Screening of Foliar Reagent Reduces Cadmium Accumulation in Wheat Grains

Shenglan Xia
Nanjing Agricultural University
Jie Wang
Nanjing Agricultural University
Zanming Chen
Nanjing Agricultural University
Hai Lan
Nanjing Agricultural University
Zhenguo Shen
Nanjing Agricultural University
Liang Shi (shil@njau.edu.cn)
Nanjing Agricultural University
Yahua Chen
Nanjing Agricultural University

Research Article

Keywords: Cadmium, EDTA, Selenium, Grain, Transport, Wheat

Posted Date: July 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-639566/v1

Abstract

Cadmium (Cd) in agricultural soils can be taken up by wheat and transferred into the grains, risking human health. In this study, we tested the effects of nineteen foliar treatments alone, and also combined treatments on the Cd concentration of grains in pot/field experiments, and the field experiment, respectively. In addition, we tested the better growth period for foliar application to inhibit Cd accumulation in wheat grains. Foliar application of Ethylenediaminetetraacetic acid (EDTA), Sodium selenite (Se) and Sodium nitroprusside (SNP) can significantly reduce Cd concentration of wheat grains, with 49.2%, 29.6%, and 28.8% decreased respectively in the field. Foliar application of EDTA, Se, Zinc sulphate (Zn), Ascorbic acid (ASA), Sodium silicate (Si) and Ammonium molybdate (Mo) can significantly reduce Cd concentration of wheat grains in different treatments, with 32.3%, 32.0%, 27.7%, 27.7%, 26.3% and 25.9% decreased respectively in the pot. Thus, foliar application of 2 mM EDTA and 2 mM Se exerted excellent effects in controlling the Cd accumulation of wheat grains for both in pot and field experiment. We were concerned about the transfer of different aboveground tissues to the grain, foliar application with 0.1 mM Se or 2 mM EDTA significantly reduced Cd concentrations in grains both in grain filling stage and heading + grain-filling stage. Spraying at the filling stage of wheat has a better effect than at the heading stage for reducing the cadmium content in grains. In addition, the relationship between Cd concentration of grains and husks were significantly positive, while the relationship between Cd concentration of grains and flag leaves was significantly negative. Cd content in wheat grains decreased may be due to the Cd accumulation of flag leaves and the decrease of Cd transport from flag leaves and husks to the grains, which in turn reduces the transport of Cd to the grains.

Introduction

Soil pollution has become a hot issue of social concern due to its impact on the safety and quality of agricultural products in recent years. Among them, Cd is one of the most dangerous environmental pollutants (Bernhoft et al. 2012). Cd is not an essential element for plant growth, but it will affect the growth and development of plants (Rizwan et al. 2016). Due to the higher mobility and bioavailability of Cd, Cd can be absorbed by plant roots and accumulated in the plant, which can lead to crop loss or even death (Rehman et al. 2019). Also, Cd can enter humans and animals from plants such as vegetables through the food chain, which has potential harm to health (Chen et al. 2018a). It was reported that Cd transport from the root to the shoot and the grains includes the following main processes: root absorption, xylem-mediated transport to the shoot, reactivation of the phloem, and finally transport to the grain (Uraguchi et al. 2012). Wheat is one of the main food crops in China. The quality and safety of wheat are related to the health of the people and the stable development of society. Among food crops, wheat is easily to absorb and accumulate Cd. Wheat can absorb Cd from soil and transport it to the aerial part of the plant through root, and distribute from aerial part to wheat grains (Naeem et al. 2010). It is important to reduce Cd accumulation of grains in wheat and other cereals for better human health. Cd in wheat grains mainly comes from the transportation of shoots, leaves and other organs (Yan et al. 2010).

Therefore, how to reduce the Cd concentrations in wheat grains has become an urgent problem to be solved.

Exogenous application of some substances can alleviate the toxicity of heavy metals to plants. Many results show that it is feasible to reduce the concentration of Cd in the edible part of crops by foliar spraying (Chen et al. 2018b; Duan et al. 2018; Gao et al. 2018). Zn is essential for plant growth and pitted against Cd at the binding sites of soil and root surface owing to the similar chemical properties of the two trace metals (Shute et al. 2006). Several studies have shown that the application of Zn can effectively reduce the concentration of Cd in wheat grains. Saifullah et al. (2016) found that foliar application of Zn at the concentration of 0.3% could alleviate the Cd toxicity of wheat and significantly reduce the Cd content in wheat grains. Nitric oxide (SNP is a donor for nitric oxide) as an important signal molecule, participates in various physiological activities of plants, and plants are easily induced to produce nitric oxide under heavy metal stress (Wang et al. 2017). Al^{3+} stress can inhibit nitric oxide biosynthesis in root tip cells of okra (Tian et al. 2017). Exogenous SNP can also reduce Cd content in wheat (Kaya et al. 2020). ASA as a reductant induce improvement in plants defense systems by increased chlorophyll content and improved the content of essential nutrients (Ahmad et al. 2018). Foliar application of ASA can significantly improve the growth and development, photosynthetic capacity and protein concentration of corn, and also reduce absorption of Cd in grains (Zhang et al. 2019). Silicon is the second most abundant element in the soil. Silicon has been proven to be beneficial to the plant growth and development of many plant species, and foliar application of Si can significantly reduce the Cd concentration in rice grains, while the mechanism may be related to Cd accumulation in the stem wall (Liu et al. 2009). Mo is a essential micronutrient for plant growth, also Mo fertilizer can increase photosynthetic rate and grain yield (Liu et al. 2010). Se is not an essential element for plant growth, but it is considered beneficial to plants (Iqbal M et al. 2015). Se can alleviate the detoxification of heavy metal in plants. The addition of Se can reduce the content of ROS in plants, thereby alleviating the toxicity of lead and Cd to plants (Kumar et al. 2012; Mroczek-Zdyrska et al. 2012). EDTA is a chemical extractant widely used to remove heavy metals. Especially when heavy metal content in soil is low, EDTA can get rid of metals in the soil by chelation (Bakht et al. 2020). EDTA is capable of decreasing metal phytotoxicity and the function in Cd uptake has already been studied in several plants (Chigbo et al. 2013; Farid et al. 2015).

Even though many studies have proven that EDTA, Se and other blocking agent can increase tolerance of crops to Cd pollution, but their impacts of foliar application on Cd accumulation in wheat grains still remain being rarely reported. The effects of different blocking agent application and the joint application need more investigation. In this study, in order to reduce Cd concentrations in wheat grains effectively, field and pot experiments were used in this experiment. The results are predicted to supply Cd blocking agent for the safe production of wheat in the Cd slightly polluted area, and to clarify the main effect of the blocking agent on the absorption of Cd by wheat.

Materials And Methods

Experimental design

The field experimental region is in Xinxiang city (N35°23'19.77" E113°59'29.69"), Henan province, China. The study area is located in the monsoon region and has a subtropical warm temperate climate. The annual average temperature is 13.8℃, and the average annual precipitation is 576.5 mm. The basic soil properties are as follows: Ammoniacal N, 36.4 mg kg^{-1}; Available P, 28.8 mg kg^{-1}; Quick-acting K, 82.5 mg kg^{-1}; Organic matter, 20.7 mg kg^{-1}. The pH and heavy metal concentrations of the soils were measured and shown in Table 1. In total, there were 19 treatments (Table 2) and each treatment was replicated three times using a randomized block design with an area of 300 m^2 (1 m×1 m) for each plot. All solutions contained 0.05% (v/v) Tween 20 as a surfactant and were applied to each plot. The middle point of each plot is marked with a label. The circular area with the label as the center of the circle (a diameter of 20 cm) are sprayed.

The pot experimental region is in the teaching and scientific research base of Nanjing Agricultural University, and it is cultivated under natural conditions in the net shed. The study area has a subtropical monsoon climate, with sufficient light and abundant rainfall. The annual average temperature is 15.7 ℃, and the average annual precipitation is 1106.5 mm. The soil was taken from the Yinmao lead-zinc mining area around in Qixia District, Nanjing City, Jiangsu Province. The basic soil properties are as follows: Ammoniacal N, 36.1 mg kg^{-1}; Available P, 30.2 mg kg^{-1}; Quick-acting K, 87.1 mg kg^{-1}; Organic matter 14.8 mg kg^{-1}. The pH and heavy metal concentrations of the collected soils were measured and shown in Table 1.There were 19 treatments (Table. 2) and each treatment was replicated three times. All solutions contained 0.05% (v/v) Tween 20 as a surfactant and were applied to each pot.

