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THE Cd:Zn RATIO IN A SOIL AFFECTS Cd TOXICITY IN SPINACH (*Spinacea oleracea* L.)

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Cadmium (Cd) is a highly soil mobile heavy metal which is toxic even at very low concentrations and is thus of potential human health concern due to its entry into the food chain via consumption of edible crops. The plant nutrition is the most economical and practicable method among all strategies for reducing the accumulation of Cd in the edible parts of the plants. This experiment investigated the effect of changing the Cd:zinc (Zn) ratio in the soil in order to minimize the accumulation of Cd in the edible parts, i.e. the leaves of spinach. Overall spinach growth was decreased in Cd contaminated soil, and Cd concentrations in spinach leaf increased with increased exposure to Cd. However, while spinach growth increased at a moderate Cd:Zn ratio of 1:10, at higher Zn soil concentrations (Cd:Zn = 1:100) plant growth was decreased, but this was also accompanied by a significantly decreased ($P < 0.05$) in the accumulation of Cd the spinach leaves. Thus the ratio of Cd:Zn in Cd contaminated soils can simultaneously decrease the adverse effects of Cd on plant growth and also minimize Cd uptake into edible plant parts.
Keywords: Cadmium, toxicity, cadmium: zinc, vegetables, health risks.

INTRODUCTION

The current population of Pakistan is almost 192 million (Economic Survey of Pakistan, 2014-15) and the water requirements for drinking and agronomic purposes are almost solely fulfilled from a single river system. The River Indus and its tributaries irrigate nearly all of the arable soils of the arid and semi-arid areas of the Pakistan. However, poor systems of irrigation distribution, a rapidly growing population, recurrent droughts and saline groundwater have all resulted in increased water shortages. Thus Pakistan has a significant water deficit issue owing to the lack of available surface storage reservoirs and ongoing depletion of ground water resources (Kashif *et al*., 2009). Thus due to the scarcity of fresh water supplies, today untreated waste water is more frequently used for irrigation. However, untreated waste water may contain number of potential contaminants including heavy metals, inorganic salts, organics and pathogens (Qadir *et al*., 2010). Continuous use of wastewater is bringing the high concentrations of Cd and elevating its level in the agricultural soil of Pakistan. Cadmium is released in soil environment through different anthropogenic activities such as atmospheric deposition, application of phosphatic fertilizers, pesticides, cement industries and metal industries (Lim *et al.,* 2013; Lee *et al.,* 2013). In particular, the high mobility of heavy metals is a major concern because of the potential for this to result in food chain contamination. Cadmium which exhibits very high mobility compared to other metals such as Pb, has the greatest potential to enter food chain in relatively high concentrations (Khodaverdiloo *et al*., 2011). Mobile Cd mainly accumulates in aerial plant parts and is transferred to animals and humans when they consume the edible parts of the food crop, especially the leaves of leafy vegetables. Excessive Cd intake is also a major concern because Cd accumulates in the body over time (McLaughlin *et al*., 1999; Kabir *et al*., 2014).

The contamination of vegetables by heavy metals; especially leafy vegetables like spinach (*Spinacea oleracea* L.) is a serious issue because of the significance of vegetables as food commodity and as a primary source of proteins, vitamins, iron, calcium and other essential minerals. The concentration of these metals in the edible parts of leafy vegetables depends mainly on the magnitude of soil contamination and the soil quality. Whereas, the innate ability of the plant to tolerate exposure to heavy metals together with the degree of soil contamination determine the overall tolerance of the plant to soil heavy metals (Sikka and Nayyar, 2012). Spinach has a relatively high potential to accumulate heavy metals such as Cd and Pb in its leaves and thus high potential to cause adverse human health effects when these leaves are consumed (Chunilall *et al*., 2004; Intawongse and Dean, 2006). High metal uptake commonly exhibited by spinach may be related to its physical structure. As broad leafy vegetable spinach has a large surface area which allows for both a high rate of transpiration and also a large surface area for the absorption of heavy metals.

