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Bioinspired Cobalt Oxide Nanoball Synthesis, Characterization, and Their Potential as Metal Stress Absorbants

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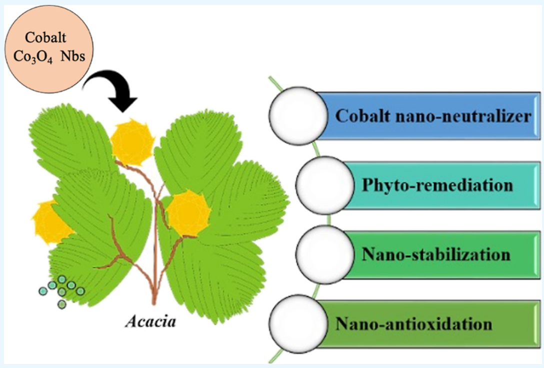
ABSTRACT: Massive accumulation of heavy metals in agricultural land as a result of enhanced levels of toxicity in the soil is an emerging global concern. Among various metals, zinc contamination has severe effects on plant and human health through the food chain. To remove such toxicity, a nanotechnological neutralizer, cobalt oxide nanoballs (Co_3O_4 Nbs) were synthesized by using the extract of *Cordia myxa*. The Co_3O_4 Nbs were well characterized via UV-vis spectrophotometry, scanning electron microscopy, and X-ray diffraction techniques. Green-synthesized Co_3O_4 Nbs were exposed over *Acacia jacquemontii* and *Acacia nilotica* at different concentrations (25, 50, 75, and 100 ppm). Highly significant results were observed for plant growth by the application of Co_3O_4 Nbs at 100 ppm, thereby increasing the root length (35%), shoot length (48%), fresh weight (44%), and dry weight (40%) of the *Acacia* species with respect to the control. Furthermore, physiological parameters including chlorophyll contents, relative water contents, and osmolyte contents like proline and sugar showed a prominent increase. The antioxidant activity and atomic absorption supported and justified the positive response to using Co_3O_4 Nbs that mitigated the heavy-metal zinc stress by improving the plant growth. Hence, the biocompatible Co_3O_4 Nbs counteract the zinc toxicity for governing and maintaining plant growth. Such nanotechnological tools can therefore step up the cropping system and overcome toxicity to meet the productivity demand along with the development of agricultural management strategies.



1. INTRODUCTION

The global agricultural food security is seriously affected by human activities and the growing human population.[1,2] *Acacia* plants belonging to Angiospermae of family Mimosaceae have a wide range of biomedical applications including breast cancer treatment,[3] anti-dengue activity,[4] damping off and root rot diseases,[5] and antiviral and antibacterial effects.[6−8] Moreover, its wood has a very high demand and is used as timber, lumber, as well as for fuel purposes in many countries throughout the world.[6,9] But, currently, *Acacia* is considered to be very susceptible and heavily loaded with heavy metals that leads to poisoning in comparison to other plants.[10,11] Soil contaminated with heavy metals is not considered safe for *Acacia* plant species as they uptake the available heavy-metal contents from the soil and cause serious health complications.[12] For the last few eras, the prevalence of heavy metals in soil has become a serious issue throughout the world which disturbs the physical, chemical, and biological features of soil with reduction in fertility and plant growth.[13,14] It has also been observed that a major cause of retarded plant growth is the industrial waste that chiefly contains lead, zinc, chromium, and cadmium which serve as the key elements behind elevated land pollution.[15,16] Among these metals, zinc as a loaded nutrient in the soil causes decline in the plant biomass, gaseous exchange, chlorophyll contents, and nutrient absorptive capacity, causing alteration in cellular structures. In the previous reports, it has been observed that heavy-metal stress has shown negative impacts on plants' vital cellular components and functions such as DNA damage, deformation of plant cells, and difference in cellular redox homeostasis, phytohormone contents, biomass, and yield.[17−19] In another report, zinc has shown negative effects including phototoxicity, modulation in abiotic response and physiological parameters, reducing photosynthetic pigment concentration, and reduction in root activity of wheat and other plants.[20−23] Although plants have their own defense system

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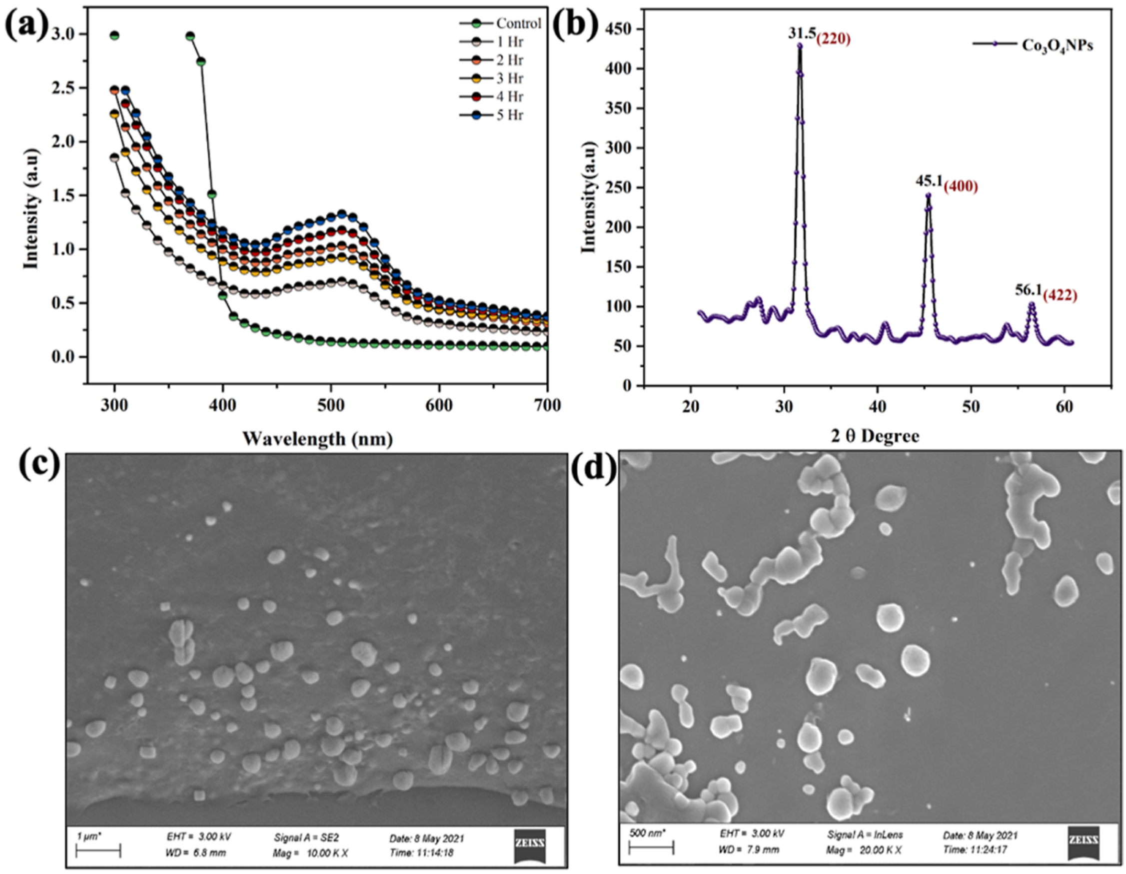

Figure 1. Characterization of Co_3O_4 Nbs from *C. myxa* leaf extract. (a) UV−vis spectra of Co_3O_4 Nbs. (b) XRD pattern of of Co_3O_4 Nbs. (c,d) Scanning electron micrographs of Co_3O_4 Nbs.

against heavy-metal stress to resist the change by cellular exudates, membrane integrity, activating heat shock proteins, metallothioneins, and organic acid within certain limits.[24,25] But, in the case of accumulation of heavy metals beyond a threshold and traditional strategies such as fertilizers, fungicides, insecticides, pesticides, use of dietaryfibers, and chelating agents are considered inadequate for coping with heavy-metal stress. Therefore, an exciting nanotechnological method of introducing nanomaterials has revolutionized the process of reducing metal stress to a great extent by absorbed heavy metals, reducing stress and enhancing the plant growth without any lethal effects.[26−28] Nanotechnology is an emerging technique that is recounting its role in the agricultural field.[29] Nanoparticles, when applied to crop plants, significantly increase the growth of plants under unfavorable environmental circumstances by triggering numerous physiological and biochemical mechanisms occurring in plants.[30−32] The small size, active movement, condensed surface area, slow-release, and higher rate of uptake make nanomaterials a good applicant as compared to outdated chemical processes.[33,34] It was detected that less mediation of NPs is mandatory that gives marked results when applied on plants as related to common salts. The application of numerous NPs makes the plants tough/lenient to abiotic stresses like scarcity, salinity, and heavy metals.[35,36] Being a step ahead, the evolution of green synthesis of nanomaterials has also widened the landscape of agricultural application.[37,38] The ecofriendly, cost effective, and surface-modified nanoparticles boosted up the compatibility and intrinsic potentials over the particles and within the biotic system.[39−41] Thereby, various biologically designed nanoparticles have shown condensed oxidative stress for reduced the uptake of heavy metals in different crops. Interestingly *Cordia myxa*, member of the family Boraginaceae, found in the east of the Mediterranean region, has shown outstanding intrinsic properties for the synthesis of efficient nanomaterials[42,43] that are diversely applied in different industrial and agricultural fields in the form of Zn NPs, Fe NPs, Co_3O_4 NPs, and so forth. However, cobalt as nanoparticles is very effective in nitrate reduction by converting it into ammonia[44,45] and also very useful for wastewater treatment.[46−48] Until now, there has not been any documented report where cobalt oxide nanoballs (Co_3O_4 Nbs) were used as treatment for metal stress such as monitoring growth in zinc-stressed *Acacia* family member plants. In this work, it was aimed to synthesize Co_3O_4 Nbs using *C. myxa* extract and characterize the particles using high-performance techniques. The synthesized nanoparticles were used as treatment for Zn-stressed *Acacia* species including *Acacia jacquemontii* and *Acacia nilotica*. Self-prepared nanoballs were treated at 25, 50, 75, and 100 ppm concentrations to monitor the growth effect, phenolic contents, antioxidant potential, and atomic absorption within two different *Acacia* plant species.

