>2</sub> NPs, Cu concentration and their interactions on measured variables based on two-way ANOVA.

Variables	Cu	TiO <sub>2</sub> NPs	Cu × TiO <sub>2</sub> NPs
Root length	48.08**	19.59**	2.91*
Shoot height	78.85**	10.39**	6.40**
Root dry weight	20.19**	4.20 ns	1.39 ns
Shoot dry weight	30.15**	12.29**	0.93 ns
Root Cu content	294.52**	1.00 ns	1.93 ns
Shoot Cu content	83.06**	17.66**	2.17 ns
Root Ti content	19.66**	264.44**	20.74**
Shoot Ti content	15.77**	218.61**	16.72**
TF for Cu	87.21**	26.55**	8.26**
TF for Ti	0.29 ns	76.56**	0.66 ns

Significance levels: \**P* < 0.05; \*\**P* < 0.01; ns, non-significant effect; TF = Translocation factor.

18.6 and 29.5 mg Cu/kg without the addition of TiO<sub>2</sub> NPs to 11.2 and 15.0 mg Cu/kg with the addition of TiO<sub>2</sub> NPs, respectively (*P* < 0.05) (Fig. 3B). The translocation factor (TF) is defined as the ratio of the metal content in shoots to the metal content in roots. Upon the co-exposure scenario, the TF of Cu in soybean plants was significantly affected by the factors of Cu concentration, TiO<sub>2</sub> NPs, and their interactions (*P* < 0.05) (Table 2). When Cu in the solutions was at levels of 1 and 2 mg/L, the TF was significantly decreased from 40.5% and 37.9% without the addition of TiO<sub>2</sub> NPs to 19.3% and 13.3% upon co-exposure to TiO<sub>2</sub> NPs, respectively (*P* < 0.05) (Fig. 4A).

The Ti contents in the soybean roots and shoots were significantly affected by the Cu concentration, the co-presence of TiO<sub>2</sub> NPs, and by their interactions (*P* < 0.05) (Table 2). When Cu was present at concentrations of 1 and 2 mg/L in the solutions, the Ti contents in the roots

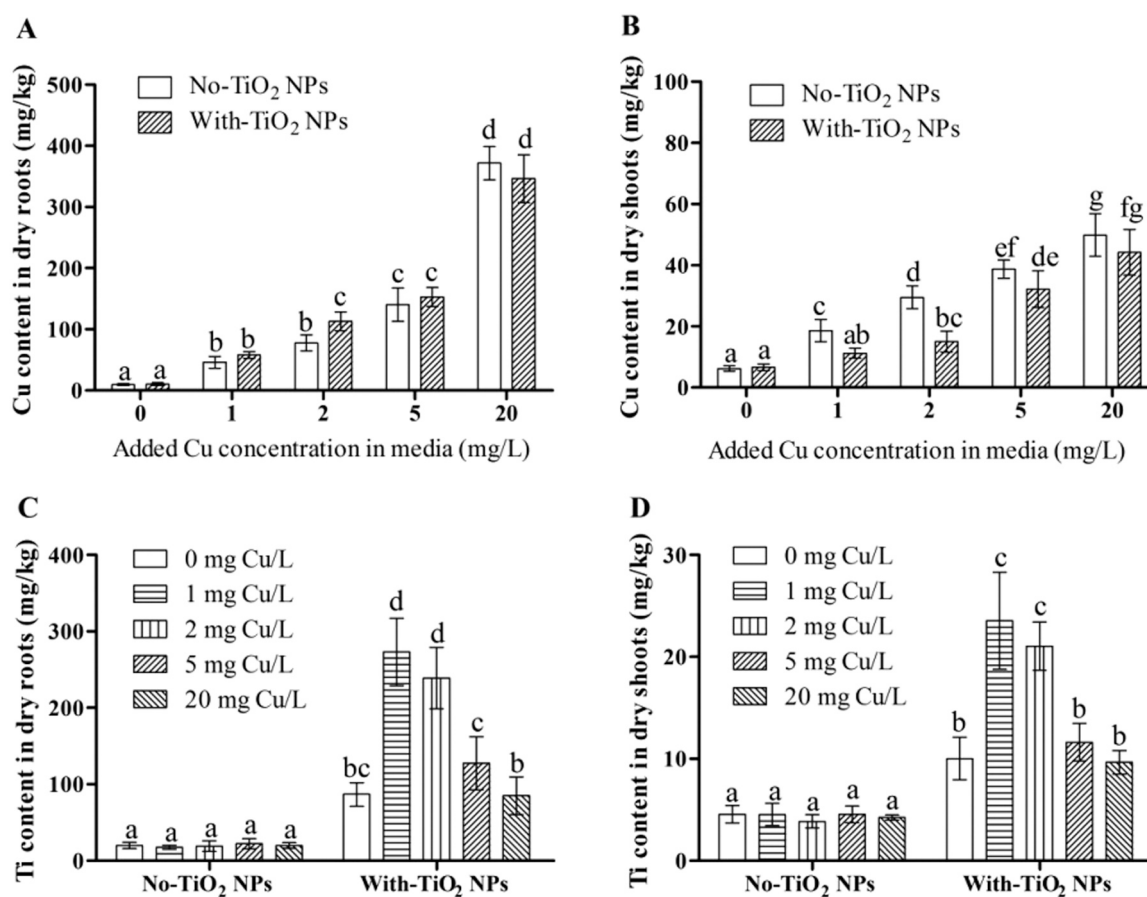
and shoots were significantly higher than those in case of addition of 0, 5, and 20 mg Cu/L in the solutions (*P* < 0.05) (Fig. 3C and D). Furthermore, the factor of Cu concentration ranging from 1 to 20 mg/L did not significantly affect the TF for Ti in the soybean plants (*P* > 0.05) (Fig. 4B).

