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Nanoparticles enhanced morpho-physiological growth of wheat in contaminated soil by restricting cadmium mobility and accumulation

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Nanotechnology has obtained importance over conventional technologies to enhance crop growth, yield and environmental sustainability. Cadmium (Cd) is one of the most ecotoxic heavy metals that directly enter the food chain and accumulate in different human body organs such as kidneys, lungs, and liver. Cadmium (Cd) contamination in agricultural soils has now become a big issue, which affects food quality and causes a serious threat to human health and the environment. Nanoparticles (NPs) are effectively used to remediate Cd-contaminated soils, due to having a large surface area and high adsorption capacity. The present study evaluated the effects of three NPs i.e., iron (Fe), silicon (Si) and zinc (Zn) NPs on the growth, and physiology of plants as well as the accumulation of Cd in different parts of plants. The collected soil was spiked with different Cd levels (0, 5, 10, 20 mg kg^{-1}), and NPs were applied at 60 mg kg^{-1} of soil prior to sowing. At maturity, plants exhibit significant increases in shoot length by 14, 12, 10% and 48, 30, 29% in root length in treatments amended with Fe, Si and Zn-NPs at 5 mg kg^{-1} Cd contamination levels. The grain yield was enhanced by 78% at 5mg kg^{-1} Cd level amended with Fe-NPs as compared to the control (no NPs application). The results revealed that NPs have a beneficial effect on stomatal conductance, transpiration rate, and photosynthetic rate compared to control. The Cd concentration in various plant parts viz. roots, shoot and grains decreased by 36%, 35% and 69% after the application of NPs. However, this reduction was more distinct at 5 mg kg^{-1} Cd with Fe-NPs followed by Zn-NPS and Si-NPs. Overall, it was concluded that NPs play a significant role in the enhancement of plant biomass, increase in nutrient availability, and decrease the Cd accumulation in different plant parts.
Keywords: Soil contamination, crop production, nanomaterials, metal toxicity.

INTRODUCTION

Cadmium (Cd) is considered one of the most toxic heavy metals with a high dispersion rate and persistence in the environment. It is classified as a class-I human carcinogen that poses a significant risk to human health by causing renal failure, reproductive effects, and cancers (Shahriar *et al.*,2020). Several anthropogenic activities, including smelting, mining, application of phosphatic fertilizer and untreated industrial wastewater are main sources of Cd and lead to soil contamination and crop accumulation (Mu *et al.*, 2019). The main site for Cd entry into the plants is plant roots, and a Cd-induced decrease in root length ultimately hinders plant growth and yield. The Cd accumulation in the plants negatively affects photosynthesis which in turn gives lower plant growth and yield and causes leaf chlorosis (Hussain *et al.*, 2019). Various studies have reported that Cd is one of the reasons why food production is significantly affected by Cd toxicity (Wang *et al*., 2020). Murtaza *et al.* (2017) and Khan *et al.* (2020) have reported that Cd toxicity reduces the uptake of essential nutrients like iron (Fe), manganese (Mn), and zinc (Zn), decline the antioxidant enzyme activities and induces structural changes. It imposes damage to membranes, nucleic acid, proteins and impedes the photosynthetic functions that lead to reduced plant growth and yield (Emamverdian *et al.*, 2020).

Nanotechnology is an emerging tool utilized in various fields of science, i.e., physics, chemistry, and biological sciences. The promising results reported in these fields have compelled scientists to use this technology in environmental remediation and enhancing agricultural productivity. Nanomaterials or specifically nanoparticles (NPs) play a significant role in the remediation of metals contaminated soils, plant protection and management of farms by providing nutrition (Duhan *et al.*, 2017). Recently, NPs have attained more attention for the remediation of contaminated soil and water (Rizwan *et al.*,