The field and pot experiment were carried out for two years. The wheat was planted from October to June during each year. The wheat varieties in field and pot are aikang58 and sukemai1 respectively. Agricultural production activities were carried out according to local practice. Foliar application was carried out at either booting, heading, or grain filling stages twice each stage. To ensure uniform application, the solution was sprayed on each plot to fully wet the surface of leaves.

Sample treatment and data analysis

Wheat yield was determined at maturity by manual harvesting; the grains were air-dried and then measured. These samples were oven-dried at 80℃ to a constant weight, and then shelled with a small huller. The concentrations of Cd and other elements in grains were determined by Inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000, PerkinElmer).

The Cd transfer factor (TF) of wheat under different treatments were calculated according to the following formula:

Transfer factor ($TF_{B\text{-}A}$) = Cd content of A/Cd content of B

Analysis of univariate ANOVA was performed using SPSS. The level of significance was set at $P < 0.05$.

Results

Effects of different foliar application on Cd concentration in wheat grains

The results show that foliar application of EDTA, Se, Zn, ASA, Si and Mo can significantly reduce Cd concentration of wheat grains in different treatments, with 32.3%, 32.0%, 27.7%, 27.7%, 26.3% and 25.9% decreased respectively in the pot experiment (Fig. 1). Foliar application of EDTA, Se and SNP can significantly reduce Cd concentration of wheat grains in different treatments, with 49.2%, 29.6%, and 28.8% decreased respectively in the field experiment (Fig. 4). There was no significant difference in Fe, Mn, Pb and Cu concentrations of wheat grains following six treatments with control in the pot/field experiment (Fig. S1, S2, S3 and S4). Foliar spray of EDTA and Se exerted excellent effects in controlling the Cd accumulation in wheat grains for both pot and field experiments. Also, the treatments with the different combined inhibitor have different effects on the cadmium content in wheat grains. Foliar spray of Se+Zn and Si+EDTA combined treatments can significantly reduce the Cd content in wheat grains by 39.1% and 40.2%, respectively (Fig. 5). Also, foliar applications of pot experiment have no significant difference on thousand grains weight of wheat at different growth stages (Fig. S5).

Effects of different foliar applications on Cd concentrations in different organs of wheat

There were great differences of Cd concentrations in different organs of wheat plants. Among different organs of above ground, the wheat grains displayed the lowest Cd concentration by 0.19-0.28 mg kg^{-1}. Cd concentration in grains was much lower than that in husks, and Cd in uppermost internodes was generally lower than that in other internodes, and Cd in flag leaves was generally lower than that in other leaves (Fig. 2). Cd concentrations in middle leaves (second and third leaves), lower part of leaves, middle internodes (second and third internodes) and lower part of stalks were not influenced by foliar application (Fig. 2). Foliar application reduced Cd concentrations in husks by 5.8-37.4%, and increased Cd concentrations of EDTA, Se, Zn, ASA, Si and Mo in flag leaves by 35.9%, 38.6%, 39.1%, 31.4%, 39.0% and 32.5%, respectively in pot experiment. Inhibition efficiency of EDTA and Se on Cd accumulation in grains was significantly greater than that of other treatments in pot and field experiments (Fig. 1 and 4).

Effects of different foliar application on translocation factors of Cd in different organs of wheat

Foliar application with different foliar application slightly decreased TF of Cd from the husks to grain ($TF_{husk\text{-}grain}$) (Fig. 3). The TF of Cd from flag leaves to the grain ($TF_{leaf\text{-}grain}$) decreased, and the TF of ASA, Citric Acid (CA), EDTA, Mo, Humic acid (HA), Potassium dihydrogen phosphate (K), Melatonin (MT), Se, Si and Zn decreased significantly (Fig. 3). Foliar application with different foliar application slightly decreased TF of Cd from the uppermost internodes to grain ($TF_{husk\text{-}grain}$). The TF of Cd from the flag leaves to husks ($TF_{leaf\text{-}husk}$) decreased, and the TF of ASA, CA, EDTA, Gibberellin (GAs), Glutathione (GSH), Mo, HA, Lanthanum (III) chloride (La), Se, Si, Quinine, SNP and Zn decreased significantly (Fig. 3). The relationship between Cd concentration of grains and middle leaves, uppermost internodes were significantly positive, while the relationship between Cd concentration of grains and flag leaves was significantly negative (Table. 3).

Cd content in wheat grain at different growth stages

Cd concentrations were different among different treatments of wheat plants. It can be seen that spraying Se, EDTA can significantly reduce the Cd content in wheat grains no matter in heading stage, grain filling stage or headin+grain-filling stage, and the effect on reducing Cd concentration in different growth stages is relatively consistent, in order: heading+grain-filling stage > grain filling stage > heading stage (Fig. 6). The results showed that spraying Se or EDTA at the grain filling stage and heading+grain-filling stage had the best effect on reducing Cd concentration in wheat grains (Fig. 6). Foliar application with Se and EDTA efficiently reduced Cd concentrations in grains by 13.2-24.7% and 15.0-24.6%, respectively. Foliar application of Se and EDTA had no significant effect on other elements content (Fig. S4). The relationship between Cd concentration and Zn or Fe concentration was significantly negative, while the relationship between Zn concentration and Mn concentrations were significantly positive (Table S1). Foliar application with 0.1 mM Se and 2 mM EDTA significantly reduced Cd concentrations in grains at the grain filling stage and heading+grain-filling stage (Fig. 6).

Cd concentrations in different organs of wheat at different growth stages

The Cd concentration in different organs of wheat had great differences. The highest Cd concentration was found in uppermost node, which was about 0.9-1.5 mg kg^{-1}. Cd concentration in grains was much lower than that in rachises, Cd concentration in uppermost internodes was generally lower than that in flag leaves (Fig. 7). Foliar application with Se and EDTA efficiently reduced Cd concentrations in husks and rachises by 1.8-35.6% and 9.6-35.4%, respectively. At the heading stage foliar application with 2 mM EDTA significantly reduced Cd concentrations in husks (Fig. 7). At the grain filling stage, foliar application with 0.1 mM Se and 2 mM Se significantly reduced Cd concentrations in husks, and foliar application with 2 mM EDTA significantly reduced Cd concentrations in rachises (Fig. 7). At the heading+grain-filling stage, foliar application with 0.1 mM Se and 2 mM EDTA significantly reduced Cd concentrations in husks and rachises, and foliar application with 0.1 mM EDTA significantly reduced Cd concentrations in husks (Fig. 7). However, Cd concentrations in flag leaves, uppermost internodes, uppermost sheath, and uppermost nodes were not influenced by Se and EDTA (Fig. 7).

Translocation factors of Cd in different organs of wheat at different growth stages

Foliar application with 0.1 mM Se and EDTA except 0.1 mM Se at the heading stage significantly decreased TF from the flag leaves to rachises ($TF_{leaf\text{-}rachis}$) (Fig. 8). Foliar application with Se and EDTA had no significant effect TF of Cd ions from rachises to grains ($TF_{grain\text{-}rachis}$), TF of Cd ions from flag leaves to rachises ($TF_{leave\text{-}rachis}$) and TF of Cd ions from uppermost internodes to flag leaves ($TF_{internode\text{-}leaf}$) (Fig. 8). The relationship between Cd concentration of grains and rachises, husks were significantly positive, while the relationship between Cd concentration of grain and flag leaves was significantly negative (Table. 4).

Discussion

Cd content in wheat grains

We found that foliar application of EDTA was capable of reducing Cd concentrations in wheat grains in the pot (Fig. 1). Plant stems and leaves can also directly absorb mineral nutrients through foliar spray and transport them to other tissues and organs (Li et al. 2020). There are few studies about foliar application of EDTA, while EDTA were applied in combination with contaminated soil. Adding EDTA may decrease Cd concentrations of grains, straws and roots in plant (Xu et al. 2010; Guo et al. 2014; Mahmud et al. 2019). Our study suggest that Cd concentration can be reduced by uppermost 24.6% through foliar spraying of EDTA treatment. EDTA and other chelators to detoxify cadmium is possible, and has been shown to have therapeutic benefits for humans and animals after completion of established protocols (Bernhoft et al. 2013). Addition of EDTA results in increase in plant growth parameters and dry matter stress tolerance index in a soil polluted with cadmium (Guo et al. 2014). The addition of EDTA in the soil increases the solubility of Cd, but the separation of Cd-EDTA limits the effectiveness of free Cd^{2+} on the root surface, which ultimately reduces the plant absorption of the metal (Najeeb et al. 2011; Wei et al. 2012; Custos et al. 2014).