In Pakistan, spinach has been extensively grown in the vicinity of the industrial areas of Faisalabad (Murtaza *et al*., 2010) and these areas are precisely where the scarcity of freshwater has seen a growing reliance on Cd contaminated wastewater for irrigation (Qadir *et al*., 2010). The ingression of heavy metals from wastewater and different other anthropogenic sources is increasing at an alarming rate in soils surrounding peri-urban areas especially in industrial cities of Pakistan. Soil is a sink for these heavy metals which will ultimately reach groundwater, plants, animals and humans. Thus in such areas it is vital to urgently minimize the entry of highly toxic heavy metals such as Cd in to the food chain and thereby decrease the associated human health risks. One potential way to reduce the entry of Cd into edible plant parts is via the addition of essential micronutrient like Zn (Koleli *et al*., 2004). For example, Wu and Zhang (2002) previously investigated the competitive relation between Cd and Zn and showed that in theory an antagonistic interaction between Cd and Zn existed which could be used to decrease the phytoavailability of Cd to spinach. Knowledge of contamination of spinach with Cd from the industrial areas of Faisalabad and use of Cd:Zn ratio to ameliorate the toxicity of Cd in this important vegetable is not yet established. Thus here we practically examine the effect of increasing Zn concentrations in a Cd contaminated soil on reducing the levels of Cd accumulated in the leaves of spinach when cropped on the contaminated soil.

MATERIALS AND METHODS

***Control Soil*:** The uncontaminated soil used in this study was collected from the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan and was used as control. The soil was air-dried, ground with wooden roller, and passed through a 2 mm sieve prior to any physicochemical analyses. A representative soil sub-sample (1 kg) was retained for measuring pH_s, EC_e, lime content ($CaCO_3$), organic matter (OM) content, soluble cations and anions, cation exchange capacity (CEC) and phosphorus following standard methods (Page *et al*., 1982). Particle-size analysis of the soil carried out following hydrometer method (Bouyoucos, 1962) and textural class was designated following the system of International Society of Soil Science. The concentrations of ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable Cd, Pb and Zn in soil were also determined with atomic absorption spectrophotometer (AAS, Thermo AA®, Solar-Series) (Table 1).

***Spiked Soil*:** The control soil was artificially contaminated with Cd (3 mg kg^{-1}) using $CdCl_2.2H_2O$. The Cd contaminated soil was also amended with Zn at two Cd;Zn ratios of 1:10 (Cd @ 3 mg kg^{-1} + Zn @ 30 mg kg^{-1}) and 1:100 (Cd @ 3 mg kg^{-1} + Zn @ 300 mg kg^{-1}). The Cd-spiked soil alone and Cd-spiked soil amended with two different concentrations of Zn were then equilibrated for a 4 weeks at field capacity to allow redistribution of Cd and Zn into different soil phases.

Table 1. Selected physiochemical properties of the control soil.

Soil characteristics	Value
Textural class	Sandy clay loam
pH_s	7.16
EC_e (dS m^{-1})	3.25
SAR (mmol $L^{-1})^{1/2}$	1.69
TSS ($mmol_c$ L^{-1})	32.5
$CaCO_3$ (%)	1.78
Organic matter (%)	0.72
AB-DTPA extractable	
Cd (mg kg^{-1})	0.08
Pb (mg kg^{-1})	1.25
Zn (mg kg^{-1})	2.45
Total (HNO_3 & $HClO_4$; 3:1)	
Cd (mg kg^{-1})	1.82
Pb (mg kg^{-1})	13.55
Zn (mg kg^{-1})	27.5

***Experimental design*:** The growth experiment was conducted in the glasshouse at University of Agriculture Faisalabad, Pakistan. Four treatments with three replicates of each treatment were conducted in a completely randomized design (CRD). Metal-spiked soils (10 kg) were filled in glazed pots internally lined with polythene sheet. The recommended dose of NPK fertilizer for spinach (85-62-50) was applied in the form of urea, diammonium phosphate (DAP) and sulfate of potash (SOP). Urea, DAP and SOP were used as the sources of nitrogen, phosphorus and potassium, respectively. The four treatments employed were T_0(control (uncontaminated soil)), T_1(Cd contaminated soil (3 mg kg^{-1}), T_2(Cd contaminated soil + 30 mg Zn kg^{-1} (1:10 ratio) and T_3(Cd contaminated soil + 300 mg Zn kg^{-1} (1:100 ratio).

