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Metal(loid) oxides and metal sulfides nanomaterials reduced heavy metals uptake in soil cultivated cucumber plants[☆]

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ABSTRACT

Agricultural soil is one of the main sink for both heavy metals and nanomaterials (NMs). Whether NMs can impact heavy metals uptake or bioaccumulation in plants is unknown. Here, cucumber plants were cultivated in a multi-heavy metals contaminated soil amended with four types of NMs (SiO_2, TiO_2, ZnS and MoS_2) separately for four weeks. Physiological and biochemical parameters were determined to investigate the impact of NMs on plant growth. Inductively coupled plasma mass spectrometry was employed to determine the metal content in plants. Results showed that none of the tested NMs impacted plants biomass, but all the NMs showed different degrees of reduction in heavy metals bioaccumulation in plant roots, stems and leaves. However, four NMs showed different degrees of reduction in macro and micro nutrients uptake. MoS_2 decreased the bioaccumulation of heavy metals (As, Cd, Cr, Cu, Ni, Al, Ti and Pb) for 36.4–60.6% and nutrients (Mg, Fe, K, Si and Mn) for 40.1%–50.1% in roots. Exposure to MoS_2 NMs also significantly increased 23.4% of Si in leaves, 205.6% and 83.9% of Mo in roots and stems, respectively. In general, the results of this study showed promising potential for NMs to reduce uptake of heavy metals in crop plants, especially MoS_2 NMs. However, the negative impacts of perturbing nutrients uptake should be paid attention as well.

1. Introduction

Due to the large amount of pollutants discharge from mining and manufacturing industries, soil contamination is becoming an important issue globally (Cachada et al., 2018). Unlike organic contaminants, metals or metalloids do not undergo microbial or chemical degradation and will be persist for a long time in soil (Bolan et al., 2014). Crops grown in heavy metal contaminated soil might produce unsafe food, posing threats to human health (Zhu et al., 2007). Using soil amendments to decrease the bioavailability or mobility of metal/metalloid is an important strategy to reduce metal accumulation in crops (González et al., 2013). A variety of materials have been used as immobilization amendment, such as phosphate compounds, liming materials, organic composts, metal oxides and biochars (Bolan et al., 2014; Yue et al., 2019).

Recently, some engineered nanomaterials (NMs) have been applied for environmental remediation due to their capacities for heavy metal absorption and high surface area (Lehman and Larsen, 2014). For example, the core (iron oxide NMs)-shell (mesoporous silica) structure enable the separation of the nanocomposites from water using permanent magnet (Chen et al., 2009). Magnetic mesoporous silica nanocomposites have been used for heavy metals (Cr and Cu) decontamination (Lin and Haynes, 2009; Chen et al., 2011). ZnS NMs was used for the removal of Pb^{2+} and Hg^{2+} from aqueous solutions (Pala and Brock, 2012). MoS_2 can adsorb heavy metal ions due to the strong Lewis acid and base soft-soft interactions (Wang and Mi, 2017). Silicon nanoparticles have been shown to reduce chromium (Cr) accumulation in Pisum sativum seedlings (Tripathi et al., 2015). TiO_2 NMs have been reported to significantly reduce heavy metals uptake in benthic organisms (Corbicula fluminea) (Fan et al., 2018). However, most of these studies were conducted in water body, only a few studies focused on contaminated soil remediation using NMs. It was recently reported that Fe_3O_4 NMs reduced heavy metals (As, Pb, Zn, Cd and Cu)

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uptake and alleviated their toxicity in plant seedling (Konate et al., 2017; Praveen et al., 2018).

On the other hand, engineered nanomaterials are regarded as emerging contaminants, which started to be produced in past two decades (Stark et al., 2015). The un-intended co-existence of heavy metals and nanoparticles in soil will be potentially increased due to the increasingly released NMs to agricultural soils through waste water irrigation and sewage sludge reuse. Therefore, to investigate the impact of NMs on crop plant performance is imperative. Most studies used uncontaminated soil to conduct the experiments, and little is known about the impact of NMs on heavy metal uptake and impact on plant growth in contaminated soil. Our hypothesis is that nanoparticles may reduce the uptake and bioaccumulation of heavy metals in plant, by binding or complexing with heavy metals, and thus promoting the plant growth.

In this study, we choose two commonly used adsorbents (SiO_2 and TiO_2 NMs) and two uncommonly used, but exhibiting great potential as adsorbents (ZnS and MoS_2 NMs) to test their performance on heavy metals uptake in plant grown in previously contaminated soil. Another reason for selecting those NMs is due to their wide application in various industries and their potential release into environment is estimated to increasing. Cucumber is an economically important vegetable crop. Meanwhile, cucumber (Cucumis sativus) have been reported to be sensitive to a wide range of contaminants (Gorsuch et al., 1991). Thus, cucumber plants were selected as model system to conduct the experiment. In this study, cucumber plants were allowed to grow in heavy metals contaminated soils collected from mining area for 4 weeks. Four types of NMs (SiO_2, TiO_2, ZnS, MoS_2) were amended in soil with a final dosing as 100 mg/kg. Physiological and biochemical response of cucumber plants to these NMs were measured. Meanwhile, the effect of NMs on heavy metals (As, Cd, Cr, Pb) and nutrient elements (Fe, Ca, K, Mg, Zn) uptake and bioaccumulation in cucumber plants were also determined. The approached data will be valuable for the risk assessment of heavy metals, NMs, or heavy metals/NMs co-exposure in environment.

