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

Simultaneous reduction of arsenic and lead bioaccumulation in rice (*Oryza sativa* L.) by nano-TiO_2: A mechanistic study

Xinyi Wu[a, b], Wenyi Jia[a], Jing Hu[a], Xuefeng Yu[a], Chuanhao Yan[a], Jason C. White[c], Junfeng Liu[a], Yu Yang[d], Xilong Wang[a, *]

[a] Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

[b] The Bartlett School of Sustainable Construction, University College London, WC1H 0QB, London, UK

[c] The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA

[d] Department of Civil and Environmental Engineering, University of Nevada, Reno, NV 89557, USA

Corresponding author. Email address: xilong@pku.edu.cn (X. Wang)

Supplementary Information

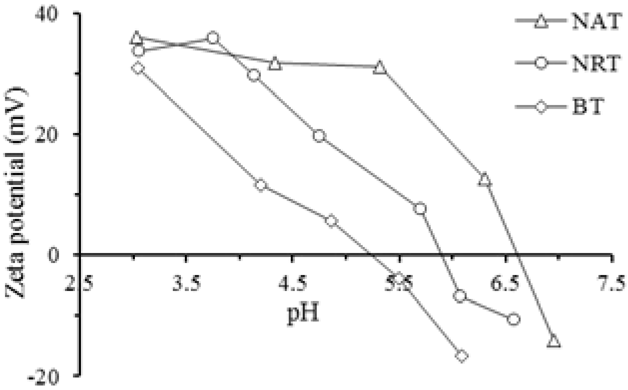

Fig. S1 Zeta potential of three types of TiO_2 as a function of pH.[5]

Text S1. Arsenic and lead content determination in plant tissues

The rice shoots and roots after DCB-extraction were dried for 15 min at 105 °C, and then at 70 °C until a constant weight was achieved. The dry biomass (DW) of roots and shoots was determined. The dried rice tissues were ground to powder in a mortar with the assistance of liquid nitrogen. Plant powder (0.2-0.4 g) was weighed into a digestion tube and immersed in concentrated nitric acid (5 mL) for pre-digestion overnight. On the next day, digestion tubes were heated for 40 min at 60 °C, and then for additional 6 h at 120 °C. After the digestion tubes were cooled to room temperature, H_2O_2 (30%, 2 mL) was added and heated again at 120 °C until the solution volume was approximately 2 mL and clear. The digestion solution was then completely cooled and diluted to 15 mL with Milli-Q water. The arsenic and lead content in rice tissues was determined by ICP-OES are presented as the mass fraction of dry weight.

Text S2. Analysis of the activity of antioxidant enzymes and malondialdehyde content

About 0.2 g fresh root or shoot tissue was ground in liquid nitrogen and quickly homogenized by ice-cold PBS buffer (100 mol L^{-1}, 1.8 mL, pH 7.8). After being centrifuged for 10 min at 3000 rpm and 4 °C, the supernatant was stored at 4 °C for later analysis. The activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and malondialdehyde (MDA) content was analyzed with SOD assay kit (WST-1 method), CAT assay kit (visible light), POD assay kit (for plants) and MDA assay kit (TBA method) that were purchased from Nanjing Jiancheng Bioengineering Institute, respectively, and all were analyzed by a spectrophotometer (Thermo Scientific Multiskan GO, Finland). Both the enzyme activity and MDA content in rice tissues are presented on the basis of unit fresh weight.

Text S3. Iron plaque extraction

To analyze the retention of arsenic and lead in iron plaque, a dithionite–citrate–bicarbonate (DCB) solution was used to extract iron plaque from the root surfaces.[1] In brief, fresh roots were immersed in solution (30 mL) that contained $NaHCO_3$ (0.125 mol L^{-1}), $Na_3C_6H_5O_7{\cdot}2H_2O$ (0.03 mol L^{-1}), and (0.6 g $Na_2S_2O_4$) at ambient temperature (20-25 °C) for 60 minutes. Then, the roots were rinsed three times with Milli-Q water and all eluents were pooled in the DCB extracts. The final solution volume was fixed to 50 mL with Milli-Q water. The content of iron, arsenic and lead in DCB extracts was synchronously measured by ICP-OES.