2. RESULTS AND DISCUSSION

The Co_3O_4 Nbs were synthesized using *C. myxa* as a bioreducing agent. The cobalt Nbs were well characterized with the help of UV−vis spectrometry, scanning electron microscopy (SEM), and X-ray diffraction (XRD). Overall, the biological synthesis, characterization, and application of Co_3O_4

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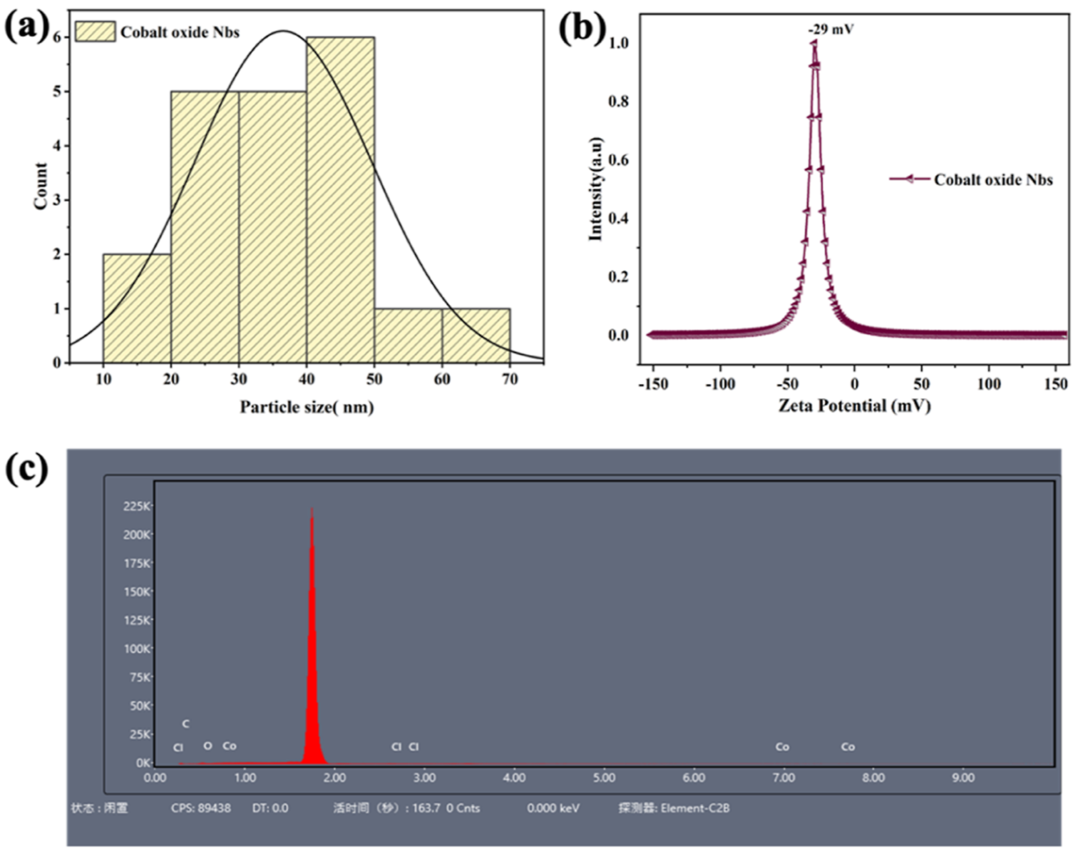

Figure 2. (a) Size-distribution histograms of Co_3O_4 Nbs obtained from SEM nanographs. (b) Zeta potential of Co_3O_4 Nbs. (c) EDS chromatographic pattern of Co_3O_4 Nbs.

Nbs for the mitigation of Zn-stressed *Acacia* species have shown good performance in the current research work. It primarily overcomes the metal stress, such as zinc, and promotes growth over different treatments with elevated antioxidant potentials.

2.1. Characterization of Co_3O_4 Nbs. UV–vis spectroscopy was used for the initial confirmation of cobalt Nbs synthesis by a biological method. The control group showed no peak (green line) as precursors of cobalt nanoparticles due to the lack of a reducing agent and reaction mixture started. The initial color of the extract was light yellow, which turned to dark brown as it mixed with the precursors of cobalt oxide, representing the formation of cobalt nanoballs. The reaction mixture primarily showed a weak absorbance in UV–vis spectra. With the passage of time, from 1 to 5 h, the peaks became sharper and clearer and showed an absorption band at 508 to 510 nm (Figure 1a. The synthesis of cobalt Nbs occurred when extract of the plant (*C. myxa*) was added to a cobalt chloride ($CoCl_2$) solution;[49] the color of the solution transformed from yellow to brown because of the reduction of $CoCl_2$ solution to cobalt ions (Co^+).[26] The absorption band in the range 300–700 nm is attributed to the superficial plasmon band of the cobalt nanostructure. The current results support and confirm the previous absorption spectral profile.[50−52] The crystal structures of cobalt Nbs were determined by XRD. The diffraction peaks at 2θ degrees of 31.5, 45.1, and 56.1° were indexed as the (220), (400), and (422) planes of $Co_3 O_4$ Nbs, respectively. The (220) plane showed a stronger peak than the other peaks, indicating that $Co_3 O_4$ Nbs had better growth along the *Z*-axis direction. As the *C. myxa*-based green synthesis had revealed bit larger peaks, thus gives a small sized particle at a glance. Mathematically, the lattice constants *a* and *c* prove the hexagonal wurtzite structure of the biologically synthesized $Co_3 O_4$ Nbs in relation.[39,41,53] The average ball size obtained for $Co_3 O_4$ Nbs was 25 nm in the green synthesis method. On the basis of the diffraction peaks, it is clear that the formed $Co_3 O_4$ Nbs were in a hexagonal phase with lattice coefficients of a = 4.36 Å and c = 4.95 Å, which were recorded with the standard hexagonal Wurtzite crystalline $Co_3 O_4$ Nbs with the reported JCPDS card no. 00-089-7102. Furthermore, the XRD pattern does not demonstrate the additional peaks of any mineral and no distinguishing peaks of *C. myxa*.[54] The absorption peak of the green synthesized Co_3O_4 Nbs was broad, which can be ascribed to the poly-isolated nature of the Co_3O_4 Nbs.[55] By means of the diffraction peaks, no impurities were noticed in the synthesized $Co_3 O_4$ Nbs sample, and the sharpness of the diffraction peak shows that the product was well crystallized (Figure 1b). Furthermore, the asymmetrical absorption spectrum of Co_3O_4 Nbs designates the size variation and anisotropy of nanoparticles. The absorption band at 2924 cm^{-1} is assigned to the vibrations of secondary amines. The broad band at 3432 cm^{-1} is due to the stretching of O–H groups. Moreover, the peak at 824 cm^{-1} is characteristic of the aromatic ring. SEM examination was done to measure the surface morphology of synthesized cobalt nanoparticles at 500 nm resolution (Figure 1c). The SEM image of cobalt nanoparticles is shown at 1 μm resolution in Figure 1d. As is plain from the figure, the green nanoballs varied in shape and size, representing the nonuniform size and distribution.

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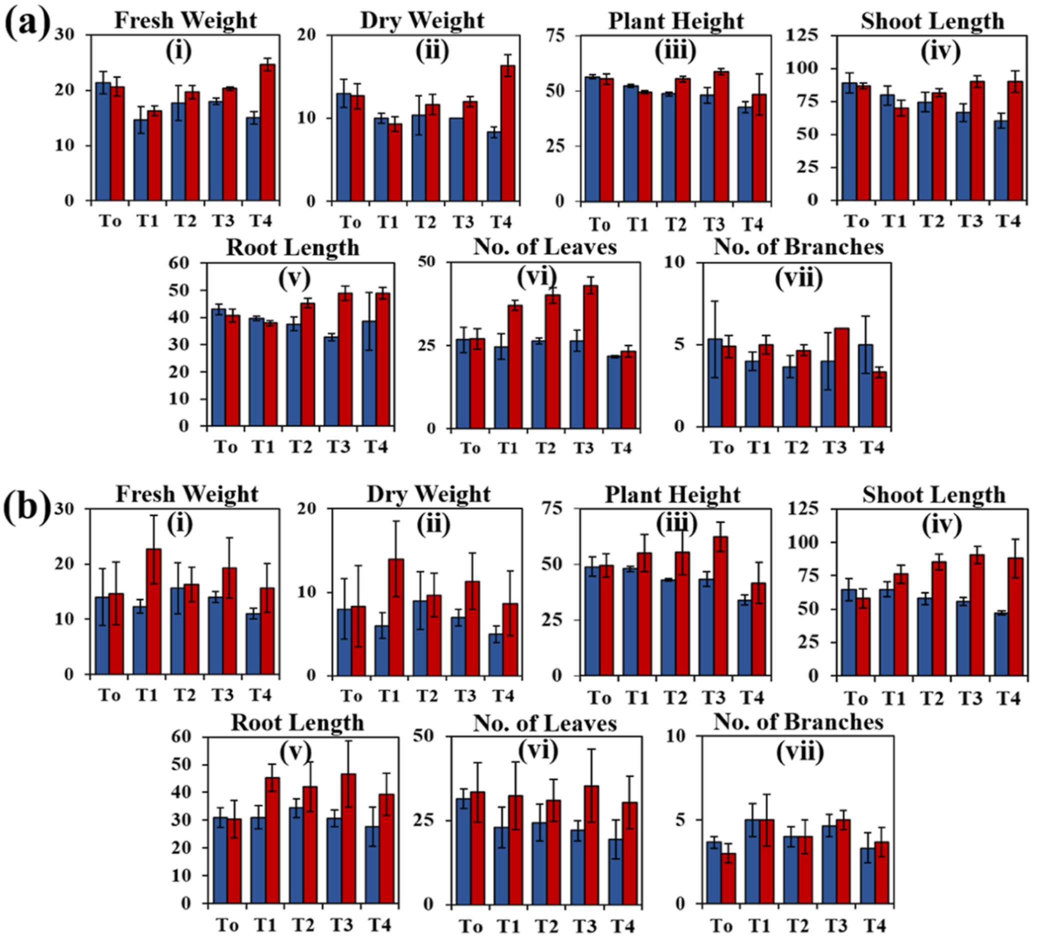

(c)

Plant species	Fresh Weight of Plant (g)	Dry Weight of Plant (g)	Plant Height (cm)	Shoot Length (cm)	Root Length (cm)	Number of Leaves	Number of Branches
A. jecquemontii	8.41	6.31	17.2	31.03	19.31	12.91	5.71
A.nilotica	21.31	16.31	30.41	35.91	33.41	35.83	4.32

Figure 3. Impact of Zn stress with and without cobalt nanoparticle treatment on morphological and physiological parameters in (a) *A. jacquemontii*, (b) *A. nilotica*, and (c) HSD values.