2. Materials and methods

2.1. Nanomaterials and soil

SiO_2 and TiO_2 NMs were purchased from Pantian Nano Inc. (Shanghai, China) and the original size is 20 and 5–10 nm, respectively. ZnS and MoS_2 NMs were purchased from Gangtian Inc. (Shanghai, China) and Bohan Inc. (Shanghai, China) and the original size is 50 and 120 nm, respectively. The hydrodynamic size for SiO_2, TiO_2, ZnS and MoS_2 are 876 ± 41.8, 801 ± 11.4, 3825 ± 492 and 1046 ± 69.7 nm, with the zeta (ζ) potential of 25.0 ± 0.666, −17.5 ± 0.476, 19.0 ± 1.485 and −29.0 ± 0.592 mv, measured via dynamic light scattering (Malvern Zetasizer Nano ZS-90), in nano pure water at pH 7 with 100 mg/L in nano pure water. The data of size and surface charge were summarized in Table 1.

The soil was collected from a field (32°09′30.29″N, 118°56′56.95″E) in a mining area in Qixia Mountain (Nanjing, China), from the top 20 cm. The soil has been contaminated by a nearby lead-zinc mining factory, which was one of the largest lead zinc deposits in East China and has been explored for more than 50 years. The collected soil was air-dried and ground to pass through a 2-mm sieve for the pot trial. ICP-MS analysis showed that the most abundance heavy metals in soil are As (65.2 ± 3.0 mg/kg), Pb (182 ± 6.0 mg/kg) and Cd (3.52 ± 2.02 mg/kg), respectively. Soil pH is 6.46 ± 0.093.

Table 1
Size and ζ Potential of nanomaterials (100 mg/L).

	Original Size (nm)	Hydrodynamic Size (nm)	Zeta Potential (mV)
SiO_2	20	876 ± 41.8	25 ± 0.666
TiO_2	5–10	801 ± 11.4	−17.5 ± 0.476
ZnS	50	3825 ± 491.8	19.0 ± 1.485
MoS_2	120	1046 ± 69.7	−29 ± 0.592

2.2. Exposure experiments

Cucumber (*Cucumis sativus*) seeds (Zhongnong 28 F1) were purchased from Hezhiyuan Seed Corp. (Shandong, China). The soil was spiked with different nanomaterials (SiO_2, TiO_2, ZnS, MoS_2) by mixing the NMs with a small portion of soil before blending the mixture with remaining soil homogeneously. Based on our previous studies and other studies used 500–1000 mg/kg NPs, which is a too high dose. Therefore, we were attempted to use a relatively low dose. The final dose of NMs in soil is 100 mg/kg. Cucumber seeds were planted in pots containing 100 g of soil. Each pot contained one seed. The plants were cultivated in a greenhouse for 28 days at 25 °C during the day and 20 °C at night. The daily light integral was 180 μmol $m^{-2}\cdot s^{-1}$ in the daytime. During the growth period, the plants were watered as needed and no additional fertilizers were applied.

2.3. Biomass and chlorophyll content analysis

At harvest, cucumber plants were thoroughly rinsed with tap water for 5 min followed by deionized water for 3 times. The fresh biomass of stem and leaf tissues was determined before oven-drying (70 °C for 72 h). Due to part of the roots was lost while washing, the root biomass was not calculated. Photosynthetic pigment analysis was performed to determine the levels of chlorophyll *a* and *b* and total carotenoids. The photosynthetic pigment contents were extracted following the protocol of Šesták et al. (1971). Briefly, 0.01 g of cucumber young leaves were mixed with 5 mL of 80% methanol for 12 h, and then the mixture was centrifuged for 10 min at 3000 rpm. A microplate Reader (Biotek Synergy H1, America) was used to measure the absorbance of chlorophyll *a* and *b* and carotenoids in the methanolic extracts at 666, 653, and 470 nm, respectively.

2.4. Total phenolics content

The total content of phenolic compounds were determined with the method of Singleton and Rossi (1964). Specifically, 50 μL of the methanolic extract was diluted with 450 μL of deionized (DI) water, and then 250 μL of 2 M Folin–Ciocalteu reagent and 1.25 mL of 20 g/L Na_2CO_3 were added to the mixture. After centrifugation, the absorbance of the supernatant was determined at 735 nm with UV spectra. Quality assurance and quality control include duplicated samples at 10%.

2.5. Lipid peroxidation

Lipid peroxidation in leaves was measured by the Thiobarbituric Acid Reactive Substances (TBARS) assay (Jambunathan, 2010). Malondialdehyde, which is the final product of fatty acid degradation, is an indicative of lipid peroxidation. Briefly, 0.2 g of fresh cucumber leaves were mixed with 4 mL of 0.1% trichloroacetic acid (TCA); the mixture was then centrifuged at 10,000 rpm for 15 min. A 1-ml aliquot of the supernatant was mixed with 2 mL of 20% TCA and 2 mL of 0.5% thiobarbituric acid (TBA); then, the mixture was heated in water bath at 95 °C for 30 min. After cooling, the visible light absorbance was measured at 532 nm and 600 nm (Biotek

Synergy H1, America). Lipid peroxidation was expressed as μmol MDA equivalents 1 g^{-1} of fresh weight.

