Adsorption (2024) 30:1419–1435
https://doi.org/10.1007/s10450-024-00511-5

Nano-water hyacinth protein adsorbent as soil amendment alleviates cadmium stress in common bean seedlings by improving soil enzymes and mitigating oxidative stress

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Received: 8 May 2024 / Revised: 5 June 2024 / Accepted: 6 June 2024 / Published online: 9 July 2024

Abstract
Heavy metal pollution is a serious environmental problem. Most of the current techniques used to mitigate the toxic effects of heavy metals have limitations. This creates an urgent need to explore safer and more efficient methods to address these toxic effects. This study investigates the potential of nano-water hyacinth protein (nano-WHP) as an adsorbent and soil amendment to mitigate cadmium pollution. Nano-WHP is derived from water hyacinth protein and immobilized on nano-chitosan. The Cd adsorption capacity and removal efficiency of nano-WHP were determined. Nano-WHP was applied as a soil amendment to examine its impact on soil enzyme activity and the growth of common bean plants under Cd stress. Nano-WHP could remove 96% of Cd with an adsorption capacity of 150 mg Cd g^{-1}. When used as a soil amendment under Cd stress, nano-WHP positively influenced soil enzyme activity, enhancing soil health and promoting the growth of common bean plants. The growth of nano-WHP-treated plants increased by approximately 35% and 50% in the first and second stages, respectively, compared to the control group under cadmium stress. Furthermore, nano-WHP significantly reduced oxidative stress markers such as lipid peroxidation, DNA oxidation, protein oxidation, and H_2O_2 levels, with reductions of about 90.63%, 85.13%, 79.35%, and 81.85%, respectively, compared to untreated plants. This reduction in oxidative stress markers is attributed to the lower availability of Cd and the heightened activity of the antioxidant machinery in nano-WHP-treated plants. These results establish a foundation for the formulation of sustainable and economically feasible methodologies to mitigate Cd contamination.

Keywords DNA oxidation · Lipid peroxidation · Nano-chitosan · Protein oxidation

1 Introduction

Abiotic stress significantly impacts plants in their environment, adversely affecting plant growth and global crop productivity. Heavy metal stress, along with salt and drought, is particularly dangerous, inducing various negative effects at the cellular, physiological, and molecular levels. The toxicity of crops to heavy metals not only hampers crop production but also poses threats to human health and the ecosystem [32]. Among heavy metals, cadmium (Cd) is considered the most toxic, exhibiting toxicity in both high and low concentrations [39].

Cadmium's high solubility in water facilitates its entry into plant cells through the water stream. Additionally, it competes with essential elements like potassium (K), calcium (Ca), and iron (Fe) on transmembrane transporters, significantly reducing the absorption rate of these essential elements in the presence of higher Cd concentrations [72].

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Oxidative stress represents one of the most toxic effects of cadmium on plant cells, as it generates reactive oxygen species (ROS) that subsequently deteriorate macro-biological molecules such as lipids, proteins, and nucleic acids [73]. Activating the antioxidant machinery can shield plants from the toxic effects of ROS induced by heavy metals [26].

Various strategies have been explored to enhance plant tolerance against heavy metal stress, including the use of hyperaccumulating species, plant growth-promoting rhizobacteria, plant hormones, and biochar [27, 39]. In recent years, the use of biochar and other soil amendments has gained popularity to address Cd pollution. The main goal is to chelate cadmium and convert it into an unavailable form, mitigating its toxic effects on soil microbial communities and plants [40, 42, 54, 91, 98]. The efficiency of biochar depends on its components, especially metal-chelating proteins such as phytochelatins and metallothioneins, which chelate heavy metals and sequester them into an unavailable form [5].

Water hyacinth (*Eichhornia crassipes*) is a pervasive invasive aquatic herb with abundant biomass. While its mechanical removal is performed to mitigate challenges in water bodies, the biomass poses ecological hazards if not repurposed effectively [35]. Water hyacinth biomass holds potential for applications such as animal feedstock, biofuels, biochar, and compost [43, 57]. Notably, water hyacinth biochar has gained attention for its efficacy in heavy metal adsorption from aqueous solutions [17, 20, 61, 70].

