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

Silicon reduces the uptake of cadmium in hydroponically grown rice seedlings: why nanoscale is more effectively than silicate?

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Contents

S1. Supplemental Methods

S1.1 Components of Kimura B solution

The Kimura B solution contains the following macronutrients including 0.37 mM $(NH_4)_2SO_4$, 0.55 mM $MgSO_4 \cdot 7H_2O$, 0.18 mM KNO_3, 0.18 mM KH_2PO_4, 0.37 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.09 mM K_2SO_4 and the micronutrients 50 µM Fe(II)-EDTA, 1 µM $ZnSO_4 \cdot 7H_20$, 1 µM $CuSO_4 \cdot 5\ H_2O$, 5 µM $MnSO_4 \cdot H_2O$, 10 µM H_3BO_3, 0.5 µM $Na_2MoO_4 \cdot 2H_20$, 100 µM NaCl, 0.2 µM $CoSO_4 \cdot 7H_2O$.

S1.2 Determination of antioxidant enzyme activities

SOD: The activity of SOD was determined in a 1.0 ml reaction containing 0.1 ml of plant tissues extracts in 50 mM potassium phosphate buffer, pH 7.0, 75 µM nitro-blue tetrazolium (NBT), 13 mM methionine, 0.1 mM EDTA, and 75 µM riboflavin [1]. The reaction mixture was irradiated using two 15 W fluorescent lamps for approx. 15 min. A micro-tube containing the reaction mixture without added plant tissues extracts was used as the control and kept under the same fluorescent lights. Another micro-tube containing only plant tissues extracts was maintained in the dark and considered the blank. The activity of SOD was calculated based on the difference in NBT reduction between the control and the leaf sample tested. One unit of SOD activity was the amount of enzyme required to inhibit 50% of the initial reduction of NBT under fluorescent light.

POD: The activity of POD was based on oxidation of guaiacol using hydrogen peroxide according to a reported method with minor modifications [2]. Each 1.0 ml reaction contained 50 mM phosphate buffer, pH 6.8, 15 mM guaiacol, 0.1 ml of plant tissues extracts, and 5 mM H_2O_2. The addition of H_2O_2 initiated the reaction and the increase in absorbance at 470 nm was measured over 1 min. The activity of POD was calculated using the extinction coefficient (26.6 $mM^{-1}\ cm^{-1}$). One unit of POD activity was defined as the amount needed to oxidize 1 µmol guaiacol per min under the assay conditions.

CAT**:** Total CAT activity was estimated by measuring the rate of disappearance of H_2O_2, as described by Aebi (1984) [3]. Each reaction contained 50 mM potassium phosphate buffer, pH 6.8, 1 mM EDTA, 15 mM H_2O_2, and 20 µl crude enzyme extract. CAT activity was determined by the reduction in absorbance at 240 nm using an extinction coefficient (36 $M^{-1}\ cm^{-1}$). One unit of CAT activity catalyzed the degradation of 1 µmol H_2O_2 per min under the assay conditions.

MDA: Fresh plant tissues samples were prepared as the ground using liquid nitrogen and homogenized in 1 mL of 10% trichloroacetic (TCA) solution. The supernatants were separated by centrifugation at 10000 rpm for 10 min at 4 °C. Then the supernatants were mixed 0.5% TBA and followed by boiling water bath for 1 h. Then the mixtures were quickly cooled by ice bath. After centrifugation at 8000×g for 10 min at 4 °C, the absorbances of the supernatant at 532 nm (A532) and 600 nm (A600) were measured. The content of MDA was expressed as nmol/g fresh weight.

S1.3 Determination of Cd and Si concentration in plant tissues

0.25 g of dry samples were treated with 5 mL mixed acid (nitric acid and perchloric acid 3:1, v/v) in a Teflon bottle. After a night of soaking, the digestion solution was heated at 120 ℃ for 30 min followed by 180 ℃ for 30 min in a microwave reaction system. Following digestion, 2.5 mL aqua regia and 1 mL hydrofluoric acid were added to the digested extracts to dissolve any remaining material. The digested extracts were diluted to 50 mL using ultrapure water and then measured for the concentrations of Cd and Si.