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2019a). The NPs are different from other materials because of their distinctive characteristics like small size, high surface area and large sorption capacity (Hussain *et al.*, 2019; Sohail *et al.*, 2021). Various studies have reported that the use of NPs is suitable to remediate various kinds of contaminants like Cd, chromium (Cr) and lead (Pb) (Zhu *et al.*, 2019; Zhang *et al.*, 2018). However, the remediation efficacy of NPs and their effects on the growth and metabolic functions vary from plant to plant. The NPs can improve modern agriculture by remediating metal contaminated soils (Mahakham *et al.*, 2017). Previously, several studies have been conducted on the use of NPs to alleviate heavy metal stress in hydroponics and agar media (Venkatachalam *et al.*, 2017; Konate *et al.*, 2017). Micronutrients or essential heavy metals-based NPs are used as nano-fertilizer due to their slow release and small sizes they provide nutrients to plants and help in metals immobilization (Farooqi *et al.*, 2021). Now, the attention is changed towards the utilization of metal nanoparticles, particularly zinc oxide nanoparticles (ZnO-NPs) and iron oxide nanoparticles (FeO-NPs) used in agriculture for the remediation of metal-contaminated soils (Rizwan *et al.*, 2019a; Munir *et al.*, 2018). Zinc oxide NPs give 80% Zn to plants as Zn is an essential nutrient for plants that's why ZnO-NPs are considered a rich source of Zn nutrition and also have a higher adsorption capacity to immobilize the heavy metal (Gui *et al.*, 2015; Rossi et al. 2018). According to US Food and Drug Administration, ZnO-NPs have been identified as a secure food supplement (Sharifan *et al.*, 2020). The application of nano zero-valent iron (nZV-Fe) to contaminated soils reduces the bioavailability of metals and improves plant growth (Gil Diaz *et al.*, 2017). Similarly, ZnO NPs combined with MgO or FeO-NPs application has beneficial effects on wheat growth and leads to an increase in grain yield (Lian *et al.*, 2022). Silicon NPs (Si-NPs) also have the same beneficial effects as they increase plant vigor and photosynthesis under stressed environmental conditions (Cui *et al.*, 2017; Mousavi *et al.*, 2018). Kim *et al*. (2017) found that Si application stimulates the antioxidant activities in plants by the alleviation of reactive oxygen species (ROS) induced oxidative stress.

Wheat is a major cereal crop and is extensively used as a staple food around the world. It has higher nutritional value for humans as well as animals owing to its compatibility under distinct environmental conditions (FAO, 2014). Wheat crop can tolerate numerous stress factors/ conditions, i.e., biotic and abiotic factors such as drought, salinity, metals toxicity and temperature. As wheat crop can tolerate metal contamination, they may accumulate a large amount of Cd in edible parts of the plant, particularly in grains (Zhuang *et al.*, 2021). Therefore, some immobilization/ metal binding methods are required to be explored for decreasing the Cd accumulation in the wheat plant, especially in grains to reduce health impacts on consumers, i.e., humans.

To explore the metals remediation/ immobilization capacities of different metal-based NPs (Fe, Si and Zn) a pot experiment was performed in Cd-contaminated soil. It was hypothesized that the application of different metal-based NPs may reduce the bioavailable/exchangeable Cd concentration in the soil. These NPs were selected since they also provide nutrients to the soil and enhance the nutritional status of the soil. Several studies showed the beneficial effects of NPs by seed priming and foliar application but their direct application on the soil at real conditions is not fully explored. Therefore, this study was performed in the soil to check the efficiency of different NPs for the immobilization of Cd from contaminated soil, and plant accumulation and its effects on the grain yield.

MATERIALS AND METHODS

Soil collection and spiking**:** The soil was collected from the experimental field area, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF), Pakistan (31° 26 18 10 N; 73° 04 09 68 E). Before analysis for soil physicochemical properties, the soil was dried, ground using the wooden tool and sieved through < 2 mm stainless steel sieve. The physiochemical properties were cited in Table 1. The textural class was determined by the hydrometer method (Bouyoucos, 1962) and pH of the saturated paste was measured by pH meter. After that, it was spiked with different Cd levels, i.e., 0, 5, 10 and 20 mg kg^{-1} Cd using $CdSO_4$ as a Cd source dissolved in distilled water (DW). Spiked soil was incubated at field conditions for 60 days with periodic mixing to attain homogeneity and acclimatization.

Table 1. Physico-chemical properties of soil used in the experiment.

Characteristic	Unit	Value
Textural class		Sandy clay loam
Sand	%	48.14
Silt	%	21.92
Clay	%	27.84
SP %	%	31.02
pH	-	7.75
EC	dS m^{-1}	1.96
Soluble ions		
TSS	mmol$_c$ L^{-1}	19.6
CO_3^{2-}	mmol$_c$ L^{-1}	Not detected
HCO_3^-	mmol$_c$ L^{-1}	1.78
Cl^-	mmol$_c$ L^{-1}	5.57
$Ca^{2+} + Mg^{2+}$	mmol$_c$ L^{-1}	12.84
SO_4^{2-}	mmol$_c$ L^{-1}	11.25
Na^+	mmol$_c$ L^{-1}	5.76
SAR	(mmol L^{-1})$^{1/2}$	2.27
CEC	cmol$_c$/kg	3.87
AB-DTPA extractable Cd	mg kg^{-1}	0.037

TSS = Total soluble salts, SAR = Sodium adsorption Ratio, SP % = Saturation percentage

***Experimental plan and setup*:** Before the start of the experiment, 10 kg of Cd-contaminated soil was filled in polyethylene-lined pots. The test crop wheat (Punjab-2008) was sown with eight seeds per pot in triplicates. After the seed's emergence, the plants were thinned to four plants per pot of uniform size with thorough crushing and mixing of the remaining thinned plants in respective pots. A total of 16 treatments with three replicates were set out in factorial design (2-way ANOVA). Spiked soil with four levels of Cd (0, 5, 10, 20 mg kg^{-1}) and amended with NP0 (no NPs) and Fe, Si and Zn-NPs at the rate of 60 mg kg^{-1} for each NPs.

