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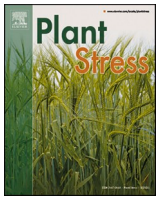

Application of SiO_2 nanoparticles to address CdS NPs contamination in spinach

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ABSTRACT

Rising cadmium (Cd) contamination poses significant threats to crop productivity, quality, and human health. To address this, nano-enabled techniques have recently gained attention for their potential to enhance crop yields and remediate contamination due to heavy metals. This study explores the efficacy of silicon dioxide nanoparticles (SiO_2 NPs) in mitigating the effects of cadmium sulfide (CdS) NPs in spinach. Field experiments were conducted growing spinach plants subjected to cultivation with 1 mg/L CdS NPs contamination, with foliar application of SiO_2 NPs at concentrations of 1, 20, and 100 mg/L. The phenotypic, biochemical, and metabolic responses of the plants to stress conditions were examined following exposure to CdS and SiO_2 for four weeks. The results showed that SiO_2 NPs increased the fresh and dry weights of both roots and shoots. Furthermore, CdS NPs exposure reduced chlorophyll content by 66.76 %, whereas SiO_2 NPs co-exposure increased chlorophyll levels by up to 42 % compared to the CdS NPs and control groups. However, elevated malondialdehyde (MDA) levels were observed in leaves for the CdS-only group and roots for all treatments indicating oxidative stress was most pronounced for the CdS case. Results demonstrated that SiO_2 application significantly reduced Cd accumulation in spinach by up to 34.92 %. Also, enhanced mineral accumulations were recorded in both roots and shoots, whereas decreased levels were found in the co-exposure groups, except for Zn. The exposure to SiO_2 resulted in upregulation of metabolites including galactonic acid, d-aspartic acid and others, and UDP-d-galactose was downregulated in the group exposed only to CdS NPs. The upregulation of these metabolites by SiO_2 NPs demonstrates their mitigating effect against CdS NPs induced stress. This work enhances understanding of phenotypic and metabolic alterations induced in spinach by CdS and SiO_2 NPs, and independently and through their co-exposure. Overall, our findings indicate that Cd contamination can be reduced in spinach using SiO_2 NPs when applied at low levels, and the mechanisms are discussed.

1. Introduction

Cadmium (Cd) is a hazardous contaminant in the natural environment. It is a heavy metal that poses a significant risk for the environment and human health (Subašić et al., 2022). It can enter the soil through industrial discharge, fertilizer application, and sewage sludge applications (Grobelak et al., 2024). Cd is bioavailable and can be taken up by plants especially in acidic soils. Plants can obtain these trace metals from their surroundings via soil-root transport and foliar absorption from the atmosphere (Lin et al., 2024). At high levels, it can disrupt soil microbial communities, reduce soil fertility, and affect plant growth (Giller et al., 2009; Ren et al., 2022). In plants, Cd can be taken up through the roots and accumulate in the edible parts of the plants such as their leaves, roots, and shoots. It can also affect the plant growth and development by affecting photosynthesis, nutrient uptake, and other physiological processes in plants resulting in stunted growth, chlorosis, and reduced yields (Haider et al., 2024). For humans, Cd can lead to severe health problems including kidney damage, bone demineralization, respiratory

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issues, and an increased risk of cancer (Nordberg et al., 2018). Previous research has highlighted the potential phytotoxicity and health risks associated with exposure to Cd ions in plants (Harris and Taylor, 2001). For instance, the application of $CdCl_2$ to wheat foliage impacted wheat biomass and grain yields; however, with the consequence of significantly elevating Cd content in wheat tissues (Li et al., 2020). In other crops such as maize, the uptake of CdS nanoparticles (NPs) has been shown to have an inhibitory effect on seedlings (Ullah et al., 2023). Furthermore, exposure to Cd significantly increased Cd levels in various tissues of chili (Ouyang et al., 2023), while CdS NPs had no negative phenotypic effects on soybean (Tian et al., 2020a). However, little attention to-date has been given to Cd NPs in general whether released from technological processes or natural sources (Večeřová et al., 2019). Their fate and toxicity to plants, and subsequently to human health via direct or indirect consumption, remains underexplored.

Silica nanoparticles (SiO_2 NPs) offers considerable potential in nano-enabled agriculture, serving as fertilizers, pesticides, carriers, or sensors, owing to their low toxicity, high biocompatibility, and positive impact on plant growth (Tian et al., 2020b). SiO_2 NPs exhibit the capability to mitigate the uptake and accumulation of Cd and arsenic (As) in crops (Liu et al., 2014). The mechanism underlying the inhibition of Cd and As uptake by rice through SiO_2 NPs is currently understood to occur in two ways. Firstly, silicon (Si) is cross-linked with cell wall hemicelluloses in plants (Ma and Yamaji, 2015), with the increased Si leading to more Si complexes in the cell wall. The resulting negative charge enhances the binding of heavy metals and inhibits their transport within the plant (Ali et al., 2019). Secondly, since the uptake channels for Cd and As in plants coincide with those for Si, the increased Si competes for the uptake and binding sites, thereby reducing their accumulations (Cui et al., 2017). In comparison to soil broadcasting, foliar application is emerging as a more targeted, scalable, efficient, and bioavailable strategy for delivering engineered nanomaterials (ENMs) in agriculture (Kah et al., 2019). Regarding SiO_2 NPs, their interaction with specific environmental pollutants during the foliar application process may influence the uptake and accumulation of pollutants in plants (Qu et al., 2018). However, currently there is limited understanding of how SiO_2 NPs specifically affects the accumulation of NPs in plants.

Metabolomics serves as a potent and comprehensive tool for delving into the cellular metabolic responses of plants under external stresses (Zhao et al., 2016). This approach has been effectively utilized to investigate how plants respond to NPs and/or pollutants. For instance, Zhao et al. conducted research on the reaction of plants to NPs, elucidating the specific detoxification mechanisms employed by lettuce exposed to nanopesticides (Zhao et al., 2016). Subsequent studies have applied metabolomics to uncover the molecular mechanisms underlying plant exposure to SiO_2 NPs (Tian et al., 2020b). These findings strongly indicate that metabolomics stands out as a useful tool for analyzing the subtle effects of ENMs or pollutants on plants, and sheds light on the underlying mechanisms.

Spinach (*Spinacia oleracea*) is a popular vegetable with a global consumption of 32 million tones yearly (Martin, 2021). Hydroponic cultivation of spinach with water culture and leaf fertilizer is popular for its precise nutrient control and water efficiency. However, uptake of Cd in this plant is of significant concern and steps need to be taken to ensure that the Cd is prevented from exceeding acceptable levels. Application of SiO_2 NPs has been shown to reduce Cd in other plants (Ali et al., 2019), and thus needs to be tested for spinach, which is the focus of this study. Careful testing and vigilant monitoring are necessary to ensure the sustainability and safety of plant and human health.

The objectives of this study are: (1) to examine the uptake and accumulation patterns of SiO_2 NPs and Cd in spinach, (2) assess the combined phytotoxic effects of SiO_2 NPs (applied foliarly) and CdS NPs (via hydroponic methods) on spinach plants accounting for both morphological and physiological effects, (3) investigate SiO_2 NPs enhancement on the metabolic reprograming of CdS NPs and explore the impact of foliar application of SiO_2 NPs on Cd accumulation and nutrients, and (4) analyze metabolic profiles to uncover signals, defense mechanisms, and damage responses. This work potentially provides a novel strategy for mitigating Cd accumulation in crops by using SiO_2 NPs. SiO_2 NPs show great promise for enhancing food safety. SiO_2 NPs improve plant growth, yield, soil water retention, and disease resistance. However, potential toxicity at high concentrations and long-term effects on soil health, microbes, ecosystems, and human health require further study.