### 3.5. Residual TiO<sub>2</sub> NPs in suspensions

The amount of TiO<sub>2</sub> NPs remaining in suspension decreased over time (Fig. 5). No matter whether Cu was co-present in the suspension, the sedimentation of TiO<sub>2</sub> NPs occurred mainly within the first 12 h of incubation (Fig. 5). Furthermore, it is worth to note that after 48 h of co-exposure the amount of residual TiO<sub>2</sub> NPs in suspension tended to be reduced with an increasing content of Cu applied in the solution (Fig. 5). As specific values, at levels of 5 and 20 mg Cu/L, there were around 28% and 22%, respectively, of the initially added amount of TiO<sub>2</sub> NPs remaining in the suspensions after 48 h of incubation. These levels were significantly lower than the residual amount of Ti without the addition of Cu to the suspension (around 41% still present in suspension after 48 h of equilibrium, *P* < 0.05) (Fig. 5).

## 4. Discussion

Even though in the current study the TiO<sub>2</sub> NPs at a concentration of 10 mg/L did not show significantly adverse effects on the growth indices (plant height and dry weight) of soybean seedlings, it was found that the TiO<sub>2</sub> NPs could aggravate the toxic effects of the co-existing heavy metal Cu on the growth of the soybean seedlings (Fig. 2). Especially when Cu was present at concentrations of 1 and 2 mg/L, the co-presence of TiO<sub>2</sub> NPs significantly aggravated the phytotoxicity of Cu, relative to the



**Fig. 3.** The Cu and Ti contents in roots and shoots of soybean seedlings in the treatments with or without the addition of 10 mg/L of TiO<sub>2</sub> NPs. Data are expressed as mean ± SD (*n* = 3). Different letters indicate significant differences among different treatments tested by one-way ANOVA and *t*-test (*P* < 0.05).

