

# Seed priming and soil application of zinc decrease grain cadmium accumulation in standard and zinc-biofortified wheat cultivars

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## ABSTRACT

**Context.** Cadmium (Cd) is a toxic metal for both plants and humans. Wheat grown on Cd-contaminated soils may accumulate toxic levels of Cd in grains. **Aim.** This study aimed to compare soil zinc (Zn) application and seed Zn-priming for decreasing grain Cd concentration in standard and Zn-biofortified wheat cultivars grown on Cd-spiked soil. **Methods.** Standard (Jauhar-2016) and Zn-biofortified (Zincol-2016) wheat cultivars were grown in pots filled with Cd-spiked soil (8 mg Cd kg<sup>-1</sup>). The tested Zn treatments were un-primed, hydro-primed, and Zn-primed seeds with and without soil Zn application at 8 mg kg<sup>-1</sup>. **Key results.** Zinc treatments significantly mitigated the toxic effects of Cd on the growth and physiological parameters of both cultivars. As compared to control, all Zn treatments significantly increased Zn and decreased Cd concentration in grains of the cultivars. On average, the maximum increase in grain Zn concentration over control was approximately 36% with Zn-priming + soil Zn. The same treatment, as compared to control, decreased grain Cd concentration by 42% in Zincol-2016 and 35% in Jauhar-2016. Grain Cd concentration was within the permissible level ( $\leq 0.2$  mg kg<sup>-1</sup>) in Jauhar-2016 at all Zn treatments and in Zincol-2016 at Zn-priming + soil Zn. **Conclusion.** Soil Zn application, seed Zn-priming, and their combination were effective in decreasing grain Cd accumulation in wheat grown on Cd-contaminated soil. **Implication.** Zinc treatments, especially the combination of soil Zn application and seed Zn-priming, should be recommended for wheat grown on Cd-contaminated soil.

**Keywords:** accumulation, biofortification, cadmium, calcareous soil, seed priming, soil application, wheat, zinc, zincol-2016.

## Introduction

At above-permissible levels, cadmium (Cd) is toxic for both plants and humans. In plants, Cd disturbs several key physiological processes like ion metabolism, photosynthesis, and mineral uptake (Khan *et al.* 2017). It affects photosynthesis by disturbing photosystems, photosynthetic pigments, carbon dioxide (CO<sub>2</sub>) reduction pathways, and electron transport systems (Parmar *et al.* 2013). Moreover, it inhibits plant growth by disrupting enzyme activities, protein synthesis, and hormone balance (Alharby *et al.* 2021). In humans, it causes oxidative stress that directly weakens the antioxidant defence system. It also damages DNA (Singh *et al.* 2021) and causes carcinogenesis and serious diseases like cardiovascular disease, and osteoporosis (Bimonte *et al.* 2021). Therefore, it is important to restrict Cd uptake from soil and its accumulation into plant edible parts.

Metal mining, wastewater irrigation, automobile exhaust, and phosphate fertilisers are the major anthropogenic sources of soil Cd contamination (Cai and Li 2022). From soils, Cd is accumulated in edible plant parts and ultimately enters the human food chain (Suhani *et al.* 2021). One clear strategy to save plant-based food from Cd exposure is to remediate contaminated soils through physical, chemical, and biological approaches (Liu *et al.* 2018). However, soil remediation techniques are expensive and mostly impractical for large-scale

soil remediation projects. Another strategy, especially for marginally Cd-contaminated soils, is to increase the supply of zinc (Zn) to crop plants (Rizwan *et al.* 2017; Umar and Hussain 2020). Zinc is essential for plant metabolism and acts as a co-factor for some important enzymes. According to Hart *et al.* (2002), the root uptake, shoot transport, and grain loading of both Zn and Cd are facilitated by common transporters in plants. Therefore, these metals compete for uptake from the root and translocation to grains. Apart from this direct competition, Zn can also decrease Cd stress on plants in many other ways. These may include increasing the photosynthetic rate (Hussain *et al.* 2018), decreasing oxidative stress (Hassan *et al.* 2005), and balancing the nutrients (Rizwan *et al.* 2017).

There are significant genotypic variations in the extent of Zn effects on Cd accumulation in wheat (Zhou *et al.* 2020; Ali *et al.* 2022). In previous studies, it was reported that Zn-biofortified wheat can take up and accumulate more Cd in grains as compared to standard wheat (Hussain *et al.* 2019; Zia *et al.* 2020). Moreover, it is well known that both soil and foliar Zn applications increase Zn and decrease Cd accumulation in wheat grains (Murtaza *et al.* 2017; Forster *et al.* 2018; Zhou *et al.* 2020). Another Zn treatment, seed Zn-priming, is a more cost-effective technique of Zn application to cereals grown in calcareous soils (Harris *et al.* 2008). However, the effectiveness of seed Zn-priming relative to soil Zn application is unknown for decreasing grain Cd accumulation in Zn-biofortified and standard wheat cultivars. In the present study, therefore, our key objective was to compare soil Zn application and seed Zn-priming for decreasing grain Cd concentration in standard (Jauhar-2016) and Zn-biofortified (Zincol-2016) wheat cultivars grown in Cd-spiked soil.