Nanoball size-distribution histogram obtained from the SEM images showed a large variation in the particle size. Figure 2a shows the dispersion of nanoballs. The nanoball sizes ranged from 10 to 80 nm with an average diameter size of 34.5 nm. Zeta potential (ZP) measurement, an analytical technique used to determine the surface charge in the colloidal solution of Nbs with values >+25 and <−25 mV, generally has a high degree of stability. If the value of ZP fluctuates from normal, then it will lead to aggregation, coagulation, or flocculation due to van der Waals interparticle attraction.[56,57] Our green synthesized Co_3O_4 Nbs retained the ZP value of −29 mV that resides in the stable range (Figure 2b). This represents that the high stability of Co_3O_4 Nbs matched with the previous work reported.[58−60] Energy-dispersive X-ray spectroscopy (EDS) is a powerful technique that was used to conduct elemental analysis of the green synthesized nanoballs. Figure 2c shows the presence of cobalt, chloride, oxygen, and carbon that confirmed the presence of cobalt in the synthesized nanoballs.[61−63]

2.2. Zinc Stress with Morphological and Physiological Analyses of *Acacia*.

2.2.1. Fresh Weight. A statistically significant ($p < 0.05$) decline was observed in the fresh weight of plants (g) in *A. jacquemontii* treated only with zinc (Zn) metal applied at different treatment levels as T3 < T2 < T4 < T1 (15.6, 17.1, 29.6, and 31.2%), respectively, in different concentrations as compared to the control T0. The results showed that maximum decline was observed at the T1 level of treatment (31.2%) in the fresh weight of plants when treated with 25 ppm of Zn metal. Furthermore, the maximum and significant increase in the fresh weight of plants was also noticed with the T4 (19.3%) treatment which was 100 ppm of Co_3O_4 Nbs along with the same concentration of metal as compared to the control plants (Figure 3ai. However, the fresh weight of plants showed a significant decline ($p < 0.05$) at T3 < T2 < T1 (1.6, 4.8, and 20.9%), respectively, as compared to that of the control plants. A significant ($p < 0.05$) decrease was observed in the fresh weight of plants (g) in *A. nilotica* treated only with Zn metal applied in different treatment levels of T1 < T4 (11.9 and 21.4%) when compared to that in control plants. At treatment level T3, there was no change in the fresh weight of plants applied with Zn metal only as compared to that of control T0. However, the treatment level at T2 showed an

Table 1. Mean Squares of Studied Parameters Obtained after Two-Way Factorial ANOVA for Two Species of *Acacia*[a]

two-way factorial ANOVA									
Acacia jecquemontii	SOV	Df	PH	NL	NB	SL	RL	FWP	DWP
	NPs	1	0.3 ns	598.53***	0.03 ns	32.03*	177.63*	19.20 ns	6.53 ns
	level	4	78.25 ns	146.97***	0.71 ns	90.47 ns	26.13 ns	17.36 ns	6.53 ns
	NPs*level	4	240.05**	82.70*	4.78 ns	1195.53***	144.80*	56.87**	38.20***
	error	20	35.4	20	3.97	115.23	44.6	8.46	4.77
	SOV	Df	PH	NL	NB	SL	RL	FWP	DWP
Acacia nilotica	NPs	1	653.33*	885.63*	3.1E33 ns	3564.3***	1044.3*	140.83 ns	86.70 ns
	level	4	213.03 ns	13.2 ns	3.45 ns	131.42 ns	29.08 ns	17.5 ns	9.11 ns
	NPs*level	4	69.67 ns	13.47 ns	0.25 ns	543.38*	17.72 ns	24.00 ns	14.61 ns
	error	20	110.67	153.63	2.23	155.03	133.53	54.33	31.83

[a]SOV: source of variation; NPs: nanoparticles; Df: degree of freedom; PH: plant height; NL: number of leaves; NB: number of branches; SL: shoot length; RL: root length; FWP: fresh weight; DWP: dry weight. Scatter plots of root, stem, and leaf (Figure 3a,b,c) were generated to observe the linearity of changes in the amount of Zn in the root, stem, and leaf. With 1 unit increase in the amount of Zn in the root, 0.12 times increase of Zn in the stem was observed.[68] 1 unit increase of Zn amount in the leaf may be due to 1.67 times increase of Zn in stem. 1 unit increase of Zn amount in the root can cause 0.019 times increase in the Zn amount in the leaf.

increase in the fresh weight of plants (11.9%) as compared to the control. The maximum and significant increase in the fresh weight of plants was noticed as the level of Nbs increased in the medium level in the order of T1 > T3 > T2 > T4 (54.5, 31.7, 11.3, and 6.8%), respectively, as compared to that of the control plants (Figure 3bi). In the present work, the obtained results show that the increase in the treatment level of heavy metal caused a huge decline in the fresh weight of selected plants that was estimated as (29.6%) in *A. jacquemontii* and (21.4%) in *A. nilotica*. To reduce the effect of heavy metal on the fresh weight of plants, cobalt Nbs were applied to the medium, as the results showed a huge increase in fresh weight in *A. jacquemontii* (19.3%) and *Acacia nilotica* (31.7%). The maximum increase in fresh weight (34−74%) and dry weight (22−36%) of *Acacia* species was also observed by the combined application of cobalt Nbs and Zn metal. Heavy-metal pollution decreases the root length, shoot length, fresh weight, and dry weight of plants, which might be due to the destructive effects of heavy metals on the photosynthetic pigments of plants.[10] It was also reported earlier that Zn stress inhibits plant growth by suppressing the activity of the antioxidative enzyme.[64] Mean squares obtained after the analysis of variance (ANOVA) of fresh weight (56.87) are presented in Table 1.

2.2.2. Dry Weight. Similarly, a statistically significant ($p < 0.05$) reduction was detected in the dry weight of plants (g) in *A. jacquemontii* treated only with Zn applied at various treatment levels as T2 < T1 and T3 < T4 (20.5, 23 and 23, 35.9%, respectively) in different concentrations as compared to that in the control T0. Maximum decline was seen at the T4 level of treatment (35.9%) in the dry weight of plants when treated with 100 ppm of Zn metal. The maximum and significant increase in the dry weight of plants was noticed at T4 (28.8%) treatment level, which was 100 ppm of Co_3O_4 Nbs, along with same concentration of metal compared with the control plants (Figure 2aii). However, the dry weight of plants showed a significant decline ($p < 0.05$) in the order T3 < T2 < T1 (5.2, 7.8, and 26.3%, respectively) as compared to that of the control plants. A significant ($p < 0.05$) decline was calculated in the dry weight of plants (g) in *A. nilotica* treated only with Zn applied in changed treatment levels that were in the order T3 < T1< T4 (12.5, 25, and 37.5%, respectively) when compared to that of control plants. At treatment level T2, there was an increase (12.5%) in the dry weight of the plants applied with zinc metal only as compared to that in the control T0. The maximum and significant increase in the dry weight of plants was noticed as the level of Nbs reduced in the medium that was in the order T4 < T2 < T3 < T1 (4, 16, 36, and 68%, respectively), as that of control plants (Figure 3bii). An important variation in the properties of different concentrations (25, 50, 75, and 100 mg/L) of Co_3 O_4 Nbs synthesized from *C. myxa* on biomass on *Acacia nilotica* and *A. jacquemontii* under controlled and metal-stressed conditions has also been observed. From our results, it is recognized that under Zn stress, the biomass of the control and untreated normal plants was reduced which may be due to protoplasm dehydration and absolute turgidity reduction, leading to the decline in photosynthesis, cell division, and growth mean squares obtained after the ANOVA of dry weight (38.20), which are given in Table 1.

2.2.3. Plant Height. A statistically significant decline ($p < 0.05$) was observed in the plant height (cm) of *A. jacquemontii* treated only with zinc (Zn) applied in the order T1 < T2 < T3 < T4 (7.1, 13.5, 14.7, and 24.2%, respectively) in different concentrations as compared to that in control T0. When comparing T4 plants with control plants, the maximum reduction in plant height (24%) was noticed in plants which were treated with 100 ppm of Zn. Maximum and significant increases in plant height were observed in T3 (6%) that were treated with 75 ppm of Co_3O_4 Nbs along with the same concentration of metal compared with the control plants (Figure 3aiii). However, the plant height showed a decline at the T1 and T4 treatment levels (10.8 and 12.6%), respectively. At 75 ppm of Nb treatment, a 6% increase was observed in the plant height than that of control plants. The higher concentration of Zn caused an adverse effect by getting absorbed by the roots of *Acacia* species and also translocating into the shoot system that ultimately decreased the efficiency of the leaf and plant height; besides these, it also inhibited the plant growth. These observed findings were confirmed with the results obtained by ref 65.

A significant ($p < 0.05$) decrease was observed in the plant height (cm) of *A. nilotica* treated only with Zn metal applied in different concentrations that were T1 < T2 < T3 < T4 (2, 12.2, 12.2, and 30.6%, respectively), as compared to the control T0. At the T4 level, the maximum reduction (30.6%) in height was observed when compared to the height of the control plants. Maximum and significant increase in plant height was noted as

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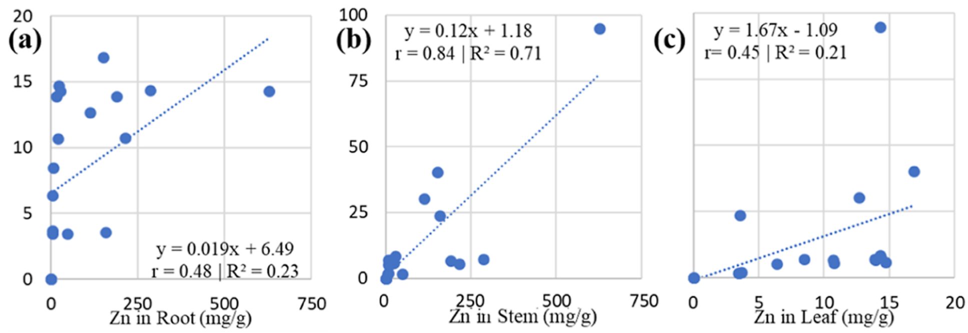

(d)

A. Jecquemontii	*PH*	NL	NB	SL	RL	FWP
NL	0.49					
NB	0.18	0.26				
SL	0.78	0.35	0.15			
RL	0.39	0.34	-0.17	0.58		
FWP	0.42	0.20	0.09	0.51	0.51	
DWP	0.50	0.17	0.02	0.62	0.59	0.90

A. nilotica	*PH*	NL	NB	SL	RL	FWP
NL	0.70					
NB	0.43	0.45				
SL	0.72	0.53	0.27			
RL	0.74	0.73	0.23	0.69		
FWP	0.72	0.70	0.24	0.62	0.75	
DWP	0.72	0.68	0.16	0.63	0.77	0.9[illegible]

Figure 4. Paired scatter plots showing the correlations along with simple linear regression equations for the amount of Zn in (a) root, (b) stem, and (c) leaf. (d) Pearson's correlation and absolute critical value $r = \pm 0.361$ beyond which the calculated correlation is found significant (p value <0.05).

the level of NPs increased in the medium that was up to the T3 level, that was T3 > T2 > T1 (25.4, 11.3, and 10%, respectively). The maximum increase in plant height was observed at the T3 level 75 ppm of Co_3O_4 Nbs along with the same concentration of metal compared with the control plants. However, with further increase in the Nb level at T4, 16.1% decline in the plant height was observed as compared to that in the control plants (Figure 3biii). The present results revealed that as the treatment levels of heavy metal increased, the plant height showed a decline up to 24.2% in *A. jacquemontii* and 30.6% in *A. nilotica*. To remove the metal stress in plants, foliar spray of cobalt Nbs was applied that showed a significant increase in plant height as 6 and 25.4% increase were calculated in *A. jacquemontii* and *A. nilotica,* respectively. Mean squares obtained after the ANOVA of plant height (240.05) are presented in Table 1. It was observed that the treatment levels of Nbs to plants enhanced plant growth in heavy-metal-contaminated soil.