Foliar spraying is widely used in the production of Se-enriched agricultural products. Foliar application of Se had significant reduction of cadmium on the wheat seeds (Fig. 1). It also found that the Se content is higher when sprayed with sodium selenate than with sodium selenite in rice grains (Chen et al. 2002). But sodium selenate has greater toxicity and environmental risks, we commonly used sodium sulfite for bio-enhancement. Foliar application with sodium selenite greatly decreased Se content in wheat gain (Fig. 6). Studies have found that the addition of Se can reduce the content of ROS in plants, thereby alleviating the toxicity of Cd to plants (Kumar et al. 2012). Many studies have shown Se can effectively reduce the Cd translocation from roots to shoots in rice, and alleviate the toxicity of Cd by promoting plant growth (Lin et al. 2012; Cao et al. 2013; Hu et al. 2014; Wan et al. 2016; Gao et al. 2018; Wan et al. 2019). Se can inhibit the absorption, transport and distribution of heavy metals, and convert toxic heavy metals into non-toxic Se complexes: Se reduces its toxicity by regulating the subcellular distribution of Sb and Cd. We found that foliar application with Se efficiently reduced Cd concentrations in grains by 13.2-24.7%. For the antagonistic effect of Se and Cd, applying Se to rice grown on cadmium-contaminated soil can reduce the content of cadmium in the rice. This can reduce the Cd entering the human body through the food chain, and at the same time improve the nutritional quality of rice, which is an effective way to control cadmium pollution.

Also, the results of the present study indicated that foliar application of Zn, ASA, Si and Mo had significant reduction of Cd on the wheat grains in the pot experiment and foliar application of SNP had significant reduction of Cd on the wheat grains in the field experiment (Fig. 1 and 4). Some previous studies indicated that foliar application of Se, Zn and Si can decrease Cd concentrations in wheat or rice planted in Cd contaminated soil (Liu et al. 2009; Saifullah et al. 2016; Chen et al. 2018; Lv et al. 2019; Wu et al. 2020). Zn plays a critical role in different metabolic processes and Zn treatment had advantage effect on wheat growth and yield on Cd contaminated soil (Cao et al. 2013; Lv et al. 2019; Saifullah et al. 2016). Si applied to Cd-treated plants improve plant dry matter or grain yield (Rizwan et al. 2012; Naeem et al. 2014; Gao et al. 2018). Foliar application with exogenous ASA decreased Cd uptake and minimized the impacts of Cd on maize (Wang et al. 2017; Zhang et al. 2019). Molybdenum (Mo) is an essential element for plant growth and development. Modified molybdenum ore treatment could reduce Cd concentrations in all parts of the rice plant, but had no significant effect on rice yields (Li et al. 2019). Under Cd stress, foliar application of SNP can increase the yield of chickpeas and reduce Cd accumulation (Kumari et al. 2011). It is reported that exogenous SNP can alleviate Cd stress and significantly reduce the Cd content in the roots and stems in wheat plants (Kaya et al. 2020). Inhibitor alone or combinations have different effects on the cadmium content, while Cd concentrations after treatment with Zn, ZnMn and ZnP decreased by 19.0-32.6%, 36.6-55.8% and 25.7-49.1%, respectively (Lv et al. 2018). Foliar spray of Se+Zn and Si+EDTA combined treatments can significantly reduce Cd content in wheat grains by 39.1% and 40.2%, respectively (Fig. 5). In general, our results are similar to the results reported.

The selection of foliar spraying period has a great influence on the absorption and distribution of Cd in plants. It can be seen that spraying at the best time is of great significance to reduce Cd content in wheat grains, but different foliar spraying treatments may have different effects (Fig. 6). It is considered that the later spraying period is beneficial to the accumulation of Se in rice and maize (Wang et al. 2013; Deng et al. 2017). It can be seen that spraying at the best time is of great significance to reduce Cd content in wheat grains, but different foliar spraying treatments may have different effects. Our results showed that spraying Se or EDTA at the grain filling stage and heading+grain-filling stage had the better effect than booting the stage on reducing Cd concentration in wheat grains (Fig. 6). However, the effect of spraying $ZnSO_4$ at booting stage on reducing Cd content in wheat grain was significantly better than that at tillering stage, jointing stage, heading stage and filling stage (Saifullah et al. 2016). There are two pathways that Cd accumulation in rice grain. One way is Cd remobilization from leaves via phloem, and another way is direct root-absorbed Cd via xylem during maturation (Rodda et al. 2011). It showed that 60 % of the final grain Cd content was remobilized from vegetative tissue accumulated by the plant before flowering, while 40 % of the grain Cd came from the root via xylem during grain-filling stage (Rodda et al. 2011). This show that Zn may reduce wheat grain Cd from leaves via phloem Pre-flowering, while Se and EDTA may reduce wheat grain Cd from root uptake at the grain-filling stage.

Cd content in different parts of wheat

There is different Cd content in rice plant parts, then Se reduced the accumulation of Cd in rice husks, stems, leaves and grains, but did not reduce the accumulation of Cd in rice roots (Chen et al. 2014). Part of the nutrients in the grains are absorbed from the vegetative organs. Foliar application of Mn can inhibit Cd transport from root to shoot, then to grain at mature stage (Xu et al. 2016; Huang et al. 2017). Foliar application of Zn can inhibit Cd transport from root to flag leaves then to the first node, and at last from rachises to grain (Lv et al. 2018). Except in husks and rachises, Cd concentrations in flag leaves, uppermost internodes, uppermost sheath, and uppermost nodes were not influenced by Se and EDTA (Fig. 7). The effect of Se and EDTA on Cd content in different parts are different (Fig. 7).

Wheat vegetative organs can temporarily store photosynthetic products before and after flowering, and then transport them to the grain at the later stage of grain filling. Rachis is the organ connecting the stem and the grains. Nutrients in wheat vegetative organs are finally transported to the developing grains through rachises in panicles. Cd content in rachises and Cd transportation ratio from rachises to grains both closely correlated with Cd concentration in rice grains (Liu et al. 2017). After different concentration of Se and EDTA was sprayed onto wheat leaves, Cd content in both husks and rachises at maturity stage was decreased by 10.2-30.3% and 2.5-36.2%, respectively. Foliar application with different concentration of Se and EDTA except 2 mM Se decreased the TF of Cd from uppermost internodes to rachises, as well as significantly decreased TF of Cd from the flag leaves to rachises (Fig. 8). In addition, the relationship between Cd concentration of grains and uppermost internodes, husks were significantly positive, while the relationship between Cd concentration of grains and flag leaves was significantly negative (Table. 3 and 4). Thus, Cd content of wheat grains decreased may be due to the Cd accumulation of flag leaves and the decrease of Cd transport from flag leaves and husks to the grains, which in turn reduces the transport of Cd to the grains.

Effects of different spraying treatments on other elements content in wheat grains

The relationship between Cd and Zn concentration was significantly negative, while the relationship between Zn concentration and Mn concentrations were significantly positive (Table. S1). This result show that Zn and Cd may indicate antagonism. The competition between Cd^{2+} and Zn^{2+} may result in increasing Zn concentration and decreasing Cd concentration (Hart et al. 2002; Duan et al. 2018; Lv et al. 2019). Cd^{2+} and Zn^{2+} share a common transport system at the root cell plasma membrane in wheat, then Zn competes with Cd for these heavy metal binding sites and blocks the transport of Cd (Hart et al. 2002). Therefore, the increase of Zn concentration may lead to decrease of Cd concentration in wheat grain. Mn treatment reduced Cd accumulations in root and shoot of rice (Xu et al. 2016; Yin et al. 2017). Cd is mainly transported into root cells through nonselective cation channels, while Mn is primarily carried into root cells via positive transporters, thus Mn may inhibit Cd uptake and translocation in rice seedlings by competing ionic channels and carrier proteins (Xu et al. 2016; Huang et al. 2017; Zhang et al. 2018). Further studies investigating the molecular mechanisms of this process would be helpful for a better understanding of this effect.