Table S1. Arsenic and lead concentrations in the digestion solution for rice seedlings with and without exposure and the method detection limits of As and Pb by ICP-OES.

Element concentration	Root without exposure	Shoot without exposure	Root or shoot under exposure	MDL	Unit
As	0.8	1.1	300-3500	4.6	μg/L
Pb	0.7	0.6	410-90000	3.1	μg/L

Table S2. The final arsenic and lead concentrations in the exposure solution without plant and TiO_2 after 7-day plant-exposure experiment.

Element concentration	Initial conc. in the AsPb2 system	Final conc. in the AsPb2 system	Initial conc. in the AsPb10 system	Final conc. in the AsPb10 system	Unit
As	0.99	0.98	0.99	0.97	μg/L
Pb	2.02	2.00	9.97	9.97	μg/L

Text S4. Sorption of arsenic and lead by three types of TiO_2 normalized by their surface area

To further compare sorption affinity of arsenic and lead by the tested TiO_2, the solution concentration reductions of arsenic and lead in the sorption systems were normalized by surface area of the amended TiO_2, according to the following equation (Eq. 1).

$$S = (C_{control} - C_{treatment})/(A_{TiO2} \times C_{TiO2}) \times 10^6 \tag{1}$$

where, S presents the sorbed concentration of arsenic or lead adsorbed on unit surface area of TiO_2, with an unit of μg m^{-2}; $C_{control}$ and $C_{treatment}$ are the arsenic and lead concentrations in the non-TiO_2 control and TiO_2-amended treatments, respectively, with an unit of mg L^{-1}; A_{TiO2} and C_{TiO2} are surface area and amended dose of TiO_2, with unit of g m^{-2} and mg L^{-1}, respectively.

The normalized results are shown in Figure S1 below. At a given amendment level of TiO_2, arsenic and lead concentrations sorbed on unit surface area of NRT are significantly higher than those on NAT, indicating the stronger contaminant affinity by NRT than NAT. This can be attributed to the higher zeta potential of NRT compared to NAT at the solution pH of 4.0 (Fig. S6). When the amended dose of TiO_2 increased, the normalized concentrations of arsenic and lead sorbed on all types of TiO_2 significantly deceased. This suggests that at the medium to high levels of amendment, stronger TiO_2 aggregation occurred, leading to lesser active sorption sites for contaminants. The abovementioned findings were observed in both AsPb2 and AsPb10 sorption systems. However, when the co-exposed lead concentration increased from 2 to 10 mg L^{-1}, concentrations of arsenic and lead sorbed on all types of TiO_2 significantly increased. Such sorption enhancement of both arsenic and lead to TiO_2 could result from the formation of a ternary surface complex that contains As, Pb, and Ti.[6] The reason why sorption strength of arsenic and lead by BT was more strongly enhanced could be a result of its larger particle size than nano-TiO_2, such that it was more conductive to the formation of surface complex.

Importantly, it should be noted that although the sorbed amount of arsenic and lead on unit surface area of BT at all amended levels and nano-TiO_2 at 10 mg L^{-1} was significantly higher than that on nano-TiO_2 at 100 and 1000 mg/L in the AsPb10 system, their effectiveness in removing arsenic and lead from nutrient solution and reducing metal accumulation in plant is very limited due to their total small surface area. Consequently, NAT and NRT at 1000 mg L^{-1} are highlighted due to their ability to minimize arsenic and lead accumulation in rice plant.