Water hyacinth biochar has been introduced as a soil amendment to enhance soil health and plant growth, particularly under stress conditions [21, 107]. Its ability to absorb heavy metals is attributed to its high content of metal-binding proteins, such as phytochelatins and metallothioneins [75]. However, a concern associated with biochar use is the potential introduction of contaminants into the soil. Therefore, it is preferable to employ purified soil amendments [102]. Concerns arise from factors such as variability in feedstock and raw materials, where contaminants from industrial waste or polluted areas may be retained in the biochar during pyrolysis [56, 95, 100].

The use of nano-materials loaded with adsorbent materials holds significant importance in pollutant remediation, offering advantages over traditional biochar [78]. In this study, we investigate the potential of purified protein from water hyacinth to adsorb cadmium and serve as a soil amendment by immobilizing it on nano-chitosan. The immobilization of proteins on nano-chitosan enhances their stability and activity [104]. Nano-materials, especially nano-chitosan particles, serve as excellent immobilization supports [67], demonstrating effectiveness in this role [3].

Nano-chitosan particles have gained attention across scientific and industrial fields due to their unique properties, including a large surface area, biocompatibility, ease of modification, enhanced mechanical strength, improved mass transfer, cost-effectiveness, antimicrobial properties, and pH sensitivity. These properties make nano-chitosan versatile and valuable in various applications, including biocatalysis, biomedicine, and environmental remediation [1, 90, 96].

Furthermore, nano-chitosan demonstrates promising benefits in agriculture, contributing to enhance plant growth, development, and tolerance to environmental stresses such as drought, salinity, and heavy metal toxicity [23]. As a soil amendment, nano-chitosan improves soil structure and fertility, enhances water retention, reduces soil erosion, and promotes nutrient availability for plants. The multifaceted advantages of nano-chitosan position it as a valuable material with diverse applications in agriculture and environmental remediation [2].

Most of the current techniques used to mitigate the toxic effects of heavy metals on water, soil, and plants have certain limitations [37]. Biochars themselves can be sources of contamination with heavy metals and pathogenic microbes [111]. Adding external rhizobacteria can disturb the environmental equilibrium in the soil [87]. Other synthetic materials have negative effects on the environment and human health. This underscores the urgent need to explore safer and more efficient ways to mitigate the negative effects of heavy metals on water, soil, and plants [37].

From this perspective, we aimed to establish a foundation for formulating safe sustainable and economically feasible methodologies to mitigate cadmium (Cd) contamination. Our study is based on the hypothesis that water hyacinth proteins possess a notable capability to efficiently capture and bind metals. When these proteins are immobilized on nano-chitosan, they gain higher stability and a larger surface area, enhancing their effectiveness in adsorbing metals. Additionally, the prepared nano water hyacinth protein (nano-WHP) is natural and safe for use, as it is composed of natural protein immobilized on nano-chitosan, which is biocompatible and non-toxic to the environment and living organisms.

To our knowledge, this is the first study to evaluate the adsorption of cadmium using nano water hyacinth protein. We assessed the cadmium adsorption capacity of the prepared water hyacinth protein and the impact of treating soil with nano-WHP on improving soil health and enhancing Cd tolerance in common beans.

2 Material and methods

2.1 Preparation of nano-water hyacinth protein

2.1.1 Water hyacinth protein extraction

The water hyacinth protein extraction process, following the methodology of Yifru et al. [105] is detailed as follows: Water hyacinth leaves were gathered from a canal in Sofia Village, Zagazig Governorate, Egypt. The collected leaves underwent

destalking and were thoroughly washed using running tap water. Subsequently, the cleaned leaves were immersed in distilled water at a ratio of 2:1 (weight to volume) for 30 min. The soaked leaves were macerated using an electric blender. To solubilize leaf proteins, the resulting slurry's pH was adjusted to pH 9.0 with 0.1 M NaOH. The slurry was then filtered through cheesecloth. For protein coagulation, 0.1 M HCl was added to the filtrate until its pH reached 2.0. The coagulated mixture underwent centrifugation at 3800g for 10 min. The resulting pellets were collected, dried at 60°C, and stored for use as water hyacinth protein (WHP).