## Materials and methods

The surface layer (0–15 cm) of uncontaminated soil was collected from the Agricultural Farm (30.273°N, 71.514°E) of Bahauddin Zakariya University, Multan, Pakistan. The collected soil was air-dried, ground, sieved (through a 2 mm sieve), and thoroughly mixed. A representative sub-sample of the soil was analysed for physical and chemical properties by following the standard procedures. Soil texture was loamy with 44% sand, 26% silt, and 30% clay, as determined by Hydrometer Method (Gee and Bauder 1979). The soil was non-saline (with an electrical conductivity [EC] of 2.69 dS m<sup>-1</sup> determined in saturated soil paste extract) but alkaline in reaction (with pH 7.8 determined in the saturated soil paste). Determined by following the Walkley and Black method (Nelson and Sommers 1996), the soil had 0.53% organic matter. Calcium carbonate in the soil was 49.8 g kg<sup>-1</sup> as determined by the acid dissolution method (Loeppert and Suarez 1996). Plant available Zn and Cd were, respectively,

0.64 mg kg<sup>-1</sup> and 0.02 mg kg<sup>-1</sup> determined on an atomic absorption spectrophotometer (240FS AA, Agilent, Santa Clara, USA) after extraction by the diethylenetriaminepentaacetic acid (DTPA) method (Soltanpour and Workman 1979).

A known amount of soil was spiked at 8 mg Cd kg<sup>-1</sup> by spraying a solution of CdCl<sub>2</sub> followed by thorough mixing. The Cd-spiked soil was then covered with a polyethylene sheet and was allowed to equilibrate for 4 weeks (Lair *et al.* 2008). After that, 36 polyethylene-lined pots were each filled with 8 kg of Cd-spiked soil.

Before seed sowing, 60 mg N kg<sup>-1</sup> as urea ((NH<sub>2</sub>)<sub>2</sub>CO), and 20 mg P kg<sup>-1</sup> and 25 mg K kg<sup>-1</sup> as potassium di-hydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) were applied to pots in solution form. As per the treatment plan detailed in the following paragraph, two soil Zn rates (0 and 8 mg Zn kg<sup>-1</sup>) were applied as a solution of Zn sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O). After nutrient application, the soil in each pot was thoroughly mixed.

There were 12 treatments comprising all combinations of two wheat cultivars (standard Jauhar-2016 (pedigree: KAUZ/PASTOR//V.3009) and Zn-biofortified Zincol-2016 (pedigree: OASIS/SKAUZ//4 × BCN/3/2 × PASTOR/4/T) and six Zn treatments [(1) un-primed seeds/control, (2) hydro-primed seeds, (3) Zn-primed seeds, (4) un-primed seeds + soil Zn, (5) hydro-primed seeds + soil Zn, and (6) Zn-primed seeds + soil Zn]. For hydro-priming, the seeds were soaked in aerated distilled water at 1:3 ratio for 12 h. In the same conditions, the seeds for Zn-priming were soaked in 4 mM ZnSO<sub>4</sub>·7H<sub>2</sub>O. The treatments in the glasshouse were laid out in 2-factorial completely randomised design with three replications.

Seven healthy seeds were sown in each pot. After germination, plants were thinned to four per pot at the four-leaf stage. Plants were irrigated with distilled water to maintain moisture contents at 90% of the field capacity. At emergence, weeds were manually removed from the pots. To ensure randomisation, the positions of pots in the glasshouse were randomly changed each week. Additional splits of N (each at 60 mg kg<sup>-1</sup>) were applied as urea at stem elongation and booting stages.

At the anthesis stage (Feekes 10.5), a fully expanded uppermost leaf was selected from each plant for measuring chlorophyll contents (SPAD [Soil Plant Analysis Development] values) and photosynthetic parameters. Chlorophyll contents (SPAD values) were measured by using the SPAD-502 (Minolta, Osaka, Japan) meter. Photosynthetic rate, stomatal conductance, and transpiration rate were measured with CIRAS-3, a portable photosynthesis system (PP Systems, Amesbury, USA).

Two wheat plants from each pot were harvested at booting (Feekes 10) and the remaining two were harvested at full maturity (Feekes 11). After harvesting at maturity, plants were separated into stems, leaves, chaff, and grains. All plant parts were washed with distilled water to remove

dust particles and then blotted dry with tissue papers. The plant samples were then oven-dried at 70°C for 48 h. Grains from the spikes were threshed by hand. Thousand-grain weights were calculated from the grain yield and the number of grains per pot. After the final harvest, post-harvest soil samples were collected from the pots for the determination of plant-available Zn and Cd by the DTPA method (Soltanpour and Workman 1979).

All plant samples were ground in a metal-free grinder followed by acid digestion in a di-acid mixture of nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) (Jones and Case 1990). Zinc and Cd concentrations were analysed in the digests by using an atomic absorption spectrophotometer (240FS AA, Agilent, Santa Clara, USA). The total plant contents of Zn and Cd at two crop stages were calculated by multiplying dry weights with the concentrations of the respective element in each plant part.

The collected data were statistically analysed by using *Statistix 8.1*® for windows (Analytical Software, Tallahassee, USA). For the assessment of treatment effects, a two-way analysis of variance (ANOVA) test was run followed by Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$  (Quinn and Keough 2002). The interactive effect of wheat cultivars and Zn treatments was significant only for grain Cd concentration. Therefore, only the significant main effects were presented for the other parameters, and the interactive effect was presented for grain Cd concentration.