Conclusion

As a method of reducing Cd content in wheat grains, foliar application is feasible. Foliar application of Se and EDTA can effectively decrease the Cd content of wheat grains in the field/pot experiment. The grain filling stage is the better period of foliar spraying of Se and EDTA to minimize the Cd content in the wheat grains. Also, it may be due to the Cd accumulation of flag leaves and the decrease of Cd transport from flag leaves and husks to the grains, that in turn reduces the transport of Cd to the grains.

Declarations

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical Approval and Consent to participate

Ethics approval and Consent to participate was not required for this research.

Consent to Publish

The data in this article has been approved by all authors for publication.

Authors Contributions

Shenglan Xia and Jie Wang analyzed soil physical, chemical properties and metal element contents in plant and soil samples. Hai Lan found and arranged the pollution assessment standard. Hai Lan and Zanming Chen finished statistical analysis. Shenglan Xia, Hai Lan and Zanming Chen planted and harvested wheat. Liang Shi and Zhenguo Shen were two major contributors in writing the manuscript. Liang Shi and Yahua Chen provided experimental programs and ideas. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Funding

This research was financially supported by the National Key Research and Development Program (2016YFD08007003), the China Agriculture Research System of MOF and MARA (CARS-10-B24; CARS-23-A03), the National Natural Science Foundation of China (31901180, 31800525), China Postdoctoral Science Foundation (2019M651845), the Priority Academic Program Development of Jiangsu Higher Education institutions and Special Fund Project of Fundamental Scientific Research Funds for Central Universities of Nanjing Agricultural University (KYQN202061).

References

1. Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Alam P (2018) Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. Protoplasma 255(1): 79–93.
2. Bakhta J, Ullaha R, Shafib M (2020) Effect of Chelator EDTA on Phyto-Remediation of Cadmium, Chromium and Lead and their Effect on Growth of Sunflower (*Helianthus annuus* L.). Pak j sci ind res Ser B: biol sci 63B (1):22-29.
3. Bernhoft RA (2013) Cadmium toxicity and treatment. The Scientific World Journal 2013:394652.

4. Cao F, Wang N, Zhang M et al (2013) Comparative study of alleviating effects of GSH, Se and Zn under combined contamination of cadmium and chromium in rice (*Oryza sativa*). Biometals 26(2): 297-308.
5. Chen HP, Yang XP, Wang P, Wang ZX, Li M, Zhao FJ (2018a) Dietary cadmium intake from rice and vegetables and potential health risk: a case study in Xiangtan, southern China. Sci Total Environ 639:271–277.
6. Chen LC, Yang FM, Xu J et al (2002) Determination of selenium concentration of rice in china and effect of fertilization of selenite and selenate on selenium content of rice. J Agric Food Chem 50(18):5128-30.
7. Chen M, Cao L, Song X et al (2014) Effect of Iron Plaque and Selenium on Cadmium Uptake and Translocation in Rice Seedlings (*Oryza sativa*) Grown in Solution Culture. J Agric Biol 16(6):1159-1164.
8. Chen R, Zhang C, Zhao Y et al (2018b) Foliar application with nano-silicon reduced cadmium accumulation in grains by inhibiting cadmium translocation in rice plants. Environ Sci Pollut Res 25(3): 2361-2368.
9. Chigbo C, Batty L (2013) Effect of EDTA and citric acid on phytoremediation of CrB[a]P-co-contaminated soil. Environ Sci Pollut Res Int 20:8955–8963.
10. Custos JM, Moyne C, Treillon T et al (2014) Contribution of Cd-EDTA complexes to cadmium uptake by maize: a modelling approach. Plant and soil 374(1-2): 497-512.
11. Deng X, Liu K, Li M et al (2017) Difference of selenium uptake and distribution in the plant and selenium form in the grains of rice with foliar spray of selenite or selenate at different stages. Field Crops Res 211: 165-171.
12. Duan MM, Wang S, Huang DY et al (2018) Effectiveness of simultaneous applications of lime and zinc/iron foliar sprays to minimize cadmium accumulation in rice. Ecotoxicol Environ Saf 165: 510-515.
13. Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I et al (2015) Exogenous application of ethylenediamminetetraacetic acid enhanced phytoremediation of cadmium by *Brassica napus* L. Int J Environ Sci Technol 12:3981–3992.
14. Gao M, Zhou J, Liu H et al (2018) Foliar spraying with silicon and selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. Sci Total Environ 631: 1100-1108.
15. Guo J, Feng R, Ding Y et al (2014) Applying carbon dioxide, plant growth-promoting rhizobacterium and EDTA can enhance the phytoremediation efficiency of ryegrass in a soil polluted with zinc, arsenic, cadmium and lead. J Environ Manag 141: 1-8.
16. Hart JJ, Welch RM, Norvell WA et al (2002) Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. Physiol Plantarum 116(1): 73-78.
17. Hu Y, Norton GJ, Duan G et al (2014) Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. Plant and Soil 384(1-2): 131-140.

18. Huang Q, An H, Yang Y et al (2017) Effects of Mn-Cd antagonistic interaction on Cd accumulation and major agronomic traits in rice genotypes by different Mn forms. Plant Growth Regul 82(2): 317-331.
19. Iqbal M, Hussain I, Liaqat H et al (2015) Exogenously applied selenium reduces oxidative stress and induces heat tolerance in spring wheat. Plant Physiol Biochem 94:95-103.
20. Kaya C, Ashraf M, Alyemeni MN, Ahmad P (2020) Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. Physiol Plantarum 168(2): 345-360.
21. Kumari A, Sunita S, Kumari S (2011) Nitric oxide induced alleviation of toxic effects of short term and long term Cd stress on growth, oxidative metabolism and Cd accumulation in Chickpea. Braz J Plant Physiol 22(4): 271-284.
22. Li JL, Liang JM, Chen BH, Li YT, Wang JJ (2019) Effect of integrative remediation measures, based on foliar fertilizer and several passivation materials, on Cd accumulation in rice. J Agric Environ Sci 38(10): 2338-2345.
23. Li N, Feng A, Liu N et al (2020) Silicon application improved the yield and nutritional quality while reduced cadmium concentration in rice. Environ Sci Pollut Res 27: 20370–20379.
24. Lin L, Zhou W, Dai H et al (2012) Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. J Hazard Mater 235: 343-351.
25. Liu CP, Li FB, Luo CL, Liu XM, Wang SH, Liu TX, Li XD (2009) Foliar application of two silica sols reduced cadmium accumulation in rice grains. J Hazard Mater 161:2-3.
26. Liu H, Hu C, Sun X et al (2010) Interactive effects of molybdenum and phosphorus fertilizers on photosynthetic characteristics of seedlings and grain yield of Brassica napus[J]. Plant and Soil 326(1-2):345-353.
27. Liu Y, Zhang CB, Zhao YL, Sun SJ, Liu ZQ (2017) Effects of growing seasons and genotypes on the accumulation of cadmium and mineral nutrients in rice grown in cadmium contaminated soil. Sci Total Environ 579:1282-1288.
28. Lv GH, Wang H, Xu C et al (2019) Effectiveness of simultaneous foliar application of Zn and Mn or P to reduce Cd concentration in rice grains: a field study. Environ Sci Pollut Res 26(9):9305-9313.
29. Mahmud JA, Hasanuzzaman M, Nahar K et al (2019) EDTA reduces cadmium toxicity in mustard (*Brassica juncea* L.) by enhancing metal chelation, antioxidant defense and glyoxalase systems. Acta Agrobot 72: 1722.
30. Mroczek-Zdyrska M, Magorzata W (2012) The Influence of Selenium on Root Growth and Oxidative Stress Induced by Lead in *Vicia faba* L. minor Plants. Biol Trace Elem Res 147(1):320-328.
31. Naeem A, Ghafoor A, Farooq M (2015) Suppression of cadmium concentration in wheat grains by silicon is related to its application rate and cadmium accumulating abilities of cultivars. J Sci Food Agric 95(12): 2467-2472.
32. Najeeb U, Jilani G, Ali S et al (2011) Insights into cadmium induced physiological and ultra-structural disorders in Juncus effusus L. and its remediation through exogenous citric acid. J Hazard Mater

186(1): 565-574.