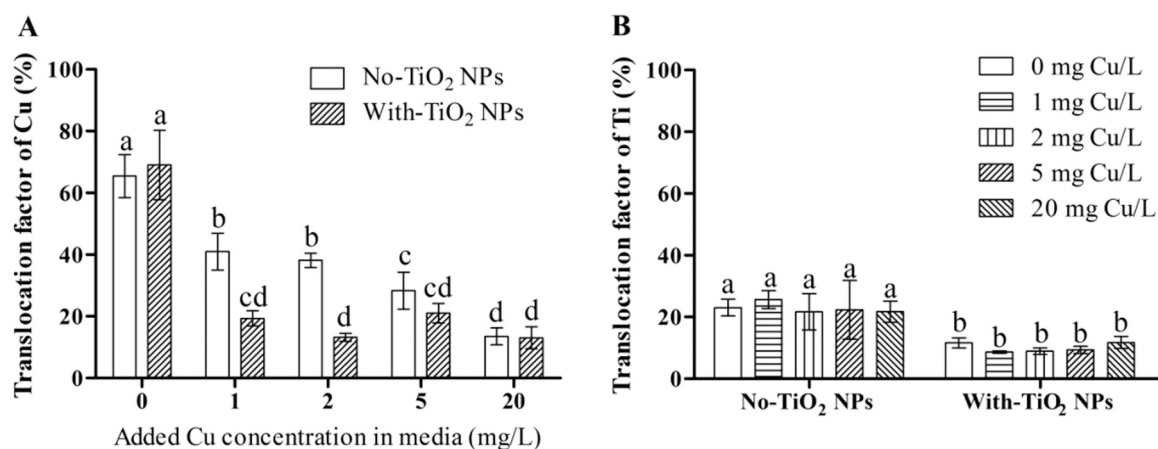


Fig. 4. Translocation factors of Cu (A) and Ti (B) in the soybean seedlings. Data are expressed as mean  $\pm$  SD ( $n = 3$ ). Different letters indicate significant differences among different treatments tested by one-way ANOVA and *t*-test ( $P < 0.05$ ).

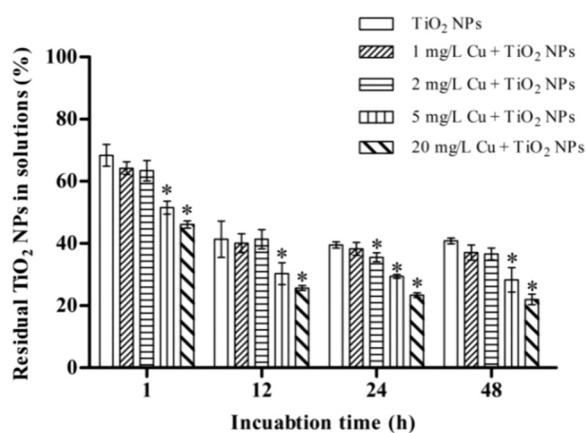


Fig. 5. Percentages of suspended TiO<sub>2</sub> NPs in the culture medium in the presence of varying amounts of Cu within the 48 h of incubation, relative to the initial TiO<sub>2</sub> NPs concentration (10 mg/L). The data are expressed as mean  $\pm$  SD ( $n = 3$ ). The black asterisks indicate a statistically different percentage of residual TiO<sub>2</sub> NPs in suspension ( $P < 0.05$ ) as compared to the control (the treatment without the addition of Cu).

phytotoxicity induced by Cu alone ( $P < 0.05$ ) (Fig. 2). Similarly, Lian et al. (2020) found that TiO<sub>2</sub> NPs (100 mg/L) significantly aggravated the toxicity of Cd to maize, with a much lower plant biomass in the co-exposure scenario than in the scenario with Cd exposure alone. The authors attributed the synergistic toxic effects of TiO<sub>2</sub> NPs and Cd to reciprocally promoting accumulation of Ti and Cd in maize plants, which subsequently induced a higher oxidative stress and synergistically aggravated the loss of plasma integrity. Fan et al. (2011) also found that TiO<sub>2</sub> NPs at a considerably safe concentration remarkably enhanced the toxicity of Cu to organisms (*Daphnia magna*). The authors attributed the aggravation of toxicity in the co-exposure of TiO<sub>2</sub> NPs and Cu both to the improved bioaccumulation of Cu in daphnids, and to the competition of TiO<sub>2</sub> NP with Cu to bind with metallothionein, which is a cysteine-rich protein and plays an important role in detoxifying excessive metals in organisms. In our study, it was also observed that the co-exposure of TiO<sub>2</sub> NPs and Cu at levels of 1 and 2 mg/L significantly promoted the contents of Ti and Cu in roots, as compared to the individual TiO<sub>2</sub> NPs or Cu exposure (Fig. 3A and C). However, the reduced accumulation of metal ions in the co-exposure of NPs in plants due to the formation of NP-metal complexes has also been widely reported by some other studies (Sharifan et al., 2020; Katiyar et al., 2020; Cai et al., 2017). Although the precise mechanisms underlying the penetration of NPs

