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ORIGINAL PAPER

Effects of Applying Different N Sources on Cd Accumulation, Mineral Micronutrients, and Grain Yield of Durum Wheat

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Abstract
This study aimed to investigate the effects of different nitrogen sources on Cd concentration in durum wheat grains in soils with low and high Cd contamination. *Triticum turgidum L*. durum, cv. Balcali-2000 was sown as test plant material in plastic pots containing 3.2 kg of soil. Low (0.5) and high Cd (5.0 mg Cd kg^{-1} soil) were added to the culture media in the form of $3(CdSO_4).8H_2O$. Nitrogen was also added in the form of $Ca(NO3)_2.4\ H_2O$ in low, sufficient, and high concentrations. In addition, a foliar application of 0.5% urea was used as a further nitrogen supply. The results showed that the total nitrogen content and the Cd concentration of the grains increased with increasing nitrogen application. This increase was more pronounced with a combination of soil nitrogen and foliar urea. While the Cd concentration in the grains was 354 µg kg^{-1} at low soil Cd concentration and insufficient nitrogen supply, the Cd concentration in the grains increased by 40% to 498 µg kg^{-1} at low Cd concentration and high nitrogen supply. This increase in Cd concentration in the grains was 32% higher under high nitrogen applications than at high Cd-insufficient conditions. In addition, foliar application of urea to durum wheat leaves at low soil Cd concentrations increased the Cd concentration in the grains from 354 µg kg^{-1} to 484 µg kg^{-1}. This study showed that different treatments and amounts of nitrogen sources can affect the uptake and accumulation of Cd in wheat grains at different Cd levels.

Keywords Durum wheat · Cd accumulation · Nitrogen supply · Foliar · Urea

1 Introduction

Potentially toxic metals (PTMs) pollution of soil is a significant environmental problem that poses a threat to human health and the ecosystem. PTMs are widespread pollutants in the soil environment, including arsenic, chromium, mercury, lead, nickel, iron, copper, zinc, and cadmium (Lai et al. 2013). Some of these PTMs, Fe, Cu, Zn, and Mn, are essential elements that plants require in small amounts for growth and development. These micronutrients are involved in all metabolic and cellular functions, and plants have varying requirements for them. However, in high concentrators, these elements can become toxic to plants (Hänsch and Mendel 2009; Kopittke et al. 2017). Unlike these elements, cadmium (Cd) is a non-essential, highly toxic environmental pollutant that is widely distributed in soil, water, and the atmosphere. Because of its high toxicity and mobility, Cd is an important industrial pollutant that is phytotoxic even at low concentrations (Chakravarty and Srivastava 1992; Das et al. 1997), and it is most active in soil so that it can be taken up and accumulated by plants. Therefore, it noticeably affects plant growth and development. Cd enters the soil through geogenic and anthropogenic processes (Boussen et al. 2013). PTM contamination of soils is mostly associated with human activities, such as industrial activities, mining, agricultural practices (Zhou et al. 2017), and sewage sludge contamination. The safety limit for total Cd concentrations in cultivated areas has been reported to be 3 mg kg^{-1} soil, while this limit for non-cultivated areas is about 0.1 mg kg^{-1} soil (Alloway 1990). In comparison, Cd concentrations in agricultural soils have been reported to range from 0.01 to 0.7 mg kg^{-1} (Szolnoki et al. 2013). However, Cd concentrations in plants vary greatly among plant species and varieties within cereals. Wheat generally accumulates more Cd than other cereals regularly, and it is in the following order: rye < barley < oats < wheat (Jansson