33. Rehman MZ, Rizwan M, Rauf A, Ayub MA, Ali S, Qayyum MF, Waris AA, Naeem A, Sanaullah M (2019) Split application of silicon in cadmium (Cd) spiked alkaline soil plays a vital role in decreasing Cd accumulation in rice (*Oryza sativa* L.) grains. Chemosphere 226:454-462.
34. Rizwan M, Ali S, Adrees M, Rizvi H, Zia-ur-Rehman M, Hannan F, Qayyum MF, Hafeez F, Ok YS (2016) Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. Environ Sci Pollut Control Ser 23:17859-17879.
35. Rizwan M, Meunier JD, Miche H et al (2012) Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination. J Hazard Mater 209: 326-334.
36. Rodda MS, Li G, Reid RJ (2011) The timing of grain Cd accumulation in rice plants: the relative importance of remobilization within the plant and root Cd uptake post-flowering. Plant and Soil 47(1-2): 105-114.
37. Saifullah, Javed H, Naeem A, Rengel Z, Dahlawi S (2016) Timing of foliar Zn application plays a vital role in minimizing Cd accumulation in wheat. Environ Sci Pollut Res 23(16):16432-9.
38. Salt DE, Smith RD, Raskin I (1988) Annual Review of Plant Physiology and Plant Molecular Biology. Phytoremediation 49: 643–668.
39. Shute T, Macfie SM (2006) Cadmium and zinc accumulation in soybean: a threat to food safety. Sci Total Environ 371:63–73.
40. Kumar M, Bijo AJ, Baghel RS et al (2012) Selenium and spermine alleviate cadmium induced toxicity in the red seaweed Gracilaria dura by regulating antioxidants and DNA methylation. Plant Physiol Biochem 51:129-38.
41. Tian QY, Sun DH, Zhao MG, Zhang WH (2007) Inhibition of nitric oxide synthase (NOS) underlies aluminum-induced inhibition of root elongation in *Hibiscus moscheutos*. New Phytol 174(2):322-331.
42. Uraguchi S, Fujiwara T (2012) Cadmium transport and tolerance in rice: perspectives for reducing grain cadmium accumulation. Rice 5(1):5.
43. Wan Y, Wang K, Liu Z et al (2019) Effect of selenium on the subcellular distribution of cadmium and oxidative stress induced by cadmium in rice (*Oryza sativa* L.). Environ Sci Pollut Res 26(16): 16220-16228.
44. Wan YN, Yu Y, Wang Q, Qiao YH, Li HF (2016) Cadmium uptake dynamics and translocation in rice seedling: Influence of different forms of selenium. Ecotox Environ Safety 133:127-34.
45. Wang J, Wang Z, Mao H et al (2013) Increasing Se concentration in maize grain with soil- or foliar-applied selenite on the Loess Plateau in China. Field Crops Res 150(Complete):83-90.
46. Wang Z, Li Q, Wu W, Guo J, Yang Y (2017) Cadmium stress tolerance in wheat seedlings induced by ascorbic acid was mediated by NO signaling pathways. Ecotox Environ Safe 135:75-81.
47. Wang ZF, Li Q, Wu WG, Guo J, Yang YL (2017) Cadmium stress tolerance in wheat seedlings induced by ascorbic acid was mediated by NO signaling pathways. Ecotox Environ Safety 135:75-81.

48. Wei JL, Lai HY, Chen ZS (2012) Chelator effects on bioconcentration and translocation of cadmium by hyperaccumulators, Tagetes patula and Impatiens walleriana. Ecotox Environ Safety 84:173-178.
49. Wu C, Dun Y, Zhang ZJ, Li ML, Wu GQ (2020) Foliar application of selenium and zinc to alleviate wheat (*Triticum aestivum* L.) cadmium toxicity and uptake from cadmium-contaminated soil. Ecotox Environ Safety 190:110091.
50. Xu WH, Li YR, He JP et al (2010) Cd uptake in rice cultivars treated with organic acids and EDTA. J Environ Sci (China) 22(3): 441-447.
51. Xu Y, Yang YX, Li WH et al (2016) Effects of manganese concentrations and transporters on uptake and translocation of cadmium in rice seedlings. J Agro Environ Sci 35(8): 1429-1435.
52. Yan YF, Choi DH, Kim DS et al (2010) Absorption, translocation, and remobilization of cadmium supplied at different growth stages of rice. J Crop Sci Biotechnol 13(2):113-119.
53. Yin XH, Zou HL, Fang YY et al (2017) Effects of manganese fertilizer on absorption and accumulation of Cd in rice. Environ Sci Technol 40(8): 8-12.
54. Zhang KP, Wang GY, Bao MC, Wang LC, Xie XY (2019) Exogenous application of ascorbic acid mitigates cadmium toxicity and uptake in Maize (*Zea mays* L.). Environ Sci Pollut Res 26(19):19261-19271.
55. Zhang S, Lu ZY, Tang Q et al (2018) Effects of foliar modulators on cadmium accumulation and transport of nutrient elements in rice. J Agro Environ Sci 37(11): 2507-2513.

Tables

Table 1. pH and heavy metal concentrations in pot and field soil

Types	pH	Cd (mg kg^{-1})	DTPA-extracted Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})
Field	7.22 ± 0.03	1.47 ± 0.02	0.81 ± 0.02	92.17 ± 2.22	66.91 ± 0.24	32.00 ± 1.27	925.14 ± 19.78
Pot	7.02 ± 0.02	3.37 ± 0.02	1.74 ± 0.04	120.23 ± 9.51	98.01 ± 0.47	97.90 ± 0.81	1687.14 ± 10.94
Standards*		0.3		120	250	100	

*Soil environmental quality risk control standard for soil contamination of agricultural land GB 15618, 2018

Table 2. Foliar application treatments and concentrations in the pot and field experiments

Treatments	concentration	Treatments	concentration
Deionized water (Control)			
Ascorbic acid (ASA)	0.2 mM	Lanthanum (III) chloride (La)	5 mM
Citric Acid (CA)	4 mM	Melatonin (MT)	0.1 mM
Calcium chloride (Ca)	5 mM	Sodium selenite (Se)	2 mM
Ethylenediaminetetraacetic acid (EDTA)	2 mM	Sodium silicate (Si)	2 mM
Gibberellin (GAs)	10 μM	Proline (Pro)	10 mM
Glutathione (GSH)	0.2 mM	Quinine	0.5 mM
Ammonium molybdate (Mo)	1 mM	Sodium nitroprusside (SNP)	0.1 mM
Humic acid (HA)	2 g/L	Tetraethylammonium chloride (TEAC)	2 mM
Potassium dihydrogen phosphate (K)	20 mM	Zinc sulphate (Zn)	5 mM

Table 3. Correlation analysis of Cd content in wheat grains and different wheat tissues in the pot experiment

	rachis	flag leaves	middle leaves	lower part of leaves	uppermost internodes	middle internodes	Lower part of stalks
Grain	0.697**	-0.805**	0.704**	0.276	0.678**	-0.167	0.244
Husk		-0.437	0.668**	0.275	0.771**	0.309	0.437
Flag leaves			-0.425	-0.174	-0.650**	0.232	-0.087
Middle leaves				0.454	0.487*	0.007	0.341
Lower part of leaves					0.029	0.051	0.354
Uppermost internodes						0.246	0.075
Middle internodes							0.177

Table 4. Correlation analysis of Cd content in wheat grains and different tissues in the pot experiment

	Rachis	Husk	Flag leaves	Uppermost internodes	Uppermost sheathes	Uppermost nodes
Grain	0.740**	0.670*	-0.787**	0.314	0.206	0.453
Rachis		0.690**	-0.741**	0.121	-0.06	0.750**
Husk			-0.512	-0.096	-0.057	0.413
Flag leaves				0.026	-0.103	-0.498
Uppermost internodes					0.325	0.242
Uppermost sheathes						-0.038