2.2.4. Shoot Length. A statistically significant ($p < 0.05$) reduction was observed in the shoot length (cm) of *A. jacquemontii* treated only with Zn applied at treatment levels in the order T1 < T2 < T3 < T4 (10.4, 16.1, 25, and 32.2%, respectively) in different concentrations as compared to that in the control T0. The maximum and significant increase in shoot length was observed at T3 > T1 (3.8 and 3.4%), respectively, that were treated with different levels of Co_3O_4 Nbs along with the same concentration of metal compared with the control plants (Figure 3aiv). However, the shoot length showed a significant decline ($p < 0.05$) at T1 > T2 (19.5 and 6.1%, respectively), than that in the control plants. A significant ($p < 0.05$) decline was detected in the shoot length (cm) of *A. nilotica* treated only with zinc metal applied in different treatment levels that were in the order T2 < T3 < T4 (9.8, 13.4, and 26.9%, respectively) as compared to that in the control T0. However, the treatment level at T1 showed a minute increase in shoot length (0.5%) as compared to that in the control. The maximum and significant increase in shoot length was observed as the level of Nbs increased in the medium that was in the order T3 > T4 > T2 > T1 (55.4, 50.8, 46.2, and 30.8%, respectively), compared to the control plants (Figure 3biv). The present results showed a significant difference in shoot lengths between the two selected plants when applied only with zinc and cobalt NPs. A maximum decline in shoot length (32.2%) of *A. jacquemontii* and 26.9% decline in *A. nilotica* were estimated. To alleviate the metal stress in both selected plants, foliar spray of cobalt Nbs was applied, and an increase in shoot length of *A. jacquemontii* (3.8%) and a maximum increase in *A. nilotica* (55.4%) were noticed. We also found an obvious variation in the effectiveness of 25, 50, 75, and 100 mg/L concentrations of Co_3O_4 Nbs. However, no lethal effects were observed even at higher concentrations.[66] It was also found that Co_3O_4 Nbs synthesized by green methods did not show noxiousness at higher concentrations and gave a positive response to plant growth and metal-stress alleviation. Mean squares obtained after the ANOVA of shoot length (1195.53) are presented in Table 1.

2.2.5. Root Length. A statistically significant ($p < 0.05$) decline was observed in the root length (cm) of *A. jacquemontii* treated only with Zn metal applied at treatment levels T1 < T4 < T2 < T3 (7.7, 10, 12.3, and 24%, respectively) in different concentrations as compared to the control T0. The maximum decline was noticed at the T3 level of treatment (24%) in the root length when treated with 75 ppm of Zn metal. The maximum and significant increase in root length was detected at T3, T4 > T2 (20.4, 20.4, and 11.4%, respectively) that were treated with different levels of Co_3O_4 Nbs along with the same concentration of metal compared with the control plants (Figure 3av). However, the root length showed a significant decline ($p < 0.05$) at T1 (6.5%) than that of control plants. A non-significant ($p < 0.05$) decline was calculated in the root length (cm) of *A. nilotica* treated only with Zn metal applied in different treatment levels, that is, T1 and T3 showed no difference in root growth applied with Zn metal only as compared to the control T0. However, the treatment level at T2 showed an increase in the root length (10.7%) as compared to the control. While the root length was noticed to be reduced at the treatment level of T4 (10.7%) when compared to that in the control. The maximum and significant increase in root length was noticed as the level of Nbs increased in the medium that was in the order of T3 > T1 > T2 > T4 (53.8, 49.4, 38.4, and 29.6%, respectively), as compared to that of the control plants (Figure 4bv. In the present work, the results showed that the increase in metal concentration in the medium caused adverse effect in the growth of root in both selected plant species; maximum decline was in the root length of *A. jacquemontii* (24%) and (10.7%) in *A. nilotica*. To reduce the hazardous effect of zinc, spray of cobalt Nbs was applied in the medium, and the obtained results showed a maximum increase in the root length of *A. jacquemontii* (20.4%) and (53.8%), respectively. Mean squares obtained after the ANOVA of root lengths (144.80) are presented in Table 1. The application of $Co_3 O_4$ Nbs on plants preserved and increased the root growth under both irrigated and metal-stressed conditions, demonstrating that nanoparticles can supply essential nutrients to the plants and benefit their growth and development.[67]

2.2.6. Number of Leaves. A statistically significant decline ($p < 0.05$) was observed in the number of leaves of *A. jacquemontii* treated only with Zn metal in the order T1 < T2 < T3 < T4 (7.4, 12.7, 12.7, and 18.7%, respectively) in different concentrations as compared to the control T0. When T4 plants were compared with control plants, the maximum reduction in the number of leaves (18.7%) was noticed in plants which were treated with 100 ppm of Zn. The maximum and significant increase in the number of leaves was observed in T3 > T2 > T1 (59.2, 48, and 37%, respectively) that were treated with different treatment levels of Co_3O_4 Nbs along with the same concentration of metal compared with the control plants (Figure 3avi). However, the number of leaves showed a decline at the T4 treatment level (13.5%) compared to the control plants. A significant ($p < 0.05$) decrease was observed in the number of leaves of *A. nilotica* treated only with zinc metal applied in different concentrations that were in the order of T2 < T1 < T3 <T4 (22.3, 26.5, 29.7, and 38.3%, respectively), as compared to that of the control T0. Maximum and significant decline in the number of leaves was noted as the level of Nbs increased in the medium that was T4 > T2 > T1(9, 6.9, and 3%, respectively), compared to the control plants. However, the number of leaves were increased due to Nbs level at T3 (6%), as compared to that in the control plants (Figure 3bvi). The present research showed that the increase in zinc concentration caused a huge decline in the number of leaves in *A. jacquemontii* (18.7%), and *A. nilotica* showed the maximum reduction (38.3%) in leaves as compared to *A. jacquemontii*. For the alleviation of metal stress in both selected plant species, foliar spray of cobalt Nbs was applied, and the results showed an enormous difference between the plants species. In *A. jacquemontii,* at the T3 treatment level of cobalt Nbs (59.2%), the increase in number of leaves was calculated as compared to *A. nilotica*. The highest level of Nbs increased the number of leaves in the presence of heavy-metal contamination by 5−14 and 9−22%. Mean squares obtained after the analysis of number of leaves (82.70) are shown in Table 1.

2.2.7. Number of Branches. A significant decline ($p < 0.05$) was observed in the number of branches of *A. jacquemontii* treated only with zinc metal applied with T2 > T1, T3 > T4 (31.1, 24.9, 24.9, and 6.1%, respectively) in different concentrations as compared to the control T0. Maximum and significant increase in the number of branches were observed at T3 > T1 (22.4 and 2%, respectively) that were treated with different treatment levels of Co_3O_4 Nbs along with the same concentration of metal compared with that for the control plants (Figure 3avii). However, the number of branches showed a significant decline ($p < 0.05$) at T2 < T4 (4.6 and 32%, respectively) than that of the control plants. A significant ($p < 0.05$) increase was observed in the number of branches in *A. nilotica* treated only with zinc metal applied in different treatment levels that were in the order T1 > T3 > T2 (36.2, 27.2, and 8.9%, respectively), as compared to that of the control T0. However, for the treatment level at T4, a reduction (9.2%) in the number of branches was observed as compared to that in the control. Maximum and significant increase in the number of branches was noticed as the level of Nbs increased in the medium that was in the order T1, T3 > T2 > T4 (66.6, 66.6, 33.3, and 22.3%, respectively) compared to the control plants (Figure 3bvii). In our present work, results of the effect of heavy-metal treatment on the number of branches showed that increase in metal concentration caused a decrease in the number of branches by up to 31.1% in *A. jacquemontii,* while there was no difference in *A. nilotica*. While *A. nilotica* showed an increase in the number of branches when cobalt NPs were applied to cope with the metal stress; maximum increase in the number of branches was 66.6% when compared to *A. jacquemontii*. While the maximum increase by inoculation of cobalt Nbs was 20% (shoot length) and 35% (root length) in the refined plants in the contaminated soil. The co-application of zinc metal and cobalt Nbs enhanced the number of branches by 13−27 and 36−45%, respectively, in comparison to the untreated stress-exposed plants. Mean squares obtained after the ANOVA of the number of branches (4.78)are shown in Table 1.

2.3. Correlation. Plant height (cm) in *A. jacquemontii* was observed to be positively significantly (<0.05) correlated with the number of leaves (0.49), number of branches (0.18), shoot length (0.78), root length (0.39), fresh weight (0.42), and dry weight (0.50) (Figure 4a−c). With the increase in the number of leaves, there was a significant increase in the number of branches, shoot length, root length, fresh weight, and dry weight of the plants, showing positive correlation among them, while there was no significant difference observed with increase in the number of branches on plant development. A positively significant difference was detected where with shoot length increase, there was increase in root length (0.58), fresh weight (0.51), and dry weight (0.62) (Figure 4d). An increase in fresh weight of the plants exhibited a positive correlation with dry weight (0.90); as fresh weight increased, dry weight also increased. The present results showed that there was significant correlation among various growth parameters between *A. jacquemontii* and *A. nilotica* when treated with zinc metal along with cobalt NPs. While there was no significant correlation as the number of branches increased in both the studied plant species. Plant height (cm) in *A. nilotica* was observed to be positively significantly (<0.05) correlated with number of leaves (0.70), number of branches (0.43), shoot length (0.72), root length (0.74), fresh weight (0.72), and dry weight (0.72). As the number of leaves increased, there was significant increase in the number of branches, shoot length, root length, fresh weight, and dry weight of the plants, showing positive correlation among them.[69] However, there was no significant difference noticed with an increase in the number of branches on plant growth. A positively significant difference was

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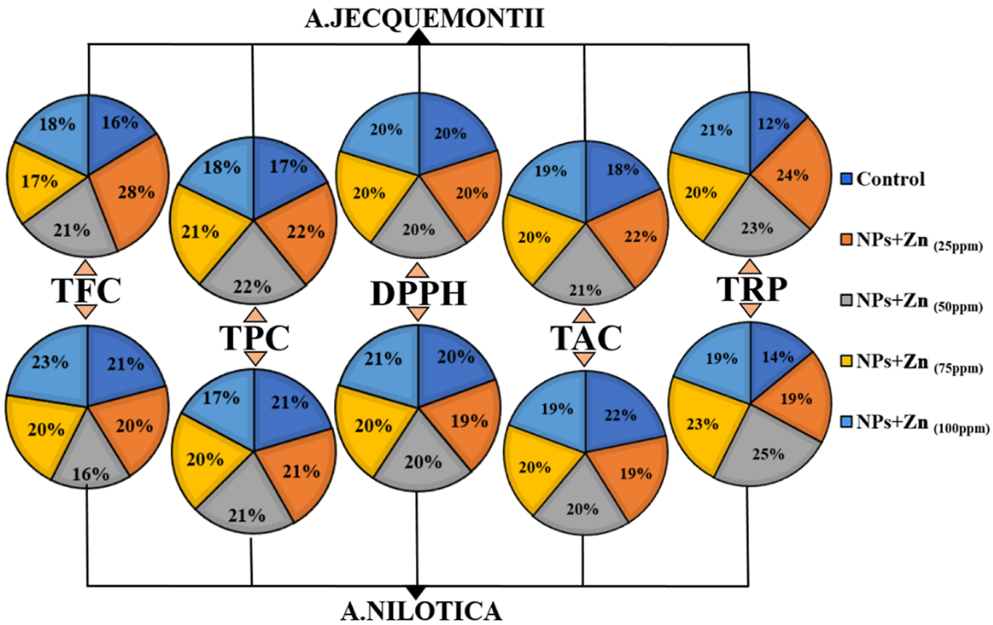

Figure 5. Pie plots of antioxidants [TPC, TFC, DPPH radical scavenging activity, TAC, and TRP] under five treatments in two *Acacia* species.