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2004). Cd enters wheat plants mainly through the roots, and Cd uptake by wheat varies depending on soil type, interactions with other elements, and wheat variety (Gao et al. 2022; Liu et al. 2015). Therefore, Cd accumulation in wheat can cause serious growth and development problems and affect grain yield and quality (Aydemir et al. 2022). Durum wheat, compared to bread wheat, tends to accumulate more Cd in the grains. Durum wheat is grown worldwide (Oleson 1994) for its high protein and fiber content, low glycemic index, and high vitamin content (Olmos et al. 2003), and is also widely used in food production. According to the Food and Agriculture Organization of the United Nations (FAO), durum wheat is the second most widely grown wheat variety in the world after common wheat. Therefore, on account of the accumulation of Cd in wheat is a growing concern because of the potential health risks to people who consume wheat products. Cd accumulation in wheat is influenced by a variety of factors, including genetic ability, soil properties, and environmental conditions. Several studies have shown that soil N fertilization has a significant impact on Cd availability and accumulation in wheat (Ishikawa et al. 2015; Li et al. 2013). It is reported that nitrogen application increased Cd concentration in grains and decreased Zn concentration in grains, and there were significant annual variations in Cd accumulation in grains (Gao and Grant 2012; Özkutlu and Kara 2018). In addition, a positive relationship between Cd phyto-availability and nitrogen (N) concentration in wheat grains is currently reported in Sweden, Australia, Canada, the United Kingdom, the United States, and New Zealand (Li et al. 2011). Gao (2010) found that Cd concentration in grains increased with the application of $(NH_4)_2SO_4$ and urea fertilizer in both field and pot experiments (Gao et al. 2010). Manure management is widely considered to be the most economical and time-saving method of reducing Cd accumulation in crops, resulting in less interference with annual crop production (Li et al. 2011; Yang et al. 2016a, b; Aydemir et al. 2023). N is the most important macronutrient and energy substrate element and plays an important role in plant growth, biomass and yield formation, especially in photosynthesis, nutrient partitioning, and, above all, in the development of metabolic processes (Makino 2011). The research results showed that the type of nitrogen fertilizer, soil type, tillage methods, and application methods affect the Cd concentration in wheat grains. Therefore, the selection of an appropriate nitrogen source, application timing, and placement is very important in increasing or minimizing the amount of Cd in wheat grain. In this study, an attempt was made to investigate the effects of soil and foliar N applications on Cd concentration in durum wheat grains. The results are informative for evaluating Cd-N interactions and N management strategies to determine the risks to food security without compromising soil sustainability.

2 Materials and methods

2.1 Greenhouse Experiment

The plants were grown at the experimental greenhouse condition of Cukurova University. The characteristics of the soil used in the experiment: soil having a clay loam texture and low organic matter content (1.5%). In addition, the pH of the soil are (7.7), organic matter content (0.7%) and calcareous (14% $CaCO_3$). The extractable P concentration of the soil was 2.28 mg kg^{-1} (Oleson et al. 1994). The soil of the DTPA-extractable Cd and Zn were 0.005 and 0.1 mg kg^{-1} determined by using the methods given in and Lindsay and Norvell (1978), respectively. As experimental plant materials, durum wheat (*Triticum turgidum* L. durum, cv. Balcali-2000) were sown in plastic pots containing 3.2 kg soil and grown under greenhouse conditions. The greenhouse condition was set to 22 $^0C \pm 1$ with an air conditioner, lighting 14 h day and 10 h night. Humidity was 60%. The experimental design was carried out in a completely randomized block with factorial combination treatment (soil and foliar doses) with four replicates in a total of 48 plastic pots.

Basic fertilizers added were phosphorus (P) 100 mg P kg^{-1}, potassium (K) 125 mg K kg^{-1} soil in the form of KH_2PO_4, and as the soil used for the experiment Zn deficient (Kaur 2021), zinc (Zn) 1.0 mg Zn kg^{-1} in the form of $ZnSO_4.7H_2O$. Durum wheat was grown in low and high Cd (0.5 and 5.0 mg Cd kg^{-1} soil) in the form of $3(CdSO_4).8H_2O$ and as N rates N150 (inadequate), N300 (adequate), and N600 (high) mg N kg^{-1} in the form of $Ca(NO_3)_2.4\ H_2O$. All these nutrients and basic fertilizers were homogeneously mixed with 3.2 kg of soil prior to putting the mixture into plastic pots. Ten durum wheat seeds (Balcalı-2000 genotype) were put in at a depth of 1.5–2.0 cm in each pot, and the number of plants was thinned to four per pot 7 days after seedlings emerged. The pots were watered daily with deionized water to maintain the water level at 60–80% of soil water holding capacity. In addition, foliar applications of 0.5% urea (w/v) fertilizer with 0.03% Tween-20 (w/w) were applied 6 times with 6 days intervals during tillering, stem elongation, boot, and heading/flowering stages of wheat. The first treatment of foliar urea fertilizer occurred during tillering (4 leaves, 29 days old), and the last application occurred during flowering (65 days old) stages. The plants were harvested at the grain maturity period (Day 156). After harvesting, the grains in spikes were separated from the husks and weighed to evaluate the grain yield for each treatment.