Figures

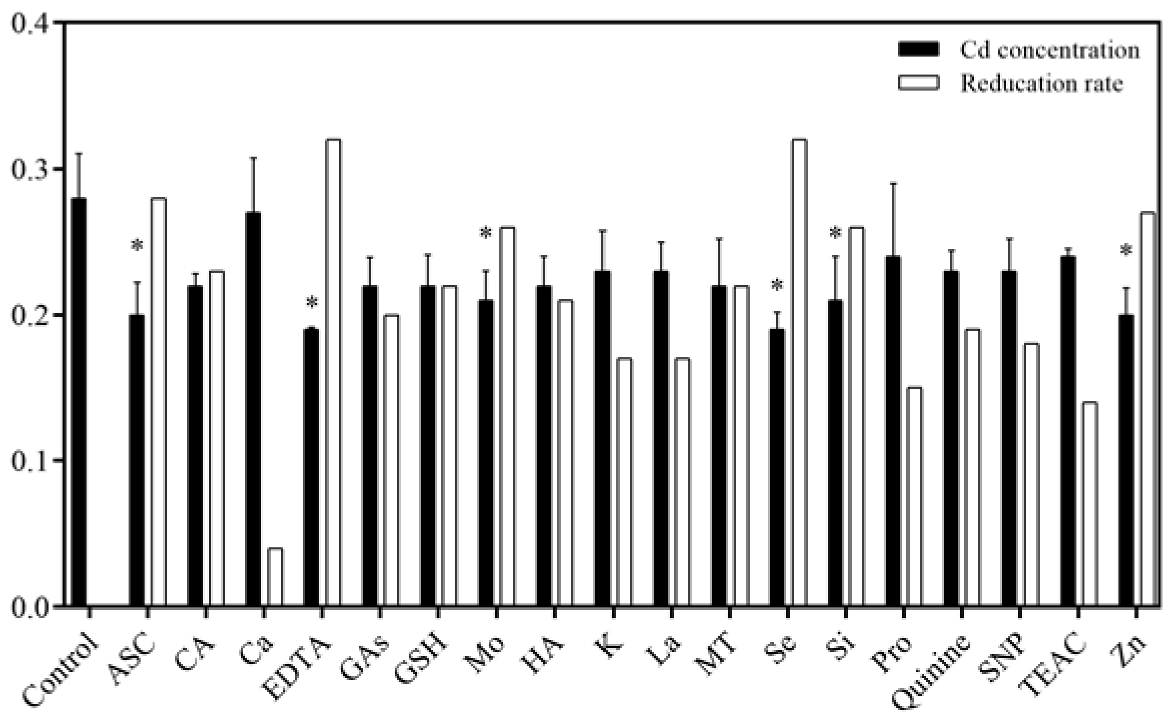

Figure 1

Effects of different spraying treatments on Cd content of wheats in the pot experiment.

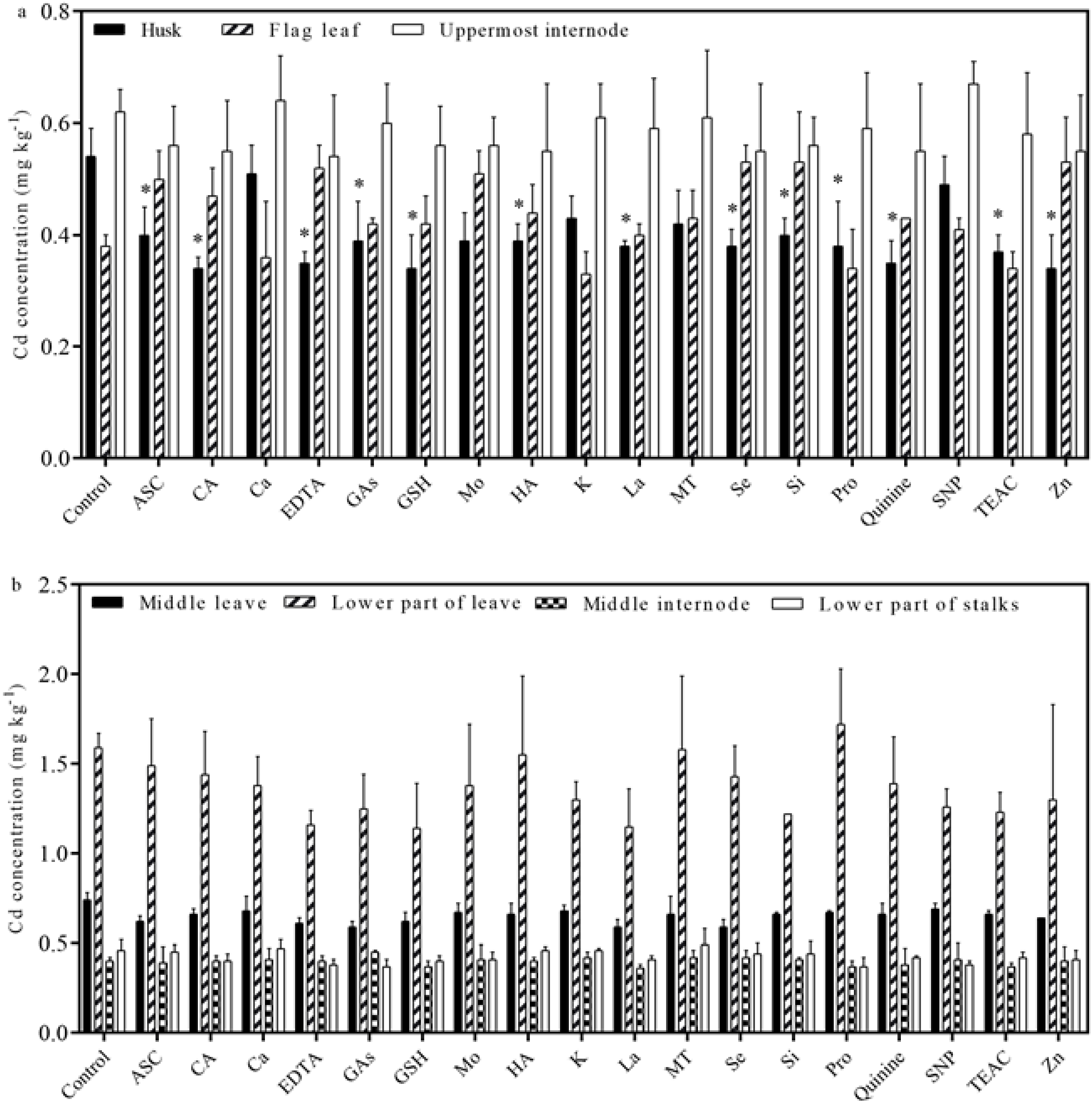

Figure 2

Effects of different spraying treatments on Cd concentration in different tissues of wheat.

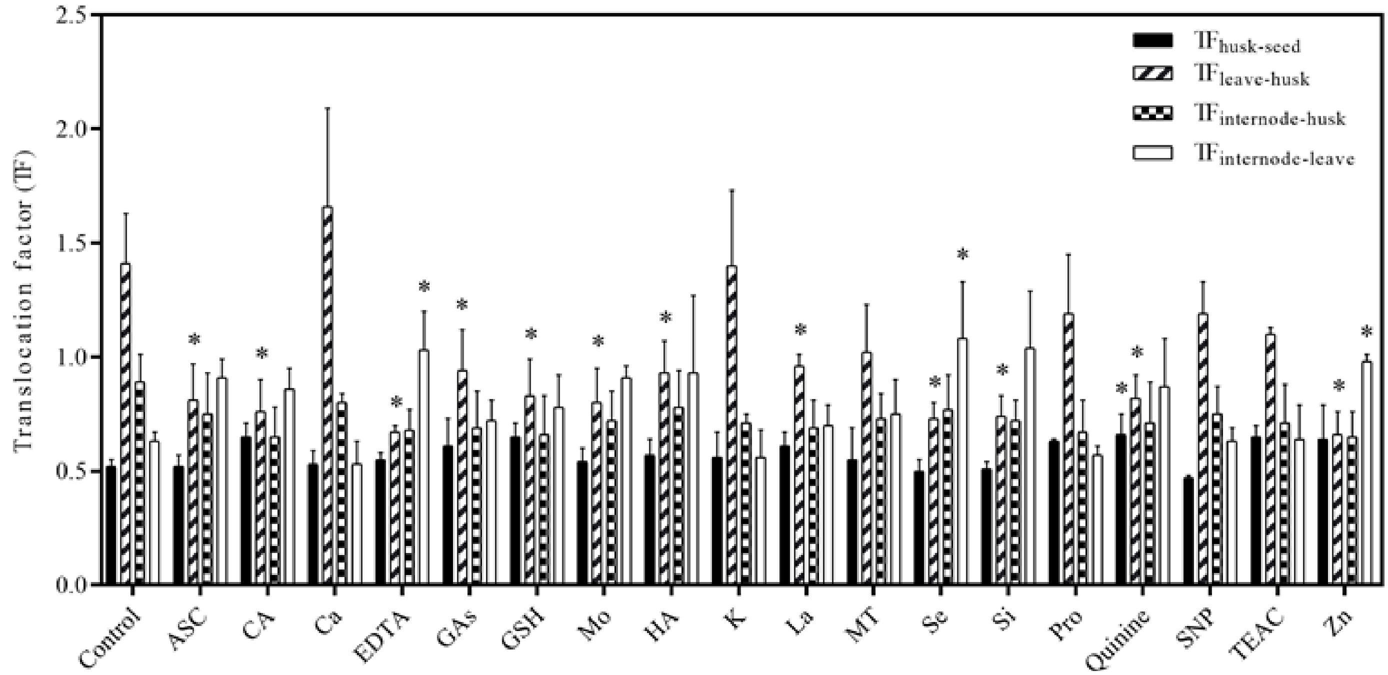

Figure 3

Effects of different foliar application on the Cd translocation factor in different tissues of wheat.