S1.4 Subcellular distribution of Cd and Si in roots

The subcellular distribution of Cd and Si were determined using the differential centrifugation technique as reported by a previous study [5]. Plant roots were homogenized with a pre-cooling buffer (Tris-HCl, 50 mM, pH 7.5) containing 0.25 M sucrose and 1 mM dithiothreitol. The homogenate was centrifuged at the speed of 3000 r/min for 15 min under 4 °C. The obtained precipitate was referred to as cell wall. The supernatant was centrifuged at the speed of 15000 r/min for 30 min under 4 °C for further separation. The resultant supernatant and deposition were designated as the soluble and organelle fractions. The separate cell wall and organelle fractions were added to the Teflon bottle and then digested with HNO_3 and $HClO_4$ (3:1, v/v), while the soluble fraction was used directly for Cd analysis.

S2. **Supplemental Results**

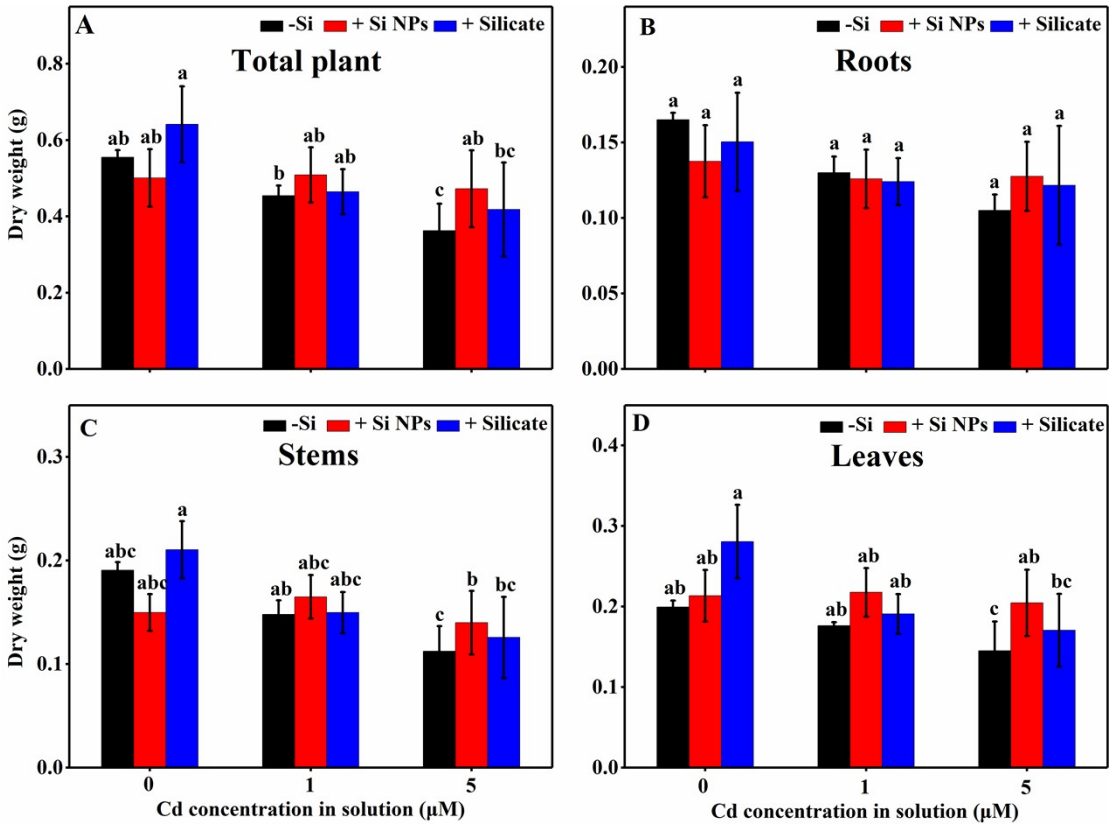

Fig. S1 The dry weight of total plant (A), roots (B), stems (C) and leaves (D)in the rice seedlings pre-treated with or without Si NPs and silicate under 1 and 5 μM Cd stress. Error bars represent the standard deviation of three independent experiments. Different letters indicate significant differences among different treatments ($p < 0.05$) according to one-way ANOVA followed by Tukey's test.