2. Materials and method

2.1. Spinach growth

Spinach seeds, bought from a local market (Shandong, China), were submerged in 2 % hydrogen peroxide solution for 30 min to ensure surface sterilization, followed by three washes with ultrapure (UP) water. Subsequently, the seeds were individually placed in soft foam and maintained in darkness at a temperature of 25 ± 2 °C. After four days, exposure to light for two days initiated full sprouting. Upon completion of sprouting, the seeds were transferred into a plastic beaker with a lid and a small hole, containing 25 % Hoagland nutrient solution, and placed in the same foam utilized for germination. The cultivation phase was then carried out in a controlled greenhouse setting with conditions set at 25 °C temperature, 65 % relative humidity, and a light intensity of 31.6 μmol/m2/s. The photoperiod consisted of 14 h of illumination followed by 10 h of darkness.

2.2. Exposure cases

There were eight treatments in total: (1) no exposure of NPs blank control (CG), (2) CdS NPs (1 mg/L), (3) CdS (1 mg/L) and SiO_2 (1 mg/L) (co-exposure low), (4) SiO_2 low (1 mg/L), (5) CdS (1 mg/L) and SiO_2 (20 mg/L) (co-exposure medium), (6) SiO_2 medium (20 mg/L), (7) CdS (1 mg/L) and SiO_2 (100 mg/L) (co-exposure high), and (8) SiO_2 high (100 mg/L). Each treatment was conducted with four replicates. The concentrations of SiO_2 NPs were selected based on established protocols from prior ecotoxicological and agronomic studies, which demonstrate dose-dependent efficacy in mitigating abiotic stress and enhancing plant resilience (Cai et al., 2023; Faisal et al., 2024; Manzoor et al., 2023). The SiO_2 NPs suspensions with a final concentration of 1, 20 and 100 mg/L were freshly prepared via dissolving SiO_2 NPs in UP water and sonicated in a water bath with ice for 30 min before application. During spraying, the leaves were held in one hand, and the vacuum spray bottle was operated in the other hand so that the droplets were evenly sprinkled onto leaf surfaces and to reduce the droplet fall. The spraying amount for each group was 10 mL, with three applications administered at intervals of 8 days

Spinach was harvested after 7 weeks for further analysis. The plants underwent thorough rinsing with UP water followed by immersion in 20 mmol ethylenediaminetetraacetic acid (EDTA) for 30 min to eliminate any adhering ions, and then given three rinses with UP water prior to analysis. The height of the spinach and root length were measured, and the plants were separated into two parts: underwater and above-water parts (roots and shoots) and the fresh biomass weight of root and shoots were measured. Then, the plants were put in an oven (72 h at 70 °C) to dry, and the dry biomass of underwater and above-water parts were measured.

2.3. Cd and other mineral analysis

A known amount of oven-dried shoots and roots were digested in H_2O_2 and plasma HNO_3 (pure) (v/v: 4:1) using a Microwave Digester (CEM Corporation, American). Cd, Na, Ca, Mn, Fe, Cu, and Zn were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS, ELAN DRC II, and PerkinElmer, USA). The translocation factor (TF) was calculated based on metal concentrations between the shoots and

roots (TF = C_{shoots}/C_{roots}), with C_{shoots} representing the Cd content in the above-ground portions (shoots), and C_{roots} denoting the Cd in the below-ground portion (roots) of the spinach.

2.4. Microscope observations

Root and leaf samples underwent a freeze-drying process, accomplished by gradually warming the samples to room temperature within a bell jar evacuated to a pressure of 2.6 Pascal using a rotary pump. These dried samples, comprising both roots and leaves, were then affixed to stubs. The examination of these samples was conducted without any coatings. A JEOL 5400 LV Scanning Electron Microscope (SEM) was employed operating in low-vacuum mode at 15 kV, utilizing a back-scattered electron detector. For elemental analysis, an attached KEVEX Sigma Energy Dispersive X-ray Spectrometer (EDS) was employed. For Transmission Electron Microscopy (TEM) EDS mapping analysis, plant tissue samples underwent a preparation process that involved slicing them into thin transverse sections (TS). These sections were placed onto a 400-mesh copper grid coated with a carbon substrate.

2.5. Relative chlorophyll and MDA level

Chlorophyll levels were assessed using the Soil Plant Analysis Development (SPAD) meter (SPAD-502Plus). Malondialdehyde (MDA) concentrations were determined spectrophotometrically with A003–1 assay kits following the protocols provided by the manufacturer (Nanjing Jiancheng Bioengineering Institute, China).

2.6. Metabolite analysis

Samples stored at −80 °C were thawed and brought up to room temperature (22 ± 2 °C). A 1.5 mL Eppendorf tube was used to mix a small sample volume with 100 μL of l-2-chlorophenylalanine (0.06 mg/mL) dissolved in methanol, serving as an internal standard. The tubes were vortexed for ten seconds. Following this, a 400 μL of an ice-cold mixture of methanol and acetonitrile (2:1, vol/vol) was added. After vortexing for 1 min, the mixtures were ultrasonicated for 10 min in an ice water bath and then stored at −40 °C for 8 h. Following storage, samples were centrifuged (1300 rpm, 4 °C) for 10 min. A 150 μL volume of the supernatant from each tube was collected using crystal syringes, then passed through 0.22 μm microfilters, and transferred to liquid chromatography (LC) vials. These vials were stored at −80 °C for LC-MS analysis. Quality control (QC) samples were prepared by pooling aliquots from all individual samples. Detailed information on LC-MS/MS analysis and data processing is provided in supplementary material (text 1).

2.7. Statistical and metabolomics analysis

The experimental results were derived from the average of four replicates and are presented as mean ± SD (standard deviation). Statistical significance was assessed using Tukey's test following one-way analysis of variance (ANOVA) across the entire dataset, with differences considered significant at $p < 0.05$. Metabolomics analysis was performed utilizing the MetaboAnalyst 6.0 online tool (https://www.metaboanalyst.ca). Graphical representations were created using Origin, SRPLOT (https://bioinformatics.com.cn/en), and Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

3. Result

3.1. Characterization of CdS and SiO_2 NPs

SiO_2 and CdS NPs are analytical reagents. As such, the characterization of CdS NPs was the same as in the previous report, by Ullah et al., 2023. The XRD pattern of the SiO_2 NPs displayed a diffraction peak centered at $2\theta = 23°$, corresponding to the (100) lattice plane (*PDF#44–1394;* Fig. S1A). The diffracted peaks observed in the analysis indicate that the material possesses a crystalline structure. This finding suggests that the arrangement of atoms within the material is ordered. Consequently, the presence of these peaks provides strong evidence for its crystalline nature. The crystallite size of the SiO_2 NPs was determined using the Debye-Scherrer equation ($D = k\,\lambda/\beta\,\cos\theta$) (Chaki et al., 2013), revealing a crystallite size of 0.70 nm. Scanning electron microscope-energy dispersive X-ray analysis (SEM-EDX) spectrum confirms that the NPs primarily consist of Cd and S (Fig. S1B and D). Combining transmission electron microscope (TEM) and ImageJ software analysis, the average particle size was determined to be 6.41 nm (Fig. S1C and E).

3.2. Effect of SiO_2 on phenotypic parameters

This study investigates the effects of CdS NPs exposure and SiO_2 NPs spraying in terms of the growth parameters of spinach. It was found that the medium dose of SiO_2 (20 mg/L) enhanced the shoot fresh weight (FW) by 78.35 % compared to the sample treated with only CdS NPs. SiO_2 for low and high doses reduced the shoot FW (Fig. S2 A and B). Root FW shows a significant increase after spraying SiO_2 for low, medium, and high doses by 1.93 %, 87.79 %, and 34.30 %, respectively. Similarly, the shoot DW for a medium dose increased, by 45.16 %; however, low and high doses resulted in decreases. Similar results were observed for root DW (Fig. S2C and D). The root lengths were increased in all groups except for the co-exposure high dose (Fig. S2E). Plant height was significantly inhibited in co-exposure groups compared to CG and CdS NPs group ($p < 0.05$, Fig. S2F). Results indicate a substantial increase in root length under CdS and SiO_2 NPs. Root examination reveals significant alterations in root structures after CdS exposure, characterized by a pronounced thinning, fragility, and easy to breakage. Additionally, Cd observed in the tips of roots was correlated with blackened tips, which is believed to be caused by the Cd. CG and application of SiO_2 NPs displayed robust and verdant leaf coloration, indicative of a flourishing and healthy state (Fig. S3). Notably, the dimensions of the leaves (width and length) and count were markedly hindered under CdS exposure (Fig. S4). Statistical analysis revealed a significant inhibition ($p < 0.05$) in leaf surface area and count, highlighting the adverse impact of CdS on the morphological aspects of the leaves. In conclusion, the SiO_2 NPs-only exposure case using 20 mg/L of spraying showed a significant increase in all phenotypic parameters, indicating the beneficial impact of SiO_2 on spinach.