***Crop husbandry*:** When the moisture in the pot reached field capacity (measured by weighing soil containing pots regularly until we get moisture in the soil equivalent to field capacity), 10-15 seeds of spinach (*Spinacia oleracea* L.) were sown in each pot on October 2, 2010. After germination, seedlings were thinned to 5 plants per pot. The uprooted plants were crushed and buried in their respective pots. One third of nitrogen applied as urea and the whole amount of DAP and SOP were incorporated into the pots at the time of sowing. Subsequently, the remaining portions of N were applied 15 and 25 days after germination in two equal splits. Spinach plants were irrigated with canal water as and when required. After eight week of sowing, the crop was harvested. Subsequently, leaves were washed with deionized water, air-dried and ground to a fine powder in a Wiley mill fitted with stainless steel blades. Effect of Cd:Zn concentration on Cd uptake, root and shoot dry weights was investigated.

***Analysis*:** Total metal concentrations in the ground plant materials were determined via an atomic absorption spectrophotometer following a di-acid (HNO_3^+ $HClO_4$; 3:1) digestion method (AOAC, 1990). One gram of oven-dried plant sample was taken in a conical flask, kept overnight after adding 5 mL concentrated nitric acid and 5 mL of perchloric acid. Next day, again added 5 mL concentrated HNO_3, and digested on a hot plate till solution was clear. After digestion, the material was cooled and made the volume 25 mL with distilled water and stored in air-tight bottles for the determination of metals with atomic absorption spectrophotometer AAS, (Thermo AA®, Solar-Series). The AB-DTPA extracting solution was prepared by dissolving 79.06 NH_4HCO_3 and 1.97 g of DTPA in distilled water and making the volume of solution up to one liter. Soil (10 g) was placed in a 250 mL Erlenmeyer flask, added 20 mL of freshly prepared extracting solution, shook on reciprocating shaker at 180 cycles per minute for 15 minutes by keeping flasks open (Soltanpour, 1985), filtered and analyzed the extract for metal ions with atomic absorption spectrophotometer (Model Thermo S-Series).

$$\text{Metal (mg kg}^{-1}) = \frac{\text{metal in extract (mg L}^{-1}) - \text{metal in blank (mg L}^{-1}) \times A}{\text{weight of soil (g)}}$$

Where A is total volume made of the extract in mL.

Biological Concentration Factor (BCF), Translocation Factor (TF) and Biological Accumulation Coefficient (BAC) of Cd in spinach were calculated by following equations.

$$BCF = \frac{metal_{root}}{metal_{soil}}$$ (Yoon *et al*. 2006)

$$BAC = \frac{metal_{shoot}}{metal_{soil}}$$ (Cui *et al*., 2007; Li *et al*., 2007)

$$TF = \frac{metal_{shoot}}{metal_{root}}$$ (Li *et al*., 2007; Cui *et al*., 2007)

All data were subjected to statistical treatment following analysis of variance (ANOVA) technique using statistic 8.1 software and treatment means were compared using least significant difference (LSD) (Steel *et al*., 1997).

RESULTS AND DISCUSSION

***Effect of Cd:Zn ratio on dry matter yield of spinach*:** The highest shoot dry weight of 5.66 g pot^{-1} was observed in the control soil. Relative to the control soil, spinach grown in the Cd contaminated soil without added Zn exhibited a decreased shoot dry weight. However, with the application of 30 mg Zn kg^{-1} to the Cd contaminated soil, a slight increase in spinach shoot dry weight was observed (Table 2). However, when the Zn concentration in Cd contaminated soil was further increased to 300 mg Zn kg^{-1}, a reduction in the dry biomass of spinach was observed. At this concentration and a Cd:Zn ratio of 1:100, the lowest shoot dry weight of 4.72 g was observed, which corresponded to a 16.6% decrease in shoot dry weight relative to the control.

A similar variation in spinach root biomass was observed in response to changes in Cd:Zn soil ratios. While root dry weight remained highest (0.87 g pot^{-1}) in control treatment, a significant reduction in root dry biomass was observed when spinach was grown in the Cd contaminated soil without the addition of Zn. However, amendment with the lower Zn levels (30 mg kg^{-1}) increased the root dry matter yield relative to the Cd contaminated soil. However further increase in Zn concentrations decreased root biomass, so that the lowest root dry weight (0.60 g pot^{-1}) was observed at a Cd:Zn ratio of 1:100 which was equivalent to 31.5% decrease relative to the control treatment.