***Crop husbandry*:** The recommended doses of nitrogen (N), phosphorus (P) and potassium (K) were applied at each pot at the rate of 120:90:60 kg ha^{-1} respectively. Urea, diammonium phosphate (DAP) and sulfate of potash (SOP) were used for N, P and K. Nitrogen was applied in three equal splits while P and K were applied in a single split at the time of sowing. Along with fertilizer application, the recommended amount of irrigation water was applied and other crop management practices like weeding, hoeing, etc. were also performed occasionally.

***Plant growth parameters*:** Growth parameters of the wheat crop were recorded at maturity. The plant's shoot length and spike length were recorded using a graduated measuring tape. Pots were saturated with water, then plants were gently removed from the pots. After thorough washing with water and drying with blotting paper, plants were separated into roots, shoots and spikes. Later, the plant parts were placed into the oven at 65 °C temperature for drying till constant weight was attained. After oven drying, the dry weights of these samples were recorded. Then these samples were separately powdered by using a high-performance electric grinder (Wiley mill with stainless steel blades) and saved for further analysis.

***Plant physiological parameters*:** Plant physiological parameters, such as chlorophyll content, transpiration rate, photosynthetic rate and stomatal conductance, were measured 60 days after sowing the crop. Chlorophyll contents were measured using a chlorophyll meter (Minolta SPAD-502 meter) by holding the leaf from the apex at three different positions. The physiological parameters were also recorded in triplicates using Infra-Red Gas Analyzer (LCi T compact photosynthesis system developed by Bioscientific laboratory, UK) in 60 days old seedlings. All the parameters were recorded soon after dawn supposing that plants were fully active at that time in the presence of sunlight.

***AB-DTPA extractable Cd*:** Ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) extractable Cd was determined from the soil extract. For soil extract preparation, 10 g of air-dried, sieved soil samples were added into falcon tubes with 20 mL extraction mixture and shaken on a shaker for 15 minutes at 250 rpm (Heidolph Promax 1020). After shaking, samples were filtered and analyzed for Cd concentrations using AAS (Rizwan *et al.*, 2019a).

***Determination of cadmium concentration in plants*:** The samples of roots, shoot and grain (0.5 g) were digested with di-acid mixture (HNO_3: $HClO_4$) at a 3:1 v/v ratio in conical flasks and stored overnight after sealing with aluminum foil (Khan et al. 2019). After this, samples were block digested using a hot plate until a clear solution was obtained. Distilled water was added to the digest to make their volume up to 50 mL and filtered. Cadmium concentrations were determined using atomic absorption spectrophotometer (AAS) (Hitachi Polarized Zeeman AAS, Z-8200, Japan).

***Statistical analysis*:** The analysis of variance (ANOVA) under factorial design was used to check the significance of the treatments. Tuckey's HSD test at $p \leq 0.05$ was performed using XLSTAT (Version: 2021-1-1).

RESULTS

***Plant growth parameters*:** The results of this study showed that wheat growth was significantly affected by different Cd-contamination levels and the maximum decrease was observed at higher contamination levels. The plant growth was enhanced at the lowest toxicity level after applying different NPs in Cd-contaminated soil (Table 2). The results indicated that shoot length and root length were significantly enhanced by 14, 12, 10% and 48, 30, 29% in treatments amended with Fe, Si and Zn-NPs at 5 mg kg^{-1} Cd contamination levels compared to control (Cd0NPs0). The wheat plant showed minimum shoot and root length at 20 mg kg^{-1} Cd level which were without any application of NPs. The spike length was significantly increased through the addition of different NPs types and decreased in treatments where no NPs were used. Iron NPs significantly enhanced the shoot and root fresh and dry weights over the control and showed the highest weight at 5 mg kg^{-1} Cd level, while the lowest was at 20 mg kg^{-1} Cd level (Table 2). The pronounced reduction in wheat grain yield was due to the Cd stress, as grain yield was decreased with increasing Cd-contamination levels. At 5 mg kg^{-1} Cd-contamination level, a significant increase in grain yield was observed by the application of different NPs in comparison with control. The grain yield was increased by 78, 62, 59% and 62.4, 59.23, 48.0 % and 53.8, 51.9, 46.1% in the treatments amended with Fe, Si and Zn-NPs at 5, 10, 20 mg kg^{-1} Cd contamination levels It was observed that Fe-NPs gave the highest grain yield at all Cd contamination levels showing Fe-NPs have more positive effects than other NPs.

***Plant physiological parameters*:** The chlorophyll contents increased significantly with the application of NPs compared to treatments that were not spiked with NPs (Fig. 1A). Application of Fe-NPs in plants spiked with 5 mg kg^{-1} Cd

Table 2. Effect of different NPs on the plant growth parameters of wheat grown under Cd stressed condition.