3.3. Effects of SiO_2 on Cd uptake and nutrient accumulation

Cd was measured across various treatment groups. In the co-exposure group, the Cd was significantly elevated compared to the CG and SiO_2 groups ($p < 0.05$), indicating a notable accumulation of Cd (Fig. 1A and B). Specifically, in the CdS treatment group, Cd accumulated to 1.01 mg/Kg in roots and 0.14 mg/Kg in shoots. Applying SiO_2 for low, medium, and high doses reduced Cd accumulation by 2.54 %, 34.92 %, and 19.07 % in roots, respectively. In shoots, Cd accumulation decreased by 24.99 % and 20.61 % for low and medium applications of SiO_2, respectively, but resulted in a 7.9 % increase for high doses of SiO_2. Samples not exposed to CdS NPs exhibited negligible Cd compared to CdS exposed groups. Furthermore, the TF was calculated for the CdS exposure groups. The TF of all treatments was greater than 0.1, indicating a relatively high level of Cd translocation. With the spraying of a low dose of SiO_2, TF decreased by 25.92 %. However, medium and high doses of SiO_2 showed an increase in TF compared to the untreated SiO_2 group. This result indicates that TF was dose-dependent on the SiO_2, and SiO_2 NPs play an important role in Cd migration from root to shoot.

The concentrations of Na, Mg, Ca, Mn, Fe, Cu, and Zn were measured in roots and shoots following exposure to CdS and SiO_2 (Fig. S5). Accumulation of Mg and Ca was found to be higher in shoots, whereas

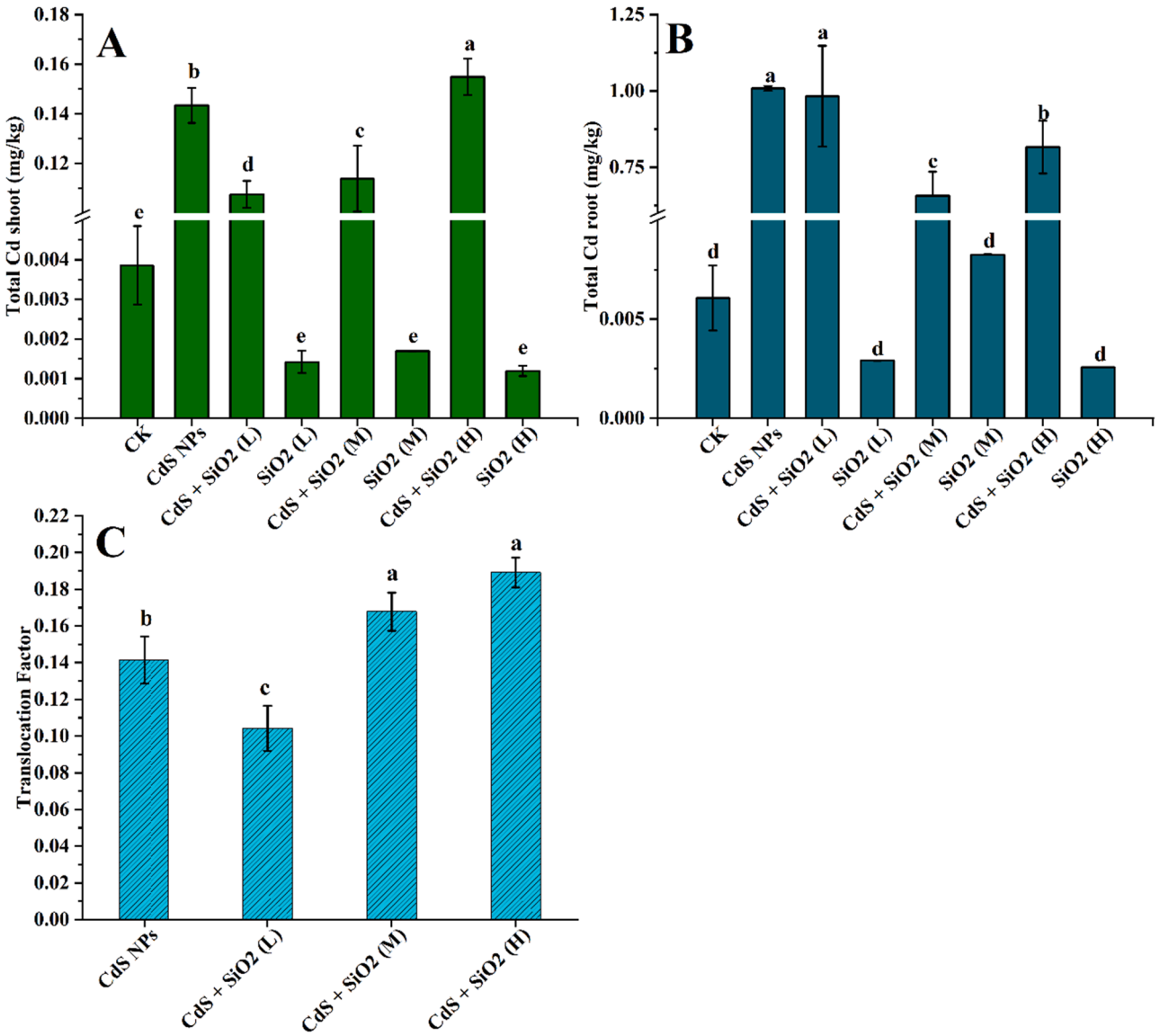

Fig. 1. Total Cd in spinach plant after CdS NPs, SiO_2 and co-exposure (A) shoots (B) roots. Translocation factor (TF) is shown in (C). The results of four replicate samples were presented as mean ± SD. Different bars represent the significant difference ($p < 0.05$).

other nutrients increased in roots (Fig. S5B and C). Mn found in roots significantly increased with the SiO_2 application, but a significant decrease was observed in shoots, except with low and medium SiO_2, indicating that Mn accumulated in roots, but its translocation to shoots was significantly inhibited. Cu showed a significant increase with the CdS NPs-only exposure case compared to other treatments (Fig. S5F). Generally, nutrient accumulation increased with the application of SiO_2, suggesting a positive impact of SiO_2 on nutrient uptake in spinach. Notably, Mn, Fe, Cu, and Zn accumulated significantly higher in roots compared to the CG (Fig. S5E and F). Furthermore, Zn levels were elevated in both co-exposure and sole exposure to CdS NPs as compared to the CG (Fig. S5 G). Overall, nutrient accumulation was higher in roots than in shoots, likely because roots are the primary site for nutrient uptake. The TF of nutrients was higher with the application of SiO_2 compared to CdS NPs alone.