2.2 Analysis of Plant Nutrient Concentrations

After the determination of grain yield, grain samples were ground, and ground samples were acid-digested (0.2 g

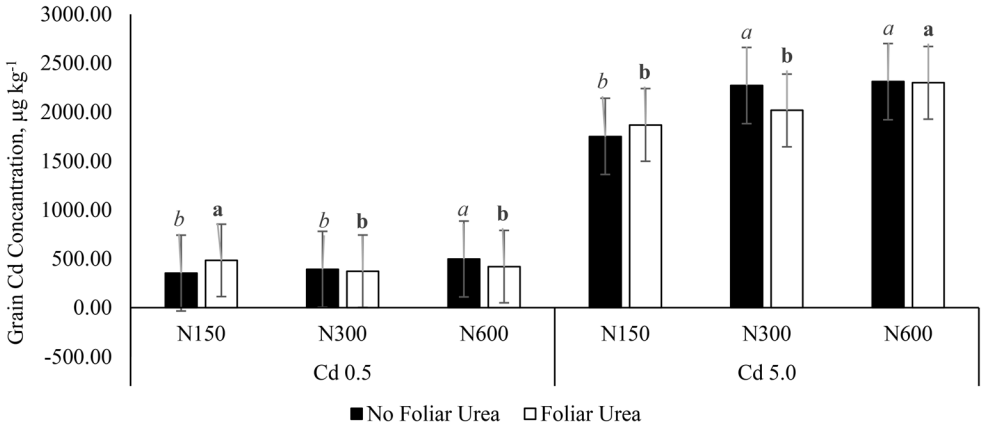


Fig. 1 Effect of foliar application of 0.5% urea and soil N supply on grain Cd concentration in durum wheat (*Triticum turgidum* L.*)* grown on soils treated with low Cd (mg 0.5 kg^{-1}) and high Cd (mg 5 kg^{-1}). Data followed by a different letter were significantly different according to Duncan's Multiple Range Test. ($p < 0.05$)

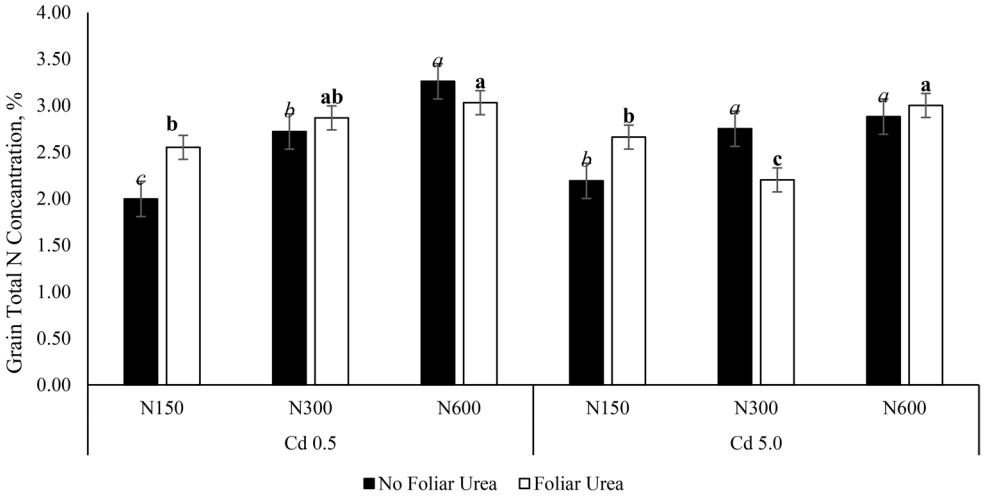


Fig. 2 Effect of foliar application of 0.5% urea and soil N supply on grain concentration of N durum wheat (*Triticum turgidum* L.*)* on soils treated with low Cd (mg 0.5 kg^{-1}) and high Cd (mg 5 kg^{-1}). Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.($p < 0.05$)

sample in 2 ml 30% H_2O_2 and 5 ml 65% HNO_3) in a closed vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). The mineral micronutrients (Fe, Cu, Mn, and Zn) and Cd were determined by ICP-AES (inductively coupled plasma-atomic emission spectrometry, Jobin Yvon-Paris). Grain N% concentrations were determined by using the Kjeldahl device (Bremner 1965). The National Institute of Standards and Technology (Gaithersburg, MD, USA) certified standard reference materials were used to verify the accuracy of element analyses.