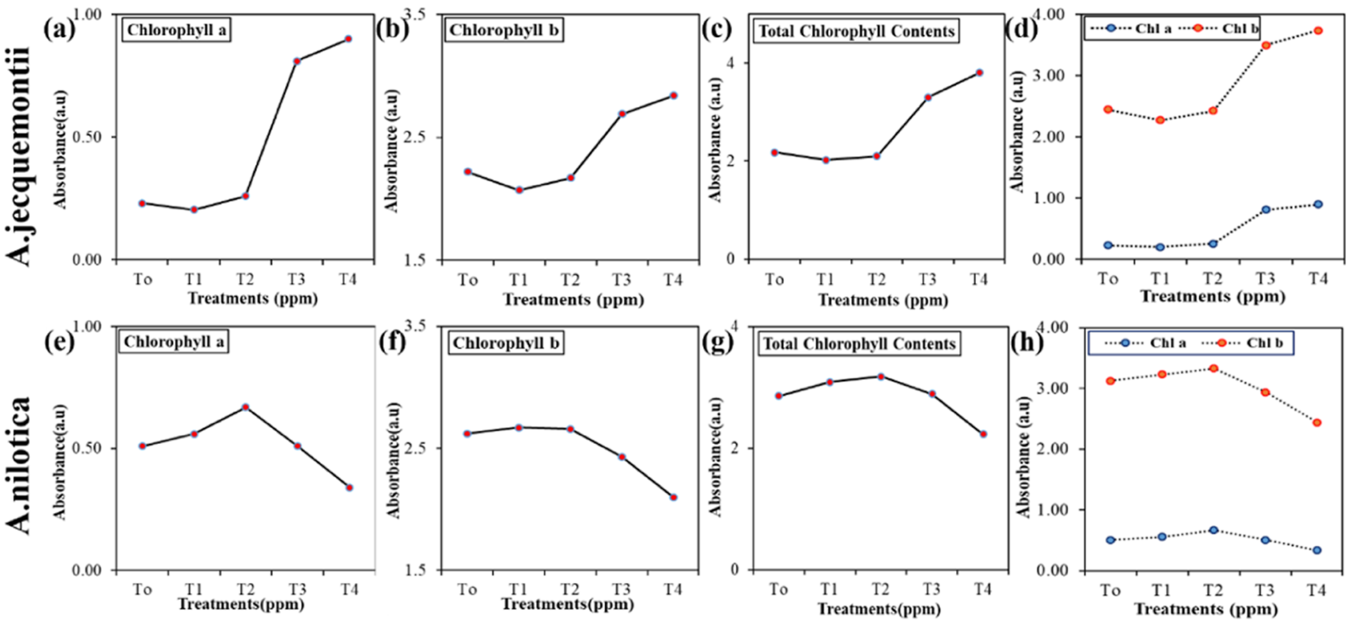

Figure 6. Variation in chlorophyll contents due to different treatments in (a−d) *A. jacquemontii* —and (e−h) *A. nilotica*—.

observed as the shoot length increases, there was increase in root length (0.69), fresh weight (0.62), and dry weight (0.63). An increase in the fresh weight of plants (0.99) showed a positive correlation with dry weight (0.99); as the fresh weight increased, the dry weight also increased.

2.4. Antioxidant Potentials. *2.4.1. Total Phenol Content.* Total phenol content (TPC) of the control group in *A. jacquemontii* was 36.53 μg/mg (17%), and the results showed that the TPC content of plants that were exposed to T1 > T2 > T3 > T4 (22, 22, 21, and 18%) was increased, respectively. While TPC content of the control in *A. nilotica* was 36.29 μg/mg (21%), and results showed that TPC content of plants that were exposed to T1 > T2 > T3 > T4 (21, 21, 20, and 17%) increased, respectively (Figure 5.

2.4.2. Total Flavonoid Content. Total flavonoid content (TFC) of the control group in *A. jacquemontii* was 10.71 μg/mg (16%), and the results showed that the TFC content of plants that were exposed to T1 > T2 > T4 > T3 (28, 21, 18, and 17%) increased, respectively. While TFC content of the control in *A. nilotica* was 23.89 μg/mg (21%), and the results showed that TFC content of plants that were exposed to T1 > T2 > T3 (20, 20, and 16%) decreased, respectively, while TFC content of T4 (23%) increased (Figure 5).

2.4.3. 2,2-Diphenyl-1-picryl hydrazyl Content. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) content of the control group in *A. jacquemontii* was 86.19 μg/mg (20%), and the results showed that DPPH content of plants showed no significant decrease or increase compared with that of the control plant. While DPPH content of the control in *A. nilotica*

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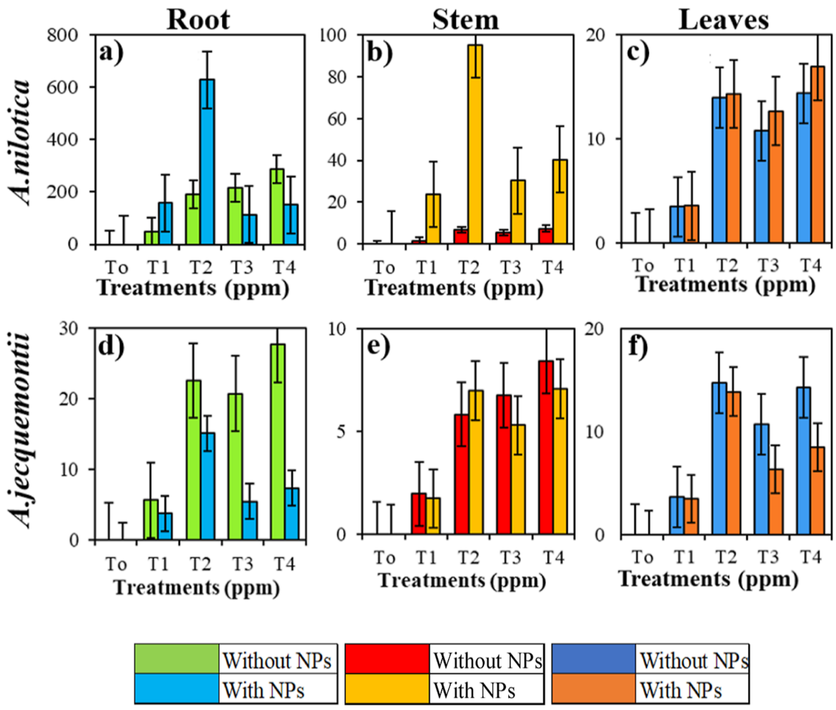

Figure 7. Illustration of Co_3O_4 Nbs influence under Zn ($\mu g\ kg^{-1}$) stress in root, stem, and leaf by atomic absorption spectroscopic analysis in (a−c) *A. nilotica* and (d−f) *A. jacquemontii*.

was 8269 μg/mg (20%), and the results showed that plants exposed to T1 > T2 > T3 > T4 (21, 20, 20, and 19%, respectively), exhibited increased DPPH content as compared to that in the control (Figure 5).

2.4.4. Total Alkaloid Content. The total alkaloid content (TAC) of the control group in *A. jacquemontii* was 131.63 μg/mg (18%), and the results presented that the TAC of the plants were significantly decreased by increase in the treatment level of NPs in the order T1 < T2 < T3 <T4 (19, 20, 21, and 22%), respectively (Figure 5). While the TAC of the control in *A. nilotica* was 174.25 μg/mg (22%), and the results exhibited a decrease in the TAC concentration up to the T3 level of applied NPs in the order T1 < T2 < T3 < T4 for plants that were exposed to 19, 20, 20, and 19%, respectively.

2.4.5. Total Reducing Potential. The total reducing potential (TRP) content of the control group in *A. jacquemontii* was 79.88 μg/mg (12%), and the results showed that the TRP contents were significantly increased from NP treatment levels of T1 > T2 > T3 > T4 (24, 23, 20, and 21%), respectively (Figure 5). While the TRP content of the control in *A. nilotica* was 87.80 μg/mg (14%), and the results revealed that TRP contents were increased in the order T1 < T2 < T3 < T4 (19, 25, 23, and 19%, respectively).

2.5. Chlorophyll Content. Chlorophyll *a* content of the control plant leaves in *A. jacquemontii* was calculated as 0.23 mg/L, and plants treated by different concentrations of cobalt nanoparticles as T1 > T2 > T3 > T4 (0.20, 0.26, 0.81, and 0.90) mg/L showed increased chlorophyll *a* content with respect to that in the control plant leaves (Figure 6a. Chlorophyll *b* content of the control plant leaves in *A. jacquemontii* was observed as 2.22 mg/L, and plants treated by different concentrations of Co_3O_4 Nbs as T1 > T2 > T3 > T4 (2.07, 2.17, 2.69, and 2.84), respectively, showed an increase in chlorophyll *b* contents with respect to that in the control plant leaves; the maximum increase (2.84 mg/L) in chlorophyll *b* was observed in plants that were treated with 100 ppm of Co_3O_4 Nbs as compared to the control plants (Figure 6b). The total chlorophyll contents of the control plant leaves in *A. jacquemontii* were estimated as 2.18 mg/L, and plants treated by different concentrations of Co_3O_4 Nbs as T1 > T2 > T3 > T4 (2.02, 2.1, 3.3, and 3.8 mg/L), respectively, showed an increase in total chlorophyll contents with respect to that in the control plant leaves; the maximum increase in total chlorophyll contents was estimated in plants that were treated by 100 ppm of Co_3O_4 Nbs as compared to that in the control plants (Figure 6c).

Chlorophyll *a* contents in the control plants of *A. nilotica* (T0) were 0.51 mg/L, and the plants treated by different concentrations of Co_3O_4 Nbs showed an increase in the order T1 > T2 > T3 > T4 (2.67, 2.89, 3.10, and 3.23 mg/L), respectively, in chlorophyll *a* contents as compared to that in the control plants (Figure 6e). The plants treated by 100 ppm of Co_3O_4 Nbs showed the maximum increase, 3.23 mg/L, in chlorophyll *a* contents as compared to that in the control plants. Chlorophyll *b* contents of the control plant leaves in *A. nilotica* were estimated as 2.62 mg/L, and plants treated by different treatment levels of Co_3O_4 Nbs as T1 > T2 > T3 > T4 (2.67, 2.66, 2.43, and 2.1 mg/L, respectively) showed an increase in chlorophyll *b* contents with respect to that in the control plant leaves; a maximum increase in chlorophyll *b* was observed in plants that were treated with 25 ppm of Co_3O_4 Nbs as compared to that in the control (Figure 6f). The total chlorophyll contents of the control plant leaves in *A. nilotica* were estimated as 2.86 mg/L, and plants treated by different levels of Co_3O_4 Nbs as T1 > T2 > T3 > T4 (3.09, 3.18, 2.89, and 2.23 mg/L, respectively), presented an increase in total chlorophyll contents with respect to that in the control plant leaves. An extreme increase in total chlorophyll contents was detected in plants that were treated by 50 ppm of Co_3O_4 Nbs as compared to that in the control plants (Figure 6g).