3.4. Microscopic observations of stomata and NP aggregates

SEM analysis shows notable differences between the stomata of CG leaves and those treated with Cd. In the CG, the stomata were observed to be wide open and turgid, indicating proper and healthy function. In contrast, the stomata in the Cd-treated leaves appear abnormal, with visible damage or irregularities around the guard cells, suggesting that Cd exposure disrupts the normal structure and function of the stomata (Fig. 2A1 and A2). On the other hand, applying SiO_2 NPs to the spinach alleviated the negative effects of Cd and restored the stomata to a better structure with a wider aperture (Fig. 2 A3). The SiO_2 NPs aggregates were observed in the stomata (Fig. S6). Examination of TEM images revealed the penetration of CdS NPs or their aggregates (depicted as black dots) had made it through the cell membrane and into the cytoplasm (Fig. S7). Following exposure to CdS NPs, EDS analysis identified of Cd and S in roots and shoots, respectively, originating from the aggregates (highlighted with a red box) (Figs. 2C and S7). This confirms the direct uptake and localization of CdS NPs by spinach roots and subsequent transportation to the shoots. Foliar exposure of SiO_2 NPs showed confirmed presence of Si through TEM-EDS mapping (Figs. 2 and S7), and aggregations in shoot and root tissues were found. The findings are corroborated by SEM-EDS, which revealed the presence of SiO_2 NPs in stomata and leaf surfaces.

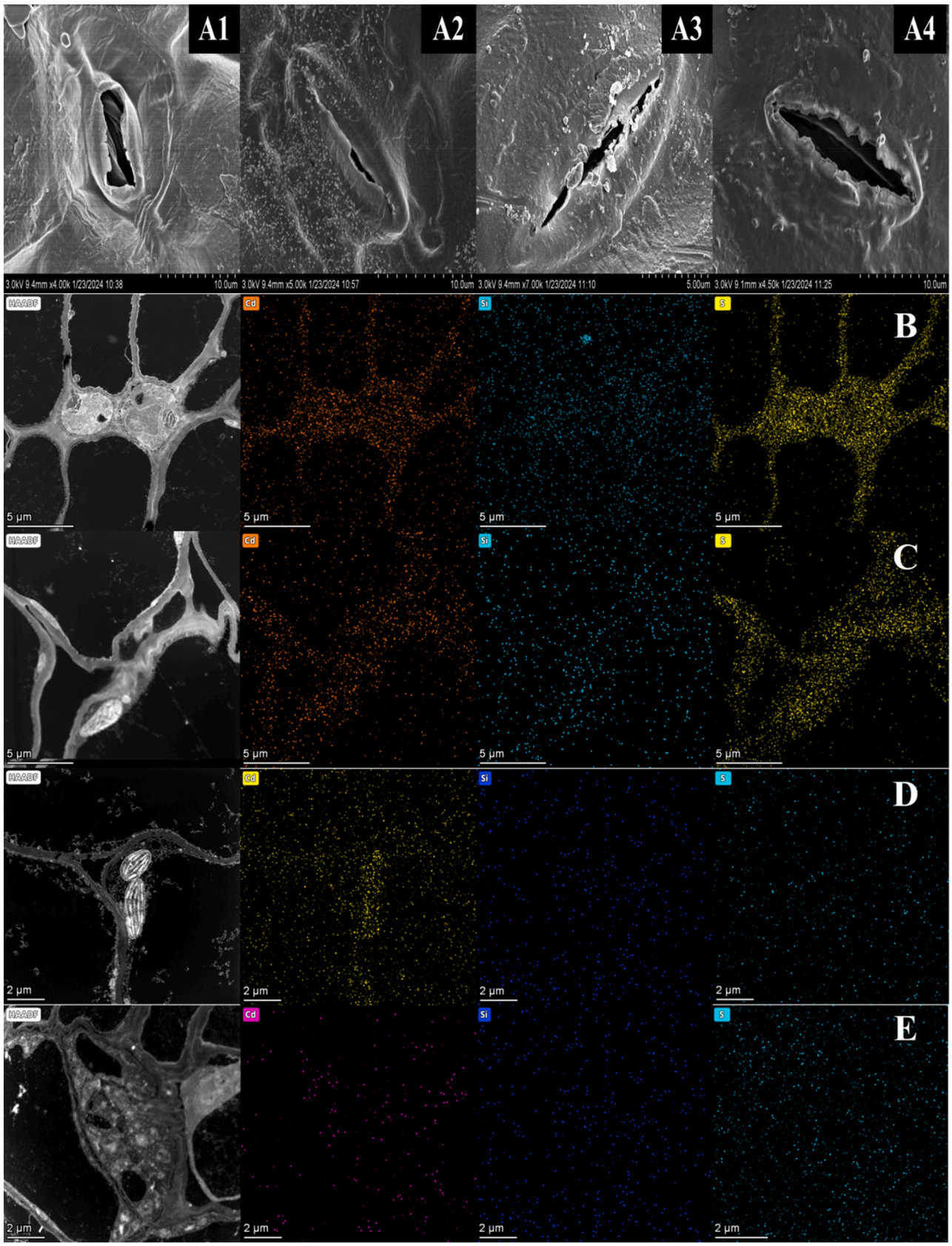

Fig. 2. SEM images of stomata (A1) CG, (A2) CdS, (A3) co-exposure, (A4) SiO_2, TEM images (B) shoots with co-exposure, (C) roots with co-exposure, (D) shoots with CdS, and (E) roots with CdS exposure.

3.5. Chlorophyll content and MDA

The exposure of CdS NPs resulted in a substantial inhibition (66.76 %) of chlorophyll content, signifying a negative impact on photosynthetic activity ($p < 0.05$; Fig. 3A). Interestingly, co-exposure with SiO_2 NPs enhanced chlorophyll contents by 22 %, 31 %, and 42 % for low, medium, and high SiO_2 NPs, respectively, compared to the exposure to only CdS NPs. Comparative analysis with the CG and SiO_2 NPs alone revealed a significant inhibitory effect of CdS NPs on chlorophyll contents. Notably, when SiO_2 NPs were applied through spraying, there was a substantial increase of 19 % in chlorophyll compared to the CG, particularly at high doses of SiO_2 NPs (Fig. 3A). SiO_2 NPs demonstrated a mitigating effect on the adverse impact of CdS NPs when co-exposed.

Generally, MDA is a byproduct during lipid peroxidation, serving as an indicative marker for cellular membrane damage. Following a single exposure to CdS NPs, MDA levels exhibit a significant increase in both leaves and roots compared to other treatments ($p < 0.05$, Fig. 3B and C). Co-exposure of CdS and SiO_2 at a medium dose in leaf tissue results in elevated MDA levels. At the same time, other treatments show a decrease, suggesting the absence of induced lipid peroxidation (Fig. 3B). Conversely, in the roots, MDA levels significantly increased across all treatments during co-exposure ($p < 0.05$, Fig. 3C). Roots were in direct contact with CdS NPs and this might increased MDA levels in roots. The application of SiO_2 NPs alleviated the adverse impact of CdS NPs stress on photosynthesis and MDA.

3.6. Metabolic response of spinach to SiO_2 under CdS NPs stress

Untargeted metabolomics can be used to investigate the metabolic processes in nano-toxicology. To unravel the metabolic response of spinach when exposed to CdS NPs hydroponically and SiO_2 NPs applied to foliage, various groups present in Section 2.2 were examined. Metabolite examination revealed the identification of 5737 unique metabolites within the spinach leaves. A one-way ANOVA analysis showed significant changes occurred in 5465 out of the 5737 (i.e. 95 %) detected metabolites in spinach leaves ($p < 0.05$; Fig. S8E). Volcano graphs show a substantial number of metabolites presented along the vertical in groups of co-exposure and exposure to only SiO_2 (Fig. S9). The PLS-DA score plot demonstrates a distinct separation between the co-exposure and single exposure to SiO_2 NPs groups as compared to the CG. Total variances along components 1 and 2 are 35.4 % and 15.6 %, respectively (Figs. 4A and S8A). Similarly, CG and SiO_2 were observed to be separated by 30.3 % along component 1 (Fig. S8B), and the co-exposure displayed a remarkable separation of 75.2 % (Fig. S8C). The detailed group-to-group comparisons are presented in Fig. S10. These findings underscore significant separation in metabolic profiles among