2.1.2 Immobilization of water hyacinth protein on nano-chitosan

The water hyacinth protein (WHP) was immobilized onto Nano-chitosan particles (NS6130-09–918) with a size range of 80–100 nm, obtained from Intelligent Materials Pvt. Ltd., USA. The immobilization procedure followed the method outlined by Badawy and Naguib [10] as follows: A 10% nano-chitosan suspension (w/v) was prepared at room temperature, following the product instructions to ensure stability. The prepared nano-chitosan suspension (100 mL) was incubated with 50g of WHP at room temperature for varying incubation periods, aiming to determine the optimal duration for maximum immobilization efficiency. After incubation, the mixture underwent centrifugation at 3800 g for 10 min. The resulting pellets were air-dried at room temperature and stored as nano-WHP. The supernatant was used for protein estimation using the Lowry assay [63] to determine immobilization efficiency, following the equation outlined by Huang et al. [45].

$$\text{Immobilization efficiency } (\%) = \frac{\text{Initail protein - Final protein}}{\text{Initial protein}} * 100$$

2.2 Cadmium removal efficiency for prepared nano-WHP in aqueous solution

2.2.1 Determination of removal efficiency and adsorption capacity

The cadmium removal efficiency and adsorption capacity of the prepared nano-WHP were evaluated in a batch system, following the method described by Wang et al. [99].

2.2.2 Evaluation the stability and reusability of prepared nano-water hyacinth protein.

In order to show the stability and reusability of the prepared nano-WHP, the adsorption–desorption cycle was repeated fifty times using the same nano-WHP using a separation column (detailed methods in the supplementary data).

2.3 Application of nano-WHP in soil for combating cadmium stress in common bean seedlings

2.3.1 Greenhouse study

The experimental setup involved plastic pots with dimensions of 25 cm in diameter and 15 cm in height, each filled with 3 kg of sandy loam soil. The pots were divided into two groups, each containing 24 pots, based on the inoculation of the soil with nano-WHP. In the first group, the soil in the pots remained untreated, serving as the control. In the second group, a blend of 15 g of nano-WHP was incorporated into each pot containing 3 kg of soil, establishing a ratio of 5 g per kg of soil (5g/kg soil). This ratio was determined from the adsorption experiment, where 5 g of nano-WHP exhibited the highest cadmium removal percentage, and further increases did not show additional benefits.

Five common bean (*Phaseolus vulgaris*) seeds (Agrimax Green Bean, Ag00310) were sown in each pot. After emergence, seedlings were thinned to two in each group. Cadmium (as $CdCl_2$) was introduced at a single dose of 100 mg/kg of soil two weeks after sowing, a concentration known to induce high toxicity in bean plants [11]. The experiment included four treatments:

Group I: Control plants (plants grown in non-treated soil without Cd treatment).
Group II: Nano-WHP Plants (plants grown in nano-WHP-treated soil without Cd treatment).
Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment).
Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment).

Plants were housed in a greenhouse with day/night temperatures of 26/16 ± 2 °C and a relative humidity of 50 ± 4%. They were irrigated as needed to maintain constant soil moisture. The experiment continued for 3 weeks after the Cd treatment. This period marked the second stage, occurring before the death of untreated plants cultivated under cadmium stress. If the experiment had continued beyond this point, no untreated plants would have survived under cadmium stress conditions. Soil and shoot samples were collected after one week, which represented the initial manifestation of chlorosis and wilting symptoms due to cadmium toxicity in Cd-exposed plants.