2.6. ICP-MS analysis for element content in plant tissues

Root, stem and leaf for metal and metalloid analysis were dried at 70 °C for 72 h. A sample of approximately 0.02 g of dried tissue was microwave-digested (Milestone, Ethos Up, Germany) in a mixture of 8 mL of H_2O_2 and 2 mL of HNO_3 (4/1 v/v) at 160 °C for 40 min. The resulting digest was diluted to a final volume of 50 mL prior to analysis. The content of Si, Ti, Zn, Mo, macro- and micronutrients (K, Ca, Mg, Cu, Na and Mn) and heavy metals (As, Pb, Cr, Cd) were quantified by inductively coupled plasma mass spectrometry (ICP-MS) (NexION-300, PerkinElmer, USA) and inductively coupled plasma-optical emission spectrometer (ICP-OES) (Optima 8300, PerkinElmer, USA). Standard reference materials from National Institute of Standards and Technology 1547 and 1570a were used to validate the digestion and analytical method obtaining recoveries between 90% and 99%. The detection limit of Si, Ti, Zn, Mo, K, Ca, Na, Mg, Cu, Ni, Al, Mn, As, Pb, Cd, and Cr quantified by ICP-MS analysis were 0.1 μg L^{-1}, 0.0008 μg L^{-1}, 0.1 μg L^{-1}, 0.001 μg L^{-1}, 1.0 μg L^{-1}, 1.9 μg L^{-1}, 0.6 μg L^{-1}, 0.1 μg L^{-1}, 0.009 μg L^{-1}, 0.02 μg L^{-1}, 0.03 μg L^{-1}, 0.03 μg L^{-1}, 0.03 μg L^{-1}, 0.0004 μg L^{-1}, 0.01 μg L^{-1} and 0.01 μg L^{-1}, respectively.

2.7. Soil pH determination

The mixture of 3 g soil and 30 mL nano pure water was shaken for 2 h at 20 °C with 200 rpm. Then the pH of the suspension was measured using a pH meter (Mettler-Toledo, Switzerland).

3. Results and discussion

3.1. Impact of NMs on photosynthetic pigments content and biomass

Results showed that SiO_2 and TiO_2 NMs had no impact on photosynthetic pigment contents in cucumber leaves (Fig. 1). The previous studies from our group also found that neither SiO_2 nor TiO_2 NMs impacted photosynthetic pigment contents and growth of maize plants grown in another agricultural soil (Mollisol) dosing at 100 mg/kg (Zhao et al., 2019). Song et al. (2013) also found that TiO_2 NMs did not exhibit negative impact on chlorophyll content and growth of three plant species in hydroponic condition. Although not statically significant, exposure to TiO_2 and MoS_2 NMs distinctly decreased carotenoids by 8.84% ($p = 0.082$) and 9.37% ($p = 0.077$) compared to control, respectively. In contrast, exposure to ZnS NMs significantly decreased carotenoids by 9.18% ($p \leq 0.05$) compared to control (Fig. 1). In addition, chlorophyll *b* content was also markedly decreased by 9.2% ($p = 0.064$) upon ZnS exposure. These results indicate that ZnS NMs have negative impact on photosynthetic pigment biosynthesis of cucumber plants. Similarly, exposure to MoS_2 NMs significantly decreased chlorophyll *b* content by 11.33% ($p \leq 0.05$) compared to control (Fig. 1). Taken together, both metal sulfide NMs (ZnS and MoS_2) inhibited photosynthetic pigment contents. The negative impact of NMs on photosynthetic pigment contents is in the order of $MoS_2 \approx ZnS > SiO_2 \approx TiO_2$. Since the leaf is not the organ which were directly exposure to NMs, the reason for the decreased photosynthetic pigments are probably because of the binding/complexing of NMs with nutrient elements (Mg and Fe) in the rhizosphere, which reduced the uptake of photosynthetic related elements by leaves and inhibited chlorophyll content. Further investigate is needed to verify this hypothesis. The changes of photosynthetic pigment content may influence the biomass accumulation. However, none of the tested NMs (SiO_2, TiO_2, ZnS and MoS_2) showed impacts on cucumber plants biomass (stem and leaf) during 4 weeks exposure (Fig. 2A), even in plants exposed to ZnS and MoS_2 NMs in which photosynthetic pigment contents were negatively impacted. Also, no visible toxicity symptoms were observed during the whole period of cultivation (Fig. 2B). Taken together, all the tested NMs generally had no impact on plant growth.

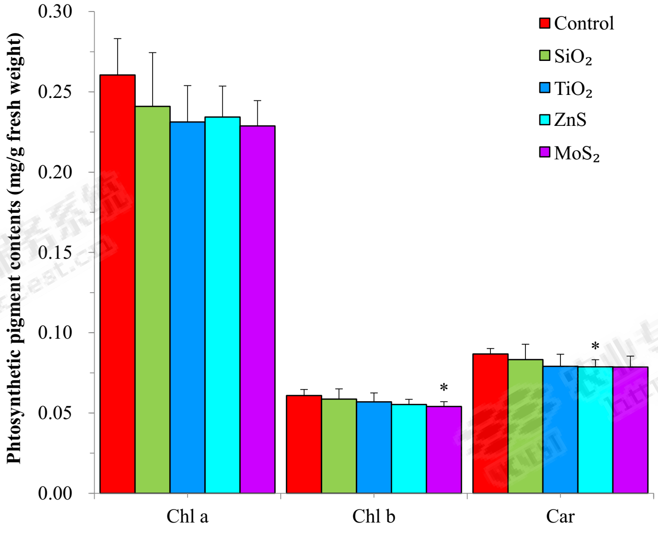

Fig. 1. Impact of Nanomaterials (SiO_2, TiO_2, ZnS and MoS_2) on photosynthetic pigment contents in cucumber plants. All data are average ± SD of four replicate. * represent statistical differences at $p \leq 0.05$ compared with control, respectively.