through root apoplastic barriers (including cell walls, suberin, cutin, and casparian strip) remain unclear, an increasing number of studies have found that NPs with a primary size larger than the size exclusion limit (SELs) of plant root apoplastic barriers can still be internalized into cytoplasm (Lian et al., 2020; Ma and Yan, 2018; Larue et al., 2012). Some possible mechanisms underlying the mutually promoted accumulation of TiO<sub>2</sub> NPs and Cu in plants have been suggested. Specifically, a first aspect to be taken into account is that electrons and electron holes can be generated on the surface of TiO<sub>2</sub> NPs, which can induce the production of reactive oxygen species (ROS) (Fenoglio et al., 2009; Guo et al., 2006). Added to that, it has to be considered that electrons and electron holes generated on the surface of TiO<sub>2</sub> NPs can recombine to recover the original constitution of TiO<sub>2</sub> NPs (Fan et al., 2016a). However, the Cu ions adsorbed onto the TiO<sub>2</sub> NPs surface can scavenge the electrons released by TiO<sub>2</sub> and thus reduce the chance of recombination (Litter, 1999), leading to more electron holes formed. Consequently, there would be increasing ROS generated when Cu ions and TiO<sub>2</sub> NPs are co-existing (Chen et al., 1997). ROS have the potential to loosen plant cell walls by unspecific cleavage of polysaccharides (Fry, 1998), which may allow TiO<sub>2</sub> NPs and Cu to penetrate into roots more easily, and further induce a higher accumulation of Ti and Cu in plants. In our study, the adsorption of Cu ions onto TiO<sub>2</sub> NPs was monitored, finding that the percentage of Cu adsorbed onto TiO<sub>2</sub> NPs, relative to the initial Cu concentration applied in solutions, was decreased with increasing initial concentrations of Cu (Table S1), while the contents of Cu adsorbed onto TiO<sub>2</sub> NPs were gradually increased with increasing initial Cu concentrations (Fig. 1). Accordingly, the increasing adsorption of Cu on TiO<sub>2</sub> NPs may result in the phenomenon of promoting the penetration of Ti together with the adsorbed Cu into the roots when Cu was at levels of 1 and 2 mg/L (Fig. 3A and C). Also relevant, the transportation efficiency from roots to shoots of the heavy metals adsorbed onto NPs would be reduced (Rossi et al., 2019, 2018; Cai et al., 2017). This is consistent with the results indicating that the co-exposure of TiO<sub>2</sub> NPs (when Cu was at levels of 1 and 2 mg/L) significantly reduced the translocation factor for Cu in soybean plants ( $P < 0.05$ ) (Fig. 4A). However, our results were not fully in agreement with the interpretation that Cu ions adsorbed onto TiO<sub>2</sub> NPs would allow more Ti and Cu to penetrate in roots. In fact, when Cu was present at 5 and 20 mg/L, the co-presence of TiO<sub>2</sub> NPs did not consistently result in a significantly higher amount of Ti and Cu contents in the soybean roots, as compared to the individual exposure of TiO<sub>2</sub> NPs or Cu ( $P > 0.05$ ) (Fig. 3A and C). This is in line with the finding that there was no statistically significant differences of phytotoxicity induced by Cu at 5 and 20 mg/L upon co-exposure with the TiO<sub>2</sub> NPs as compared to the treatments of Cu exposure alone ( $P > 0.05$ ) (Fig. 2).