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ACS Omega 2023, 8, 5836−5849

2.6. Atomic Absorption Spectroscopic Analysis. Maximum absorption of zinc metal was observed in plant roots that were treated with 100 ppm (T4) of metal as compared to the control plants in *A. nilotica*. While maximum absorption of Nbs was observed in plants that were treated by 50 ppm of cobalt Nbs along with zinc when compared with the control. 65% (μg kg^{-1}) increase in absorption of zinc was calculated in plants that were treated with Nbs (Figure 7a. Maximum absorption of zinc was calculated in stems that were treated with 75 ppm (T3) of metal when compared with the control plant, while maximum translocation of zinc was calculated in stems that were treated with 50 ppm (T2) of cobalt Nbs along with the same concentration of metal (Figure 7b). The translocation of zinc from root to stem was 55% (μg kg^{-1}) reduced in plants treated with 50 ppm of zinc metal. Maximum increase in absorption of zinc metal was observed in the leaf of *A. nilotica* when compared to the control plants (Figure 7c). Plants treated with 50 and 100 ppm showed maximum translocation of zinc in the leaves, while maximum translocation of Nbs was calculated in plants treated with 100 ppm of cobalt Nbs along with zinc. The results showed that there was about 45% (μg kg^{-1}) decrease in the translocation of zinc from root toward leaf due to the application of cobalt Nbs as a foliar spray. Zn concentrations accumulated in *Acacia* shoots were lower than those reported, ranging from 131 to 425 mg/kg under the effect of various absorbing agents and different treatment levels of zinc. Cultivating *Acacia* plants to clean the contaminated soil of heavy metals, especially Zn, was confirmed by the results obtained. The maximum translocation of zinc was estimated in plants that were treated with 50 ppm (T2) when compared to the control plants in *A. jacquemontii* roots, while maximum translocation of Zn was estimated in plant roots that were treated with 100 ppm (T4) along with cobalt Nbs applied to plants as compared to the control plants (Figure 7d). There was 37% (μg kg^{-1}) increase in the translocation of zinc metal in the roots of *A. jacquemontii*. The maximum increase in the absorption of zinc in stems was observed in plants that were treated with 75 and 100 ppm (T3 and T4) when compared to the control plants (Figure 7e). While there was maximum translocation of zinc observed in plants treated with 100 ppm of cobalt Nbs when compared to the control plants. Maximum translocation of zinc was observed in leaves of *A. jacquemontii* when treated with 50 ppm of cobalt Nbs as a foliar spray. The estimated translocation in leaves was 42% (μg kg^{-1}) increased as compared to that in roots and stems of plants (Figure 7f).

3. MATERIALS AND METHODS

3.1. Reagents and Chemicals. All chemicals and reagents for the biological synthesis of Co_3O_4 Nbs such as $CoCl_2$ hexahydrate ($CoCl_2 \cdot 6H_2O$), *C. myxa*, ethanol (CH_3CH_2OH), methanol (CH_3OH), sodium hydroxide (NaOH), and DPPH were purchased from Sigma Company.

3.2. Preparation of Plant Extract. The leaves of *C. myxa* were collected from the trees found in Lal Suhanra National Park, Bahawalpur, Pakistan. The leaves were washed with water to remove debris and air dried for 7 days. Then, the dried leaves were ground with an electric grinder to make a refined powder. The aqueous extract of leaves was prepared by mixing 20 g of crushed leaves with 100 mL of distilled water in a 250 mL conical flask and boiling it on a hot plate at 80 °C for 4 h with constant stirring. The extract of leaves was filtered by using Whatman filter paper for eliminating particulate material and to get a clear solution and stored at 4 °C for synthesis of nanoparticles by the biological method.

3.3. Green Synthesis of Co_3O_4 Nbs. The synthesis of Co_3O_4 Nbs was carried out by adding 10 mL of aqueous extract of *C. myxa* in a 5 mM solution of (90 mL) $CoCl_2$ with pH 5. Later, 400 mL distilled water and 20 mL ethanolic plant extract were added to 47.36 g of $CoCl_2$ in a reagent bottle and heated at 70 °C for 4 h on a hot plate with constant stirring (model, MSH-20A). After 4 h, the solution turned dark brown which confirmed the Co_3O_4 Nbs formation. The well-developed Co_3O_4 Nbs were gained by centrifugation (6000 rpm) for at least 20 min, and the thus-obtained Co_3O_4 Nbs pellet were separated and dried in a hot air oven for 24 h at 80 °C. Black colored powder of Co_3O_4 Nbs was formed as a final product.

3.4. Characterization Studies of Co_3O_4 Nbs. *3.4.1. UV–Visible Spectrometry.* UV–visible spectrometry was carried out for the confirmed synthesis of Co_3O_4 Nbs by the green synthesis method, and reduction of cobalt ions and their validation were monitored by constant sampling of aliquots from time to time for the measurement. The spectra were recorded after 30 min difference of all aliquots as a function of time retortion on a UV spectrophotometer (Epoch-BioTek Instruments USA) having the range of 300–700 nm.

3.4.2. XRD Studies. To govern the size and structural properties of the Co_3O_4 Nbs crystals, diffracted intensities of the dried powder of Co_3O_4 Nbs were recorded at 40 kV and 30 mA current at 0.3 s per count with a 20–80 scanning range by using an X-ray diffractometer (D8, Advance, Germany).

3.4.3. Scanning Electron Microscopy. The morphological and microstructure of the prepared nanoballs were detected by SEM (Quanta 250, 30 kV, FEI, Czech Republic, magnification up to 2000 times).

3.5. Experimental Design. Seeds of two selected plant species *A. nilotica* and *A. jacquemontii* were collected from Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. The seeds were treated with a solution of 1% sodium hypochlorite and then washed several times with distilled water. Plastic pots possessing a diameter of 15 cm and a length of 24 cm were filled with homogenized soil. There were 10 seeds in each pot during the sowing season from January to March 2022. The heavy metal zinc was applied to the soil having the same concentration as Co_3O_4 Nbs. When seedlings emerged, application of the treatment in different groups with intervals of zinc along with Co_3O_4 Nbs as a foliar spray was done after 10 days; the untreated plants were given distilled water only, and these plants served as controls. Samples were collected for analysis after 30 days of germination. The overall design was as follows. As the control T0 odd mean of positive and negative control without Nbs treatment, treatment T1 having 25 ppm Co_3O_4 Nbs + Zn, treatment T2 with 50 ppm Co_3O_4 Nbs + Zn, treatment T3 with 75 ppm Co_3O_4 Nbs + Zn, and treatment T4 having 100 ppm Co_3O_4 Nbs + Zn.

3.6. Determination of Morphological Parameters. After the completion of treatments, plants were harvested and washed with distilled water, and their morphological parameters such as plant height (cm), root length (cm), and shoot length (cm) were measured by using a scale; number of leaves and number of branches of the plants were counted visually. The fresh weight of plants was noted by using a digital weight balance, and then plant samples were placed in the oven at 70 °C for 3 days to obtain the dry weight.

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ACS Omega 2023, 8, 5836–5849

3.7. Estimation of Photosynthetic Pigments. Chlorophyll *a* and chlorophyll *b* contents in the leaves of Co_3O_4 Nbs-treated plants were measured. The leaves were extracted in acetone (80%) and after filtration of prepared samples, the absorbance was measured at 663 and 645 nm by a spectrophotometer. The observations were noted by using the following formula

$$\text{Chl } a = [12.7\ (A \text{ at } 663) - 2.69\ (A \text{ at } 645)] \times V/1000W\ (\text{mg}\cdot\text{g}^{-1})$$

$$\text{Chl } b = [22.9\ (A \text{ at } 645) - 4.68\ (A \text{ at } 663)] \times V/\ 1000\ W\ (\text{mg}\cdot\text{g}^{-1})$$

where V is the volume of supernatant (25 mL), W is the weight of leaves used for extraction of pigment (0.25 g), and A is the absorbance.

The chlorophyll contents of the leaf sample were expressed as mg/g of fresh leaf. Chlorophyll *a* and *b* ratio = chlorophyll *a*/chlorophyll *b*.

3.8. Estimation of Antioxidants. The DPPH method was used to test the radical scavenging potential of green synthesized cobalt nanoparticles as well as of the plant extract by the spectrophotometric technique. DPPH solution was prepared by adding 6.7 mg of DPPH to 20 mL of methanol. Briefly, to 70 mL of DPPH solution in a 96-well plate, 10 mL of the green synthesized Co_3O_4 Nbs (25, 50, 75, and 100 mg/mL) and plant extract were added separately. After incubation for 1 h, the absorbance was checked at 517 nm. A decreasing amplitude of the signal at the selected wavelength confirmed a high radical scavenging activity. Ascorbic acid was taken as the positive standard, while DPPH solution without samples was the negative control. The percent scavenging of DPPH was precalculated by using the following formula

$$\%\ \text{scavenging} = [\text{ABc} - \text{ABs}/\text{ABc}\}] \times 100$$

where ABs and ABc denote absorbance of the sample and absorbance of the control, respectively. Extracts of samples were prepared to estimate the TFC and TPC. Leaf samples (0.2 g each) were ground in methanol (16 mL) having 1% HCl at 24 °C for 24 h. The procedure was repeated twice, and methanol extract was collected after centrifugation at 4000 rpm. The diluted extract (0.5 mL) was moved to polypropylene tubes consisting of 5% $NaNO_3$ (0.15 mL) and 2 mL of distilled water. After 5 min, 10% $AlCl_3\cdot 6H_2O$ (0.5 mL) was added extra, followed by the addition of 1 mL of (1 M) NaOH. After 15 min, the absorbance was taken at 415 nm. Measurements were made by comparison with the standard curve of gallic acid. Fresh plant leaf samples were taken and ground with liquid nitrogen and standardized with phosphate buffer (0.5 M, pH 7.8) and filtered. The mixture was centrifuged at 4 °C at 12,000*g* for 10 min and then the supernatant was collected. This supernatant was used for additional analyses.

3.9. Determination of Heavy Metals. Plant samples (0.5 g) were dried, crushed, and digested with acids, including sulfuric acid and hydrogen peroxide (3:1), in digestion tubes. The overnight digested samples were placed on an electrical hot plate for 1 h at 80 °C. After that, the temperature was increased to 120 ± 10 °C. The same process was repeated two to three times until the samples turned to a transparent solution. The transparent solution was filtered and collected in an acid-washed volumetric flask, and distilled water was added to it to make up the volume to 50 mL. The solution was subjected to atomic absorption spectrophotometry (AAS, Germany) as previously described. The bioaccumulation factor was calculated by using the following formula: bioaccumulation factor = metal in root dry weight DW/metal in soil dry weight DW.

3.10. Statistical Analysis. Statistical analysis of data was performed by using two-way ANOVA suited for CRD and correlation coefficient. Mean values were compared using Statistic 8.1 (Analytical Software, Cary, NC, USA). A significant difference at $P \leq 0.05$ among treatments was evaluated by using Tukey's honest significant difference (HSD).