3.2. Impact of NMs on leaf lipid peroxidation and total phenolics

Nanoparticle can either directly interact with root, or the released ions from NPs can be taken up by root and induce metabolic changes. To test whether NMs or ions induce lipid peroxidation, malondialdehyde (MDA) content, which is the reaction product of membrane lipid peroxidation, were determined. Results showed that MDA content was unchanged by all types of NMs (Fig. 3A), indicating no lipid peroxidation occurred by any of the tested NMs exposure. Navarro et al. (2012) found that CdSe/ZnS caused oxidative stress in *Arabidopsis thaliana* plants hydroponically cultivated. The different findings are possibly because of the growth media, NMs in soil will undergo aggregation and binding with soil mineral and organic matters, and get less chance to be directly taken up by plants and cause phytotoxicity. In addition, total phenolics, which are class of compounds with the ability of scavenge reactive oxygen species (ROS) and are indicators of the antioxidant stress responses, were unchanged by all types of NMs (Fig. 3B). The unchanged MDA level and total phenolics suggest that the plants had no stress response upon exposure to all types of NMs.

3.3. The uptake and translocation of Si, Ti, Zn and Mo in cucumber tissues

Results showed that Si, Ti, Zn in plants root exposed to different types of NMs were not significantly higher than control (Fig. 4), indicating no uptake of NMs or the released ions were taken up by cucumber plants. The results also revealed that the solubility of SiO_2, TiO_2, ZnS are extremely low in soil, or the released ions were bound with soil clay minerals lowering their bioavailability. Also, the huge surface area of NPs make it have many active points which

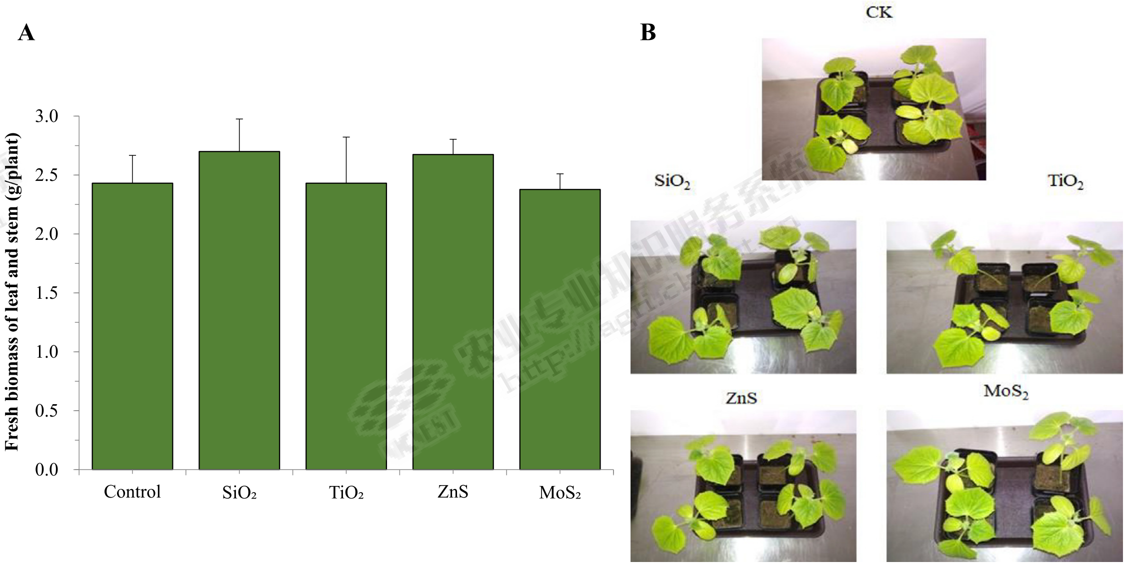

Fig. 2. Cucumber plant above-ground tissues biomass (A) and cucumber images (B).

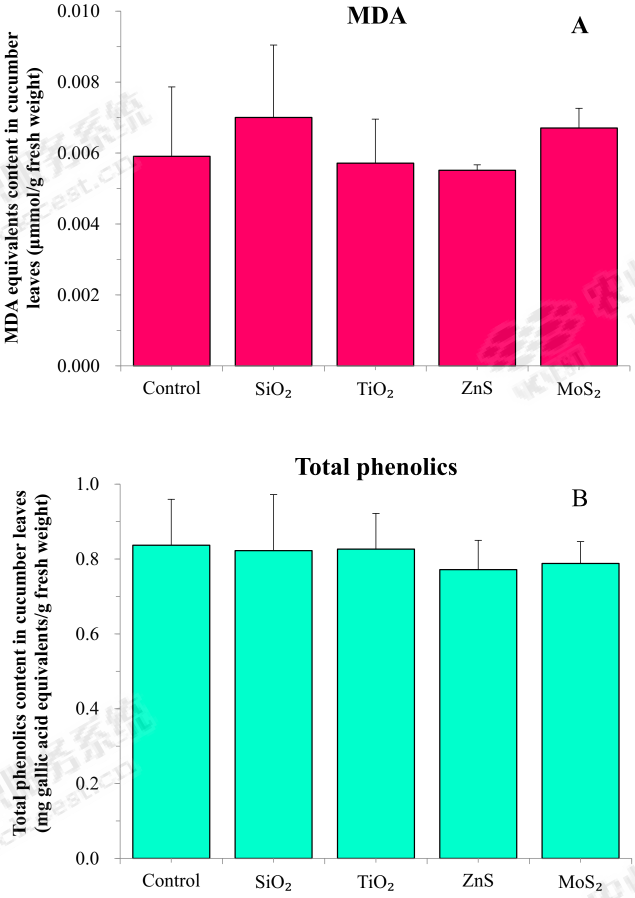

Fig. 3. Impact of Nanomaterials (SiO_2, TiO_2, ZnS and MoS_2) on MDA and total phenolics contents in cucumber plants. All data are average ± SD of four replicate.

can easily binding with soil minerals or organic matter, and inhibit their uptake and translocate by plants (Song et al., 2013). In above ground tissues (stem and leaf), Si and Ti were also not detected to be higher than that in control, indicating no translocation from root to upper part tissues (Fig. 4), whereas Zn was observed to be lower than that in control. Aggregation to larger particles which is much bigger than the root pore size is not easy to be taken up by root and translocate to upper part tissues.