2.3.2 Changes in soil enzymes activity

The assessment of soil enzyme activities is crucial for evaluating soil health [109]. In this study, β-glucosidase, urease, acid phosphatase, and dehydrogenase activities were determined according to the methods of Sanchez-Hernandez et al., [80] and Kaur and Kaur [53] (detailed methods in the supplementary data file).

2.3.3 Changes in growth parameters

We determined the shoot fresh and dry weights to calculate the live fine fuel moisture (LFFM) of shoots according to the following equation:

$$LFFM = \frac{Freshweight - Dryweight}{Dryweight} X100$$

The change in growth percent was calculated according to the change in dry weight compared to the control according to the following equation:

$$Change\ in\ growth\% = \frac{treatment\ dry\ weight - control\ dry\ weight}{control\ dry\ weight} \times 100$$

We also determined some leaf growth parameters for the lower leaf, including leaf variables including leaf relative water content (RWC), leaf moisture (LM), and leaf dry matter content (LDMC) according to the following equations, respectively:

$$RWC(\%) = \frac{Leaf\ fresh\ weight - Leaf\ dry\ weight}{Leaf\ turgid\ weight - Leaf\ dry\ weight} \times 100$$

$$LM(\%) = \frac{Leaf\ fresh\ weight - Leaf\ dry\ weight}{Leaf\ dry\ weight} \times 100$$

$$LDMC = \frac{Dry\ leaf\ weight}{Turgid\ leaf\ weight}$$

2.3.4 Changes in antioxidant machinery and oxidative stress markers

Oxidative stress markers In this study, oxidative stress and damage in plant cells were assessed using various indicators. Hydrogen peroxide (H_2O_2), a representative reactive oxygen species in plant cells, was determined through its reaction with potassium iodide, following the method outlined by Alexieva et al. [4]. Lipid peroxidation, a key marker of oxidative damage, was quantified by measuring malondialdehyde (MDA) using the thiobarbituric acid (TBA) method, as reported by Li [58]. Protein oxidation was assessed through the measurement of tyrosine using an ELISA kit (Nikken SEIL Co., Ltd., Japan), following the procedures described by Kato et al. [51]. To evaluate DNA oxidation, the levels of 8-hydroxydeoxyguanosine (8-OHdG) were measured in prepared leaf extracts using an ELISA Kit (E-EL-0028) from Elabscience Biotechnology Inc., United States, according to the product manual. The decrease in the oxidative stress markers was calculated from the following equation:

$$Oxidative\ Stress\ Marker\ Descreas\ percent = \frac{Content\ in\ group\ III - Content\ in\ group\ IV}{Content\ in\ group\ III} \times 100$$

where, Group III: Plants grown in non-treated soil with Cd treatment. Group IV: Plants grown in nano-WHP-treated soil without Cd treatment.

Antioxidant activity–Non-enzymatic antioxidant activity The assessment of free radical scavenging capacity in shoots was conducted using the DPPH radical scavenging assay, following the method proposed by Blois [14]. The determination of non-enzymatic antioxidant compounds, namely total phenols and flavonoids, involved extracting these compounds from common bean shoots based on the procedure outlined by Campbell and Ellis [16]. The quantification of phenolic content was achieved through the Folin-Ciocalteu assay. Additionally, the determination of flavonoid content employed an aluminum chloride (AlCl3) assay, following the procedure described by Pallab et al. [74].

Antioxidant enzymes activity Superoxide dismutase (SOD) activity was evaluated using the method of Beyer and Fridovich [13], which involves assessing the reduction of nitro blue tetrazolium (NBT). Polyphenol oxidase (PPO) activity was determined through the oxidation of pyrogallol, following the procedure outlined by Kar and Mishra [49]. The activities of both soluble and cell wall-bound peroxidases were measured according to the methodology described by Saroop et al. [82].

2.4 Statistical analysis

The experiment was systematically conducted five times, employing a completely randomized design to ensure the robustness and reliability of the results. The data collected were entered into an Excel sheet to generate figures and compute the mean values along with standard deviations (SD) from the five replicates. To assess statistical differences between distinct groups, a two-way analysis of variance (ANOVA) was performed using the Statistical Package for the Social Sciences (SPSS version 17.0 for Windows).