To further interpret the joint toxicity and accumulation of TiO<sub>2</sub> NPs

and Cu in soybeans, the reciprocal effects of Cu and TiO<sub>2</sub> NPs on their fates were monitored. In this study, at a high concentration of Cu ( $\geq 5$  mg/L), the Cu adsorbed onto the TiO<sub>2</sub> NPs significantly enhanced the zeta-potential of the TiO<sub>2</sub> NP suspension, as compared to other treatments including the suspensions with the TiO<sub>2</sub> NPs alone and with the co-exposure of the NPs and Cu at 1 and 2 mg/L ( $P < 0.05$ ) (Table 1). This resulted in reduction of the electrostatic repulsion between TiO<sub>2</sub> NPs and improvement of the aggregation of TiO<sub>2</sub> NPs. Consequently, the sedimentation of TiO<sub>2</sub> NPs at a relatively high concentration of co-existing Cu ( $\geq 5$  mg/L) was higher. Similarly, Wang et al. (2019) found that only when the co-existing Pb<sup>2+</sup> was at a level higher than 2 mmol/L, the aggregation of TiO<sub>2</sub> NPs (800  $\mu$ g/L) was evidently promoted. This indicates that only when the concentration of counter-ion exceeds a certain level can evidently promote the aggregation of NPs. In this study, a high level of Cu (5 and 20 mg/L) induced a higher extent of sedimentation of TiO<sub>2</sub> NPs is likely to have reduced the bioavailability of the particles together with the adsorbed Cu ions to organisms (Torre et al., 2015; Rosenfeldt et al., 2014). Reduction of the bioavailability of TiO<sub>2</sub> NPs together with the adsorbed Cu may consequently alleviate or even neutralize their promoting role with regard to penetration of the soybean roots upon co-exposure. This elucidation seemingly well accounts for the results of non-significant mutual effects of the TiO<sub>2</sub> NPs and Cu on their contents in soybean roots in the co-exposure of TiO<sub>2</sub> NPs and Cu at 5 and 20 mg/L ( $P > 0.05$ ) (Fig. 3A and C). However, further evidence in terms of the physical-chemical interactions between TiO<sub>2</sub> NPs and co-existing heavy metals (Cu in the current study) is needed to deeply understand the underlying mechanisms of the specific impact of TiO<sub>2</sub> NPs on the phytotoxicity of such pollutants.

In summary, the co-presence of TiO<sub>2</sub> NPs resulted in the alterations of toxicity and contents of Cu ions in soybean plants. Such alterations were dependent on the initial concentration of Cu for co-exposure. At relatively lower concentrations of Cu (namely 1 and 2 mg Cu/L), the co-presence of TiO<sub>2</sub> NPs significantly enhanced the phytotoxicity of Cu ( $P < 0.05$ ), while at 5 and 20 mg Cu/L, the significantly aggravated toxicity due to the co-presence of TiO<sub>2</sub> NPs disappeared ( $P > 0.05$ ). The reciprocal effects of TiO<sub>2</sub> NPs and Cu on their fates were further monitored. The co-presence with TiO<sub>2</sub> NPs resulted in a gradual increase of adsorption of Cu ions onto TiO<sub>2</sub> NPs upon increasing Cu concentration. The adsorption of Cu onto TiO<sub>2</sub> NPs did not reduce the bioavailability of Cu and TiO<sub>2</sub> NPs for soybean roots. Instead, the contents of Ti together with the adsorbed Cu ions in soybean roots were significantly increased upon the co-exposure of TiO<sub>2</sub> NPs and Cu at 1 and 2 mg/L ( $P < 0.05$ ). However, the increase of Ti and Cu concentrations in soybean roots did not occur when Cu was applied at concentrations of 5 and 20 mg/L. This may be caused by the fact that the larger amounts of Cu adsorbed onto TiO<sub>2</sub> NPs when the Cu concentration equaled 5 and 20 mg/L, showed a higher tendency to reduce the zeta-potential of the TiO<sub>2</sub> NPs and subsequently led to a higher extent of aggregation and sedimentation of TiO<sub>2</sub> NPs, as compared to the treatments with the co-exposure of TiO<sub>2</sub> NPs and Cu at concentrations of 1 and 2 mg Cu /L. This may consequently alleviate or even neutralize the promoting toxicity and accumulation of Cu in plants upon the co-exposure of TiO<sub>2</sub> NPs and Cu at a relatively low concentration. Our results thus suggest that the potential risk of the co-exposure of TiO<sub>2</sub> NPs and Cu to edible plants needs to be carefully considered. Future study is needed to confirm whether similar interactions between TiO<sub>2</sub> NPs and the co-occurrence of metals, and specifically Cu, in a soil-plant system can be observed.