The exception is Mo. As shown in Fig. 4, exposure to MoS_2 significantly increased Mo content in root and stem, by 205.6% ($p < 0.01$) and 83.9% ($p < 0.01$), respectively, compared to control. MoS_2 aggregates have large size of more than 1000 nm, which is much bigger than the typical root surface pore size of 20 nm. Therefore, it is almost not possible for plants to uptake MoS_2 aggregates. Thus, we speculate that the Mo in stem is in the form of Mo ion, instead of MoS_2 NMs. Correspondingly, part of the detected Mo in roots should be Mo ions. Molybdenum ions are components of enzymes related to nitrate assimilation and nitrogen fixation, such as nitrate reductase and nitrogenase (Schwarz and Mendel, 2006). This may suggest that MoS_2 NP have the potential to increase nitrate assimilation. Further study is needed to verify this hypothesis.

3.4. *Effect of nanomaterials on heavy metals uptake in cucumber plants*

Since the collected soils were contaminated by a variety of toxic heavy metals (As, Cd, Cr, Cu, Ni, Al, Zn and Pb), we were curious that whether the amended nanomaterials in soil could decrease the heavy metal uptake in plant tissues due to the adsorption or complexation between NMs and heavy metals. Results showed that heavy metals content (As, Cd, Cr, Cu, Ni, Al, Zn and Pb) in cucumber root exposed to SiO_2 NMs were not significantly changed compared to control (Table 2). This indicates that mesoporous SiO_2 NMs had no impact on heavy metal bioaccumulation in cucumber plant, even previous reports showed mesoporous SiO_2 had good performance in removing heavy metals from wastewater (Sayari et al., 2005; Heidari et al., 2009; Li et al., 2011). While exposure to TiO_2 NMs resulted in a significant reduction uptake of As and Al, by 34.8% and 47.2%, respectively, in cucumber root (Table 2). In addition, exposure to ZnS NMs only significantly ($p < 0.05$) decreased Cr content in cucumber root by 43.3% compared to control (Table 2). Different from other types of NMs, MoS_2 NMs significantly ($p < 0.05$) decreased the content of almost all heavy metals (As, Cd, Cr, Cu, Ni, Al, Ti and Pb) in cucumber roots by approximately

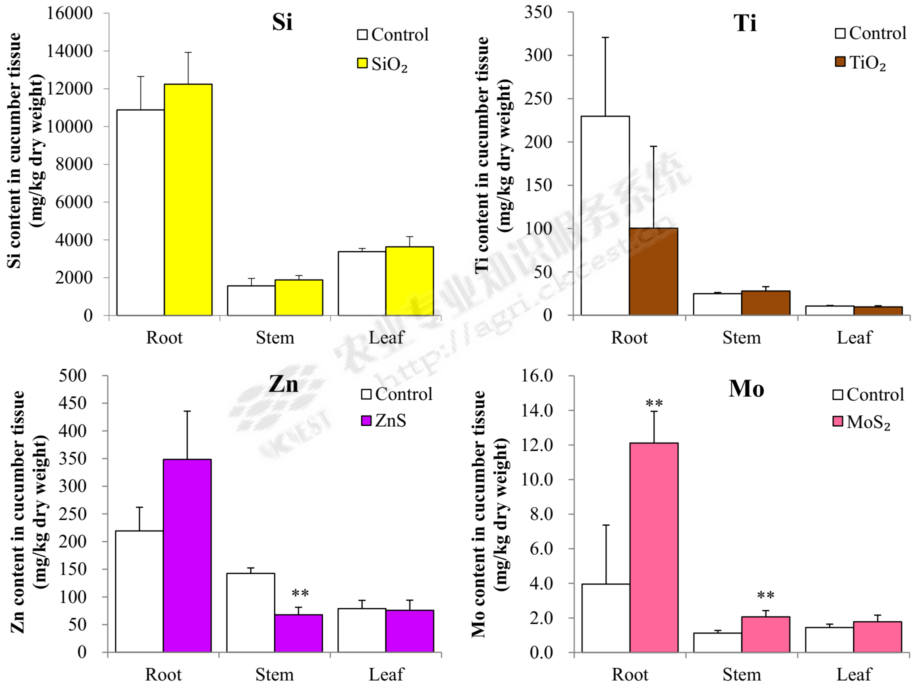

Fig. 4. Bioaccumulation of Si, Ti, Zn and Mo in root, stem and leaf of cucumber plants exposed to 100 mg/kg SiO_2, TiO_2, ZnS and MoS_2 NMs for 4 weeks. All data are average ± SD of four replicate. ** represent statistical differences at $p \leq 0.01$ compared with control, respectively.

Table 2
Heavy metal contents in cucumber tissues (mg/kg dry weight). * and ** represent statistical differences at $p \leq 0.05$ and $p \leq 0.01$ compared with control, respectively.