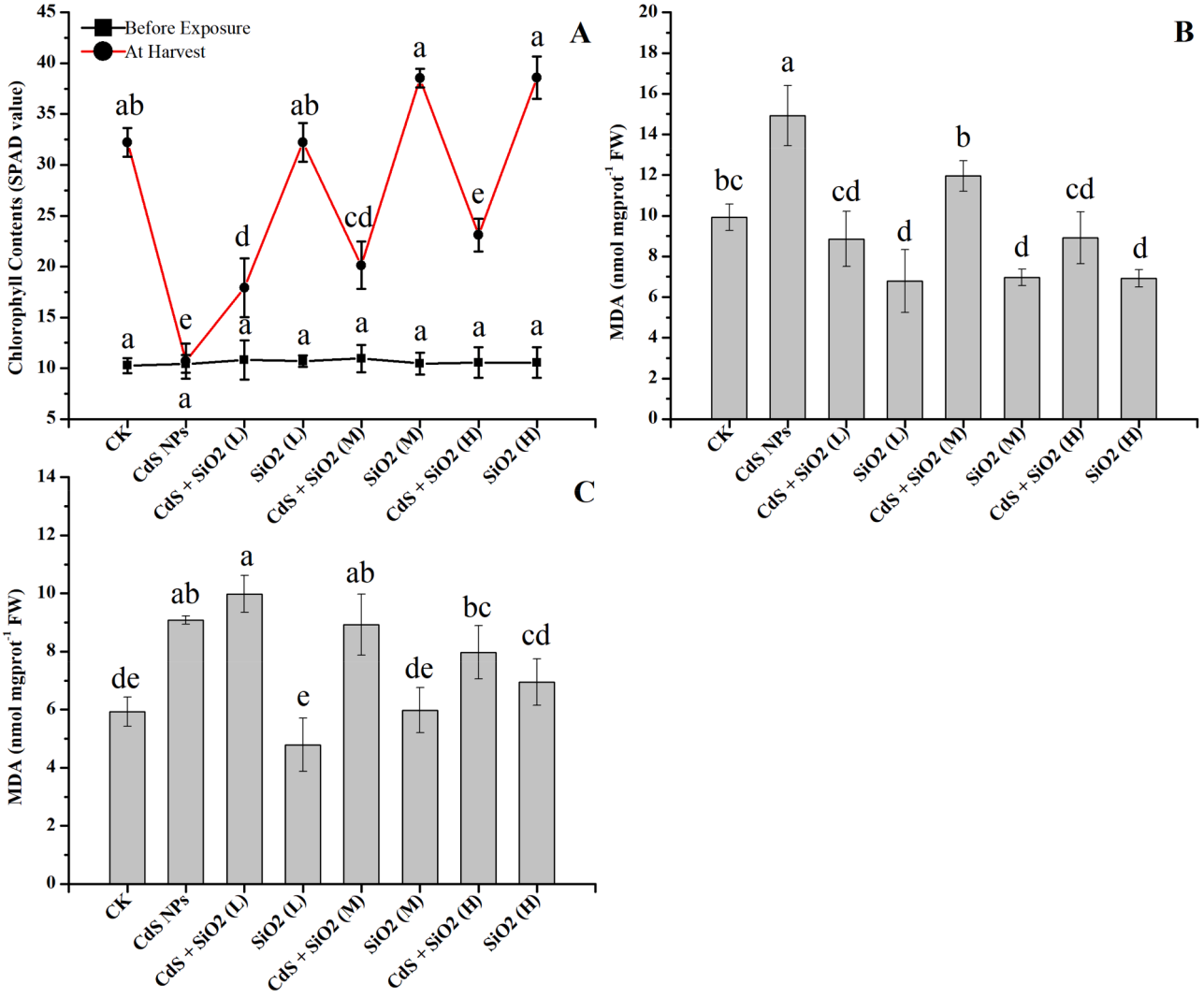

Fig. 3. (A) Relative chlorophyll content in different treatments: CdS, co-exposure and SiO_2 low, medium, and high doses for spinach, before exposure and after exposure. MDA level in (B) leaves and in (C) roots. Significant differences between the treatment means are shown by bars with different letters ($p < 0.05$). Data are reported as the mean ± SD ($n = 4$).

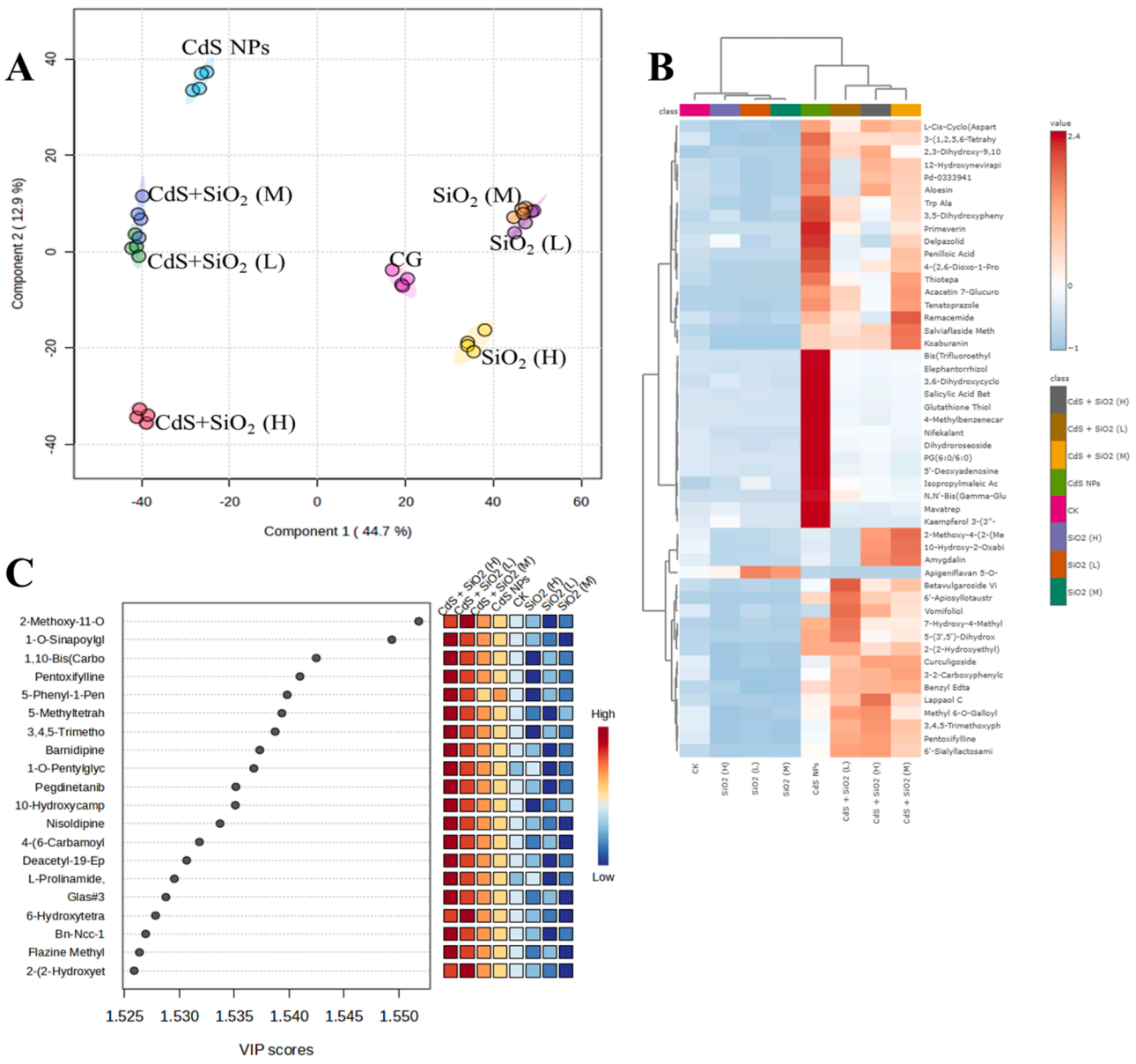

Fig. 4. (A) The PLS-DA score plot displays the separation and clustering of treatment groups based on metabolomic profiles, (B) heat map of relative abundance of the top 50 metabolites across all treatment groups. Rows represent metabolites, columns represent samples, and color gradients indicate concentration levels for comparison between treatment. (C) shows the top 20 metabolites (VIP > 1.5), highlighting the most significant metabolites for distinguishing between treatment groups, with VIP scores from the PLS-DA model indicating their importance.

the different exposure groups, as elucidated by the PLS-DA analysis. The plot of PCA scores also demonstrated the separation in the entire treatment groups with 37.6 % and 31.75 % for components 1 and 2, respectively (Fig. S11H).