Table 2. Effect of Cd:Zn soil ratio on spinach dry matter yield (g pot^{-1}).

Treatment	Shoot dry weight	Root dry weight
Control	5.66a	0.87a
Cd (3 mg kg^{-1})	5.50ab (2.83)*	0.83a (4.05)
Cd:Zn (1:10)	5.58a (1.36)	0.85a (2.68)
Cd:Zn (1:100)	4.72b (16.6)	0.60b (31.5)

Means sharing similar letter in a row or in a column are statistically non-significant at $P < 0.05$.

*Values in parenthesis are % increase (+) or decrease (-) over the respective control.

Cadmium is a highly toxic metal that can interfere with both the physiological and metabolic processes of spinach which ultimately results in retardation of growth (Murtaza *et al*., 2015). In agreement with several previous studies both shoot and root dry matter yield of spinach was decreased here in the Cd contaminated soil due to the Cd induced phytotoxicity (Kherbani *et al*., 2015; Ezhilvannan and Sharavanan, 2015). While a decrease in overall growth of spinach was generally observed upon exposure to Cd, a non-significant decrease in the shoot dry matter was observed at a Cd:Zn ratio of 1:10 compared to the Cd contaminated treatment. Thus addition of Zn to the Cd contaminated soil at the lower (30 mg kg^{-1}) level did not significantly increase the dry matter yield of spinach. However a slight positive effect on the growth was monitored compared to Cd contaminated soil. Zinc is an important micronutrient which is essential for plant growth. Thus the presence of Zn at 30 mg kg^{-1} might have slightly decreased the toxic effect of Cd by improving overall photosynthesis and chlorophyll content in the spinach (Hassan *et al*., 2005). The increased reduction in both shoot fresh and dry biomass (g pot^{-1}) at a Cd:Zn ratio of 1:100 was probably due to the toxic effect of Zn alone at this concentration (300 mg kg^{-1}) as indicated in the previous study conducted by Alia *et al*. (2015). Certainly, there was a sharp decrease in all of spinach's the growth parameters at the higher levels of Zn. At higher level, Zn has previously been reported to cause reductions in both shoot and root biomass through direct toxic effects at higher concentration and also occasionally due to antagonistic interaction in absorption of other nutrient ions necessary for plant growth. The observed decrease at a Cd:Zn ratio of 1:100 is in agreement with Alia *et al*. (2015) who reported that application of Zn (250 mg kg^{-1}) decreased the growth parameters of spinach, which is comparable to the 300 mg kg^{-1} added here for spinach at which Zn became a toxic pollutant instead of being as essential nutrient.

***Effect of Cd:Zn in the soil on the concentration of Cd and Zn in the spinach leaves*:** The Cd:Zn ratio in the soil had a significant effect at $P < 0.05$ on the concentration (mg kg^{-1}) of Cd in the spinach leaves (Table 3). Relative to the control, a significant increase in the Cd concentration of spinach leaves was observed when grown on the Cd contaminated soil. However, the highest concentration of leaf Cd (3.67 mg kg^{-1}) was observed at a Cd:Zn ratio of 1:10 (i.e. Cd @ 3 mg kg^{-1} and Zn @ 30 mg kg^{-1}). At the higher Cd:Zn ratio of 1:100 (i.e. Cd @ 3 mg kg^{-1} and Zn @ 300 mg kg^{-1}), Cd spinach leaf concentrations were significantly decreased (1.96 mg kg^{-1}) relative to both the contaminated soil and that treated with only modest amounts of Zn. Overall, a decrease in the concentration of Cd in spinach leaves of up to 46.2% was observed at a Cd:Zn ratio of 1:100. Concurrent with the significant decrease in Cd leaf levels at this ratio was significant increase in Zn leaf concentration. Thus Zn leaf concentrations were highest (343 mg kg^{-1}) at a Cd:Zn ratio of 1:100 and decreased to 48.4 mg kg^{-1} at a Cd:Zn ratio of 1:10, but were lowest (0.01 mg kg^{-1}) in the control soil which was comparable to the contaminated soil (0.15 mg kg^{-1}).

Table 3. Concentrations (mg kg^{-1}) of Cd and Zn in spinach leaves.

Treatment	Cd (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.01c	0.01c
Cd (3 mg kg^{-1})	3.29a	0.01c
Cd:Zn (1:10)	3.67a	48.4b
Cd:Zn (1:100)	1.96b	343a

Means sharing similar letter in a row or in a column are statistically non-significant at $P < 0.05$.