## Results

### Post-harvest soil zinc and cadmium

The main effect of Zn treatments influenced ( $P < 0.001$ ) the post-harvest availability of Zn in the soil (Table 1). Averaged across cultivars, DTPA-extractable Zn was increased in all the treatments with soil Zn application as compared to control (Supplementary Table S1). Soil DTPA-extractable Zn ranged from 0.61 to 0.63 mg kg<sup>-1</sup> in treatments without soil Zn and from 0.83 to 0.85 mg kg<sup>-1</sup> in treatments with soil Zn. On the other hand, the imposed treatments did not affect DTPA-extractable Cd (Table 1). On average, the DTPA-extractable Cd in the soil after the harvest was 0.13 mg kg<sup>-1</sup> (Table S1).

### Photosynthetic parameters

The photosynthetic parameters, measured in the flag leaves at anthesis, were influenced by the main effects of wheat cultivars and Zn treatments (Table 1). Averaged across Zn treatments, stomatal conductance, photosynthesis rate and transpiration rate were significantly higher in Jauhar-2016 than in Zincol-2016 (Table 2). Averaged across cultivars and as compared to control, soil Zn combined with Zn-priming and hydro-priming increased transpiration rate,

SPAD values, and stomatal conductance. However, the increase was non-significant in stomatal conductance with soil Zn + hydro-priming.

### Plant growth and grain yield

The main effects of cultivars and Zn treatments influenced shoot dry matter at booting, and stem, leaf, chaff and grain dry matter at maturity (Table 1). Averaged across Zn treatments, dry matters of stems, chaff and grains at maturity were significantly higher in Jauhar-2016 than in Zincol-2016 (Table 3). Averaged across cultivars, shoot dry matter at booting was increased by 18–25% with the treatments having soil Zn over control. Dry matters of stems, leaves, chaff, and grains at maturity were significantly increased by, respectively, 23–30%, 27–35%, 23–31%, and 18–25% with the treatments having soil Zn relative to control.

Thousand-grain weight was also affected by the main effects of cultivars ( $P = 0.005$ ) and Zn treatments ( $P < 0.001$ ) (Table 1). On average, the weight of 1000 grains was 6% higher in Jauhar-2016 than in Zincol-2016 (Table 3). Averaged across cultivars and as compared to control, 1000-grain weight was significantly increased (by 14–20%) with the treatments having soil Zn application.

### Zinc concentration and contents

The main effects of cultivars and Zn treatments influenced Zn concentration and contents in shoots at booting, and in stems, leaves, chaff and grains at maturity (Table 1). On average, Zincol-2016 had significantly higher shoot Zn concentration (~18% more) and contents (~16% more) than Jauhar-2016 at booting (Tables 4, 5). At maturity, Zn concentration in stems, leaves, chaff and grains was, respectively, 14%, 14%, 18%, and 19% higher in Zincol-2016 than Jauhar-2016.

Averaged across cultivars, grain Zn concentration varied from 25 mg kg<sup>-1</sup> in un-primed control to 34 mg kg<sup>-1</sup> in Zn-priming + soil Zn (Table 4). Averaged across cultivars and as compared to control, Zn-priming + soil Zn increased shoot Zn concentration at booting by 51%. As compared to control, Zn-priming + soil Zn increased Zn concentration in stems, leaves, chaff and grains by 64%, 45%, 48% and 38%, respectively.

Averaged across cultivars, both at booting and maturity, shoot Zn contents were higher in Zn-treated pots than in control (Table 5). However, hydro-priming also significantly increased shoot Zn contents at maturity as compared to control. Averaged across Zn treatments, Zincol-2016 had higher (~16% more) shoot Zn content than Jauhar-2016 at maturity. In the cultivars, most of the total plant Zn was accumulated in grains (62–66%) followed by stems (18–21%), chaff (9–10%) and leaves (7–8%). However, Zn partitioning in stems was greater in Jauhar-2016 than Zincol-2016 whereas Zn partitioning in grains was greater in Zincol-2016 than Jauhar-2016.