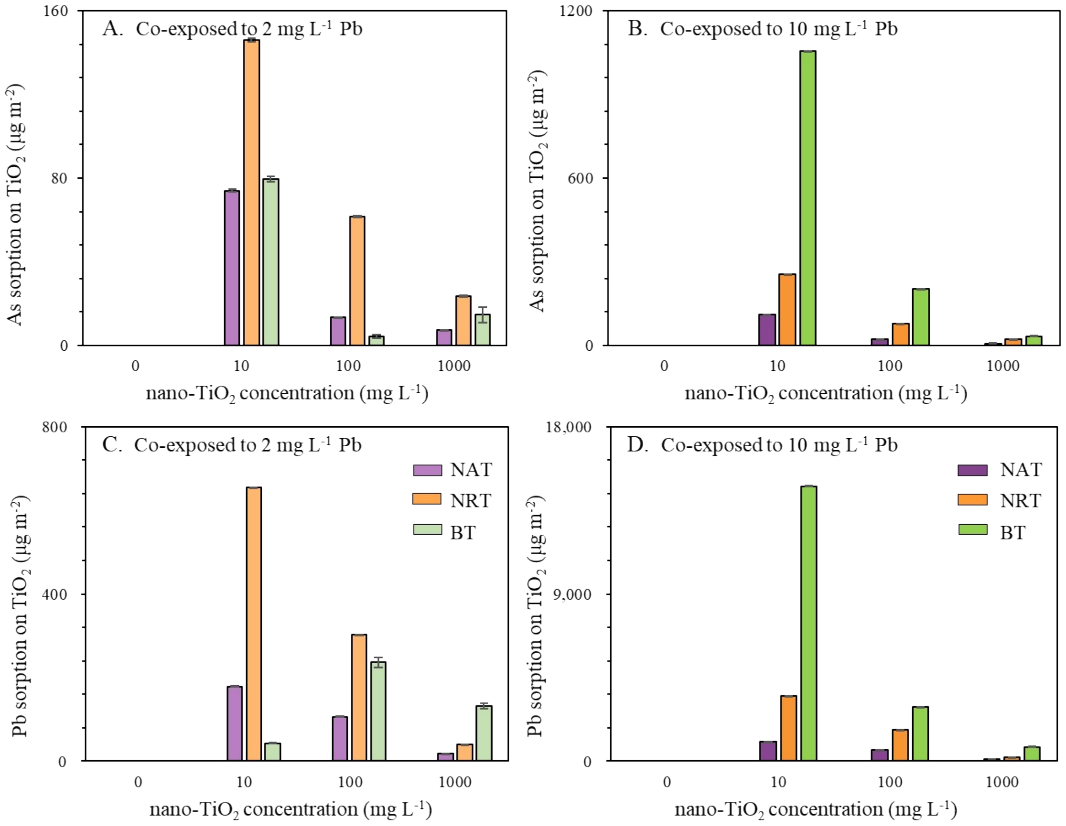

Fig. S2. Effect of TiO_2 type and amended dose on arsenic and lead sorption normalized by its surface area.

Text S5. Influence of the co-exposed lead concentration on solution arsenic concentration reduction

To explore the influence of the co-exposed lead and its concentration on freely dissolved arsenic concentration reduction in nutrient solution resulting from sorption to the amended TiO_2, the solution arsenic concentration in the arsenic exposure alone system, AsPb2 and AsPb10 systems was compared. It was shown that without TiO_2 amendment, the solution arsenic concentration in the systems in the absence and presence of 2 mg L^{-1} lead was statistically comparable during the entire exposure period. This indicates that the co-exposed 2 mg L^{-1} lead in the AsPb2 systems did not alter arsenic uptake by plant root. Conversely, the final solution arsenic concentration in AsPb10 system was 57 and 60% higher than that in AsPb2 system and the arsenic exposure alone system, respectively. This could be due to 10 mg L^{-1} lead in the AsPb10 system inducing greater stress on the plant, thereby more strongly inhibiting arsenic uptake. Accordingly, the plant biomass and the arsenic content in the plant in the AsPb10 system were significantly lower than those in the AsPb2 system and arsenic exposure alone system.