2.3 Statistical Analyses

One-way ANOVA analyzed the data with SPSS software (version 22.0). The Duncan test was used to determine meaningful differences between the means ($p < 0.05$).

3 Results

Grain Zn, Fe, Mn, Cu, and Cd and N% concentration of Durum wheat grown on soil contaminated with 0.5 mg kg^{-1} and 5 mg kg^{-1} Cd showed statistically significant differences between soil N and (foliar urea + soil N) treatments ($p < 0.05$) (Figs. 1, 2, 3 and 4). Cd concentration in grain of Durum wheat grown on soil contaminated with 0.5 mg kg^{-1} was highest with 498.00 ± 37.00 µg kg^{-1} in soil treatment (N600) and 484.00 ± 37.00 µg kg^{-1}in treatment (foliar urea + N150). The lowest Cd concentration in grain of Durum wheat grown on soil contaminated with 0.5 mg kg^{-1} was 354.00 ± 18.00 µg kg^{-1} in soil treatment (N150) and 373.00 ± 29.00 µg kg^{-1} in treatment (foliar urea + N300). In soils contaminated with 5 mg kg^{-1} Cd, the highest Cd concentration was obtained in durum wheat grains with 2312.00 ± 64.55 µg kg^{-1} in soil treatment (N600) and 2300.00 ± 132.00 µg kg^{-1} in treatment (foliar urea + N600). In contrast, the lowest Cd concentration was obtained with 1752.00 ± 85 µg kg^{-1} in soil treatment (N150) and with 1869.00 ± 32.00 µg kg^{-1} in treatment (foliar urea + N150) (Fig. 1).

The highest N concentration in soil contaminated with 0.5 mg Cd kg^{-1} was obtained with 3.26 ± 0.23 in soil treatment (N600) and 3.03 ± 0.27 in treatment (foliar urea + N600). In contrast, the lowest N% concentrations were obtained at 2.00 ± 0.12 in soil treatment (N150) and 2.55 ± 0.26 in treatment (foliar urea + N150). In soils