#### CRedit authorship contribution Statement

**Yinlong Xiao:** Conceptualization, Methodology, Writing, Funding acquisition; **Ying Du:** Investigation, writing - original draft, Funding acquisition; **Yue Xiao:** Investigation; **Xiaohong Zhang:** Resources; **Jun Wu:** Formal analysis, Resources; **Gang Yang:** Analysis, Funding acquisition; **Yan He:** Investigation; **Yaoyu Zhou:** Writing - review & editing; **Willie J.G.M. Peijnenburg:** Writing - review & editing; **Ling Luo:**

Supervision, conceptualization, resources.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112312.

#### References

- Asli, S., Neumann, P.M., 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. *Plant Cell Environ.* 32, 577–584.
- Cai, F., Wu, X.Y., Zhang, H.Y., Shen, X.F., Zhang, M., Chen, W.X., Gao, Q., White, J.C., Tao, S., Wang, X.L., 2017. Impact of TiO<sub>2</sub> nanoparticles on lead uptake and bioaccumulation in rice (*Oryza sativa* L.). *NanoImpact* 5, 101–108.
- Cao, Q., Steinman, A.D., Wan, X., Xie, L., 2018. Combined toxicity of microcystin-LR and copper on lettuce (*Lactuca sativa* L.). *Chemosphere* 206, 474–482.
- Chavan, S., Sarangdhar, V., Nandanathangam, V., 2020. Toxicological effects of TiO<sub>2</sub> nanoparticles on plant growth promoting soil bacteria. *Emerg. Contam.* 6, 87–92.
- Chen, L.X., Rajh, T., Micić, O., Wang, Z., Tiede, D.M., Thurnauer, M., 1997. Photocatalytic reduction of heavy metal ions on derivatized titanium dioxide nanoparticle surface studied by XAFS. *Nucl. Instrum. Methods Phys. Res.* 133, 8–14.
- Chen, Q., Yin, D., Zhu, S., Hu, X., 2012. Adsorption of cadmium(II) on humic acid coated titanium dioxide. *J. Colloid Interface Sci.* 367, 241–248.
- Dietz, K.J., Herth, S., 2011. Plant nanotoxicology. *Trends Plant Sci.* 16, 582–589.
- Fan, W.H., Cui, M.M., Liu, H., Wang, C., Shi, Z.W., Tan, C., Yang, X.P., 2011. Nano-TiO<sub>2</sub> enhances the toxicity of copper in natural water to *Daphnia magna*. *Environ. Pollut.* 159, 729–734.
- Fan, W.H., Liu, T., Li, X.M., Peng, R.S., Zhang, Y.L., 2016a. Nano-TiO<sub>2</sub> affects Cu speciation, extracellular enzyme activity, and bacterial communities in sediments. *Environ. Pollut.* 218, 77–85.
- Fan, W.H., Peng, R.S., Li, X.M., Ren, J.Q., Liu, T., Wang, X.R., 2016b. Effect of titanium dioxide nanoparticles on copper toxicity to *Daphnia magna* in water: role of organic matter. *Water Res.* 105, 129–137.
- Fenoglio, I., Greco, G., Livraghi, S., Fubini, B., 2009. Non-UV-induced radical reactions at the surface of TiO<sub>2</sub> nanoparticles that may trigger toxic responses. *Chem. Eur. J.* 15, 4614–4621.
- Fry, S.C., 1998. Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochem. J.* 332, 507–515.
- Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO<sub>2</sub>, ZnO, Ag, CNT, Fullerenes) for different regions. *Environ. Sci. Technol.* 43, 9216–9222.
- Guo, Z., Ma, R., Li, G., 2006. Degradation of phenol by nanomaterial TiO<sub>2</sub> in wastewater. *Chem. Eng. J.* 119, 55–59.
- Hoagland, D.R., Arnon, D.I., 1950. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stat. Circ.* 347, 25–32.
- Ji, Y., Zhou, Y., Ma, C.X., Feng, Y., Hao, Y., Rui, Y.K., Wu, W.H., Gui, X., Le, V.N., Han, Y. N., Wang, Y.C., Xing, B.S., Liu, L.M., Cao, W.D., 2017. Jointed toxicity of TiO<sub>2</sub> NPs and Cd to rice seedlings: NPs alleviated Cd toxicity and Cd promoted NPs uptake. *Plant Physiol. Biochem.* 110, 82–93.
- Katiyar, P., Yadu, B., Korram, J., Satnami, M.L., Kumar, M., Keshavkant, S., 2020. Titanium nanoparticles attenuates arsenic toxicity by up-regulating expressions of defensive genes in *Vigna radiata* L. *J. Environ. Sci.* 92, 18–27.
- Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B.M., Lu, J., Wanzer, M.B., Woloschak, G.E., Smalle, J.A., 2010. Uptake and distribution of ultrasmall anatase TiO<sub>2</sub> Alizarin red S nanoconjugates in *Arabidopsis thaliana*. *Nano Lett.* 10, 2296–2302.
- Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., Carriere, M., 2012. Accumulation, translocation and impact of TiO<sub>2</sub> nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. *Sci. Total Environ.* 431, 197–208.
- Lian, J.P., Zhao, L.F., Wu, J.N., Xiong, H.X., Bao, Y.Y., Zeb, A.R., Tang, J.C., Liu, W.T., 2020. Foliar spray of TiO<sub>2</sub> nanoparticles prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays* L.). *Chemosphere* 239, 124794.