**Table 1.** Calculated *F* values based on two-way ANOVA with interactions.

Parameters	Cultivars	Zn treatments	Cultivars × Zn treatments
Post-harvest soil DTPA-extractable Zn	0.00	155.95***	0.11
Post-harvest soil DTPA-extractable Cd	1.32	1.86	0.94
Stomatal conductance	16.67***	3.18*	0.04
Photosynthesis rate	2.74	1.95	0.13
Transpiration rate	8.38**	4.34**	0.09
Chlorophyll contents (SPAD values)	1.63	4.05**	0.26
1000-grain weight	9.22**	7.13***	0.04
Total dry matter at booting	1.53	12.98***	0.22
Stem dry matter at maturity	13.49**	14.48***	0.16
Leaf dry matter at maturity	1.90	16.05***	0.13
Chaff dry matter at maturity	4.53*	14.19***	0.86
Grain dry matter at maturity	5.27*	16.56***	0.09
Shoot Zn concentration at booting	105.08***	51.91***	2.04
Stem Zn concentration at maturity	89.48***	94.40***	1.12
Leaf Zn concentration at maturity	106.74***	66.84***	1.58
Chaff Zn concentration at maturity	128.48***	59.90***	2.18
Grain Zn concentration at maturity	200.23***	42.80***	1.25
Shoot Zn contents at booting	42.82***	61.03***	0.83
Shoot Zn contents at maturity	89.25***	149.34***	1.50
Stem Zn partitioning at maturity	9.36**	3.62*	0.27
Leaf Zn partitioning at maturity	0.80	1.26	0.04
Chaff Zn partitioning at maturity	0.01	1.57	0.22
Grain Zn partitioning at maturity	9.37**	7.09***	0.54
Shoot Cd concentration at booting	96.10***	32.10***	0.13
Stem Cd concentration at maturity	140.56***	14.26***	0.42
Leaf Cd concentration at maturity	0.02	21.97***	0.78
Chaff Cd concentration at maturity	107.14***	54.28***	1.33
Grain Cd concentration at maturity	313.04***	103.70***	8.77***
Shoot Cd contents at booting	90.31***	4.01**	0.14
Shoot Cd contents at maturity	49.83***	1.40	1.04
Stem Cd partitioning at maturity	1.32	1.71	0.97
Leaf Cd partitioning at maturity	27.85***	2.81*	0.17
Chaff Cd partitioning at maturity	0.09	0.74	0.26
Grain Cd partitioning at maturity	61.12***	27.24***	1.01

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

### Cadmium concentration and contents

On average, shoot Cd concentration at booting, and stem and chaff Cd concentrations at maturity were higher ( $P < 0.001$ ) in Zincol-2016 than in Jauhar-2016 (Table 6). Averaged across cultivars and as compared to control, all Zn-containing treatments significantly decreased shoot Cd concentration at booting, and stem, leaf and chaff Cd concentration at maturity. The decrease with hydro-priming

over control was non-significant in shoot Cd concentration at booting and in stem, leaf and chaff Cd concentration at maturity.

Grain Cd concentration ranged from 0.16 mg kg<sup>-1</sup> in Jauhar-2016 (with Zn-priming + soil Zn) to 0.35 mg kg<sup>-1</sup> in Zincol-2016 (in un-primed control) (Fig. 1). The interactive effect of cultivars × Zn treatments was significant ( $P < 0.001$ ) for grain Cd concentration (Table 1). In un-primed control, grain Cd concentrations in Zincol-2016 and Jauhar-2016

**Table 2.** Chlorophyll contents (SPAD values), stomatal conductance, photosynthesis rate, and transpiration rate in the flag leaf of two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Chlorophyll contents (SPAD values)	Stomatal conductance ( $\mu\text{mol m}^{-1} \text{s}^{-1}$ )	Photosynthesis rate ( $\mu\text{mol CO}_2 \text{m}^{-1} \text{s}^{-1}$ )	Transpiration rate ( $\text{mmol H}_2\text{O m}^{-1} \text{s}^{-1}$ )
Cultivars	Jauhar-2016	40 ± 2A	371 ± 15A	20 ± 1A	3.2 ± 0.2A
	Zincol-2016	40 ± 2A	350 ± 19B	19 ± 1B	3.0 ± 0.2B
Zinc treatments	Control (un-primed)	38 ± 2B	344 ± 17B	19 ± 1A	2.9 ± 0.1B
	Hydro priming	39 ± 2AB	349 ± 17AB	19 ± 1A	3.0 ± 0.1AB
	Zinc priming	40 ± 2AB	366 ± 18AB	19 ± 1A	3.1 ± 0.1AB
	Soil Zn (un-primed)	40 ± 1AB	361 ± 17AB	20 ± 1A	3.0 ± 0.1AB
	Hydro priming + soil Zn	41 ± 2A	367 ± 18AB	20 ± 1A	3.2 ± 0.2A
	Zinc priming + soil Zn	42 ± 2A	374 ± 19A	20 ± 1A	3.2 ± 0.1A

Separately for each main effect, different letters after means ± s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

**Table 3.** Dry matter yields (g pot<sup>-1</sup>) of two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Shoots at booting	Stems at maturity	Leaves at maturity	Chaff at maturity	Grains at maturity	1000-grain weight
Cultivars	Jauhar-2016	12.5 ± 1.3A	6.6 ± 0.7A	2.2 ± 0.3A	3.1 ± 0.4A	12.0 ± 1.1A	32 ± 2A
	Zincol-2016	12.2 ± 1.1A	6.0 ± 0.7B	2.1 ± 0.3A	3.0 ± 0.3B	11.5 ± 1.1B	30 ± 3B
Zinc treatments	Control (un-primed)	10.9 ± 0.7C	5.4 ± 0.4C	1.8 ± 0.1C	2.6 ± 0.1C	10.4 ± 0.7C	28 ± 2C
	Hydro-primed	11.6 ± 0.7BC	5.8 ± 0.4C	1.9 ± 0.1C	2.8 ± 0.2C	10.9 ± 0.6C	29 ± 2BC
	Zinc-primed	11.9 ± 0.5BC	6.0 ± 0.5BC	2.0 ± 0.1BC	2.9 ± 0.2BC	11.4 ± 0.6BC	31 ± 2ABC
	Soil Zn (un-primed)	12.9 ± 0.9AB	6.7 ± 0.4AB	2.3 ± 0.2AB	3.2 ± 0.2AB	12.3 ± 0.6AB	32 ± 2AB
	Hydro-primed + soil Zn	13.4 ± 0.7A	6.9 ± 0.4A	2.4 ± 0.2A	3.4 ± 0.2A	12.6 ± 0.5A	33 ± 2AB
	Zinc-primed + soil Zn	13.7 ± 0.7A	7.1 ± 0.7A	2.4 ± 0.2A	3.4 ± 0.3A	12.9 ± 0.6A	33 ± 2A