3 Results

3.1 Preparation of nano-water hyacinth protein and its adsorption capacity removal efficiency of cadmium in liquid solution

Figure 1A depicts that the immobilization efficiency increased with time pass till optimum incubation period. Optimal duration for achieving maximum immobilization efficiency was around 48 h. No further enhancement in immobilization efficiency was observed beyond this period.

Cadmium removal efficieny increased with increasing the amount of ano-water hyacinth protein (nano-WHP) till the concentration 5 g/L, Significantly, at a concentration of 5 g/L, nano-water hyacinth protein showcased a Cd removal efficiency of approximately 96%, with negligible alterations in efficiency beyond this concentration (Fig. 1B).

Nano-WHP exhibited an impressive adsorption capacity of about 150 mg Cd^{+2} g^{-1}, as illustrated in Fig. 1B. Furthermore, it demonstrated notable stability and reusability, retaining over 75% of its removal efficiency even after 50 treatment cycles, as shown in Fig. 1C

3.2 Effect of nano-WHP on soil enzymes under Cd stress

Figure 2 presents compelling evidence of a substantial decrease in key soil enzymes (β-glucosidase, phosphatase, urease, and dehydrogenase) under cadmium stress in the no-treatment with nano-WHP. Interstingly, treatment with nano-WHP led to a significant increase in soil enzyme activity, both in normal conditions and under Cd pollution (Fig. 2). The soil enzyme activity in the nano-WHP treated group was significantly higher than that of the control under either normal or Cd contamination conditions.