- Litter, M.I., 1999. Heterogeneous photocatalysis: transition metal ions in photocatalytic systems. *Appl. Catal. B Environ.* 23, 89–114.
- Ma, X., Yan, J., 2018. Plant uptake and accumulation of engineered metallic nanoparticles from lab to field conditions. *Curr. Opin. Environ. Sci. Health* 6, 16–20.
- Margenot, A.J., Rippner, D.A., Dumlao, M.R., Nezami, S., Green, P.G., Parikh, S.J., McElrone, A.J., 2018. Copper oxide nanoparticle effects on root growth and hydraulic conductivity of two vegetable crops. *Plant Soil* 431, 333–345.
- Meesters, J.A.J., Quik, J.T.K., Koelmans, A.A., Hendriks, A.J., van de Meent, D., 2016. Multimedia environmental fate and speciation of engineered nanoparticles: a probabilistic modeling approach. *Environ. Sci. Nano* 3, 715–727.
- Robichaud, C.O., Uyar, A.E., Darby, M.R., Zucker, L.G., Wiesner, M.R., 2009. Estimates of upper bounds and trends in nano-TiO<sub>2</sub> production as a basis for exposure assessment. *Environ. Sci. Technol.* 43, 4227–4233.
- Rosenfeldt, R.R., Seitz, F., Schulz, R., Bundschuh, M., 2014. Heavy metal uptake and toxicity in the presence of titanium dioxide nanoparticles: a factorial approach using *Daphnia magna*. *Environ. Sci. Technol.* 48, 6965–6972.
- Rosenfeldt, R.R., Seitz, F., Senn, L., Schilde, C., Schulz, R., Bundschuh, M., 2015a. Nanosized titanium dioxide reduces copper toxicity—the role of organic material and the crystalline phase. *Environ. Sci. Technol.* 49, 1815–1822.
- Rosenfeldt, R.R., Seitz, F., Zubrod, J.P., Feckler, A., Merkel, T., Luderwald, S., Bundschuh, R., Schulz, R., Bundschuh, M., 2015b. Does the presence of titanium dioxide nanoparticles reduce copper toxicity? A factorial approach with the benthic amphipod *Gammarus fossarum*. *Aquat. Toxicol.* 165, 154–159.
- Rosenfeldt, R.R., Seitz, F., Haigis, A.C., Hoger, J., Zubrod, J.P., Schulz, R., Bundschuh, M., 2016. Nanosized titanium dioxide influences copper-induced toxicity during aging as a function of environmental conditions. *Environ. Toxicol. Chem.* 35, 1766–1774.
- Rossi, L., Zhang, W.L., Schwab, A.P., Ma, X.M., 2017. Uptake, accumulation, and in planta distribution of coexisting cerium oxide nanoparticles and cadmium in *Glycine max* (L.) Merr. *Environ. Sci. Technol.* 51, 12815–12824.
- Rossi, L., Sharifan, H., Zhang, W.L., Schwab, A.P., Ma, X.M., 2018. Mutual effects and in planta accumulation of co-existing cerium oxide nanoparticles and cadmium in hydroponically grown soybean (*Glycine max* (L.) Merr.). *Environ. Sci. Nano* 5, 150–157.
- Rossi, L., Bagheri, M., Zhang, W.L., Chen, Z.H., Burken, J.G., Ma, X.M., 2019. Using artificial neural network to investigate physiological changes and cerium oxide nanoparticles and cadmium uptake by *Brassica napus* plants. *Environ. Pollut.* 246, 381–389.