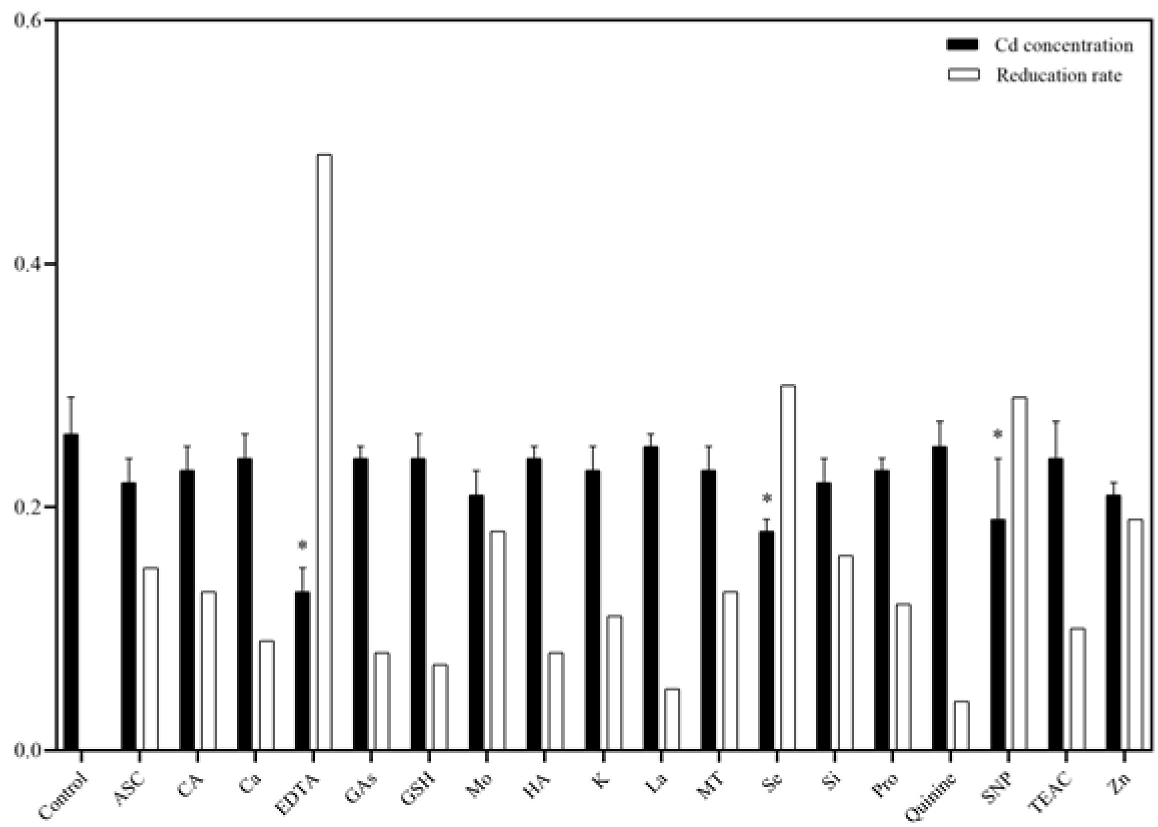

Figure 4

Effects of different spraying treatments on Cd content of wheats in the field experiment.

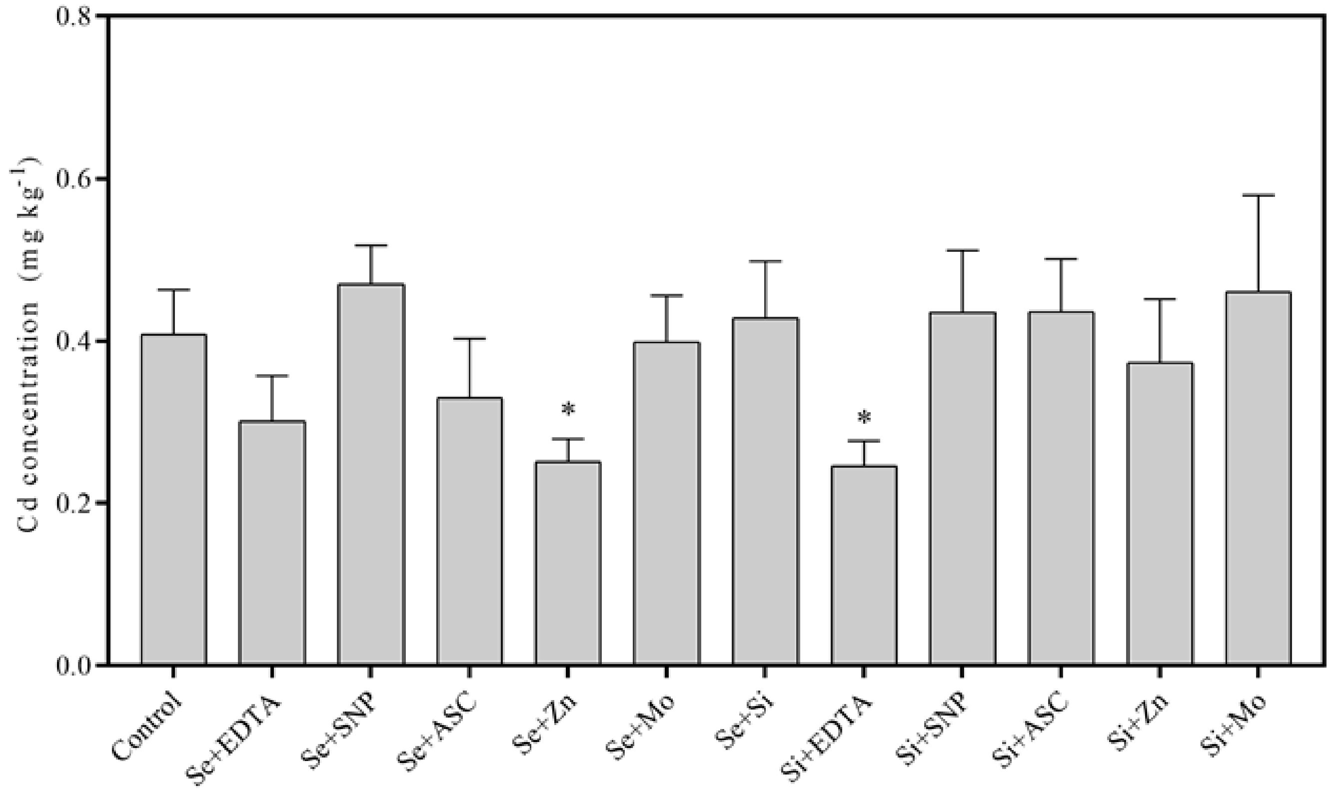

Figure 5

Effects of combined spraying treatments on Cd content of wheat grains in the field experiment.