In the arsenic exposure alone systems, only 1000 mg L^{-1} NAT and NRT significantly decreased the arsenic concentration by 32 and 57% relative to the control without TiO_2 amendment. In the systems amended with a same type and amount of TiO_2, no significant difference in the final solution arsenic concentration between the AsPb2 and arsenic exposure alone systems was observed. This was consistent with the data showing that the root and shoot arsenic content in these two systems was comparable. However, the reduction tendency of the solution arsenic concentration in the absence and presence of 2 mg L^{-1} over time was considerably different. The discrepancy can be attributed to the solution pH difference in these systems that was addressed in the main text. In contrast, with amendment of various TiO_2, both the final solution arsenic concentration and its tendency to change over time in the AsPb10 system were comparable to those in the arsenic exposure alone system. This indicates that amendment with TiO_2 not only effectively decreased solution arsenic concentration but also eliminated the negative influence of the co-exposed lead concentration increase on solution arsenic concentration reduction.

Text S6. The pH variation in exposure solution

To identify the impact of possible pH changes on contaminants sorption to TiO_2 in the plant exposure system, the pH of nutrient solution was measured daily during the exposure period, as was the zeta potential changes of the added TiO_2. Solution pH in the AsPb2 system increased slightly but significantly from 5.8 to 6.0 on the first day with or without TiO_2 amendment, then decreased to about 4.0 on the third day, and was maintained in the range of 3.5-4.0 over the last four days (Fig. S2). The AsPb10 systems exhibited a comparable pattern of solution pH variation. Conversely, in the absence of co-exposed lead, solution pH of TiO_2 treatments rapidly decreased from 5.8 to 3.3-3.5 within the first three days and was steady at 3.5-3.8 for the remainder of the exposure period.[5] Lead ions' sorption and desorption on TiO_2 during the early exposure stage may account for the slower pH decrease observed in arsenic-lead co-exposure systems compared to those exposed to arsenic alone, as detailed in section 3.5 of the main text. Although the variation profiles of solution pH differed between systems with and without lead during the exposure period, no significant difference in the final solution pH was evident among those systems.