		Spike length (cm)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Grain weight (g)
Cd0	NPs0	9.21^{c-g}	52.8^{c}	8.26^{c-e}	21.74^{e-g}	7.11^{gh}	20.55^{e-h}	7.05^{gh}	10.46^{d-f}
	Fe-NPs	9.97^{a-f}	53.7^{a-c}	9.84^{a-e}	23.58^{d-f}	7.35^{fg}	22.44^{d-f}	7.29^{fg}	12.40^{b-d}
	Zn-NPs	9.70^{b-f}	53.3^{bc}	9.26^{b-e}	21.66^{e-g}	7.67^{g}	21.16^{e-g}	7.56^{g}	11.60^{c-e}
	Si-NPs	9.67^{b-f}	53.0^{bc}	9.50^{a-e}	22.17^{e-g}	8.12^{gh}	21.43^{e-g}	8.06^{gh}	12.26^{b-d}
Cd5	NPs0	8.57^{e-g}	52.7^{c}	8.30^{c-e}	20.48^{fg}	5.98^{hi}	19.62^{f-h}	5.93^{hi}	9.03^{e-g}
	Fe-NPs	11.67^{a}	60.0^{a}	12.20^{a}	29.39^{a}	12.30^{a}	28.21^{a}	12.17^{a}	15.53^{a}
	Zn-NPs	11.10^{ab}	57.2^{a-c}	10.67^{a-d}	28.20^{ab}	11.92^{ab}	27.33^{ab}	11.81^{ab}	14.76^{ab}
	Si-NPs	10.63^{a-d}	59.3^{ab}	10.77^{a-c}	27.46^{a-c}	11.23^{a-c}	26.26^{a-c}	11.13^{a-c}	14.63^{ab}
Cd10	NPs0	8.20^{fg}	51.8^{c}	7.83^{de}	19.78^{g}	5.21^{i}	18.88^{gh}	5.08^{i}	8.70^{fg}
	Fe-NPs	10.80^{a-c}	57.5^{a-c}	11.93^{ab}	28.26^{ab}	10.20^{b-d}	27.39^{ab}	10.13^{b-d}	14.33^{ab}
	Zn-Nps	9.33^{b-g}	57.6^{a-c}	10.56^{a-d}	27.32^{a-c}	9.76^{cd}	26.00^{a-c}	9.65^{cd}	13.90^{a-c}
	Si-NPs	10.16^{a-e}	57.1^{a-c}	10.63^{a-d}	26.24^{a-d}	9.23^{d-f}	25.00^{a-d}	9.15^{d-f}	14.00^{a-c}
Cd20	NPs0	7.77^{g}	51.5^{c}	6.97^{e}	18.73^{g}	5.04^{i}	17.53^{h}	4.93^{i}	7.56g
	Fe-NPs	9.93^{a-f}	57.4^{a-c}	11.63^{ab}	26.49^{a-d}	9.52^{de}	25.57^{a-d}	9.4^{de}	13.96^{a-c}
	Zn-NPs	9.06^{c-g}	57.6^{a-c}	9.93^{a-d}	25.79^{b-d}	8.22^{e-g}	24.93^{b-d}	8.12^{e-g}	13.16^{a-c}
	Si-NPs	8.87^{d-g}	57.4^{a-c}	10.40^{a-d}	24.12^{c-e}	9.24^{d-f}	23.34^{c-e}	9.12^{d-f}	12.93^{a-d}

Upper-case letters indicate significant differences present among the treatments at $p \leq 0.05$.

Table 3. Translocation factor of Cd under different NPs treatment at different concentration of Cd.

	NPs	Cd 0	Cd 5	Cd 10	Cd 20
Root to Shoot	N0	0.728±0.122	0.912±0.054	0.759±0.073	0.641±0.039
	Fe	0.609±0.188	0.928±0.011	0.555±0.048	0.478±0.031
	Zn	0.631±0.105	0.940±0.058	0.528±0.023	0.463±0.021
	Si	0.869±0.278	1.071±0.025	0.577±0.034	0.517±0.055
Shoot to Grain	N0	0.096±0.026	0.300±0.070	0.288±0.002	0.206±0.006
	Fe	0.115±0.032	0.144±0.033	0.162±0.034	0.152±0.003
	Zn	0.107±0.046	0.145±0.013	0.178±0.016	0.160±0.006
	Si	0.083±0.014	0.126±0.024	0.160±0.011	0.168±0.012

Bold values represent mean of three replicates ±SE

Table 4. Correlation Matrix of different studied parameters.