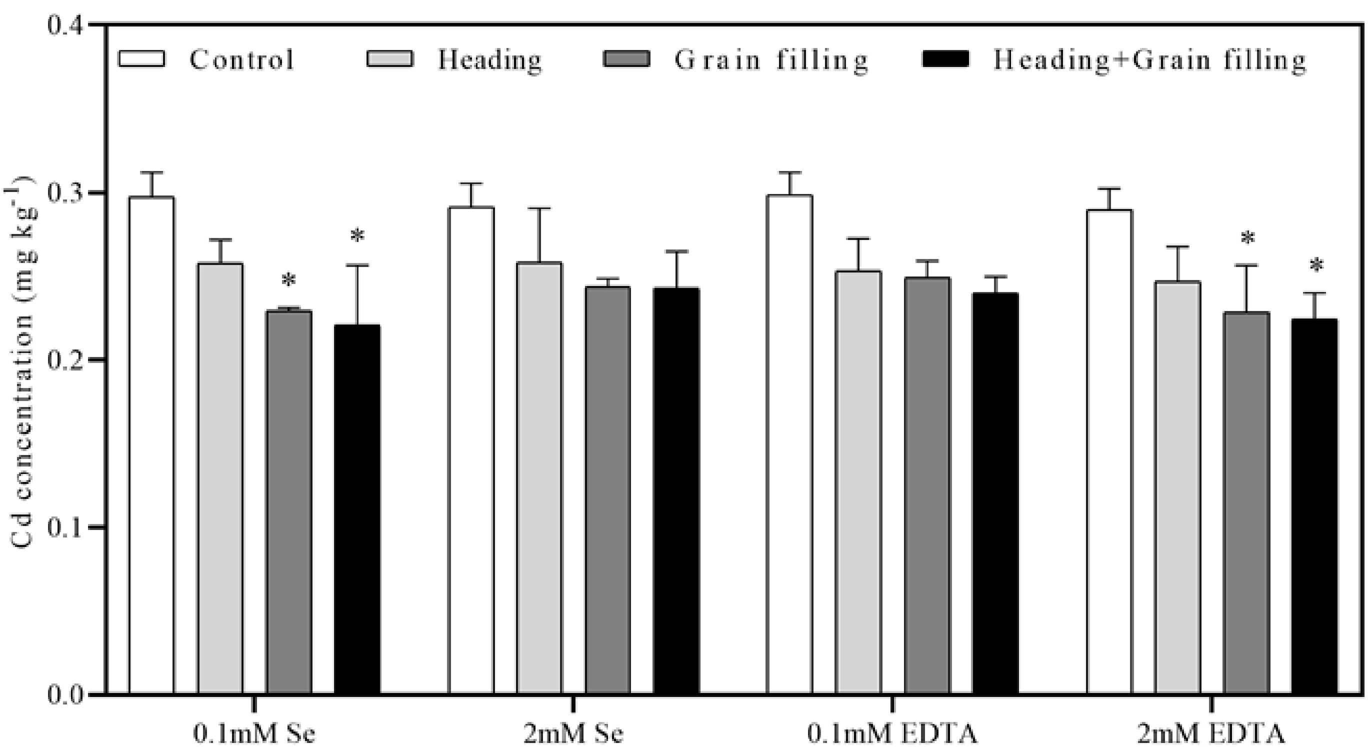

Figure 6

Cd content of wheat grains at different growth stages in the pot experiment.

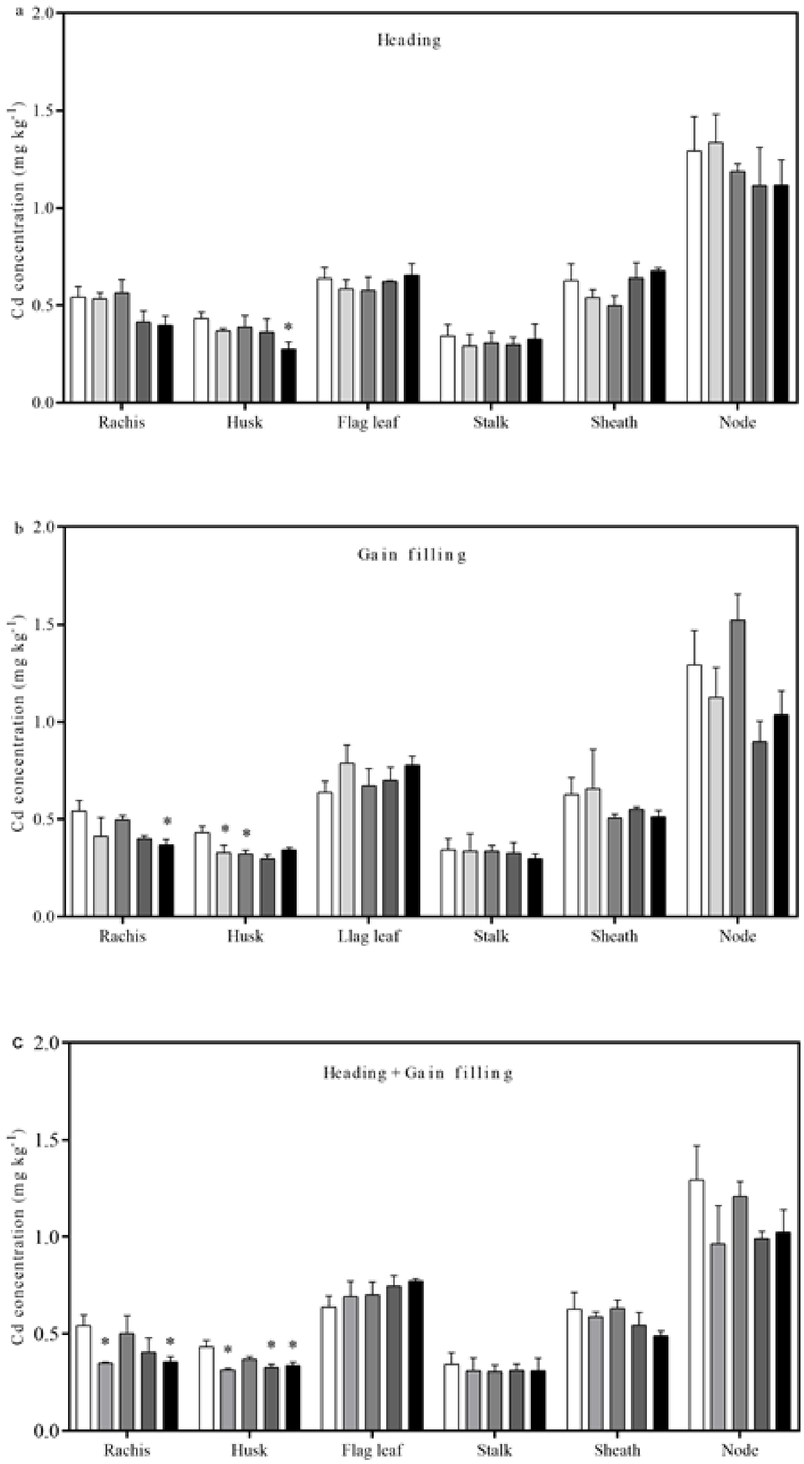

Figure 7

Cd content in different organs of wheat in the pot experiment.

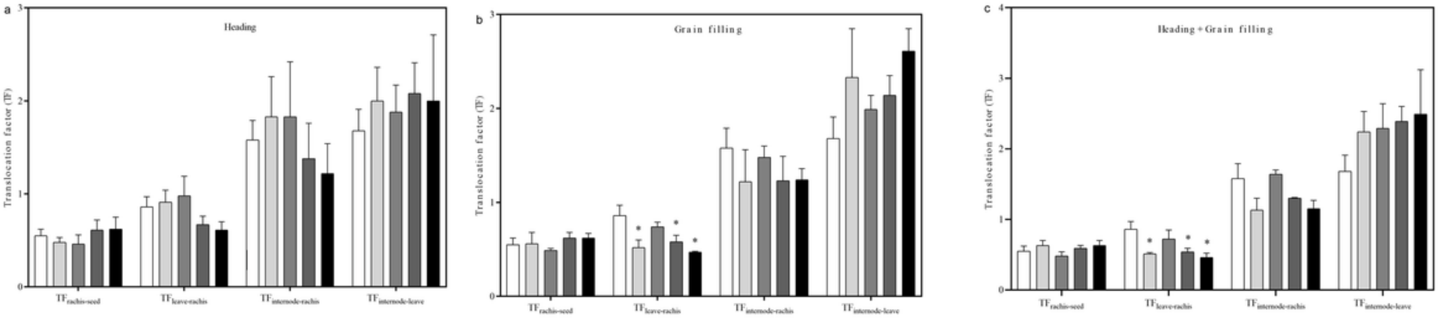

Figure 8

Effects of different foliar application on the TF Cd in different tissues of the wheat.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.doc