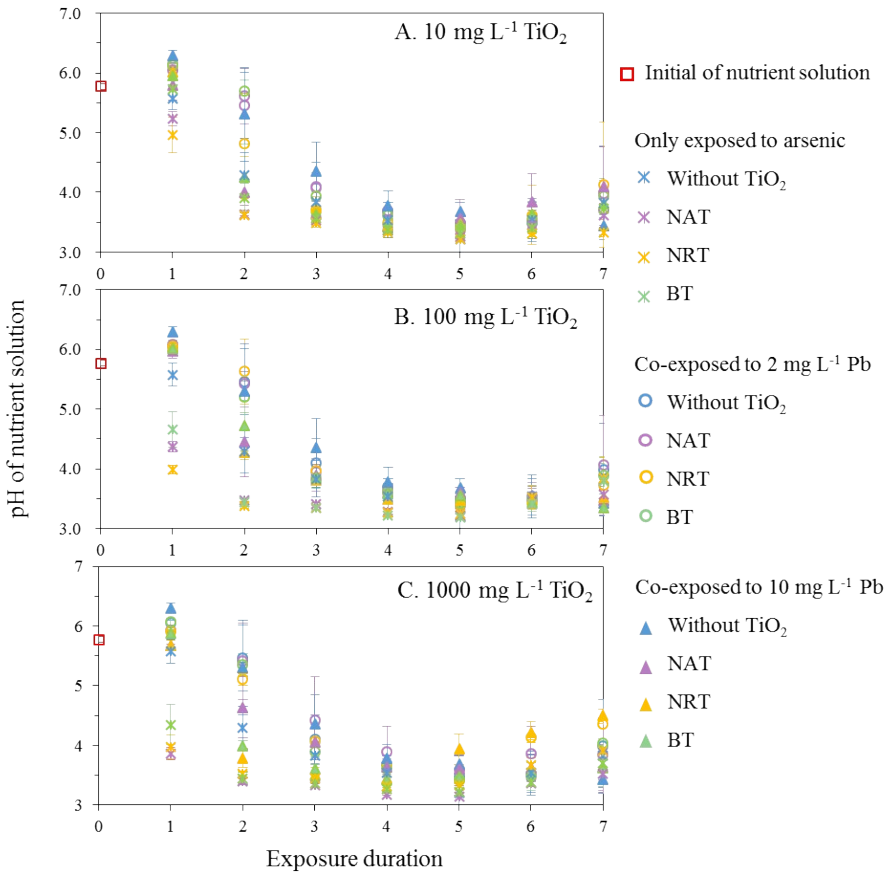

Fig. S3. The pH profile of the exposure solution. The initial solution pH in the systems only exposed to arsenic, co-exposed to arsenic and 2 mg L^{-1} lead as well as co-exposed to arsenic and 10 mg L^{-1} lead was 5.82, 5.77, and 5.76, respectively. Error bars indicate mean standard deviation.

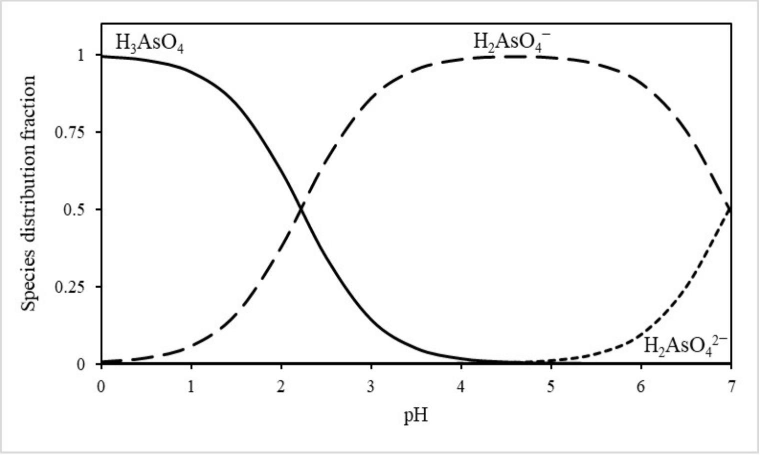

Fig. S4. Species distribution of arsenic (V) as a function of pH.

Text S7. The lead concentration in exposure solution

With regard to lead, the solution concentration in all systems with or without TiO_2 rapidly decreased by 92-98% within the first day, but then gradually increased until reaching a constant level in the range of 0.3-1.5 mg L^{-1} varied by TiO_2 treatment and lead exposure concentration (Fig. S4). This can also be attributed to the reduction in solution pH (Fig. S2). Specifically, when the solution pH decreased beginning at the second day, all amended TiO_2 became more positively charged, thereby enhancing its electrostatic repulsion with the positively charged Pb^{2+}. The sorption of lead to TiO_2 would decrease accordingly. It is also likely that a portion of lead that was initially sorbed to the TiO_2 would subsequently desorb with the decreasing pH. Therefore, lead concentration in solution gradually increased until the solution pH stabilized, at which point it reached a constant level. In addition to TiO_2, iron plaque formed on root surface can also sorb lead ions (see below), but this effect would also decrease as pH declined. Therefore, the solution lead concentration in the control without TiO_2 also increased over time.