A heatmap of the top 50 metabolites shows significant differences between groups exposed to CdS NPs and those treated only with SiO_2 NPs (Fig. 4B). In groups exposed to CdS and treated with different SiO_2 doses, the stress was clearly reduced, as evidenced by the heatmap data. Eighty-eight metabolites (VIP > 1.5) were identified as influential in distinguishing the CG group from other exposure groups (Fig. 4C and Table S1). Further analysis revealed that the CdS NPs and co-exposure at low, medium, and high concentrations discriminated 85, 78, 72, and 79 metabolites, respectively. The SiO_2 treatments discriminated 57, 47, and 48 metabolites at low, medium and high concentrations, respectively. The top 15 metabolites in each group were compared to CG and are presented in Fig. S12A–G. These results indicate that more metabolic alterations were observed in the presence of CdS NPs. Furthermore, the discriminated metabolites were involved in biological pathways. CdS NPs group perturbed 43 metabolites (Fig. S13 G) and co-exposures of low, medium, and high altered 41, 48, and 37 metabolic pathways, respectively (Fig. S13A, C and E). Similarly, exposure to only SiO_2 NPs for low, medium, and high levels resulted in 38, 46, and 38 altered metabolic pathways (Fig. S13B, D and F). Significantly altered pathways were identified for 6, 9, 4, 6, 12, and 6 for co-exposure low, medium, high and SiO_2 low, medium, and high. In contrast, only CdS NPs exposures significantly altered 7 metabolic pathways (Fig. 5). Co-exposure and SiO_2 disturbed different metabolic pathways due the presence of CdS NPs stress (Fig. S14). The findings suggest that CdS NPs and SiO_2 NPs have unique effects on the metabolic reprogramming of intermediates in spinach.

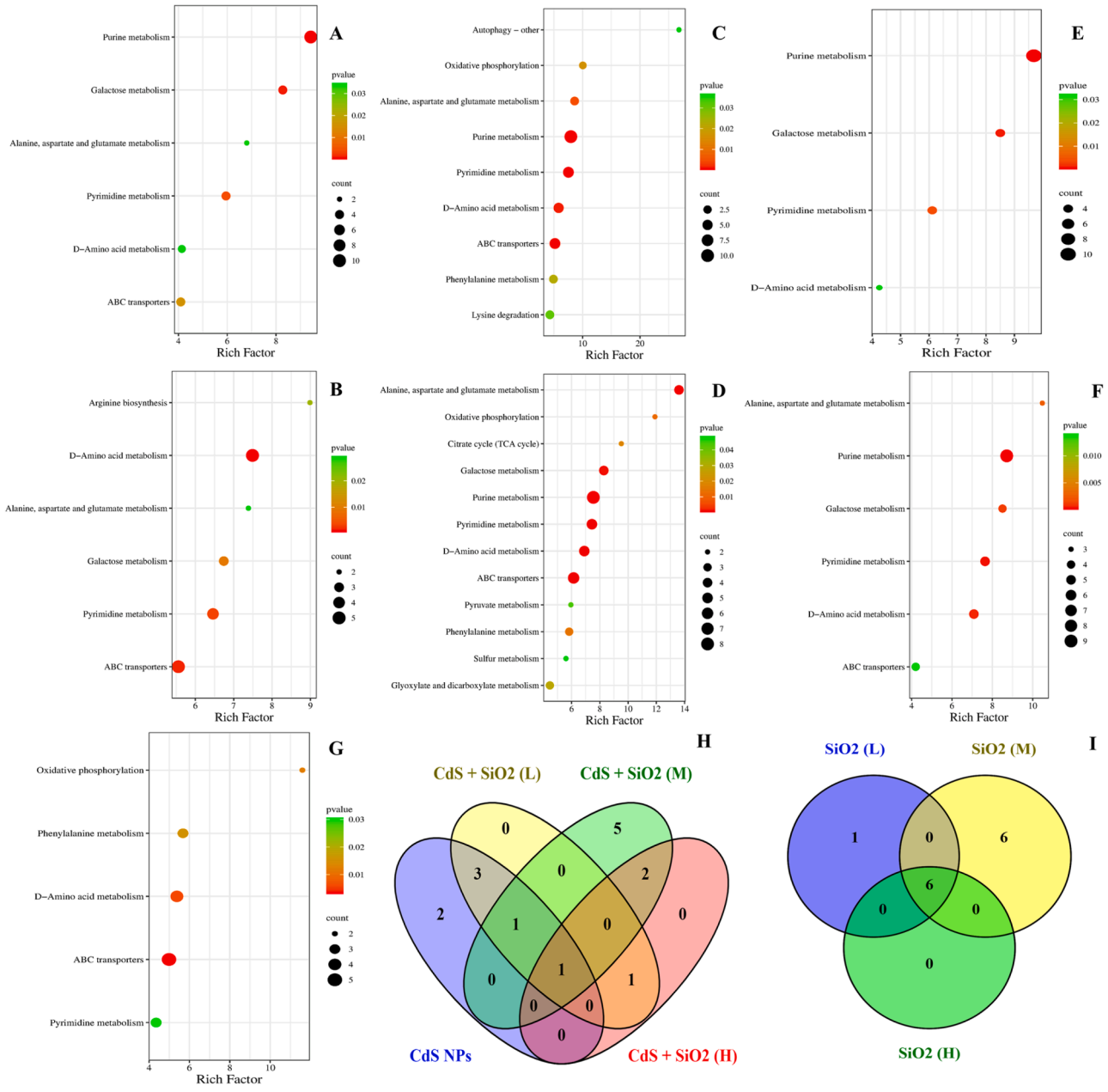

Fig. 5. Metabolic pathways induced by the discriminating metabolites in (A) CdS + SiO_2 (L), (B) SiO_2 (L), (C) CdS + SiO_2 (M), (D) SiO_2 (M) (E) CdS + SiO_2 (H), (F) SiO_2 high dose and (G) CdS VS CG. (H) overlapped metabolites in co-exposure groups and (I) only exposure to SiO_2 NPs.

4. Discussion

4.1. Uptake, physiological response of spinach

Spinach showed a significant reduction in plant biomass and leaf counts as well as increases in chlorosis of leaves after exposure to CdS NPs (1 mg/L). Cd toxicity is reported to disrupt fundamental growth processes, physiological functions, and cellular biochemical properties, primarily through oxidative stress (Rizwan et al., 2017). In previous studies, foliar application of Si has promoted plant growth and increased yields by enhancing phosphorus and carbon utilization efficiency (Dutra et al., 2023). In this study, SiO_2 NPs shows a significant increase in biomass, leaf counts, and leaf surface area (Fig. S2). Our results show that Si can improve antioxidative enzymatic activities and regulate nutrient uptake which helps the plant to mitigate against adverse stress conditions (Alam et al., 2022). Consistently, the application of SiO_2 NPs resulted in a significant increase in chlorophyll levels, which likely enhanced photosynthetic activities and led to higher production. It shows that foliar application is a viable approach as an effective agricultural strategy to mitigate against heavy metal toxicity. In other studies, applying salicylic acid as a foliar fertilizer for lettuce significantly reduced the uptake and accumulation of Cd (Tang et al., 2023). Similarly, applying biosynthetic nano-selenium as a foliar spray greatly diminished heavy metal absorption in *Brassica chinensis* (Zhu et al., 2022). Here, the application of SiO_2 significantly reduced Cd content in roots and shoots except for high SiO_2 doses (Fig. 1A, B). This might be due to the strengthening root barriers, enhancing antioxidant activity, and regulating metal transporters (Rizwan et al., 2012; Weng et al., 2020). This suggests that foliar application can reduce Cd accumulation. Furthermore, it is noteworthy that low doses of SiO_2 decreased the TF, while medium and high doses increased TF. This indicates that once Cd accumulates in the roots in the presence of SiO_2, it is more likely to be

transferred to the vegetative parts (Fig. 1C).