Separately for each main effect, different letters after means ± s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

**Table 4.** Zinc concentration (mg Zn kg<sup>-1</sup>) in two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Shoots at booting	Stems at maturity	Leaves at maturity	Chaff at maturity	Grains at maturity
Cultivars	Jauhar-2016	23 ± 4B	15 ± 3B	18 ± 2B	15 ± 2B	26 ± 3B
	Zincol-2016	28 ± 5A	18 ± 3A	21 ± 3A	18 ± 3A	32 ± 4A
Zinc treatments	Control (un-primed)	21 ± 2D	13 ± 2D	16 ± 1D	13 ± 1D	25 ± 2E
	Hydro-primed	21 ± 2CD	14 ± 2D	17 ± 1CD	14 ± 1CD	26 ± 2DE
	Zinc-primed	24 ± 3C	16 ± 2C	18 ± 2C	15 ± 2C	29 ± 2CD
	Soil Zn (un-primed)	28 ± 4B	18 ± 2B	21 ± 2B	18 ± 2B	31 ± 3BC
	Hydro-primed + soil Zn	29 ± 4AB	18 ± 2B	22 ± 2B	18 ± 3AB	32 ± 4AB
	Zinc-primed + soil Zn	31 ± 4A	21 ± 3A	23 ± 2A	19 ± 3A	34 ± 3A

Separately for each main effect, different letters after means ± s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

were 0.35 mg kg<sup>-1</sup> and 0.25 mg kg<sup>-1</sup>, respectively (Fig. 1). As compared to control, grain Cd concentration was significantly decreased with Zn treatments in both Jauhar-2016 and Zincol-2016. The maximum decrease over respective controls was 35% in Jauhar-2016 and 42% in Zincol-2016, achieved with Zn-priming + soil Zn. In Jauhar-2016, all Zn-containing treatments decreased grain Cd concentration below the permissible level of 0.20 mg kg<sup>-1</sup>. However, in Zincol-2016,

only Zn-priming + soil Zn decreased grain Cd concentration to ~0.20 mg kg<sup>-1</sup>.

The main effects of treatments and cultivars influenced shoot Cd contents at booting and maturity (Table 1). On average, Cd contents in various plant samples of booting and maturity were higher ( $P < 0.001$ ) in Zincol-2016 than in Jauhar-2016 (Table 7). Averaged across cultivars and as compared to control, shoot Cd contents at booting

**Table 5.** Zinc contents in two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Shoot Zn contents at booting ( $\mu\text{g pot}^{-1}$ )	Shoot Zn contents at maturity ( $\mu\text{g pot}^{-1}$ )	Plant Zn partitioning at maturity (%)			
				Stem	Leaf	Chaff	Grain
Cultivars	Jauhar-2016	293 $\pm$ 70B	506 $\pm$ 109B	20 $\pm$ 2A	8 $\pm$ 1A	9 $\pm$ 1A	63 $\pm$ 3B
	Zincol-2016	348 $\pm$ 87A	586 $\pm$ 132A	19 $\pm$ 1B	8 $\pm$ 1A	9 $\pm$ 1A	65 $\pm$ 2A
Zinc treatments	Control (un-primed)	225 $\pm$ 25D	390 $\pm$ 30F	18 $\pm$ 2B	8 $\pm$ 1A	9 $\pm$ 1A	66 $\pm$ 2A
	Hydro-primed	249 $\pm$ 26CD	435 $\pm$ 23E	18 $\pm$ 1B	7 $\pm$ 0A	9 $\pm$ 1A	66 $\pm$ 2A
	Zinc-primed	281 $\pm$ 34C	499 $\pm$ 30D	19 $\pm$ 2AB	7 $\pm$ 0A	9 $\pm$ 1A	65 $\pm$ 3AB
	Soil Zn (un-primed)	361 $\pm$ 40B	597 $\pm$ 48C	20 $\pm$ 1AB	8 $\pm$ 1A	9 $\pm$ 0A	62 $\pm$ 1BC
	Hydro-primed + soil Zn	383 $\pm$ 57AB	642 $\pm$ 72B	20 $\pm$ 1AB	8 $\pm$ 1A	10 $\pm$ 1A	63 $\pm$ 1BC
	Zinc-primed + soil Zn	425 $\pm$ 52A	714 $\pm$ 64A	21 $\pm$ 2A	8 $\pm$ 1A	9 $\pm$ 1A	62 $\pm$ 2C

Separately for each main effect, different letters after means  $\pm$  s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