4. CONCLUSIONS

Pollution by heavy metals has been an excessive fear in the last few decades because of their health hazards to man and other organisms when tranquil within a biological system. It is evident that phytoremediation assists in restoring a stressed environment, but it is important to continue with caution. This study demonstrated the potential of two selected *Acacia* species to remediate Zn-polluted soil. These plant species generally have the highest accumulation of Zn in their roots, meaning that the efficiency of this plant in cleaning the polluted soils is at the root stages of its growth. We successfully synthesized cobalt nanoparticles with an average size of 25 nm by using *C. myxa*. The synthesized Nbs show a remarkable response against Zn stress imposed on plant growth by alleviating the stress. Therefore, this equivalent assessment determined that the green synthesized cobalt Nbs were cost-effective, inexpensive, energy efficient, and biologically more useful. Therefore, the present work is convenient and helpful for the applications for enhancing the plant growth and removal of heavy-metal effects in plants. The present findings suggest that *Ajacquemontii* and *A nilotica* can naturally tolerate and absorb higher levels of the studied metal in their various parts and can be used for phytoremediation in contaminated soil. Further study is recommended to screen these plants for other heavy metals.

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■ REFERENCES

(1) Raj, S.; Roodbar, S.; Brinkley, C.; Wolfe, D. W. Food Security and Climate Change: Differences in Impacts and Adaptation Strategies for Rural Communities in the Global South and North. *Front. Sustain. Food Syst.* **2022**, *5*, 691191.

(2) Ramankutty, N.; Mehrabi, Z.; Waha, K.; Jarvis, L.; Kremen, C.; Herrero, M.; Rieseberg, L. H. Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. *Annu. Rev. Plant Biol.* **2018**, *69*, 789.

(3) Salih, A.; Salih, N. A.; Mohameed, M. O. EXTRACTION OF 1,2-BUTADIENE FROM THE ACACIA PLANT AND A STUDY OF ITS EFFECTS ON BREAST CANCER. *Biochem. Cell. Arch.* **2020**, *20*, 5049.

(4) Panya, A.; Yongpitakwattana, P.; Budchart, P.; Sawasdee, N.; Kronthong, S.; Paemanee, A.; Roytrakul, S.; Rattanabunyong, S.; Choowongkomon, K.; Yenchitsomanus, P. Novel bioactive peptides demonstrating anti-dengue virus activity isolated from the Asian medicinal plant Acacia Catechu. *Chem. Biol. Drug Des.* **2019**, *93*, 100.

(5) Hadii, K. F. Control of damping-off and root rot diseases of acacia tree using some biological control agents and plant extracts. *Res. Crops* **2020**, *21*, 160.

(6) Thomas, S. K.; Begum, P. M. S.; Dominic, C. D. M.; Salim, N. V.; Hameed, N.; Rangappa, S. M.; Siengchin, S.; Parameswaranpillai, J. Isolation and characterization of cellulose nanowhiskers from Acacia caesia plant. *J. Appl. Polym. Sci.* **2021**, *138*, 50213.

(7) Hendrik, A. C.; Daud, Y. Survival of Acacia leucophloea and Pterocarpus indicus Under Canopy of Acacia nilotica as Nurse Plants. *J. Penelit. Kehutanan Wallacea* **2020**, *9*, 63.

(8) Amutha, V.; Senthilkumar, B. Physical, Chemical, Thermal, and Surface Morphological Properties of the Bark Fiber Extracted from Acacia Concinna Plant. *J. Nat. Fibers* **2021**, *18*, 1661.

(9) Ribeiro, A. S.; Costa, S. M.; Ferreira, D. P.; Calhelha, R. C.; Barros, L.; Stojković, D.; Sokovic, M.; Ferreir, I. C. F. R.; Fangueiro, R. Chitosan/nanocellulose electrospun fibers with enhanced antibacterial and antifungal activity for wound dressing applications. *React. Funct. Polym.* **2021**, *159*, 104808.

(10) Dubey, S.; Shri, M.; Gupta, A.; Rani, V.; Chakrabarty, D. Toxicity and detoxification of heavy metals during plant growth and metabolism. *Environ. Chem. Lett.* **2018**, *16*, 1169.

(11) Morkunas, I.; Woźniak, A.; Mai, V. C.; Rucińska-Sobkowiak, R.; Jeandet, P. The role of heavy metals in plant response to biotic stress. *Molecules* **2018**, *23*, 2320.

(12) Sultana, B.; Anwar, F.; Przybylski, R. Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. trees. *Food Chem* **2007**, *104*, 1106−1114.

(13) Shikha, D.; Singh, P. K. In situ phytoremediation of heavy metal-contaminated soil and groundwater: a green inventive approach. *Environ. Sci. Pollut. Res.* **2021**, *28*, 4104.

(14) Chibuike, G. U.; Obiora, S. C. Heavy metal polluted soils: Effect on plants and bioremediation methods. *Appl. Environ. Soil Sci.* **2014**, *2014*, 9627.

(15) He, K.; Sun, Z.; Hu, Y.; Zeng, X.; Yu, Z.; Cheng, H. Comparison of soil heavy metal pollution caused by e-waste recycling activities and traditional industrial operations. *Environ. Sci. Pollut. Res.* **2017**, *24*, 9387.

(16) Sadh, P. K.; Duhan, S.; Duhan, J. S. Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresour. Bioprocess* **2018**, *5*, 1.

(17) Siddiqui, S. DNA damage in Cicer plant grown on soil polluted with heavy metals. *J. King Saud Univ., Sci.* **2015**, *27*, 217.

(18) Dey, U.; Mondal, N. K. Ultrastructural deformation of plant cell under heavy metal stress in Gram seedlings. *Cogent Environ. Sci.* **2016**, *2*, 1196472.

(19) Atici, Ö.; Ağar, G.; Battal, P. Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. *Biol. Plants* **2005**, *49*, 215.

(20) Mathur, S.; Kalaji, H. M.; Jajoo, A. Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. *Photosynthetica* **2016**, *54*, 185.

(21) Stambulska, U. Y.; Bayliak, M. M.; Lushchak, V. I. Chromium(VI) toxicity in legume plants: Modulation effects of rhizobial symbiosis. *BioMed Res. Int.* **2018**, *2018*, 8031213.

(22) Stanislawska-Glubiak, E.; Korzeniowska, J. Effect of Salicylic Acid Foliar Application on Two Wheat Cultivars Grown under Zinc Stress. *Agronomy* **2022**, *12*, 60.

(23) Quaglia, M.; Troni, E.; D'Amato, R.; Ederli, L. Effect of zinc imbalance and salicylic acid co-supply on Arabidopsis response to fungal pathogens with different lifestyles. *Plant Biol.* **2022**, *24*, 103922.

(24) Moreira, J. R.; Leandro, M. R.; Vespoli, L. D.; Andrade, L. F.; Pimentel, V. R.; Soares, F. S.; de Souza, S. A.; Intorne, A. C.; Silveira, V.; de Souza Filho, G. A. Essential role of extracytoplasmic proteins in the resistance of Gluconacetobacter diazotrophicus to cadmium. *Res. Microbiol.* **2022**, *173*, 103922.

(25) Chung, S.; Kwon, C.; Lee, J. H. Epigenetic control of abiotic stress signaling in plants. *Genes Genomics* **2022**, *44*, 267.

(26) Zafar, A.; Tuba, T.; Hasan, M.; Nazar, M.; Rasheed, M. N.; Mahmood, N.; Shu, X. Green-maturation of Cobalt-Oxide nano-sponges for reinforced bacterial apoptosis. *Colloids Interface Sci. Commun.* **2021**, *45*, 100531.

(27) Hasan, M.; Mehmood, K.; Mustafa, M.; Zafar, Z.; Tariq, T.; Hassan, G. H.; Loomba, L.; Zia, Z.; Mazher, M.; Mahmood, M.; Shu, X. Phytotoxic evaluation of phytosynthesized silver nanoparticles on lettuce. *Coatings* **2021**, *11*, 225.

(28) Hasan, M.; Rafique, R.; Zafar, Z.; Loomba, L.; Khan, K.; Hassan, G. H.; Khan, W. K.; Zahra, Z.; Zia, Z.; Mustafa, M.; Shu, X.; Ihsan, I.; Mahmood, M. Physiological and anti-oxidative response of biologically and chemically synthesized iron oxide: Zea mays a case study. *Heliyon* **2020**, *6*, No. e04595.

(29) Zulfiqar, F.; Navarro, M.; Ashraf, M.; Akram, N. A.; Munné-Bosch, S. Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Plant Sci.* **2019**, *289*, 110270.