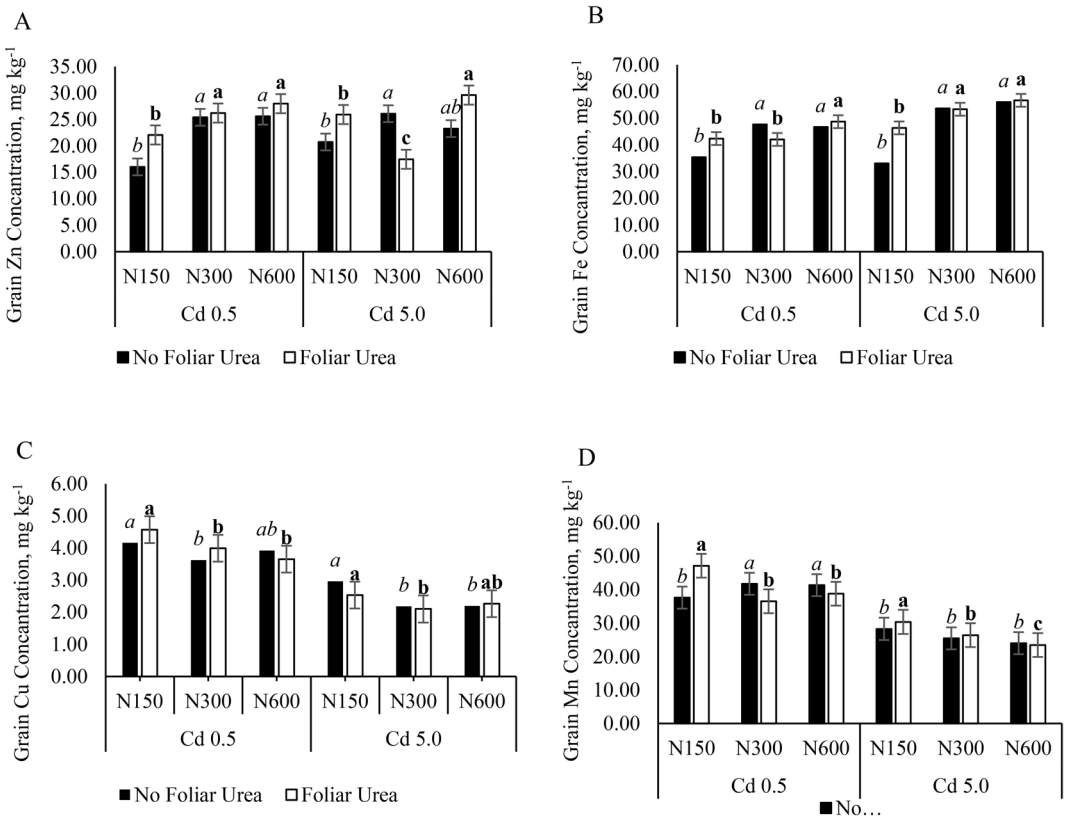


Fig. 3 Effect of foliar application of 0.5% urea and soil N supply on the concentrations of Zn (**A**), Fe (**B**), Cu (**C**), and Mn (**D**) in durum wheat (*Triticum turgidum* L.*)* grown on low Cd (mg 0.5 kg^{-1}) and high Cd (mg 5 kg^{-1}) treated soils. Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.($p < 0.05$)

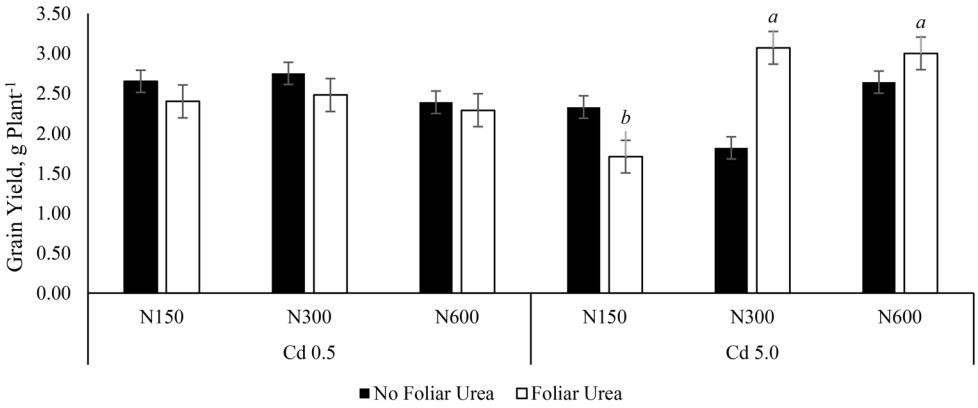


Fig. 4 Effect of foliar application of 0.5% urea and soil N supply on grain yield of durum wheat (*Triticum turgidum* L.) on soils treated with low Cd (mg 0.5 kg^{-1}) and high Cd (mg 5 kg^{-1}). Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.($p < 0.05$)

contaminated with 5 mg kg^{-1} Cd, the highest N concentration was obtained with 2.88 ± 0.20 in soil treatment (N600) and 3.00 ± 0.18 in treatment (foliar urea + N600), the lowest N% concentration was 2.19 ± 0.25 in soil treatment (N150) and 2.20 ± 0.09 in treatment (foliar urea + N300)(Fig. 2).

The highest Zn, Fe, Cu, and Mn concentrations in the soil contaminated with 0.5 mg Cd kg^{-1} were found to be 25.60 ± 2.62 (N600), 47.63 ± 1.53 (N300), 4.16 ± 0.32 (N150), 41.71 ± 2.11 (N300) in soil treatment as (mg kg^{-1}), and 27.98 ± 2.03, 48.67 ± 0.58 (foliar urea + N600), 4.57 ± 0.30, 47.10 ± 2.57 (foliar urea + N300) in treatments as (mg kg^{-1}), respectively. In contrast, the lowest Zn, Fe, Cu and Mn concentrations were 16.00 ± 1.04, 35.33 ± 1.53 (mg kg^{-1}) in the soil treatment (N150) and 22.05 ± 1.11 (mg kg^{-1}) in the treatment (foliar urea + N150), 42.00 ± 1.53 (foliar urea + N300), 3.65 ± 0.10 mg kg^{-1} (foliar urea + N600), 36.47 ± 1.12 (foliar urea + N300), respectively. The highest Zn, Fe, Cu and Mn concentrations were found in soils contaminated with 5 mg kg^{-1} Cd, 26.08 ± 2.74 (N300), 56.00 ± 1.00 mg kg^{-1} (N600), 2.96 ± 0.08 and 28.27 ± 1.31 (N150) in soil treatment and 29.60 ± 0.52, 56.67 ± 1.53, 2.26 ± 0.14 mg kg^{-1} (foliar urea + N600) and 30.35 ± 0.71