***Effect of Cd:Zn in the soil on the concentration of Cd and Zn in the spinach roots*:** The ratio of Cd:Zn in the soil had a significant ($P <0.05$) effect on the concentration of Cd in the spinach roots (Table 4). Relative to the control, there was a significant increase in the concentration of Cd in spinach roots when grown in the contaminated soil. However, as observed for shoots, the highest concentration of Cd (3.14 mg kg^{-1}) in spinach roots was observed at a Cd:Zn ratio of 1:10 . At the higher Cd:Zn ratio of 1:100, Cd concentrations decreased. Overall a decrease in the concentration of spinach root Cd of up to 37.2% was observed at Cd:Zn ratio of 1:100. As had been observed with shoots, the highest root Zn concentration (350 mg kg^{-1}) was observed at a Cd:Zn ratio of 1:100 followed by 51.4 mg kg^{-1}at a Cd:Zn ratio of 1:10 and the lowest root Zn concentration was observed in the control (0.01 mg kg^{-1}).

Table 4. Concentrations (mg kg^{-1}) of Cd and Zn in spinach roots.

Treatment	Cd (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.01c	0.02c
Cd (3 mg kg^{-1})	2.96a	0.01c
Cd:Zn (1:10)	3.14a	51.4b
Cd:Zn (1:100)	1.97b	350a

Means sharing similar letter in a row or in a column are statistically non-significant at $P < 0.05$.

***Effect of Cd:Zn on biological accumulation coefficient (BAC), biological concentration factor (BCF) and translocation factor (TF) of Cd*:** The soil Cd:Zn ratio had a pronounced influence on a range of Cd bioaccumulation and transfer coefficients for spinach (Table 5). Relative to the control, the Cd biological concentration factor (BCF) for Cd increased significantly when spinach was grown on the Cd contaminated soil. While the lowest level of applied Zn (30 mg kg^{-1} soil) increased BCF, the highest level of applied Zn (300 mg kg^{-1} soil) significantly decreased BCF. Similar behaviors were also observed for both the biological accumulation coefficient (BAC) and the translocation factor (TF) of Cd in spinach. Consistently, at the lower level of applied Zn (30 mg kg^{-1} soil) both BAC and TF were increased but at the higher applied Zn concentration both BAC and TF were decreased relative to the values observed in the Cd contaminated soil.

Table 5. Effect of Cd:Zn on biological accumulation coefficient (BAC), biological concentration factor (BCF), translocation factor (TF) of Cd.

Treatment	BCF	BAC	TF
Control	0.01	0.01	0.01
Cd (3 mg kg^{-1})	0.99	1.10	1.11
Cd:Zn (1:10)	1.05	1.22	1.17
Cd:Zn (1:100)	0.66	0.65	0.99

As expected from simple mass balance, the concentration of Cd in spinach leaves increased as the level of Cd in the soil increased. With the addition of Cd to the soil, it is expected that both the soluble and exchangeable pools of Cd in the soil would also increase and that this would ultimately increase plant uptake leading to diminished growth and development of spinach (Sikka and Nayyar, 2012). While addition of Zn at the lower level (1:10 ratio) did not significantly affected the concentration of Cd in spinach plants compared to those plants grown in the Cd contaminated soil, there was a slight increase in the concentration of Cd with the addition of Zn at a 1:10 ratio, i.e. 30 mg Zn kg^{-1} soil. This could be attributed to increased synthesis of phytochelatins (PCs) by spinach. Cadmium induced toxicity results in more significant formation of Cd forming Cd-PC complexes (Rauser, 2000). Such complexes are able to better sequester Cd in the vacuole thereby reducing Cd translocation to the shoot. However, with the addition of increasing amounts of Zn to the soil it is likely that Zn-PC complexes are competitively formed in larger amounts thus increasing the concentration of free Cd which results in the increased translocation of Cd from root to shoot. Moreover, since the Cd is chemically similar to Zn, it may be taken up by the roots through Zn transporters. At low level of Zn in growth medium, Zn transporters are not fully loaded and Cd is more likely to be transported by Zn transporter. Above these are possible explanations to the occurrence of slightly high Cd concentrations in the leaves of spinach at a 1:10 ratio of Cd and Zn.