(30) Mejias, J. H.; Salazar, F.; Pérez Amaro, S.; Hube, M.; Rodriguez, M.; Alfaro, M. Nanofertilizers: A Cutting-Edge Approach to Increase Nitrogen Use Efficiency in Grasslands. *Front. Environ. Sci.* **2021**, *9*, 52.
(31) Kalwani, M.; Chakdar, H.; Srivastava, A.; Pabbi, S.; Shukla, P. Effects of nanofertilizers on soil and plant-associated microbial communities: Emerging trends and perspectives. *Chemosphere* **2022**, *287*, 132107.
(32) Toksha, B.; Sonawale, V. A. M.; Vanarase, A.; Bornare, D.; Tonde, S.; Hazra, C.; Kundu, D.; Satdrive, A.; Tayde, S.; Chatterjee, A. Nanofertilizers: A review on synthesis and impact of their use on crop yield and environment. *Environ. Technol. Innovation* **2021**, *24*, 101986.
(33) Chaudhry, N.; Dwivedi, S.; Chaudhry, V.; Singh, A.; Saquib, Q.; Azam, A.; Musarrat, J. Bio-inspired nanomaterials in agriculture and food: Current status, foreseen applications and challenges. *Microb. Pathog.* **2018**, *123*, 196.
(34) Ioannou, A.; Gohari, G.; Papaphilippou, P.; Panahirad, S.; Akbari, A.; Dadpour, M. R.; Krasia-Christoforou, T.; Fotopoulos, V. Advanced nanomaterials in agriculture under a changing climate: The way to the future? *Environ. Exp. Bot.* **2020**, *176*, 104048.
(35) Cinisli, K. T.; Uçar, S.; Dikbaş, N. Use of nanomaterials in agriculture. *Yuz. Yil Univ. J. Agric. Sci.* **2019**, *29*, 817.
(36) Kaphle, A.; Navya, P. N.; Umapathi, A.; Daima, H. K. Nanomaterials for agriculture, food and environment: applications, toxicity and regulation. *Environ. Chem. Lett.* **2018**, *16*, 43.
(37) Hasan, M. I.; Ullah, H.; Zulfiqar, K.; Naeem, A.; Iqbal, H.; Gul, M.; Ashfaq, N.; Mahmood, N. Biological entities as chemical reactors for synthesis of nanomaterials: Progress, challenges and future perspective. *Mater. Today Chem.* **2018**, *8*, 13.
(38) Zulfiqar, H. Z.A.; Zafar, M. N.; Rasheed, Z.; Ali, K.; Mehmood, A.; Mazher, M.; Hasan, N.; Mahmood, N. Synthesis of silver nanoparticles using Fagonia cretica and their antimicrobial activities. *Nanoscale Adv.* **2019**, *1*, 1707.
(39) Luo, F. M.; Wang, L.; Huang, Z.; Wu, W.; Wang, A.; Zafar, Y.; Tian, M.; Hasan, X.; Shu, X. Synthesis of Zinc Oxide Eudragit FS30D Nanohybrids: Structure, Characterization, and Their Application as an Intestinal Drug Delivery System. *ACS Omega* **2020**, *5*, 11799.
(40) Hasan, M. Z. A.; Zafar, I.; Shahzadi, F.; Luo, S. G.; Hassan, T.; Tariq, S.; Zehra, T.; Munawar, F.; Iqbal, X.; Shu, X. Fractionation of biomolecules in Withania coagulans extract for bioreductive nanoparticle synthesis, antifungal and biofilm activity. *Molecules* **2020**, *25*, 3478.
(41) Saif, M. S. A.; Zafar, M.; Waqas, S. G.; Hassan, A.; Haq, T.; Tariq, S.; Batool, M.; Dilshad, M.; Hasan, X.; Shu, X. Phyto-reflexive Zinc Oxide Nano-Flowers synthesis: An advanced photocatalytic degradation and infectious therapy. *J. Mater. Res. Technol.* **2021**, *13*, 2375.
(42) Abbas, S. F.; Ali, I. N.; Kareem, S. H.; Muhammed, S. A. E. Green synthesis of copper nanoparticles from Cordia myxa L. leaves ethanol extract and their antibacterial activity. *AIP Conf. Proc.* **2022**, *2386*, 020028.
(43) Al-Musawi, M. H.; Ibrahim, K. M.; Albukhaty, S. In vitro study of antioxidant, antibacterial, and cytotoxicity properties of Cordia myxa fruit extract. *Iran. J. Microbiol* **2022**, *14*, 97.
(44) Wei, P.; Liang, J.; Liu, Q.; Xie, L.; Tong, X.; Ren, Y.; Li, T.; Luo, Y.; Li, N.; Tang, B.; Asiri, A. M.; Hamdy, M. S.; Kong, Q.; Wang, Z.; Sun, X. Iron-doped cobalt oxide nanoarray for efficient electrocatalytic nitrate-to-ammonia conversion. *J. Colloid Interface Sci.* **2022**, *615*, 636.
(45) Li, J.; Zhao, D.; Zhang, L.; Yue, L.; Luo, Y.; Liu, Q.; Li, N.; Alshehri, A. A.; Hamdy, M. S.; Li, Q.; Sun, X. A FeCo2O4 nanowire array enabled electrochemical nitrate conversion to ammonia. *Chem. Commun.* **2022**, *58*, 4480.
(46) Chen, D.; Pan, L.; Pei, P.; Shangwei, H.; Peng, R.; Xin, S. Carbon-coated oxygen vacancies-rich Co3O4 nanoarrays grow on nickel foam as efficient bifunctional electrocatalysts for rechargeable zinc-air batteries. *Energy* **2021**, *224*, 120142.
(47) Li, Z.; Jie, L.; Qian, L.; Lisi, X.; Longcheng, Z.; Yuchun, R.; Luchao, Y.; Na, L.; Bo, T.; Abdulmohsen, A. A.; Mohamed, S. H.; Yonglan, L.; Qingquan, K.; Xuping, S. High-efficiency ammonia electrosynthesis via selective reduction of nitrate on ZnCo2O4 nanosheet array. *Mater. Today Phys.* **2022**, *23*, 100619.
(48) Liu, Q.; Xie, L.; Liang, J.; Ren, Y.; Wang, Y.; Zhang, L.; Yue, L.; Li, T.; Luo, Y.; Li, N.; Tang, B.; Liu, Y.; Gao, S.; Alshehri, A. A.; Shakir, I.; Agboola, P. O.; Kong, Q.; Wang, Q.; Ma, D.; Sun, X. Ambient Ammonia Synthesis via Electrochemical Reduction of Nitrate Enabled by NiCo2O4 Nanowire Array. *Small* **2022**, *18*, 2106961.
(49) Hasan, M. Z.; Teng, I.; Iqbal, A.; Awan, S.; Meng, R.; Dai, Q.; Qing, D.; Deng, Y. Assessment of bioreducing and stabilizing potential of dragon's blood (dracaena cochinchinensis, Lour. S. C. Chen) resin extract in synthesis of silver nanoparticles. *Nanosci. Nanotechnol. Lett.* **2013**, *5*, 780.
(50) Hasan, M. Z. A.; Zafar, M.; Yousaf, H.; Gulzar, K.; Mehmood, S. G.; Hassan, A.; Saeed, A.; Yousaf, A.; Mazher, D.; Rongji, N.; Mahmood, N. Synthesis of Loureirin B-Loaded Nanoliposomes for Pharmacokinetics in Rat Plasma. *ACS Omega* **2019**, *4*, 6914.
(51) Hasan, M. M.; Altaf, A.; Zafar, Z.; Hassan, T.; Ali, M. S.; Mustafa, F.; Munawar, M. W.; Saif, G.; Tariq, A.; Iqbal, N.; Khan, X.; Mahmood, A.; Mahmood, N.; Shu, X. Bioinspired synthesis of zinc oxide Nano-flowers: A surface enhanced antibacterial and harvesting efficiency. *Mater. Sci. Eng. C* **2021**, *119*, 111280.
(52) Qasim, S.; Zafar, A.; Saif, M. S.; Ali, Z.; Nazar, M.; Waqas, M.; Haq, A. U.; Tariq, T.; Hassan, S. G.; Iqbal, F.; Shu, X. G.; Hasan, M. Green synthesis of iron oxide nanorods using Withania coagulans extract improved photocatalytic degradation and antimicrobial activity. *J. Photochem. Photobiol., B* **2020**, *204*, 111784.
(53) Munawar, T. Y.; Yasmeen, H.; Hasan, M.; Mahmood, H.; Hussain, A.; Ali, M. I. I.; Arshad, M. I.; Iqbal, F. Novel tri-phase heterostructured ZnO-Yb2O3-Pr2O3 nanocomposite; structural, optical, photocatalytic and antibacterial studies. *Ceram. Int.* **2020**, *46*, 11101.
(54) Farid, M. A.; Rafique, M. Y.; Irfan, M.; Hassan, S. ul; Attia, F.; Murtaza, H. Structural, electrical and dielectric properties of pyrochlore LaCrZr2-xGexO7 nanospheres. *J. Nanosci. Nanotechnol.* **2017**, *17*, 5740.
(55) Luo, F. W.; Wang, M.; Chen, Z.; Zheng, D.; Zeng, M.; Hasan, Z.; Fu, X.; Shu, X. Synthesis and efficacy of the n-carbamoylmethionine copper on the growth performance, tissue mineralization, immunity, and enzymatic antioxidant capacity of nile tilapia (oreochromis niloticus). *ACS Omega* **2020**, *5*, 22578.
(56) Dang, H. H.; Meng, M.; Zhao, Z.; Iqbal, I.; Dai, D.; Deng, D.; Lv, L. Luteolin-loaded solid lipid nanoparticles synthesis, characterization, & improvement of bioavailability, pharmacokinetics in vitro and vivo studies. *J. Nanopart. Res.* **2014**, *16*, 2347.
(57) Lv, F.; Hasan, M.; Dang, H.; Hassan, S. G.; Meng, W.; Deng, Y.; Dai, R. Optimized luteolin loaded solid lipid nanoparticle under stress condition for enhanced bioavailability in rat plasma. *J. Nanosci. Nanotechnol.* **2016**, *16*, 9443.
(58) Huang, L.; Chen, R.; Luo, J.; Hasan, M.; Shu, X. Synthesis of phytonic silver nanoparticles as bacterial and ATP energy silencer. *J. Inorg. Biochem.* **2022**, *231*, 111802.
(59) Mustafa, G.; Hasan, M.; Yamaguchi, H.; Hitachi, K.; Tsuchida, K.; Komatsu, S. A comparative proteomic analysis of engineered and bio synthesized silver nanoparticles on soybean seedlings. *J. Proteonomics* **2020**, *224*, 103833.
(60) Hasan, M. W.; Yang, Y.; Ju, X.; Chu, Y.; Wang, Y.; Deng, N.; Mahmood, Y.; Hou, Y. Biocompatibility of iron carbide and detection of metals ions signaling proteomic analysis via HPLC/ESI-Orbitrap. *Nano Res.* **2017**, *10*, 1912.
(61) Munawar, T.; Nadeem, M. S.; Mukhtar, F.; Manzoor, S.; Ashiq, M. N.; Batool, S.; Hasan, M.; Iqbal, F. Enhanced photocatalytic, antibacterial, and electrochemical properties of CdO-based nanostructures by transition metals co-doping. *Adv. Powder Technol.* **2022**, *33*, 103451.

(62) Luo, F.; Zeng, D.; Chen, R.; Zafar, A.; Weng, L.; Wang, W.; Tian, Y.; Hasan, M.; Shu, X. PEGylated dihydromyricetin-loaded nanoliposomes coated with tea saponin inhibit bacterial oxidative respiration and energy metabolism. *Food Funct.* **2021**, *12*, 9007.

(63) Wu, Z.; Ren, Y.; Liang, Y.; Huang, L.; Yang, Y.; Zafar, A.; Hasan, M.; Yang, F.; Shu, X. Synthesis Characterization, Immune Regulation, and Antioxidative Assessment of Yeast-Derived Selenium Nanoparticles in Cyclophosphamide-Induced Rats. *ACS Omega* **2021**, *6*, 24585.

(64) Chen, Q.; Zhang, X.; Liu, Y.; Wei, J.; Shen, W.; Shen, Z.; Cui, J. Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity; suppressed metal uptake and oxidative stress in rice seedlings. *Plant Growth Regul.* **2017**, *81*, 253.

(65) Pant, P. P.; Tripathi, A. K. Impact of heavy metals on morphological and biochemical parameters of shorea robusta plant. *Ekologia* **2014**, *33*, 116−126.

(66) Kumari, S.; Khanna, R. R.; Nazir, F.; Albaqami, M.; Chhillar, H.; Wahid, I.; Khan, M. I. R. Bio-Synthesized Nanoparticles in Developing Plant Abiotic Stress Resilience: A New Boon for Sustainable Approach. *Int. J. Mol. Sci.* **2022**, *23*, 4452.

(67) Liu, R.; Zhang, H.; Lal, R. Effects of Stabilized Nanoparticles of Copper, Zinc, Manganese, and Iron Oxides in Low Concentrations on Lettuce (Lactuca sativa) Seed Germination: Nanotoxicants or Nanonutrients? *Water, Air, Soil Pollut.* **2016**, *227*, 42.

(68) Di Baccio, D.; Tognetti, R.; Sebastiani, L.; Vitagliano, C. Responses of Populus deltoides × Populus nigra (Populus × euramericana) clone I-214 to high zinc concentrations. *New Phytol.* **2003**, *159*, 443−452.

(69) Kapoor, N.; Pande, V. Effect of salt stress on growth parameters, moisture content, relative water content and photosynthetic pigments of fenugreek variety RMt-1. *J. Plant Sci.* **2015**, *10*, 210−221.

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