	GW	SpL	ShL	RL	SFW	SDW	RFW	RDW	Root Cd	Shoot Cd	Grains Cd
SpL	**0.597**	**1.000**									
ShL	**0.659**	**0.500**	**1.000**								
RL	**0.820**	**0.753**	**0.669**	**1.000**							
SFW	**0.816**	**0.750**	**0.769**	**0.819**	**1.000**						
SDW	**0.830**	**0.760**	**0.770**	**0.834**	**0.995**	**1.000**					
RFW	**0.781**	**0.780**	**0.720**	**0.799**	**0.889**	**0.903**	**1.000**				
RDW	**0.780**	**0.782**	**0.718**	**0.799**	**0.888**	**0.901**	**1.000**	**1.000**			
Root Cd	-0.082	-0.302	0.146	-0.080	0.008	-0.005	-0.089	-0.095	**1.000**		
Shoot Cd	-0.347	-0.499	-0.131	-0.334	-0.282	-0.301	-0.368	-0.374	**0.921**	**1.000**	
Grains Cd	-0.472	**-0.587**	-0.225	-0.412	-0.399	-0.416	-0.487	-0.492	**0.872**	**0.961**	**1.000**
AB-DTPA	-0.451	**-0.640**	-0.235	-0.457	-0.425	-0.441	**-0.520**	**-0.526**	**0.830**	**0.920**	**0.940**

GW (Grain weight), SpL (Spike length), ShL (Shoot length), RL (Root length), SFW (Shoot fresh weight), SDW (Shoot dry weight), RFW (Root fresh weight), RDW (Root dry weight). Bold values show a significant correlation at the *0.05* level.

levels showed maximum chlorophyll contents, but these were decreased by 3 to 8% with the increasing concentrations of Cd-contamination levels from 5 to 20 mg kg^{-1}. The photosynthetic rate was increased by 74, 56, and 70% in the plants treated with Fe, Si and Zn-NPs where Cd was applied at the rate of 5 mg kg^{-1} Cd levels (Fig. 1B).

Similarly, the stomatal conductance increased significantly in different NPs amended treatments compared with their respective controls. Iron NPs improved the stomatal conductance, but it decreased with the increasing Cd concentration as it was in the order of 5 mg kg^{-1} (83%) > 10 mg kg^{-1} (43%) > 20mg kg^{-1} (26%). A similar trend was also observed for Si and Zn-NPs (Fig. 2A). The transpiration rate was significantly higher (63%) in Fe-NP treatment at 5 mg kg^{-1} Cd level compared with control (Fig. 2B). It was also observed that an increase in transpiration rate was recorded in those plants which were treated with different NPs at different levels of Cd.

***Soil AB-DTPA extractable cadmium*:** The results revealed that Cd mobility was decreased by the application of different NPs (Fig. 3). The highest concentration of AB-DTPA extractable Cd was found in the soil contaminated at 20 mg kg^{-1} Cd level with no NPs application. The extractable concentration of Cd was found to be minimum in the soil amended with Fe, Si and Zn-NPs at 5mg kg^{-1} Cd contamination levels. Overall, all the applied NPs have significantly decreased the bioavailability of Cd compared to the soil having their respective Cd-contamination levels but no NPs.

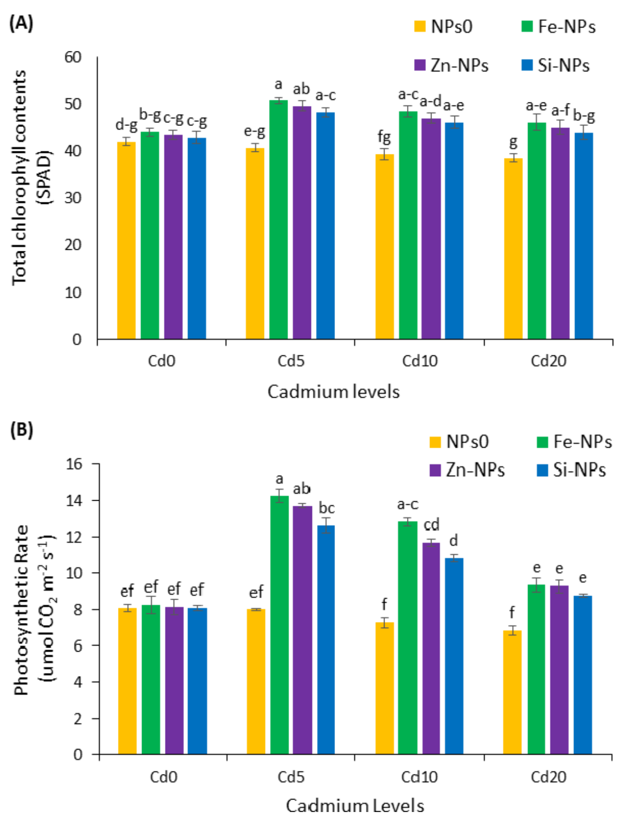

Figure 1. Effect of different NPs on chlorophyll content **(A)** and photosynthetic rate (B) of wheat grown under Cd stressed condition. Different letters on the bars indicate the significant difference present among the treatments according to HSD test at $p \leq 0.05$.