	Zn	Al	Ti	Cu	Ni	Cr	Pb	As	Cd
Control	78.8 ± 15.0	67.0 ± 11.4	10.6 ± 0.690	2.44 ± 0.231	1.88 ± 0.075	0.766 ± 0.094	0.672 ± 0.148	0.380 ± 0.046	0.118 ± 0.006
SiO_2	49.0 ± 8.35*	112 ± 43.4	11.5 ± 1.03	2.35 ± 0.369	2.22 ± 0.406	2.51 ± 1.16	0.682 ± 0.154	0.383 ± 0.041	0.139 ± 0.039
TiO_2	65.1 ± 9.50	81.8 ± 11.4	9.65 ± 1.25	1.79 ± 0.032**	1.43 ± 0.051**	0.692 ± 0.109	0.647 ± 0.155	0.334 ± 0.049	0.093 ± 0.011**
ZnS	75.8 ± 18.3	40.9 ± 6.33*	8.28 ± 0.650**	1.73 ± 0.347*	1.52 ± 0.092**	0.456 ± 0.122*	0.764 ± 0.139	0.297 ± 0.036*	0.114 ± 0.017
MoS_2	74.2 ± 15.2	27.7 ± 11.7**	8.58 ± 0.794**	1.67 ± 0.026**	1.69 ± 0.130	3.99 ± 0.685**	0.501 ± 0.094	0.299 ± 0.004*	0.093 ± 0.008**
Control	143 ± 9.87	66.1 ± 17.9	24.9 ± 1.38	13.0 ± 1.20	2.62 ± 0.268	4.26 ± 0.964	2.09 ± 0.152	4.93 ± 0.869	0.677 ± 0.135
SiO_2	58.3 ± 1.52**	92.1 ± 17.3	29.8 ± 2.80*	9.70 ± 0.990**	2.60 ± 0.105	3.98 ± 0.453	1.87 ± 0.236	2.65 ± 0.230**	0.413 ± 0.033**
TiO_2	55.7 ± 13.6**	93.7 ± 10.5	28.0 ± 4.97	10.1 ± 1.35*	2.48 ± 0.206	3.52 ± 0.741	3.39 ± 0.754*	2.43 ± 0.186**	0.356 ± 0.038**
ZnS	67.9 ± 13.5**	60.0 ± 7.98	29.1 ± 2.17*	9.64 ± 0.948*	2.79 ± 0.488	5.28 ± 1.20	1.91 ± 0.369	2.35 ± 0.354**	0.411 ± 0.036**
MoS_2	50.7 ± 13.9**	46.8 ± 10.7	26.9 ± 4.49	12.4 ± 1.68	2.45 ± 0.207	4.15 ± 0.398	2.63 ± 0.841	2.43 ± 0.350**	0.358 ± 0.047**
Control	219 ± 42.7	7720 ± 1511	230 ± 91.0	30.5 ± 6.96	7.62 ± 1.78	17.5 ± 3.66	201 ± 63.1	22.4 ± 1.85	3.89 ± 0.565
SiO_2	191 ± 19.1	8635 ± 1273	169 ± 44.0	24.7 ± 2.80	5.78 ± 1.41	12.2 ± 3.07	169 ± 41.4	23.6 ± 5.31	4.28 ± 0.405
TiO_2	514 ± 140*	4080 ± 838*	100 ± 94.5	25.0 ± 4.06	6.19 ± 1.28	24.8 ± 4.04	104 ± 67.9	14.6 ± 3.94*	3.20 ± 0.489
ZnS	348 ± 87.3	5894 ± 600	174 ± 28.0	29.4 ± 5.51	5.69 ± 1.04	9.93 ± 2.38*	160 ± 49.0	20.4 ± 5.92	3.39 ± 0.762
MoS_2	203 ± 36.2	3861 ± 538**	90.6 ± 16.7*	19.4 ± 2.83*	3.45 ± 0.470**	9.50 ± 1.98*	86.2 ± 12.4*	13.2 ± 0.802**	2.41 ± 0.310**

36.4–60.6% relative to control (Table 2). This indicates that MoS_2 NMs have stronger complex or absorption affinity with heavy metal ions compared with other NMs. Wang and Mi (2017) contribute the strong adsorption ability of MoS_2 with heavy metals to the strong Lewis acid and base soft-soft interactions, due to the abundance of exposed sulfur atoms on its surface of MoS_2 NMs.

In stems, exposure to SiO_2 NMs significantly ($p < 0.01$) reduced the content of As (46.2%), Cd (39.0%), Cu (25.4%) and Zn (59.2%) (Table 2). Similarly, exposure to TiO_2 NMs resulted in a significant decrease of the content of As (50.7%, $p < 0.01$), Cd (47.4%, $p < 0.01$), Cu (22.3%, $p < 0.05$) and Zn (61.0%, $p < 0.01$) in stem as well. In addition to SiO_2 and TiO_2 NMs, ZnS also significantly decreased the level of As (52.3%, $p < 0.01$), Cd (39.3%, $p < 0.01$), Cu (25.8%, $p < 0.05$) and Zn (52.5%, $p < 0.01$) in stem. Exposure to MoS_2 NMs significantly decreased the content of As (50.7%, $p < 0.01$), Cd (47.1%, $p < 0.01$) and Zn (64.5%, $p < 0.01$) in stem, with no impact on Cu uptake. In summary, four types of NMs generally had similar pattern on reducing heavy metals bioaccumulation in stem.