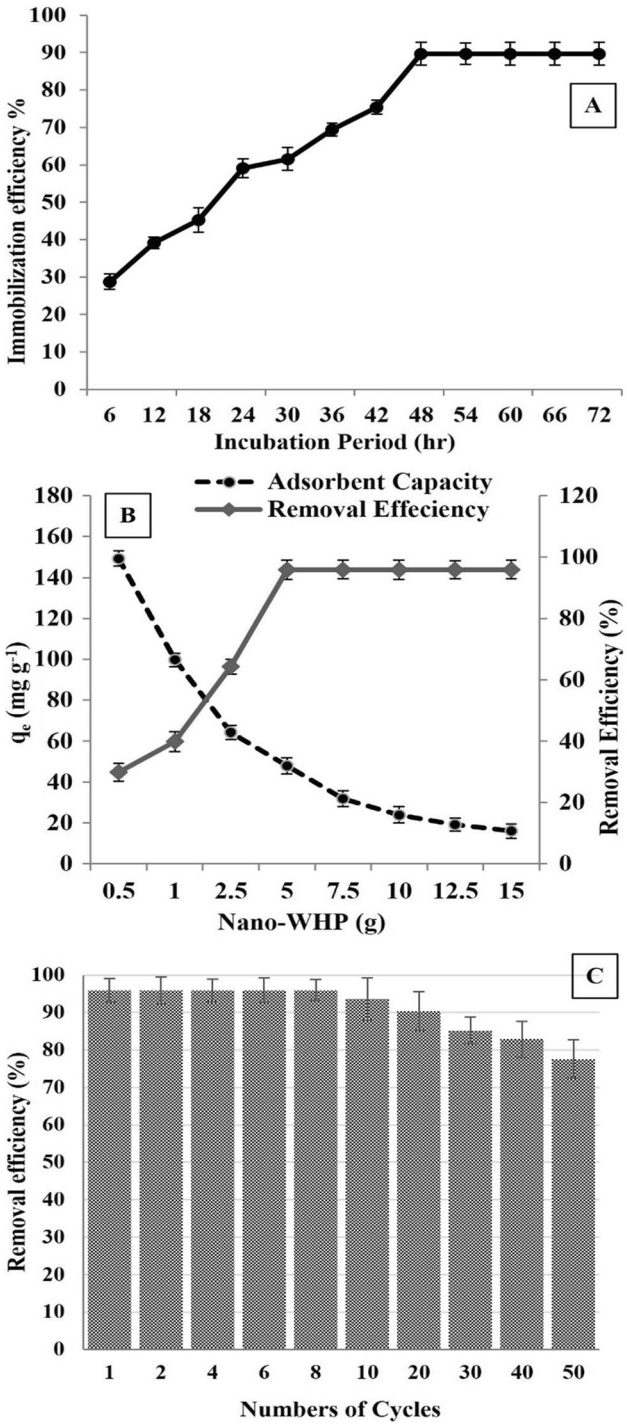

Fig. 1 Effect of immobilization time on the efficiency of water hyacinth protein immobilization on nano-chitosan. **B**. Cadmium removal efficiency and the adsorption capacity of the nano-water hyacinth protein. C. Cadmium removal efficiency through different treatment cycle

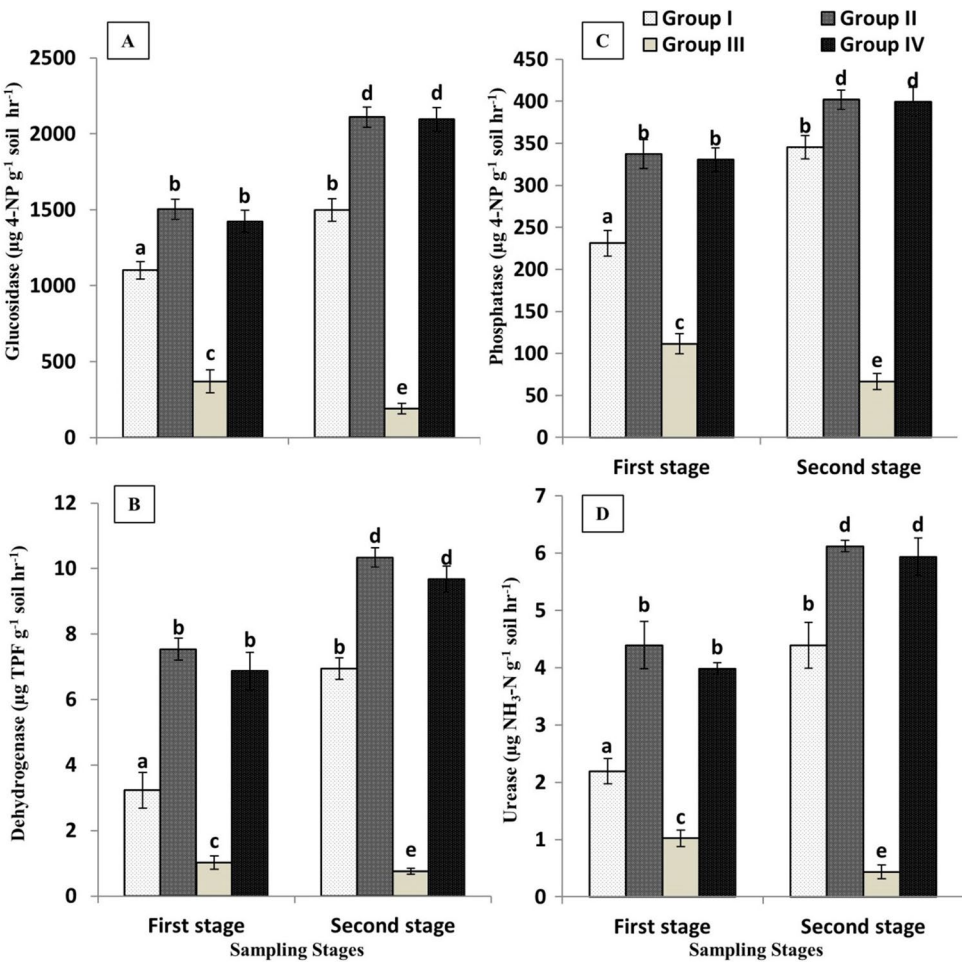

Fig. 2 Effect of nano-water hyacinth protein treatment on soil enzymes activity (glucosidase (**A**), dehydrogenase (**B**), phosphatase (**C**), and urease (**D**)) after a week (first stage) and 3 weeks (second stage) after Cd treatment. Group I: Control plants (plants grown in non-treated soil without Cd treatment); Group II: Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment); Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment); and Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment). Columns followed by different letters are significantly different according to a two-way ANOVA test ($P = 0.05$). The bars represent the standard deviation