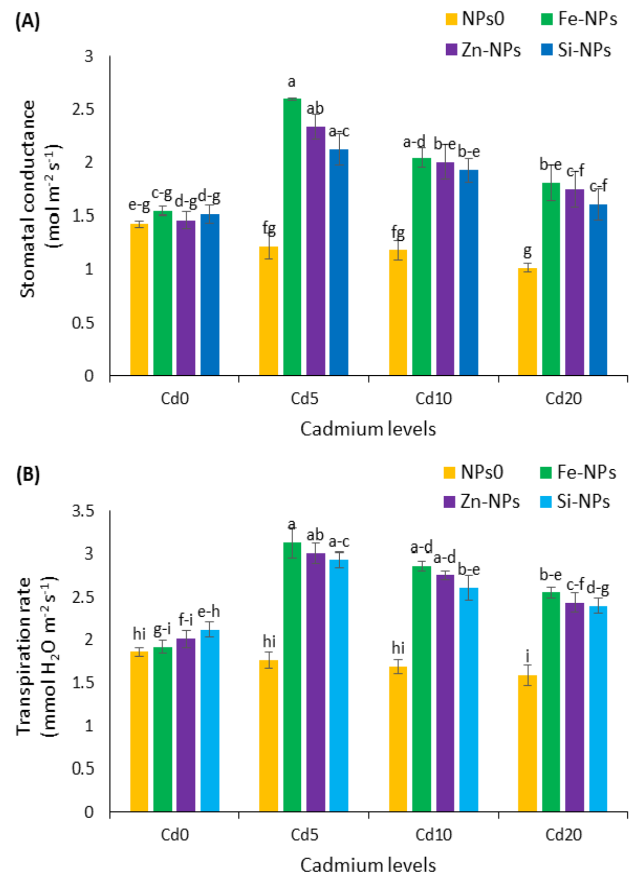

Figure 2. Effect of different NPs on stomatal conductance (A) and transpiration rate (B) of wheat grown under Cd stressed condition. Different letters on the bars indicate the significant difference present among the treatments according to the HSD test at $p \leq 0.05$.

***Cadmium concentration in wheat and its translocation to other plant tissues*:** Cadmium concentration in different parts of the plant was measured to check the efficiency of different soil-applied NPs upon the transport of Cd. Results indicated that accumulation of Cd occurs more in roots than shoots and grains. The highest Cd concentration in roots, shoots and grains was found at 20 mg kg^{-1} Cd level where no NPs were applied. However, the application of different NPs remarkably decreased the Cd concentrations in the root, shoot and grains of the test crop Fig. 4 A, B, C). Results reported a significant increment in the concentration of Cd in wheat roots, shoots, and grains with the increasing concentration of Cd. The plants treated with Fe-NPs showed a notable decrease in the concentration of Cd accumulated in root, shoot, and grains more effectively than other NPs (Si and Zn). The translocation of Cd from root to shoot was increased with increasing Cd concentrations but decreased with NPs application at low Cd. The translocation of Cd from root to shoot was maximum (0.728 mg kg^{-1}) in control and lowest (0.413 mg kg^{-1}) at 5 mg kg^{-1} Cd-contamination level amended with Fe-NPs. Similarly, the translocation from shoot to grain also decreased in treatments amended with different NPs.

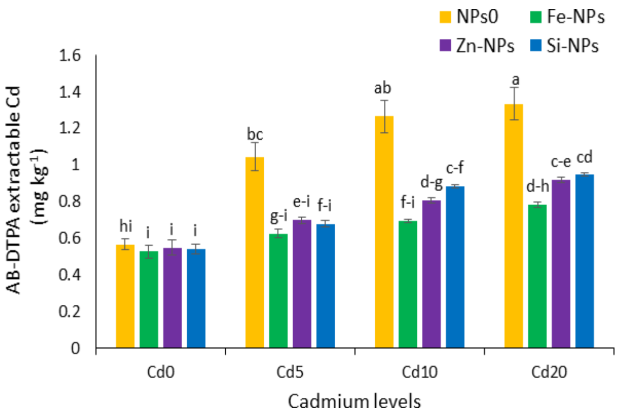

Figure 3. Effect of different NPs on AB-DTPA extractable Cd (mg kg^{-1}) under Cd stressed condition. Different letters on the bars indicate the significant difference present among the treatments according to HSD test at $p \leq 0.05$.