In case of leaves, exposure to SiO_2 NMs did not impact the uptake of heavy metals, except Zn, with a 37.8% content decrease ($p < 0.05$) compared to control (Table 2). While TiO_2 NMs resulted in a significant decrease of Cd (21.2%, $p \leq 0.01$), Cu (26.6%, $p \leq 0.01$) and Ni (23.9%, $p \leq 0.01$) content in cucumber leaves compared to control. ZnS NMs significantly reduced content of six heavy metals in cucumber leaves, including As (21.8%, $p \leq 0.05$), Cr (40.5%, $p \leq 0.05$), Cu (29.1%, $p \leq 0.05$), Ni (19.1%, $p \leq 0.01$), Al (39.0%, $p \leq 0.05$) and Ti (21.9%, $p \leq 0.01$) compared to control. MoS_2 at 100 mg/kg decreased accumulation of a number of heavy metals in cucumber leaves, by 21.3% for As ($p \leq 0.05$), 21.2% for Cd ($p \leq 0.01$), 31.6% for Cu ($p \leq 0.01$), 58.7% for Al ($p \leq 0.01$), compared to control without NMs. In contrast, Cr accumulation in cucumber leaves was significantly increased (421%, $p \leq 0.01$) by MoS_2 NMs. Combining that Cr in root was decreased by MoS_2 NMs, the results suggest that MoS_2 NMs enhanced the translocation of Cr from below ground tissues (root) to aboveground tissues (leaf). The underlying mechanism is still unknown.

Combining the data of all tissues, two metal sulfides (ZnS and MoS_2) had better performance in reducing the heavy metal uptake in cucumber plants compared to metal(loid) oxides NMs (SiO_2 and TiO_2). Moreover, MoS_2 NMs had more significant adsorption or binding capacity compared with ZnS NMs. The mechanism of the interactions between MoS_2 and adsorbed metal ions has been summarized by Wang and Mi (2017). They assume that the formation of metal-sulfur bonding could be a primary adsorption mechanism. In addition, the electrostatic attraction also contributes to the interaction between ions and MoS_2 (Zhi et al., 2016). Taken together, chemical complexation and electrostatic attraction might be the main mechanism for NMs to complex with heavy metal ions.

3.5. Effect of nanomaterials on mineral nutrients uptake in cucumber plants

The adsorption of heavy metals with NMs hindered their uptake and translocation in plant, which is beneficial for crop plant due to the reducing risks to transfer to food chain. However, NMs with high surface area and binding sites, possibly complex with cations like K^+, Ca^{2+} and Fe^{3+} as well, and thus lower down the macro- or micro-nutrients uptake by plant, which will be negative impacts for plant growth, given the fact that macro and micro nutrients play an important role in plant metabolism and function. Root is the organ for water and mineral nutrients uptake and leaf is the organ in which important metabolic processes occur, like photosynthesis and respiration. Herein, the excess or deficiency of micronutrient accumulation induced by nanomaterials in root or leaf might perturb plant metabolism and growth.

Concentrations of macro and micro nutrients including Si, Ca, Na, Mg, Fe, K, Mn and Mo found in cucumber tissues grown in soil amended with different types of NMs are shown in Table 3. Exposure to SiO_2 and ZnS generally had no impact on nutrient elements uptake in root compared to control, except that ZnS NMs decreased Mg content by 37.7% ($p < 0.05$). The weak absorbing capacity of SiO_2 and ZnS with mineral nutrient may attribute to their positive surface charge (Table 1). In comparisons, TiO_2 and MoS_2 NMs disturbed the accumulation of a number of nutrients including Si, Mg, Fe, K and Mn in cucumber root. As shown in Table 3, exposure to TiO_2 and MoS_2 NMs significantly ($p < 0.05$) reduced Si by 48.6% and 50.1%, respectively; Mg by 68.4% and 47.8%, respectively; Fe by 45.1% and 48.8%, respectively; K by 59.4% and 49.3%, respectively; Mn by 47.5% and 40.1%, respectively. This indicates that NMs are not only binding with heavy metals, but also with macro and micro nutrients. It is interesting to note that TiO_2 and MoS_2, which have negative surface charge, induced much more distribution of macro and micro nutrient uptake, compared to two positive charged NMs (SiO_2 and ZnS). Therefore, surface charge is key factor impacting the interaction between NMs and mineral nutrients. Peralta-Videa et al. (2014) also observed that CeO_2 and ZnO NPs interfered with Mg and K accumulation in soybean root. They proposed the possible underlying mechanism is that NPs stuck on the soybean root surface, blocking the channels and reducing the absorption of Mg. The disturbed nutrient elements (Si, Mg, Fe, K, Mn) act important role in structural integrity, energy storage and redox reaction (Lazar et al., 2003). For example, Mg is a part of the ring structure of the chlorophyll molecule, and the deficiency of Mg will induce chlorosis (Lazar et al., 2003). Mn ions activate several enzymes, such as decarboxylases and dehydrogenases involved in TCA cycle (Peralta-Videa et al., 2014). Therefore, although unchanged at harvest, the biomass might decrease during longer exposure due to the deficiency of these nutrients.

In the above-ground tissues, the most noticeable changes were significantly decreased potassium accumulation in leaves by 8%–17% ($p < 0.05$) when exposed to SiO_2, TiO_2 and MoS_2, compared to control (Table 3). Potassium is usually accumulated at high concentrations in plant cells, and K plays an important role in regulation of the osmotic potential of plant cells (Hedrich and Kudla, 2006). It also activates many enzymes involved in respiration and photosynthesis. Therefore, the disturbed K accumulation might negatively impact plant physiological function. As mentioned before, chlorophyll content was negatively impacted by ZnS and MoS_2, probably because nutrients elements involved in photosynthetic process, such as potasium, were negatively impacted. In addition, exposure to TiO_2, ZnS and MoS_2 resulted in the significant decrease of leaf Na accumulation by 31.5%–56.8% ($p < 0.05$) compared to control (Table 3). Peralta-Videa et al. (2014) also found that CeO_2 NPs reduced Na accumulation in soybean leaves. Sodium stimulates growth through enhanced cell expansion, and it can partly substitute for potassium as an osmotically active solute (Lazar et al., 2003). Thus, the interfered sodium and potassium uptake in leaf may negatively impact some metabolism process in leaves, like carbon fixation in photosynthesis.