These findings are consistent with other studies demonstrating that foliar spraying influences Cd for other crops (Wang et al., 2024). However, the observed trend in TF is different. It is known that Zn and Cd can exhibit antagonistic effects in terms of their accumulation in plants, with plants often preferentially taking up Zn to alleviate Cd-induced toxicity. Similarly, Fe competes with Cd for the same membrane transporters, which results in lower Cd uptake and reduced translocation to the shoots, consequently lowering Cd accumulation in plants. In this study, Zn and Fe were found to be significantly increased by spraying low and high doses of SiO_2. This shows the impact of these nutrients on Cd. Overall nutrient accumulation was enhanced by the application of SiO_2 NPs which in turn reduced Cd stress. Additionally, Si can improve the levels of both enzyme and non-enzyme based antioxidant under Cd stress, thereby mitigating Cd-induced oxidative damage. In this study, it was observed that SiO_2 application under CdS NP stress reduced the MDA levels in shoots and roots except in the case of low doses of SiO_2 NPs when compared to groups not sprayed with SiO_2 NPs (Fig. 3B). This indicates that by reducing the MDA level, it shows significant stress reduction. Overall, the application of different concentrations of SiO_2 NPs resulted in varying effects on Cd uptake, TF, nutrient accumulation, photosynthesis, and MDA levels.

4.2. Bioaccumulation mechanisms of SiO_2 and CdS NPs

Plants employ various pathways to uptake, translocate, and biotransform different NPs. The primary route for bioaccumulation of NPs in plants is through the roots. Generally, two pathways (apoplastic and symplastic) govern how NPs are taken up by roots and transported to other plant tissues (Fig. 6). NPs have been reported in the apoplastic pathways in plant roots (Avellan et al., 2017). In this study, the NPs aggregates were found in both pathways, indicating also a breach of cell membranes (Fig. 4). Cell wall pores are typically in the range of 3–20 nm, and particles larger than 20 nm have been observed within the intercellular space (Beattie and Haverkamp, 2011). Generally, one plausible explanation is that barriers previously considered rigid are more flexible and easily modifiable (Ma and Yan, 2018). In this work, the particle sizes are between 3 and 20 nm, indicating that CdS NPs would easily pass through the barrier to reach intercellular spaces. After foliar exposure, the cuticular and stomatal pathways are the main pathways for the uptake of NPs (Lv et al., 2019). According to previous studies, the estimated size of cuticles ranges from 0.6 to 4.8 nm, and the dimension of the stomatal aperture typically is approximately 25 μm in length and 3–10 μm wide (Eichert et al., 2008). The average NP size of CdS was 9.2 nm and of SiO_2 was 6.41 nm. The SiO_2 NPs were sprayed on the leaves, so possibly the main pathway for NP uptake might be the stomata. The detection of NPs and their aggregates in the stomata of leaves were observed (Fig. S7), confirming the stomatal pathway for the uptake of SiO_2 NPs.

The xylem moves water and dissolved substances, including NPs, from the roots to the shoots, facilitating NPs movement throughout the plant (Hong et al., 2014), whereas the phloem transports nutrients and other compounds typically from leaves to other parts of the plant. When NPs reach the xylem or phloem then they can move from roots to shoots and vice versa. In this study, TEM-EDS mapping shows the CdS and SiO_2 NPs in the intercellular spaces and inside the cells (Figs. 3 and S8). The presence of NPs in the intercellular space indicates that NPs can move through the epidermis via the cortex, utilizing the apoplastic pathway. For spinach exposed to CdS NPs, TEM-EDS mapping reveals the presence of the Cd elements distributed in both intercellular spaces and dispersed within the cells (Fig. S8). Elevated Cd was also detected by the ICP-MS, as shown in Fig. 2. For the SiO_2 cases, an increased occurrence of aggregates was observed in intercellular spaces; however, Cd was reduced in the cytoplasm compared to the only CdS NPs exposure. Following the application of SiO_2 NPs, the Si content in plants increased, leading to more Si complexes in the cell wall. Consequently, the resulting negative charge enhanced the binding with Cd, thereby inhibiting its transport within the plant (Ali et al., 2019). Secondly, since the uptake channels for Cd in plants coincides with those for Si, the accumulated Si competes for the uptake and binding sites of Cd, thereby reducing the accumulation (Cui et al., 2017). This suggests SiO_2 NPs may inhibit the entry of CdS NPs into the cellular cytoplasm. Overall, this result suggested that CdS NPs could be migrated and transported in spinach. However, the biological toxicity, transport mechanisms, and risks associated with CdS NPs in food chains requires careful consideration.

4.3. Effect of CdS NPs exposure and spraying of SiO_2 on spinach

Metabolomics is used to evaluate the metabolites within cells and to determine the origins of nanotoxicity by detecting alterations in the metabolite profiles. The metabolomics approach provides a comprehensive overview of metabolites that reveals distinctive changes in both

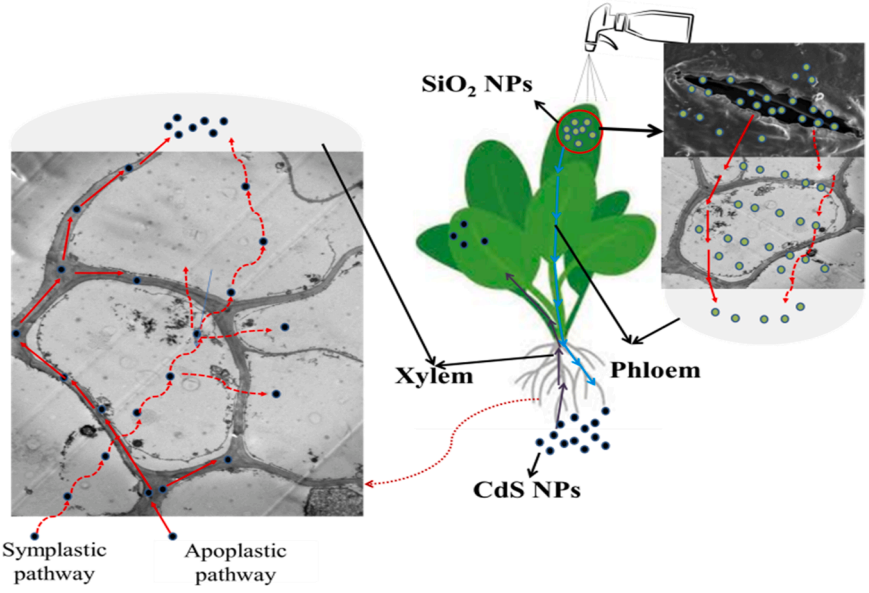

Fig. 6. Diagram illustrating the uptake and translocation mechanisms of CdS and SiO_2 NPs in spinach.

cells and tissues. TEM detected CdS NPs in leaves, suggesting their migration from roots to shoots. Their presence in leaves may potentially cause metabolic reprogramming. NPs can induce oxidative stress, prompting the activation of antioxidant defenses and influencing metabolic pathways regulated by redox signals (Rico et al., 2013). They also disrupt the balance of ions within the plant, affecting enzyme function and nutrient distribution. Moreover, NPs can modify plant hormone levels, which in turn impact growth, stress responses, and gene expression, resulting in alterations to both primary and secondary metabolic processes (Mirzajani et al., 2013). Physiological observations of the metabolic response show that by metabolic reprogramming, the plants enhance their growth and development after SiO_2 NPs spraying. Six overlapping pathways (ABC transporters, alanine, aspartate and glutamate metabolism, d-amino acid metabolism, galactose metabolism, purine metabolism, and pyrimidine metabolism) were found in the SiO_2-only application group, where pathways 1 and 6 were distinctive in the low and medium groups (Fig. 5H and I). In the co-exposure groups, only one metabolic pathway was overlapped (Fig. 5H). These changes in the metabolic pathways govern various aspects of plants including physiology, signaling, biochemistry, and defense mechanisms (Shrestha et al., 2022). Certain d-amino acid metabolism-related metabolites, d-aspartic acid, 5-amino-pentanoic acid, and N-acetyl-l-glutamic acid were found to be increased by spraying SiO_2 NPs from 45.50 to 113 % (Figs. 5 and S15–21). The same metabolites were down-regulated in the CdS NPs-only exposure by 72 % (Figs. 5 and S15). d-glutamine was increased in the CdS NPs-only exposure by 16.11 % (Fig. S17), while a decrease in co-exposure for the medium group of up to 25.77 % (Figs. 5 and S18) was found. This shows by down-regulation of d-glutamine in spinach can enhance its resistance to CdS NPs.