***Correlation matrix*: ** The collected data were subjected to Pearson (n) correlation matrix (Table 4) to find any relation among studied parameters. All the agronomic parameters had a positive significant correlation with each other. The root length was significantly positively correlated with shoot length, spike length, grain weight, and root, shoot fresh and dry weights. This showed that if the plant possesses a healthy long root, it will produce a healthy plant and greater yield. The plants which represent higher root length get access to the far-lying nutrients in the soil due to which plants also show greater shoot length and yield. The maximum shoot length results in longer spikes that lead to the production of a greater number of healthy grains which in turn provide maximum grain weight. Grain Cd and soil's AB-DTPA extractable Cd together were in highly positive significant correlation but showed a significant negative correlation with spike length. This showed that if the soil has higher exchangeable Cd, it reduces the spike length and results in higher grain Cd concentration. So, to get better yield and greater grain weight, it is required to reduce the exchangeable Cd concentration in the soil. AB-DTPA extractable Cd also showed a significant negative correlation with root fresh and dry weights. This depicts that higher exchangeable Cd hinders plant growth by reducing the root growth due to which root fresh and dry weight reduces. AB-DTPA extractable Cd was also found in significant positive correlation with root and shoot Cd concentration. This means that higher exchangeable Cd concentration in soil is responsible for higher Cd accumulation in root, shoot and grain of wheat crop.

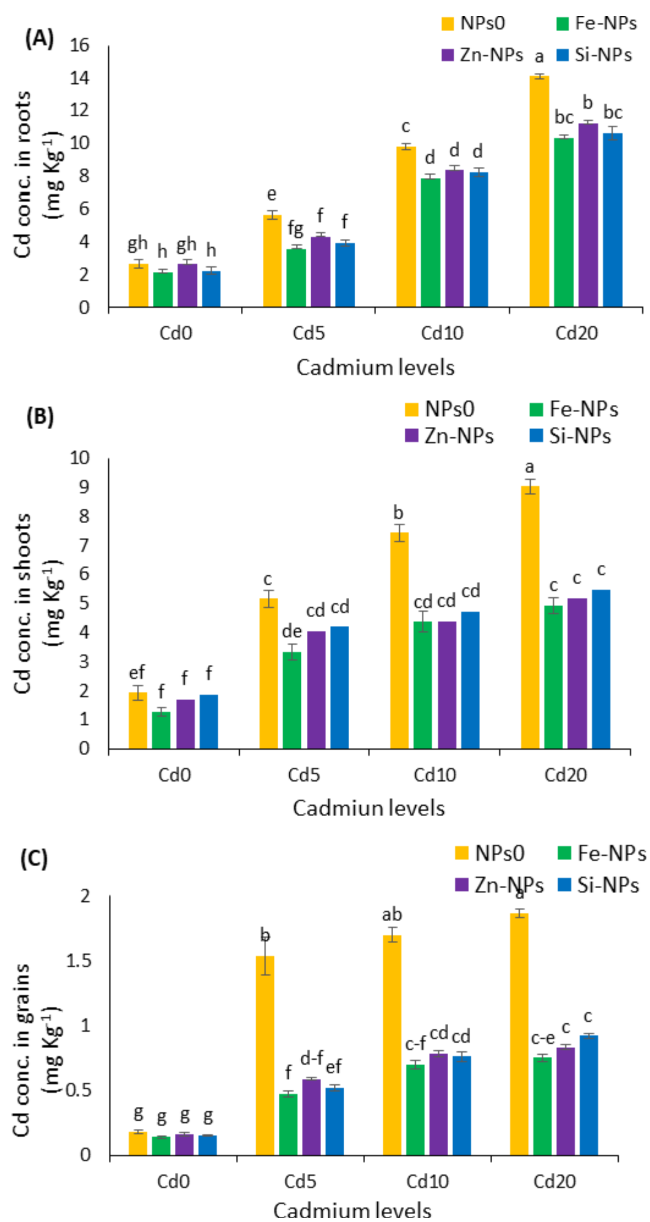

Figure 4. Effect of different NPs on metal concentration in roots (A), shoots (B) and grains (C) of wheat grown under Cd stressed condition. Different letters on the bars indicate the significant difference present among the treatments according to the HSD test at $p \leq 0.05$.

DISCUSSION

In this study, the effects of different NPs, i.e., Fe, Si and Zn-NPs were evaluated on the growth, physiology and metal immobilization/ translocation capacity of wheat exposed to various Cd-contamination levels (0, 5, 10, 20 mg kg^{-1}). The plants' growth was enhanced by the application of different

NPs showing that they have positive effects on wheat growth in Cd-contaminated soil. Many published studies have demonstrated that soil or foliar use of NPs decreased the concentration of Cd in different plants and enhanced crop growth by various mechanisms like enhancing mineral nutrition or by mitigating heavy metal stress (Chen *et al.,* 2018; Hussain *et al.,* 2018). Alzahrani *et al*. (2018) also demonstrated that Si-NPs enhanced plant growth as it alleviates the Cd stress by enhancing the K^+ content in wheat plants. The results obtained in our study showed a significant increase in plant growth by the application of different NPs as they absorbed more harmful substances due to having a small size and large surface area (Dhuan *et al.,* 2017).