In the AsPb2 systems, amendment of 100 mg L^{-1} NAT and NRT, as well as 1000 mg L^{-1} BT, markedly decreased the lead concentration by 36-43%, and 1000 mg L^{-1} NAT and NRT reduced the concentration by 70 and 77%, respectively, relative to the control (Fig. S4). The difference in the final solution lead concentration among various TiO_2 treatments aligned well with the sorption results by these materials, suggesting that the sorption process played a critical role in solution lead content reduction in the AsPb2 system (Fig. 4). Alternatively, the final solution lead concentration in the AsPb10 systems in the absence or presence of TiO_2 was statistically equivalent. This could be a result of significant lead uptake by rice seedlings in the control (without TiO_2). Approximately 92% of the lead reduction in solution in the control was accumulated in the plant, and 8% was retained in the iron plaque on root surface. The fraction of lead distributed in the plant, iron plaque and TiO_2 in the systems amended with 1000 mg L^{-1} TiO_2 were 17-34%, 2.0-2.4% and 63-80%, respectively. Hence, although amendment of 1000 mg L^{-1} NAT, NRT and BT did not significantly change the final solution lead concentration in the AsPb10 system, these amendments still significantly reduced lead accumulation in the plant tissues (Fig. 2).

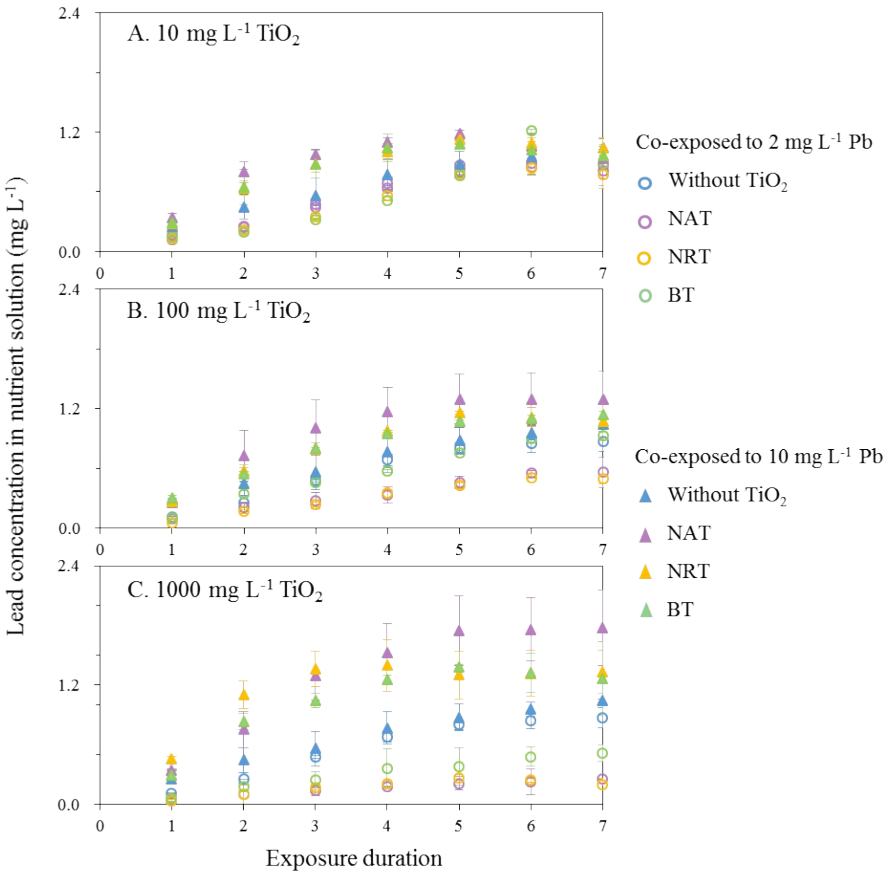

Fig. S5. The lead concentration profile in the nutrient solution during exposure. The initial arsenic concentration is 1 mg L^{-1} in all systems, and the initial concentration of co-exposed lead is 2 or 10 mg L^{-1}. The final arsenic and lead concentrations in the blank control with neither TiO$_2$ nor plant had no significant difference from the initial values. Error bars indicate mean standard deviation.