(foliar urea + N600). The lowest concentrations of Zn, Fe, Cu, and Mn were 20.71 ± 2.04, 33.00 ± 1.00 in soil treatment (N150), 2.18 ± 0.03 (N300), 24.03 ± 0.45 (N600) and 17.44 ± 0.22 (mg kg^{-1}) in treatment (foliar urea + N300), 46.33 ± 2.09, 2.10 ± 0.08, 23.47 ± 0.81 (foliar urea + N150), respectively (Fig. 3).

Grain yield of Durum wheat grown on soils contaminated with 0.5 mg kg^{-1} and 5 mg kg^{-1} Cd showed no statistically significant differences between soil-N and (foliar urea + soil-N) treatments, except for (foliar urea + soil-N) treatments on soils contaminated with 5 mg kg^{-1} Cd ($p < 0.05$) (Fig. 4). The highest grain yield of Durum wheat grown on soil contaminated with 5 mg kg^{-1} Cd was 3.07 ± 0.35 and 3.00 ± 0.72 g per $plant^{-1}$ in the (foliar urea + N300) and (foliar urea + N600) treatments, respectively. However, there were no statistically significant differences in grain yield between the two treatments.

4 Discussions

In addition, many studies report that uptake, translocation, and accumulation of Cd in plants is strongly regulated by N (Wångstrand et al. 2007; Gao et al. 2016a; Yang et al. 2016b). The results of the study showed that Zn concentration in durum wheat grains in soils contaminated with low doses of Cd increased with increasing soil N dose, while the increase in Zn concentration was even greater with additional foliar application of urea. Zn concentration in Durum wheat grains grown on soil contaminated with 0.5 mg kg^{-1} Cd increased by 60% with the N600 application compared to the N150 application. This increase was 74.88% for the foliar application of urea + N600 compared to the N150 application. Zn is a cation that interacts with almost all plant nutrients in the soil, especially with anions. In addition, Zn has a positive interaction with N in the grains (Lakshmanan et al. 2005; Rehman et al. 2018). In the current study results, it is assumed that increasing N supply increases the Zn concentration in the grains, probably by affecting the abundance of Zn transporters and the content of Zn-chelating nitrogen compounds. Moreover, increased N application to wheat increased the uptake of Zn by the roots and the translocation rate of the shoot by 300% (Erenoglu et al. 2011). It has reported that increasing the amount of N applied also increases the Zn concentration in the wheat grain (Kutman et al. 2011). The lowest Cu concentration in Durum wheat grains grown on soils contaminated with 5 mg kg^{-1} Cd occurred in (foliar urea + N300) treatment, while the highest Cu concentration in Durum wheat grains grown on soils contaminated with 0.5 mg kg^{-1} Cd occurred in (foliar urea + N150) treatment. The reduction in Cu concentration was 117.62% compared to the (foliar urea + N150) and (foliar urea + N300) treatments. At high Cd application, the Cu concentration in the grain decreased. However, this led to an increase in Cu concentration at low Cd content in the soil. Metals in the plant phloem are generally transported in bound form and not as free ions (Clemens 2006; Hazama et al. 2015; Li et al. 2020; Stephan and Scholz 1993). This shows that there is competition between Cd and Cu for binding compounds. Increased N supply in the presence of high cadmium can promote Cd absorption and mobilization of Cd by affecting the content of Cd-chelating nitrogen compounds. It can therefore be assumed that Cu cannot compete with Cd. Some studies report that cadmium accumulation in wheat shoots depends on Cd translocation from root to shoot, while accumulation in grains depends on Cd transfer from root to shoot and the direct pathway of Cd transport from root to grain via transfer from xylem to phloem in the root (Ganeshan et al. 2012; Harris and Taylor 2004, 2013;). Mn concentration in durum wheat grains in soils contaminated with low doses of Cd decreased with increasing soil N dose, while the increase in Mn concentration was higher with additional treatments (foliar urea + soil N) than with treatments (soil N). However, Mn concentration tended to decrease even more when supplemental treatments (foliar urea + soil N) and (soil N) were reduced, while Mn concentration increased in durum wheat grains in soils contaminated with low doses of Cd in response to increasing soil N treatments. Solutions must be developed to reduce Cd pollution in the food chain, which poses a global threat to sustainable agriculture and human health. Therefore, a thorough understanding of the regulatory mechanisms of N that control Cd uptake, translocation, and accumulation is also needed (Yang et al. 2020). N enhances Cd uptake by regulating NO-derived transporters for divalent cations, and cation metal-bearing transmembrane proteins play an important role in Cd uptake and translocation in plants (Clemens et al. 2013; Yamaji et al. 2013;). It has been reported that iron-regulated transporters such as transgenic plants (IRT1 and IRT2) play an important role in the uptake of Zn, Fe, and Cd (Nakanishi et al. 2006), and overexpression of OsIRT1 results in higher Zn, Fe, and Cd levels (Lee and An 2009). Moreover, Zn, Fe, Mn, and Cd are divalent cations with many similar properties and are taken up by the same channels or transporters or both (Uraguchi and Fujiwara 2013). Thus, N-regulated transporters play an important role in the sorption of Cd from the rhizosphere to the roots and in the transport from roots to shoots. The coexistence of Cd and Fe forces the transport of Cd to the upper parts of rice plants. In addition, the high nutrient demand of seeds results in lower Fe concentration in the plant body and stimulation of the Fe uptake and transport system. As a result, the rice can absorb more Cd (Nakanishi et al. 2006). Cd uptake likely occurs via ZIP transporters such as Zn/Fe-regulated