Iron NPs enhanced the length of roots and shoots under metal-stressed conditions (Alharaby and Ali, 2022). Similar results were observed in our study as wheat exhibits an increase in the length of root and shoot with the application of NPs because the NPs have a great capacity for providing nutrients to plants. Iron NPs stimulate the redox process and biosynthesis of chlorophyll which in turn stimulates the growth of wheat. The crucial constituent of chloroplast is chlorophyll, and it is directly related to the plant's photosynthetic rate (Rizwan *et al.,* 2019a). Cadmium remarkably influenced the chlorophyll content present in leaves and it was considered one of the important signs of metal toxicity to plants. Therefore, plant health might be affected by a small change in the chlorophyll content and our results indicated that chlorophyll content and photosynthetic rate were increased by the application of different NPs (Fig. 1a).

Silicon is involved in enhancing the efficiency of crops to use light energy which might be the possible cause of significant development of chlorophyll content in wheat (Adrees *et al.,* 2018; Gao *et al.,* 2018). Plants grown in Cd stressed conditions showed suppressed growth because Cd plays an important role in the increased production of ROS by disturbing the physiological and molecular mechanisms in plants (Rizwan *et al.,* 2019b). The distinguished increase in physiological parameters was observed in those plants which were grown in soil amended with different types of NPs. Mahakham *et al.* (2016) also reported similar results as plants have more ability to absorb water and nutrients due to the presence of NPs. This might be one of the possible causes of improvement in physiological parameters. Nanoparticles save the photosynthetic apparatus and help in the improvement of photosynthetic efficiency as a result give better resistance to plants against any stress (Rasheed *et al.,* 2022). The present study revealed that the application of different NPs decreased the Cd concentration in various parts of wheat as shown in Fig. 4.

Sebastian *et al.* (2018) also illustrated that the Cd content in roots and leaves of rice plants decreased significantly by the application of FeO-NPs. The use of Zn and Fe-NPs reduced the Cd in different parts of wheat particularly in grains (Rizwan *et al.,* 2019a). Silicon acts as a barrier by accumulating in leaf apoplast through the translocation from root to shoot and gives safety to plants in case of various stresses and results in increased plant growth (Silva *et al.,* 2017). The reduction in Cd concentration by the application of different NPs might be due to increased uptake of Fe, Si and Zn in the presence of Fe, Si and Zn-NPs, respectively. Therefore, Fe, Si and Zn nutrients compete with Cd, particularly at root level, and may lower the uptake of Cd because of competitive absorption by plants. Application of Fe, Si and Zn-NPs increase the Fe, Si and Zn content while these nutrients are considered as a source of fertilizer in several studies (Munir *et al.,* 2018; Gao *et al.,* 2018). Micronutrients are mostly essential to plants for their normal metabolic functions and numerous nutrients play an important role in redox reaction. Deficiency of these nutrients in the plants leads to poor growth and yellowing of leaves (Rizwan *et al.,* 2019b). In this study, plants showed better growth and vigor with the application of different NPs in Cd contaminated soil.

The application of Si to soil decreased the Cd concentration in various plants part via reducing the Cd bioavailable content (Ali *et al.*, 2019). This study showed that the Cd bioavailable content also decreased where NPs were applied while it revealed increased concentration with no NPs. The effects of NPs on the growth of plants mainly depend upon the crop species, types, doses and shapes of NPs (Noman *et al.,* 2018). Similarly, the foliar-applied Si-NPs reduced the metal concentration in rice grains and decreased the metal translocation factor from root to shoot. Cadmium translocation is reduced due to the application of NPs, and it may also be related to enhanced plant biomass (Chen *et al.,* 2018). It was found that the translocation of Cd from root to shoot was maximum in control, showing that chances of translocation were maximum. However, at low concentrations of Cd the defense system of plants may not be fully active and there are more chances for metal transfer to shoot (Khan *et al.,* 2019). It has been noted that the metal translocation from root to shoot declines when the concentration of metal is high in the root (Rizwan *et al.,* 2021). Similarly, our results depicted that translocation from shoot to grain was decreased with increasing concentration of Cd because accumulation was maximum at higher concentrations so translocation possibility might be minimum. Khan *et al.* (2019) reported that translocation from shoot to grain decreased with increasing levels of NPs. Generally, the application of different NPs could be a systematic approach to obtain good quality as well as quantity of food under Cd stressed conditions.

***Conclusions*:** The application of different NPs significantly increased plant growth, biomass, and yield with simultaneously decreasing Cd accumulation in wheat grains. The chlorophyll content and gas exchange parameters were significantly enhanced in NPs treated plants. Nanoparticles decreased the availability of Cd and serve as a source of safe food production to overcome food security challenges. Fe-based NPs performed better to immobilize the Cd at all contamination levels followed by Zn and Si-NPs. Therefore, NPs could also be used for improving the nutritional status of crops and this technique may be a good substitute for conventional technologies. However, studies at the field level along with other crops are needed for a better understanding of the mechanism of metal uptake and for confirmation of these results.

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