3.3 Effect of nano-WHP on plant growth under Cd stress

This study provides evidence for the efficacy of nano-Water Hyacinth Protein (nano-WHP) in alleviating the adverse effects of cadmium on common bean growth, as illustrated in Fig. 3D. Cadmium significantly impeded the growth of common bean seedlings, resulting in a 35% reduction compared to the control in the first stage and a more pronounced 75% decrease in the second stage. In contrast, plants treated with nano-WHP exhibited enhanced growth under normal conditions, surpassing the control by 45% and 55% in the first and second stages, respectively. Remarkably, under cadmium stress, nano-WHP-treated plants demonstrated growth increments of approximately 35% and 50% in the first and second stages, respectively, compared to the control.

In addition to assessing growth changes, we investigated shoot live fine fuel moisture (LFFM), a reliable indicator of plant health and combustibility that reflects shoot water content relative to its dry mass. Figure 3E demonstrates a significant enhancement in shoot LFFM with nano-WHP treatment. In the first stage, it increased from approximately 120% in the control to 150% under both normal and cadmium stress conditions, and in the second stage, it rose from 150 to 180%. This indicates that nano-WHP treatment contributes to increased shoot moisture, thereby aiding in biomass formation. Moreover, nano-WHP treatment significantly improved leaf growth parameters, including leaf relative water content (RWC), leaf moisture (LM), and leaf dry matter content (LDMC), under both normal conditions and cadmium contamination (Fig. 3A-C).

3.4 Effect of nano-WHP on antioxidant machinery and oxidative stress markers

The present study reveals a significant increase in oxidative stress markers H_2O_2, malondialdehyde (a product of lipid peroxidation), dityrosine (a product of protein oxidation), and 8-hydroxydeoxyguanosine (an indicator of DNA oxidation) under cadmium stress, observed in both nano-Water Hyacinth Protein (nano-WHP)-treated and non-treated plants during the first stage. However, in the second stage, these oxidative stress markers substantially decreased in nano-WHP-treated plants while significantly increasing in