Text S8. Ti content in exposed rice tissues

Although TiO$_2$ at 1000 mg L^{-1} can significantly decrease arsenic and lead accumulation in rice, it is necessary to clarify whether TiO$_2$ can be accumulated by the plant and consequently pose additional risk to food safety. Because TiO$_2$ does not readily dissolve and ionize, the titanium content in rice plant tissues can be used to characterize the behavior of TiO$_2$.[2] To thoroughly digest the recalcitrant TiO$_2$, hydrofluoric acid was further added to the digested sample described in Text S2.[3] The digestion efficiency was assessed by analyzing a mixture of 100 mg L^{-1} TiO_2 suspension and a certified reference material of Citrus Leaves (GBW10020, PR China), where the recovery of titanium was 94-103%.

The measured titanium content in exposed rice tissues is shown in Figure S1. Compared to the blank without TiO_2, amendment of various TiO_2 at all levels significantly increased root titanium content upon co-exposure to arsenic and lead. In the AsPb2 systems, root titanium content in the control was 227 μg g^{-1}, whereas it increased to 1573-2362, 1670-6160 and 19700-29700 μg g^{-1} under amendment of 10, 100 and 1000 mg L^{-1} TiO_2, respectively. Likewise, in the AsPb10 systems, root titanium content upon amendment of 10, 100 and 1000 mg L^{-1} TiO_2 was 1800-2870, 4420-7930 and 25400-32000 μg g^{-1}, much higher than that in the control (390 μg g^{-1}). It appears that the proportion of titanium amount in rice root (i.e., a product of root titanium content and dry weight of root) to the total amount of titanium amended was 13-25% in 10 mg L^{-1} TiO_2 treatments and only 1-7% in 100 and 1000 mg L^{-1} TiO_2 trials, suggesting the limited uptake and sorption of TiO_2 to root. More importantly, since the plant roots were immersed in TiO_2 suspension, a large number of TiO_2 particles adhered on root surface were likely difficult to completely remove before plant digestion for titanium content determination. Therefore, a large part of the titanium content measured in root was likely contributed from the TiO_2 particles attached to the root surface rather than from those taken up by the roots. A similar phenomenon was also reported by Hu et al.[3] After exposure coriander (*Coriandrum sativum* L.) to 400 mg L^{-1} TiO_2 suspension and careful rinsing its root with Milli-Q water, the root titanium content measured by ICP-OES was significantly higher than that of the control, but importantly, titanium was only detected by SEM-EDS on root surface rather than in the interior part. This is consistent with the observation of rice plant exposed to 1000 mg L^{-1} NAT, NRT or BT using SEM and TEM by Cai et al.[4] where it was found that only a small amount of NAT entered the intercellular space of rice roots.

Conversely, no significant difference in shoot titanium content was shown between all TiO_2 treatments and the blank without TiO_2 amendment. One reason was that shoot did not directly contact TiO_2 suspension, thus avoiding the interference of TiO_2 particles adhered to the plant surface. The other reason was that the translocation of internalized TiO_2 particles from root to shoot would be highly restrained due to the steric hindrance effect. Cai et al.[4] found that a small amount of NAT particles taken up by rice plant root were mainly in the intercellular space; they did not enter cell, indicating that the transport of TiO_2 in rice plant was through apoplastic pathway instead of symplastic route. Transport of the substances such as TiO_2 through the apoplastic pathway in rice plant would most likely be blocked by the casparian strip in endodermis, which makes it difficult to enter the vascular column and be transported to shoot. Therefore, even if a small amount of TiO_2 entered the plant root, it would likely not be transported to shoot or accumulate in rice grain and thus pose risk to food safety.