Interestingly, exposure to SiO_2 and TiO_2 NMs significantly ($p < 0.05$) increased Fe content by 58.2% and 40.6%, respectively, in cucumber leaves. Iron plays an important role as a component of enzymes involved in the electrons transfer, such as cytochromes (Lazar et al., 2003). The increased Fe accumulation in leaves is beneficial for photosynthesis process. Another interesting finding is that a significant increase of Si (23%, $p < 0.01$) in leaf was observed in presence of MoS_2 NMs (Table 3). It is known that Si plays an important role in maintain structural integrity and plants deficient in Si are more susceptible to fungal infection. The underlying mechanism is that silicon forms complexes with polyphenols and

Table 3
Mineral nutrients content in cucumber tissues (mg/kg dry weight). * and ** represent statistical differences at $p \leq 0.05$ and $p \leq 0.01$ compared with control, respectively.

	Ca	Mg	K	Si	Na	Fe	Mn	Mo
Control	28467 ± 3609	8085 ± 1304	7757 ± 98.9	3380 ± 171	111 ± 15.0	67.7 ± 16.4	49.1 ± 9.85	1.44 ± 0.201
SiO_2	30203 ± 236	8877 ± 750	7061 ± 340**	3634 ± 536	82.3 ± 19.3	107 ± 21.5*	45.9 ± 2.14	1.19 ± 0.228
TiO_2	26361 ± 1915	7933 ± 663	6879 ± 694*	3347 ± 292	76.5 ± 14.0*	95.2 ± 6.57*	45.3 ± 3.37	1.21 ± 0.206
ZnS	26629 ± 2240	7606 ± 480	7232 ± 910	4038 ± 373*	69.1 ± 21.7*	74.0 ± 19.0	44.3 ± 4.72	0.64 ± 0.069**
MoS_2	29387 ± 3062	7629 ± 803	6437 ± 916*	4171 ± 119**	48.4 ± 3.38**	71.0 ± 14.3	45.9 ± 6.93	1.78 ± 0.385
Control	19886 ± 1559	8455 ± 1126	13857 ± 1188	1566 ± 398	1374 ± 267	119 ± 25.4	20.3 ± 3.59	1.12 ± 0.155
SiO_2	23853 ± 3390	9771 ± 1048	14135 ± 1459	1879 ± 233	1811 ± 173	110 ± 18.2	23.8 ± 2.09	1.14 ± 0.104
TiO_2	21189 ± 1294	8425 ± 496	14199 ± 1669	1904 ± 249	1756 ± 254	117 ± 9.73	23.3 ± 2.66	1.03 ± 0.109
ZnS	22062 ± 1856	8738 ± 404	17375 ± 1606*	1816 ± 86	1768 ± 129*	94.7 ± 8.09	21.9 ± 1.85	0.77 ± 0.059**
MoS_2	21476 ± 2352	8312 ± 1678	15303 ± 3000	1560 ± 139	1923 ± 157*	96.5 ± 20.2	20.2 ± 1.75	2.06 ± 0.360**
Control	13707 ± 1460	3422 ± 553	2685 ± 760	10880 ± 1773	699 ± 163	7102 ± 1129	339 ± 68.4	3.96 ± 3.41
SiO_2	14958 ± 1241	2742 ± 162	1822 ± 305	12245 ± 1683	616 ± 124	7714 ± 1187	379 ± 77.6	1.13 ± 0.493
TiO_2	15257 ± 2796	1080 ± 347**	1091 ± 462*	5589 ± 1182*	664 ± 139	3897 ± 1144*	178 ± 68.5*	11.0 ± 8.42
ZnS	13694 ± 602	2131 ± 427*	1617 ± 493	8340 ± 710	562 ± 71.1	5355 ± 229	278 ± 7.25	3.70 ± 3.23
MoS_2	14071 ± 972	1781 ± 274**	1362 ± 233*	5431 ± 735**	581 ± 112	3638 ± 389**	203 ± 41.0*	12.1 ± 1.83**

serve as an alternative to lignin in the reinforcement of cell wall (Lazar et al., 2003). Therefore, these results suggest that MoS_2 might help plant resist pests or fungus through the enhancement of Si uptake in cucumber leaves.

4. Conclusions

In summary, the results of this study indicate that four types of nanomaterials dosing as low as 100 mg/kg, have the potential to reduce toxic heavy metal accumulation in cucumber plants. In the experimental conditions of this study, MoS_2 NMs impacted the accumulation of most heavy metals. Thus, the tested NMs especially MoS_2 NMs, can be applied as immobilization amendment in contaminated soils to reduce the risk of the transfer of toxic heavy metals to food chain. However, although the biomass was unchanged by NMs, the photosynthetic pigments contents were decreased by metal sulfides NMs (MoS_2 and ZnS). Additionally, NMs also inhibited the uptake of macro and micro nutrients in cucumber plants. The negative impact on beneficial nutrients involved in photosynthesis, such as Fe and Mg, may impact physiological function. The uptake and translocation of NMs or their released ions by plant tissues were not found, except MoS_2/Mo. In presence of MoS_2, the content of Si in cucumber leaves increased, which may enhance the resistance to attackers. This study provides a perspective on how emerging nanomaterials impact the heavy metals uptake and also physiological impact on plants. Both positive (reduce heavy metal accumulation) and negative (decrease nutrient element accumulation) impact should be taken into consideration when NMs were applied in environmental remediation. Cucumber plants, which is sensitive to heavy metal contaminations, can be applied with NMs, especially MoS_2, to enhance their tolerance and resistance to heavy metals.

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