**Table 6.** Cadmium concentration ( $\text{mg Cd kg}^{-1}$ ) in two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Shoots at booting	Stems at maturity	Leaves at maturity	Chaff at maturity
Cultivars	Jauhar-2016	1.01 $\pm$ 0.14B	0.94 $\pm$ 0.08B	1.89 $\pm$ 0.13A	0.63 $\pm$ 0.08B
	Zincol-2016	1.21 $\pm$ 0.14A	1.14 $\pm$ 0.09A	1.90 $\pm$ 0.17A	0.73 $\pm$ 0.10A
Zinc treatments	Control (un-primed)	1.30 $\pm$ 0.13A	1.16 $\pm$ 0.14A	2.10 $\pm$ 0.09A	0.80 $\pm$ 0.08A
	Hydro-primed	1.24 $\pm$ 0.11AB	1.11 $\pm$ 0.11AB	2.03 $\pm$ 0.07A	0.76 $\pm$ 0.08AB
	Zinc-primed	1.15 $\pm$ 0.11B	1.04 $\pm$ 0.11BC	1.89 $\pm$ 0.06B	0.71 $\pm$ 0.07B
	Soil Zn (un-primed)	1.03 $\pm$ 0.13C	1.00 $\pm$ 0.13C	1.84 $\pm$ 0.07BC	0.63 $\pm$ 0.05C
	Hydro-primed + soil Zn	1.00 $\pm$ 0.11C	0.99 $\pm$ 0.11C	1.81 $\pm$ 0.08BC	0.60 $\pm$ 0.04C
	Zinc-primed + soil Zn	0.95 $\pm$ 0.12C	0.95 $\pm$ 0.13C	1.72 $\pm$ 0.07C	0.58 $\pm$ 0.06C

Separately for each main effect, different letters after means  $\pm$  s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

were significantly decreased only by Zn-priming + soil Zn. At maturity, Zn-priming and all treatments with soil Zn significantly decreased shoot Cd contents than control.

On average, most of the total above-ground plant Cd in both cultivars was accumulated in stems (41–44%), followed by leaves (25–28%), grains (15–20%), and chaff (13–14%) (Table 7). However, grain Cd partitioning was relatively higher in Zincol-2016 than in Jauhar-2016. Averaged across cultivars, priming treatments (hydro- and Zn-priming) had a non-significant effect on grain Cd partitioning, but the treatments with soil Zn significantly decreased grain Cd partitioning as compared to control.

## Discussion

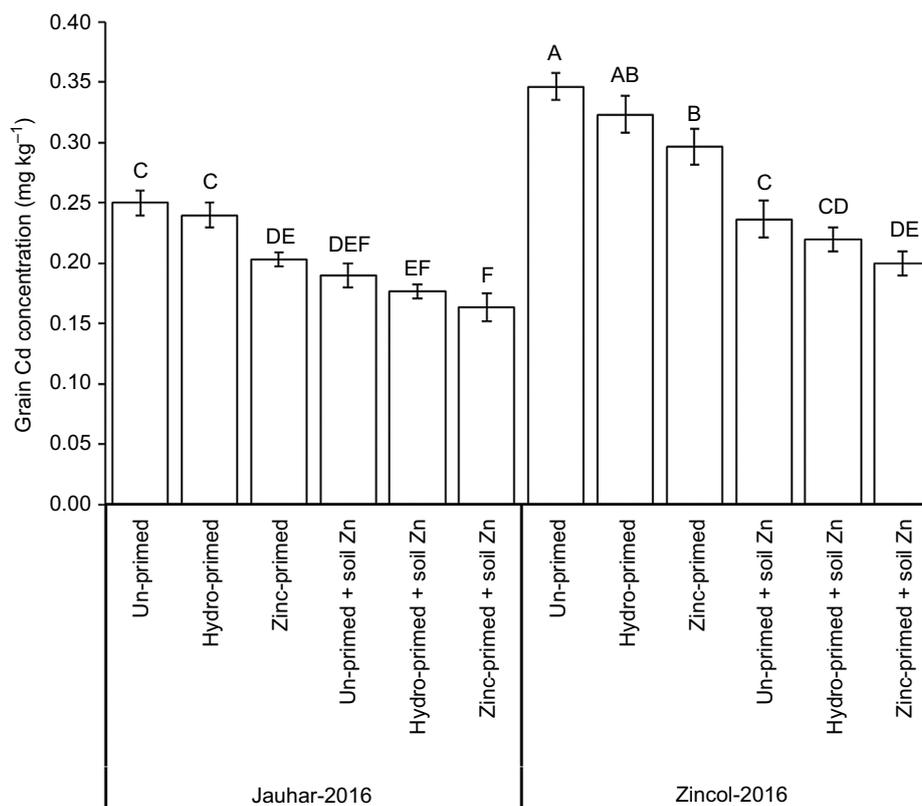
### Photosynthetic parameters and crop yield

Cadmium is a toxic heavy metal that adversely affects the photosynthetic parameters and growth of plants (Hussain *et al.* 2018). Cadmium stress hampers the synthesis of chlorophyll and also affects the functional proteins associated with photosystem-II (Abbas *et al.* 2017). Therefore, it damages photosystem-II and adversely affects the photosynthetic

parameters. Zinc has an ameliorative effect on plant Cd stress (Sperdouli *et al.* 2022). Zinc application restores the photochemical efficiency of photosystem-II and improves the photosynthetic parameters. It was previously reported that, under Cd stress, Zn application significantly increases stomatal conductance and net transpiration rate (Zhou *et al.* 2019). Similarly, in the present study, Zn-priming + soil Zn was effective in decreasing Cd stress by increasing chlorophyll contents (SPAD values), stomatal conductance, photosynthesis rate, and transpiration rate as compared to control (Table 2). However, the increase in photosynthesis rate was only marginal as compared to the control.