Amino acids are crucial for ion transport, detoxification, and enzyme biosynthesis, contributing significantly to enhanced growth and development (Kumar et al., 2023). Another pathway (alanine, aspartate, and glutamate metabolism) related to amino acids was found altered except for the co-exposure high dose case (Fig. S20). The metabolite d-aspartic acid, involved in the alanine, aspartate and glutamate metabolism pathways, was decreased for the CdS NPs-only group by 32.78 % (Fig. S15). Whereas in co-exposures of low and medium groups it was increased by 58.10 % and 25.33 %, respectively (Figs. S16 and S18). SiO_2-only exposures for low, medium, and high showed significant increases of up to 4923.50 %, 4453.01 %, 6245.50 % (Figs. S17, 19 and 21). This indicates that CdS stress caused significant reductions in d-aspartic acid to reduce stress, while SiO_2 showed significant upregulation. By production of these metabolites, spinach tolerates CdS NP stress and regulates its growth. The variations in amino acid levels may indicate disturbances in primary nitrogen metabolism. Galactose metabolism was disturbed across all the treatments (Figs. S15–21). The level of galactonic acid (organic acid) in galactose metabolism decreased by between 20 % and 59.53 % in all treatments with spraying of SiO_2. Conversely, in the CdS NPs-only exposure group, the level was increased by 128.23 %, initiating a significant upregulation (Fig. S15). Organic acids play pivotal roles in fundamental carbon metabolism processes such as respiration and photosynthesis within plant cells, serving as temporary or stored fixed carbon (Hayat et al., 2012). Organic acids are crucial for balancing redox reactions, generating and utilizing ATP, supporting membrane ion gradients, and acidifying extracellular spaces. Therefore, the significant upregulation in the organic acid and their derivatives under CdS NPs stress results from the reprogramming of the metabolic pathways.

In addition to the observed disruption in the ABC transporter, pyrimidine metabolism, and purine metabolism pathways across different treatments, there is a distinct alteration in metabolite levels within these pathways. Specifically, sulfate levels in the ABC transporter pathway showed varying degrees of upregulation with increases of 118.04 % in the SiO_2 exposure group and 216.29 % in the CdS NPs exposure group. The ABC transporter, situated in the plasma membrane of the epidermal cells, plays an essential role in transporting wax to the cuticle (Gelli and Blumwald, 1997). The observed increase in sulfate levels may be attributed to the mechanical stress exerted on cell membranes by CdS and SiO_2 NPs. Similar trends were observed for other metabolites such as adenosine, betaine, phosphate, riboflavin, and betaine. These findings suggest complex interactions between NPs exposure and metabolic pathways, highlighting potential mechanisms of cellular response and adaptation under stress conditions.

Succinic acid was involved in the disturbance of various pathways under different treatment conditions. In the co-exposure medium group, succinic acid levels decreased by 33.76 % (Fig. S18), while in the SiO_2 NPs group, it was increased by 27.72 %. Succinic acid plays several critical roles in physiology and is a key indicator of metabolic processes, particularly related to energy production and synthesizing essential cellular components. The decrease observed in the CdS exposure group indicates stress, while the response by spraying SiO_2 NPs suggests an improvement in physiological conditions, potentially promoting spinach growth and development. Most metabolic pathways exhibited disturbances in the SiO_2 medium group (Figs. S18, and 19), with reduced uptake of Cd and increased photosynthesis observed. UDP-d-galactose (UDP-Gal) is essential in plant metabolism, especially for synthesizing cell wall polysaccharides and glycoproteins. UDP-Gal was significantly decreased (100 %) for the CdS NPs-only exposure (Fig. S15) but by spraying SiO_2 NPs an up to 373-fold increase was observed (Figs. S14–19). UDP-Gal levels can increase in response to various stressors. For example, plants might elevate UGE gene expression during abiotic stresses to preserve cell wall structure and function. Certain environmental conditions or treatments can reduce UDP-Gal synthesis (Hou et al., 2021). In this study, the downregulation of UDP-Gal observed after exposure to CdS NPs indicates plant stress. However, SiO_2 application mitigates the CdS-induced stress by up-regulating UDP-Gal, thereby enhancing plant metabolism. This suggests that SiO_2 NPs acts to reduce the stress induced on the plant by CdS NPs. These up and down alterations in these metabolites can lead to detoxification. In summary, these shifts in metabolites provide detailed insights into how plants react to exposure of both CdS and SiO_2 both separately and in combination, and emphasizes the importance of different doses of SiO_2 NPs to address CdS NPs stress in spinach.

5. Conclusion

The study was seeking to determine whether CdS NP toxicity could be reduced by application of SiO_2 NPs and sought to evaluate the impacts in terms of phenotypic characteristics. Our results indicate that indeed SiO_2 application resulted in improvements in phenotypic parameters for the spinach treatments studied. Both FW and DW increased compared to the CdS NPs exposed group. Additionally, the yellowish discoloration caused by CdS NPs partially recovered following SiO_2 NP treatment. Analysis confirmed the accumulation of Cd in shoot and root tissues in CdS NP-exposed treatments. However, upon application of SiO_2 NPs at low, medium and high doses, it was shown that the low SiO_2 NPs exposure acted to reduce the uptake of Cd into the roots, but the TF was higher at medium and high doses of SiO_2 NPs. Significant increases in relative chlorophyll levels were also noted with SiO_2 exposures. SEM-EDS revealed the presence of SiO_2 on leaf surfaces and stomata, while CdS and SiO_2 NP aggregates were detected in intercellular spaces and cytoplasm indicating both apoplastic and symplastic pathways were active. Elemental composition analysis through TEM-EDS mapping confirmed the presence of Cd, Si, and S. The MDA level was observed to be increased in leaves with CdS exposure, with root MDA levels increased across all treatments. Metabolite analysis using LC-MS/MS revealed significant changes in 95.25 % of metabolites. Altered metabolites were classified as amino acids, carbohydrates, defense, energy and organic acids. Different metabolic pathways, including ABC transporters, alanine, aspartate and glutamate metabolism, d-amino acid metabolism, galactose metabolism, purine metabolism, and pyrimidine metabolism, were significantly altered by SiO_2 as the plant adapted to

manage stress induced by CdS NPs. In conclusion, this study illustrates that application of SiO_2 NPs in spinach can have a positive impact on detoxification through metabolic reprogramming; however, care must be taken to apply the appropriate concentration of SiO_2 for effective mitigation of contaminants.

CRediT authorship contribution statement

Hameed Ullah: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yanqing Sheng:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Wenjing Wang:** Resources, Investigation. **Zheng Wang:** Investigation, Data curation. **Huiyi Yang:** Writing – review & editing. **Steven Dobbie:** Writing – review & editing.

Declaration of competing interest

This manuscript has been seen by all co-authors, and its submission has been approved by all co-authors. All the authors declare that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100811.

Data availability

Data will be made available on request.

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