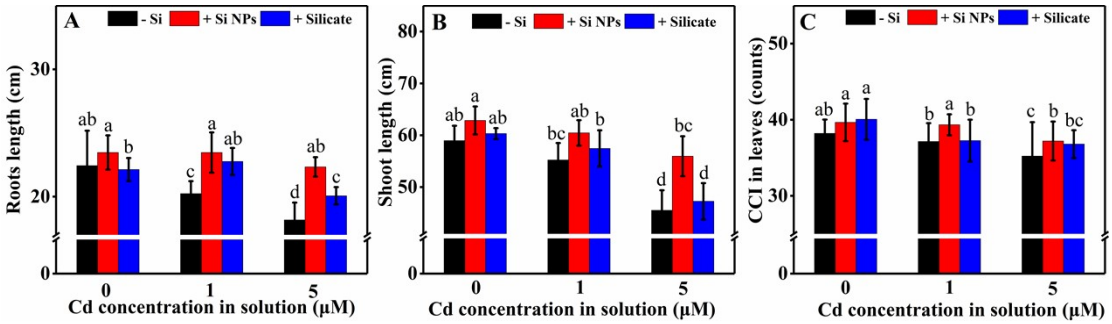

Fig. S2 Roots (A) and shoot (B) length and CCI (C) of rice seedlings pre-treated with or without Si NPs and silicate under 1 and 5 μM Cd stress. Error bars represent the standard deviation of three independent experiments. Different letters indicate significant differences among different treatments ($p < 0.05$) according to one-way ANOVA followed by Tukey's test.

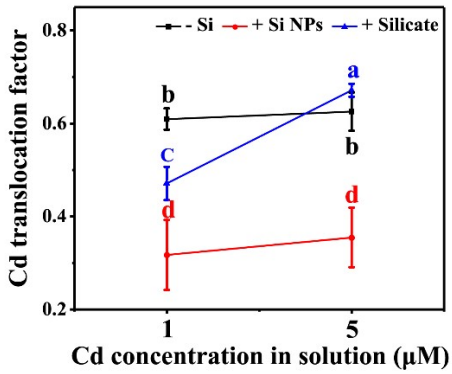

Fig. S3 Cd translocation factor from roots to shoots of rice seedlings pre-treated with or without Si NPs and silicate under 1 and 5 μM Cd stress. Different letters indicate significant differences among different treatments ($p < 0.05$) according to one-way ANOVA followed by Tukey's test.

S3. Supplemental Tables

Table S1. FTIR absorption bands for different fractions of cell walls and their corresponding functional groups according to the previous literature [6-10].

Absorption band (cm^{-1})	Main Assignments	Assigned cell wall component
1740	C=O stretching vibration of alkyl ester	Pectin
1620	COO^- symmetric stretch	Pectin
1431	COO^- symmetric stretch	Pectin
1370	CH_2 bending	Cellulose/Hemicellulose
1243	C-O stretching	Pectin
1160	O-C-O stretching	Cellulose
1130	O-C-O asymmetric stretching	Hemicellulose
1075	C-O\C-C stretching	Hemicellulose
1019	C-O\C-C stretching	Pectin
921	C-H bending	Cellulose/Hemicellulose

Table S2 Primers for RT-PCR analysis of the genes.

Gene	Forward primer 5'→3'	Reverse primer 5'→3'
OsNramp1	CGACTAAGCTTAAGAAGCCGCACTAGTATG	CCGGTCTAGAAGGGTACTACACGGGTGGCT
OsNramp5	CAGCAGCAGTAAGAGCAAGATG	GTGCTCAGGAAGTACATGTTGAT
OsHMA3	AGAACAGCAGGTCGAAGACG	ATTGCTCAAGGCCATCTGCT

Table S3. Principal component analysis (PCA) of absorbances at picked peaks from average spectra of root cell walls of Si NPs or silicate treated rice seedlings exposed to 0, 1 and 5 μM Cd.

Wavenumber (cm^{-1})	PC1	PC2	PC3	PC4	PC5
1740	**0.330**	**-0.092**	**0.286**	**0.440**	**-0.658**
1620	**0.339**	**-0.006**	**0.014**	**-0.045**	**-0.093**
1431	**0.311**	**-0.007**	**-0.817**	**0.454**	**0.126**
1370	**0.332**	**-0.027**	**-0.206**	**-0.607**	**0.063**
1243	**0.339**	**0.024**	**0.124**	**0.029**	**0.209**
1160	**-0.068**	**-0.984**	**0.015**	**0.022**	**0.139**
1130	**0.331**	**0.072**	**0.360**	**0.284**	**0.560**
1075	**0.335**	**-0.131**	**-0.020**	**-0.142**	**-0.247**
1019	**0.337**	**-0.011**	**0.245**	**-0.010**	**0.259**
921	**0.338**	**-0.023**	**-0.039**	**-0.358**	**-0.185**
Percentage of variation	**86.357**	**9.914**	**2.202**	**0.852**	**0.314**

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