transporter-like proteins, and increased tissue N concentrations may also increase the amount of ZIP transporters in roots (Erenoglu et al. 2011). N management appears to be a promising agronomic strategy for wheat biofortification with Zn. Adequate N supply has been shown to effectively increase Zn concentration in wheat grain, especially when the Zn available to the plant in the soil is high enough (Cakmak et al. 2010; Kutman et al. 2010). According to the experimental results, a decrease in Mn uptake and Mn transport were observed in the presence of increasing Cd. Similar results were reported by many authors (Cataldo et al. 1981; Jarvis et al. 1976; Zhang et al. 2014). This can be explained by the fact that the uptake or transport of Mn into shoots is not favoured in the presence of Cd.

The experimental results showed that the grain yield of durum wheat was not affected by Cd uptake, and the amount of Cd had no toxic effects. On the contrary, the highest increase in grain yield was obtained at the highest Cd concentration in the grain. This increase was obtained when (foliar urea + N300) and (foliar urea + N600) were applied to Durum wheat grown on soils with the highest Cd contamination of 5 mg kg^{-1}. The lowest grain yield was observed when (foliar urea + N150) was applied. Comparing the applications of (foliar urea + N300) and (foliar urea + N600) with the application of (foliar urea + N150), the grain yield of Durum wheat increased by 79.53%. Moreover, there was no positive correlation between percent N concentration in grain of durum wheat, Cd concentration and grain yield. Increasing N fertilization increases Cd uptake and accumulation in plants grown under field conditions where Cd is freely available. Numerous studies reported that N fertilizer, regardless of its form, has a positive relationship with Cd accumulation in plants such as durum wheat (Li et al. 2011; Wångstrand et al. 2007; Gray et al. 2002; Mitchell et al. 2000). However, it can be explained that N fertilizer has a mechanism that causes detoxification effects in plants by improving the nutritional status of plants, providing product growth, and increasing ion exchange reactions in the soil. Thus, it can be said that N protects plants from Cd toxicity, so that even if Cd accumulation is increased, there is no loss of yield.

5 Conclusion

This study presents innovative findings on the relationship between different nitrogen (N) applications and cadmium (Cd) uptake in wheat grains. Most of the studies conducted so far have focused on Cd uptake and the effects of soil N application on wheat yields. However, there are virtually no studies on the effects of foliar N application on Cd uptake and yield, and all studies have focused on foliar application of microelements. It is therefore expected that this study will lead to future research on the uptake of potentially toxic metals in plants through foliar application of different nitrogen sources.

The research results suggest that nitrogen application can affect the uptake and accumulation of Cd in wheat grains, although the direction and magnitude of this effect may depend on the method of nitrogen application, the amount of nitrogen applied, and the Cd contamination of the soil. In addition, future research could focus on investigating the molecular and physiological mechanisms underlying the interaction between N and Cd in wheat grains and the effects of different N sources and applied amounts on Cd accumulation.

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