Photosynthetic parameters are closely associated with crop yield and its components (Yang *et al.* 2022). In this study, all the treatments with soil Zn increased the shoot dry matter at booting, and stem, chaff, and grain dry matter at maturity (Table 3). In addition, 1000-grain weight was also increased by soil Zn, which agrees with Hussain *et al.* (2019).

The difference in photosynthetic rate and yield between Jauhar-2016 and Zincol-2016 might be because of the genetic differences between these cultivars (Table 2). Jauhar-2016 was specifically developed for high grain yield (Ali *et al.* 2019) and it had, as compared to Zincol-2016, higher



**Fig. 1.** Grain Cd concentration in standard (Jauhar-2016) and Zn-biofortified (Zincol-2016) wheat as affected by Zn treatments. Error bars represent  $\pm$  standard deviations ( $n = 3$ ). The letters above bars indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

**Table 7.** Cadmium contents in two wheat cultivars grown in Cd-spiked soil and supplied with Zn treatments.

Main effect	Levels	Shoot Cd contents at booting ( $\mu\text{g pot}^{-1}$ )	Shoot Cd contents at maturity ( $\mu\text{g pot}^{-1}$ )	Plant Cd partitioning at maturity (%)			
				Stem	Leaf	Chaff	Grain
Cultivars	Jauhar-2016	12.6 $\pm$ 0.7B	14.6 $\pm$ 0.7B	42 $\pm$ 2A	28 $\pm$ 2A	13 $\pm$ 1A	17 $\pm$ 2B
	Zincol-2016	14.7 $\pm$ 0.8A	16.0 $\pm$ 0.6A	43 $\pm$ 2A	25 $\pm$ 2B	13 $\pm$ 1A	19 $\pm$ 2A
Zinc treatments	Control (un-primed)	14.2 $\pm$ 1.5A	15.2 $\pm$ 1.4A	41 $\pm$ 1A	25 $\pm$ 3A	14 $\pm$ 1A	20 $\pm$ 2A
	Hydro-primed	14.3 $\pm$ 1.2A	15.4 $\pm$ 1.4AB	41 $\pm$ 2A	25 $\pm$ 2A	14 $\pm$ 1A	20 $\pm$ 1A
	Zinc-primed	13.7 $\pm$ 1.2AB	14.8 $\pm$ 1.2B	41 $\pm$ 3A	26 $\pm$ 2A	14 $\pm$ 1A	19 $\pm$ 2A
	Soil Zn (un-primed)	13.2 $\pm$ 1.2AB	15.5 $\pm$ 1.1C	43 $\pm$ 2A	27 $\pm$ 3A	13 $\pm$ 1A	17 $\pm$ 1B
	Hydro-primed + soil Zn	13.3 $\pm$ 1.3AB	15.6 $\pm$ 1.1C	43 $\pm$ 2A	28 $\pm$ 2A	13 $\pm$ 1A	16 $\pm$ 1B
	Zinc-primed + soil Zn	13.0 $\pm$ 1.4B	15.2 $\pm$ 0.7C	44 $\pm$ 2A	28 $\pm$ 3A	13 $\pm$ 1A	15 $\pm$ 1B

Separately for each main effect, different letters after means  $\pm$  s.d. of each parameter indicate significant differences based on Tukey's HSD test ( $P \leq 0.05$ ).

stomatal conductance, photosynthetic rate, transpiration rate, shoot dry matter and 1000-grain weight (Tables 2, 3). On the other hand, Zincol-2016 is Zn-biofortified wheat that was developed for high Zn accumulation in grains (Hussain *et al.* 2019; Lowe *et al.* 2022). In the present study, Zincol-2016 had a 19% higher grain Zn concentration than Jauhar-2016 (Table 4).

### Zinc and cadmium accumulation

Zinc biofortification is an effective approach to fulfil the Zn requirements of the increasing population (Prahara *et al.* 2021). Both genetic and agronomic Zn biofortification are useful approaches to combat Zn hunger (Ishfaq *et al.* 2021). For agronomic Zn biofortification, researchers have suggested soil Zn application (Hussain *et al.* 2012), foliar Zn spray (Ishfaq

*et al.* 2022) and seed Zn-priming (Hassan *et al.* 2019). In our study, averaged across cultivars, Zn-priming and soil Zn increased grain Zn concentration at maturity as compared to control (Table 5). Rizwan *et al.* (2019) and Hussain *et al.* (2018) recommended the application of Zn oxide nanoparticles to increase shoot Zn concentration. Tavares *et al.* (2015) reported that Zn addition increased stem, husk, and leaf Zn concentration under Cd stress. In our study, on average, Zn-priming and the treatments with soil Zn application were effective in increasing grain Zn concentration over control (Table 4). This might be because the Zn-priming provides Zn and improves crop growth only during the initial stages of a crop, whereas soil Zn application may provide a continuous supply of Zn throughout the crop lifecycle. In our study, post-harvest soil analysis clearly showed higher Zn availability in soil applied with soil Zn (Table S1).