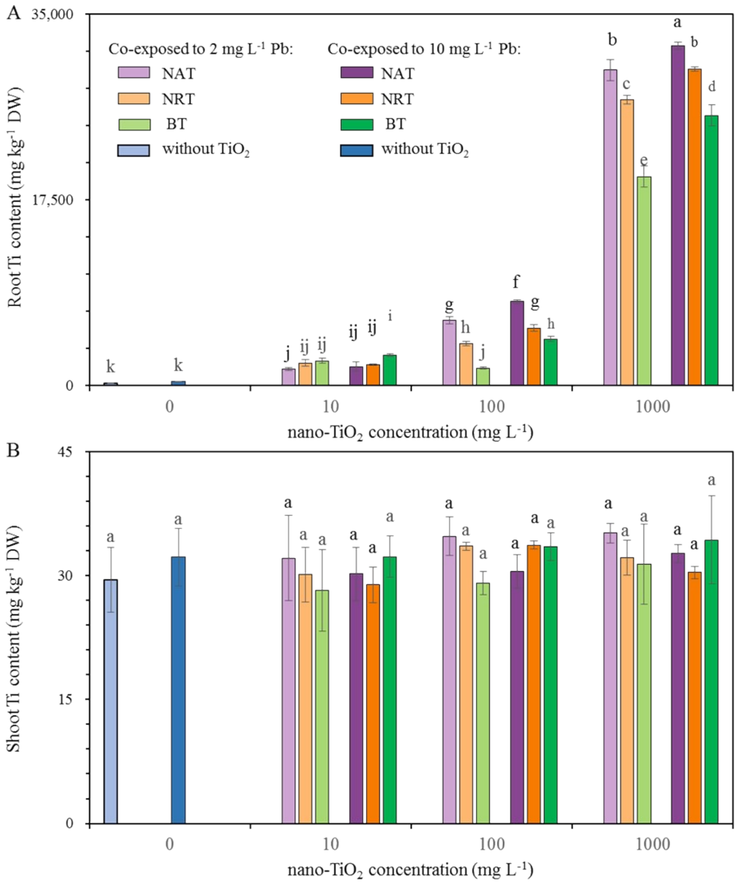

Fig. S6. Titanium content in roots and shoots upon co-exposure of arsenic and lead with varying types and doses of TiO_2 amendment. Error bars indicate mean standard deviation. Bars with the different letters are significantly different ($p < 0.05$).

References

1. W. J. Liu, Y. G. Zhu, F. A. Smith and S. E. Smith, Do phosphorus nutrition and iron plaque alter arsenate (As) uptake by rice seedlings in hydroponic culture? *New Phytol.*, 2004, **162**, 481-488.
2. B. Asadishad, S. Chahal, A. Akbari, V. Cianciarelli, M. Azodi, S. Ghoshal and N. Tufenkji, Amendment of agricultural soil with metal nanoparticles: Effects on soil enzyme activity and microbial community composition. *Environ. Sci. Technol.*, 2018, **52**, 1908-1918.
3. J. Hu, X. Y. Wu, F. Wu, W. X. Chen, J. C. White, Y. Yang, B. Wang, B. S. Xing, S. Tao and X. L. Wang, Potential application of titanium dioxide nanoparticles to improve the nutritional quality of Coriander (*Coriandrum Sativum L.*). *J. Hazard. Mater.*, 2020, **389**, 121837.
4. F. Cai, X. Wu, H. Zhang, X. Shen, M. Zhang, W. Chen, Q. Gao, J. C. White, S. Tao and X. Wang, Impact of TiO_2 nanoparticles on lead uptake and bioaccumulation in rice (*Oryza Sativa L.*). *NanoImpact*, 2017, **5**, 101-108.
5. X. Y. Wu, J. Hu, F. Wu, X. Y. Zhang, B. Wang, Y. Yang, G. F. Shen, J. F. Liu, S. Tao and X. L. Wang, Application of TiO_2 nanoparticles to reduce bioaccumulation of arsenic in rice seedlings (*Oryza Sativa* L.): A mechanistic study. *J. Hazard. Mater.*, 2021, **405**, 124047.
6. S. Hu, M. Nie, J. lu and C. Jing, Cadmium effect on arsenate adsorption on TiO_2, *Environ. Chem.*, 2011, **30**, 739-746.