- Sebastian, A., Nangia, A., Prasad, M.N.V., 2019. Cadmium and sodium adsorption properties of magnetite nanoparticles synthesized from *Heveabrsiliensis* Muell. Arg. bark: relevance in amelioration of metal stress in rice. *J. Hazard. Mater.* 371, 261–272.
- Servin, A.D., White, J.C., 2016. Nanotechnology in agriculture: Next steps for understanding engineered nanoparticle exposure and risk. *NanoImpact* 1, 9–12.
- Sharifan, H., Wang, X.X., Guo, B.L., Ma, X.M., 2018. Investigation on the modification of physicochemical properties of cerium oxide nanoparticles through adsorption of Cd and As(III)/As(V). *ACS Sustain. Chem. Eng.* 6, 13454–13461.
- Sharifan, H., Moore, J., Ma, X.M., 2020. Zinc oxide (ZnO) nanoparticles elevated iron and copper contents and mitigated the bioavailability of lead and cadmium in different leafy greens. *Ecotoxicol. Environ. Saf.* 191, 110177.
- Tan, L.Y., Huang, B., Xu, S., Wei, Z.B., Yang, L.Y., Miao, A.J., 2017. Aggregation reverses the carrier effects of TiO<sub>2</sub> nanoparticles on cadmium accumulation in the waterflea *Daphnia magna*. *Environ. Sci. Technol.* 51, 932–939.
- Thounaojam, T.C., Panda, P., Panda, P., Mazumdar, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., Panda, S.K., 2012. Excess copper induced oxidative stress and response of antioxidants in rice. *Plant Physiol. Biochem.* 53, 33–39.
- Torre, C.D., Balbi, T., Grassi, G., Frenzilli, G., Bernardeschi, M., Smerilli, A., Guidi, P., Canesi, L., Nigro, M., Monaci, F., Scarcelli, V., Rocco, L., Focardi, S., Monopoli, M., Corsi, I., 2015. Titanium dioxide nanoparticles modulate the toxicological response to cadmium in the gills of *Mytilus galloprovincialis*. *J. Hazard. Mater.* 297, 92–100.
- Wang, D., Wang, P., Wang, C., Ao, Y., 2019. Effects of interactions between humic acid and heavy metal ions on the aggregation of TiO<sub>2</sub> nanoparticles in water environment. *Environ. Pollut.* 248, 834–844.
- Wang, J., Dai, H., Nie, Y., Wang, M., Yang, Z., Cheng, L., Liu, Y., Chen, S., Zhao, G., Wu, L., Guang, S., Xu, A., 2018b. TiO<sub>2</sub> nanoparticles enhance bioaccumulation and toxicity of heavy metals in *Caenorhabditis elegans* via modification of local concentrations during the sedimentation process. *Ecotoxicol. Environ. Saf.* 162, 160–169.
- Wang, X.X., Sun, W.J., Zhang, S., Sharifan, H., Ma, X.M., 2018a. Elucidating the Effects of cerium oxide nanoparticles and zinc oxide nanoparticles on arsenic uptake and speciation in rice (*Oryza sativa*) in a hydroponic system. *Environ. Sci. Technol.* 52, 10040–10047.
- Wang, Y., Peng, C., Fang, H.X., Sun, L.J., Zhang, H., Feng, J.B., Duan, D.C., Liu, T.T., Shi, J.Y., 2015. Mitigation of Cu(II) phytotoxicity to rice (*Oryza sativa*) in the presence of TiO<sub>2</sub> and CeO<sub>2</sub> nanoparticles combined with humic acid. *Environ. Toxicol. Chem.* 34, 1588–1596.