Cadmium, a toxic metal, has a chemical resemblance with the essential micronutrient Zn. It is well known that Zn application decreases the harmful effects of Cd on plants grown on Cd-contaminated soil (Wang *et al.* 2018; Hussain *et al.* 2019; Khan *et al.* 2019; Ali *et al.* 2022). In plants, both Zn and Cd are transported through the same carriers (*ZRT1*, *YSL*, and *ZI* proteins) (Zheng *et al.* 2012, 2018), therefore, Zn application in Cd-contaminated soil decreased Cd uptake from soil to root (Hart *et al.* 2002). Wu *et al.* (2020) found Zn application decreased stem, leaf and chaff Cd concentration in wheat grown in Cd-stressed conditions. In our study, both Zn-priming and soil Zn significantly decreased grain Cd concentration, with more decrease with the combined Zn-priming + soil Zn (Table 6). Additionally, all the Zn treatments significantly decreased stem, leaf, and chaff Cd concentration as compared to control.

Various studies compared Zn application methods like soil Zn application (Abbas *et al.* 2017; Forster *et al.* 2018), foliar Zn application (Hussain *et al.* 2019; Wu *et al.* 2020), and seed Zn-priming (Rizwan *et al.* 2019; Ali *et al.* 2022) for increasing grain Zn concentration under Cd stress. Soil factors such as pH, organic matter, cation exchange capacity, clay contents and availability of other competitive elements affect the phytoavailable fractions of Zn and Cd (Antoniadis *et al.* 2017). These factors also affect the effectiveness of various Zn application methods. In alkaline soils, phytoavailable Zn and Cd are much lower than in low pH soils. In such conditions, soil Zn application significantly increases shoot Zn uptake and decreases Cd uptake. However, a major portion of the added Zn may be specifically adsorbed in alkaline soils (Riaz *et al.* 2020). Higher seed Zn contents in Zn-primed seeds may partly fulfil Zn requirements during seed germination. These Zn enriched seeds, therefore, accelerate the Zn transporter family (*ZIP*) that restricts the Cd uptake within the plants (Kiran *et al.* 2020). Soil Zn application may increase the plant-available Zn in soil that in combination with seed Zn-priming may decrease the Cd uptake in alkaline soils. Therefore, in our study seed

Zn-priming + soil Zn resulted in the highest decrease in the grain Cd concentration in both cultivars.

In Jauhar-2016, grain Cd concentration was above the permissible limit ( $0.2 \text{ mg kg}^{-1}$ ) in only control and hydro-priming (Fig. 1). On the other hand, in Zincol-2016, grain Cd concentration was above the permissible limit in all treatments except with Zn-priming + soil Zn. Zincol-2016 is Zn-biofortified wheat (PARC 2017) and it was specifically bred for high grain Zn concentration. Therefore, it has a higher ability to uptake Zn from the soil. High Cd concentration in Zincol-2016 might be because of the enhanced expression of specific genes controlling the similar transporters of Zn and Cd (*ZIP*, *YSL*, and *ABC transporters*) (Song *et al.* 2017; Umar and Hussain 2020). Therefore, Zincol-2016 and other high Zn cultivars may accumulate above-permissible levels of Cd in grains when grown on Cd-contaminated soils.

Qaswar *et al.* (2017) also found that Zn application significantly decreases grain Cd concentration in Zincol-2016. However, they reported a relatively higher decrease in grain Cd concentration in Zincol-2016 as compared to the tested standard wheat cultivar. This might be because of genetic differences in the standard wheat cultivars used in the previous and present studies. Moreover, various soil factors may also have contributed to this response, including the differences in the types and levels of soil contaminants. In this study, uncontaminated soil was artificially contaminated with a low level of Cd ( $8 \text{ mg Cd kg}^{-1}$ ). In contrast, Qaswar *et al.* (2017) used naturally contaminated soil that had toxic levels of many heavy metals.

The highest contents of plant total Cd are partitioned in roots, followed in order by stems, leaves, grains and chaff (Cai *et al.* 2020). Similar observations were made in the present study (Table 7). However, the cultivars had different pattern of leaf and grain Cd partitioning. Grain Cd partitioning was relatively higher in Zincol-2016 than in Jauhar-2016. This is because relatively more Cd was partitioned in leaves of Jauhar-2016 than Zincol-2016. Averaged across cultivars and as compared to control, grain Cd partitioning was decreased in all the treatments with soil Zn. This can be linked to the effect of Zn on Cd transport through all plant barriers from roots to the developing grains. The effects of Zn on Cd transport in plants are discussed above. Moreover, the findings of this study suggest work at the subcellular level to explain the Zn-mediated changes in Cd translocation and compartmentalisation in cereal crops.

## Conclusions

Soil Zn application and seed Zn-priming, especially if combined, proved effective strategies for increasing grain yield and grain Zn concentration, and for decreasing grain Cd concentration under Cd contamination. Averaged across treatments, Zincol-2016 had higher grain Cd and

Zn concentrations than Jauhar-2016. In Zincol-2016, only Zn-priming + soil Zn was effective in decreasing grain Cd concentration within the permissible levels ( $\sim 0.20$  mg Cd kg<sup>-1</sup>). On the other hand, in Jauhar-2016, all Zn-containing treatments decreased grain Cd concentration below the permissible limit. Therefore, seed Zn-priming and soil Zn application are important Zn management strategies for standard and Zn-biofortified wheat cultivars grown on Cd-contaminated soils.

## Supplementary material

Supplementary material is available [online](#).

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**Data availability.** The data that support this study will be shared upon reasonable request to the corresponding author.

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