In contrast, at the higher Zn concentration (300 mg kg^{-1}) there was a significant decrease in the concentration of Cd. Previous studies have indicated that one option to decrease Cd accumulation in plants is to effectively improve plant Zn nutrition (Qiu *et al*., 2005; Aravind *et al*., 2009). Since both Zn and Cd have almost identical ionic radii, the addition of Zn might result in active competition with Cd for the same membrane binding sites and transport systems, thereby resulting in reduced Cd accumulation in spinach. The interaction of Cd with Zn in plants is based on the substitution of Cd with Zn and decreasing the concentration of Cd to below phytotoxic levels in spinach leaves. The application of higher soil Zn concentrations decreased soil Cd phytoavailability and subsequently decreased the uptake of Cd by the plants. This is in agreement with the antagonistic relationship previously reported (Hassan *et al*., 2005). Results of the present study indicated that there was a sharp decrease in the concentration of Cd in spinach leaves at 1:100 Cd:Zn ratio corresponding to a 46.2% decrease in the concentration of Cd compared to a Cd:Zn ratio of 1:10.

***AB-DTPA extractable Cd and Zn concentration in post-harvest soil analysis*:** The ratio of Cd:Zn in the soil had a highly significant ($P < 0.05$) effect on the concentration of AB-DTPA extractable Cd and Zn in the post-harvest soil samples (Table 6). Relative to the control, there was a significant increase in the concentration of AB-DTPA extractable Cd (0.62 mg kg^{-1}) in post-harvest soil sample of Cd contaminated soil. However, a significant decrease in the concentration of Cd was observed at a Cd:Zn ratio of 1:10 . At the higher Cd:Zn ratio of 1:100 (i.e. Cd @ 3 mg kg^{-1} and Zn @ 300 mg kg^{-1}) AB-DTPA extractable Cd concentrations increased and the highest concentration of 0.87 mg kg^{-1} was monitored. The concentration of AB-DTPA extractable Zn was the highest (57.9 mg kg^{-1}) at the higher Cd:Zn ratio of 1:100 followed by 3.64 mg kg^{-1} at a Cd:Zn ratio of 1:10 (i.e. Cd @ 3 mg kg^{-1} and Zn @ 30 mg kg^{-1}) and lowest in the control treatment.

Table 6. AB-DTPA extractable concentrations (mg kg^{-1}) of Cd and Zn in post-harvest soil samples.

Treatment	Cd (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.09d	0.14c
Cd (3 mg kg^{-1})	0.62b	0.15c
Cd:Zn (1:10)	0.45c	3.64b
Cd:Zn (1:100)	0.87a	57.9a

Means sharing similar letter in a row or in a column are statistically non-significant at $P < 0.05$.

From the above result it is evident that with the increase of soil contamination there was an increase in the concentration of AB-DTPA extractable Cd. The concentration of AB-DTPA extractable Cd decreased significantly with the addition of lower level of Zn in soil (1:10), reflecting an increased uptake of Cd by spinach leaves as the values of BAC and BCF increased in the present study. However, higher concentration of Zn (i.e. 300 mg kg^{-1}) at 1:100 Cd:Zn significantly increased the concentration of AB-DTPA extractable Cd in soil samples compared to Cd-spiked soil. At higher level of Zn, Zn transporters are fully occupied and cannot take up Cd resulting in the decreased accumulation of Cd in spinach and increased AB-DTPA extractable soil Cd.

***Conclusion*:** At the highest level of Zn applied in this study (300 mg kg^{-1}) an antagonistic relation between Cd and Zn was observed. At the lower level of applied Zn (30 mg kg^{-1}) Cd toxicity was alleviated and the dry matter yield of spinach was increased. However, the lower level of applied Zn in combination with a contaminated Cd soil (3 mg kg^{-1}) did not decrease the Cd leaf concentrations. A higher level of Zn (300 mg kg^{-1}) corresponding to a Cd:Zn ratio of 1:100 did however decrease the Cd leaf concentrations in spinach by 46.2% relative to the Cd:Zn ratio of 1:10. This study highlights the importance of choosing a suitable Cd:Zn ratio which can effectively decrease the damaging effects of Cd on plant growth and simultaneously minimize Cd uptake in to edible parts of plants when grown in Cd stressed soils.

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