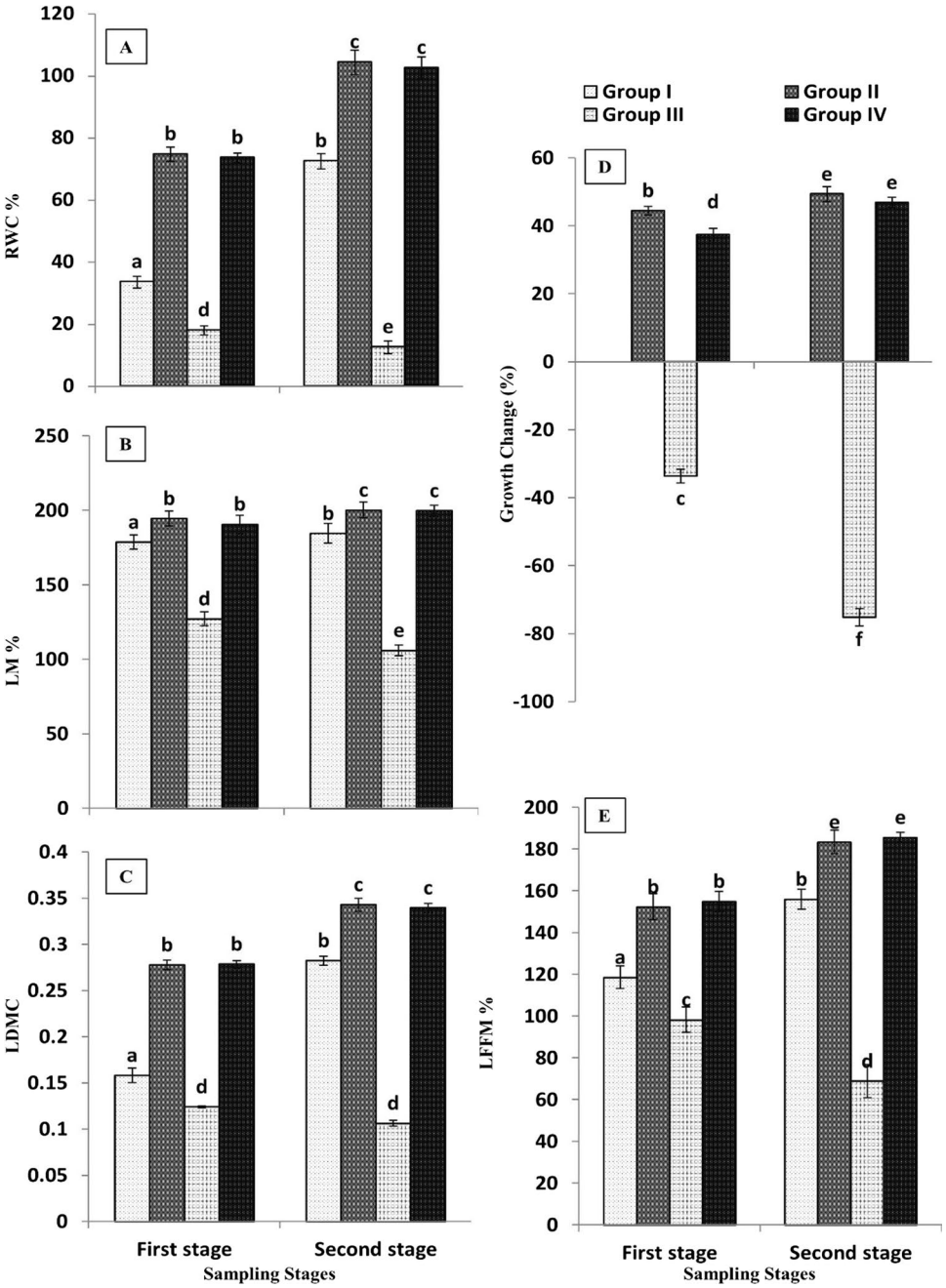

Fig. 3 Effect of nano-water hyacinth protein treatment on growth parameters (leaf relative water content (RWC) (**A**), leaf moisture (LM) (**B**), leaf dry matter content (LDMC) (**C**), growth change percent (**D**), and shoot live fine fuel moisture (LFFM) (**E**)) after a week (first stage) and 3 weeks (second stage) after Cd treatment. Group I: Control plants (plants grown in non-treated soil without Cd treatment); Group II: Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment); Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment); and Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment). Columns followed by different letters are significantly different according to a two-way ANOVA test ($P = 0.05$). The bars represent the standard deviation

non-treated plants. The application of nano-WHP led to a remarkable reduction in lipid peroxidation (by 90.63%), DNA oxidation (by 85.13%), protein oxidation (by 79.35%), and H_2O_2 levels (by 81.85%) compared to plants without nano-WHP treatment (Table 1, Fig. 4).

The treatment with Nano-WHP significantly boosted the levels of antioxidant enzymes (as shown in Fig. 5) and non-enzymatic antioxidant compounds, including radical scavenging capacity, phenols, and flavonoids (as illustrated in Fig. 6) during both the initial and subsequent stages. Conversely, the untreated group exhibited a minor increase in antioxidant enzyme levels during the first stage compared to the control, but during the second stage, the activity of antioxidant enzymes dramatically decreased, dropping significantly lower than that of the control (as depicted in Fig. 5).

4 Discussion

Protein immobilization is a well-known technique for improve the protein use and stability [71]. Nano-particles are promising immobilization material due to its

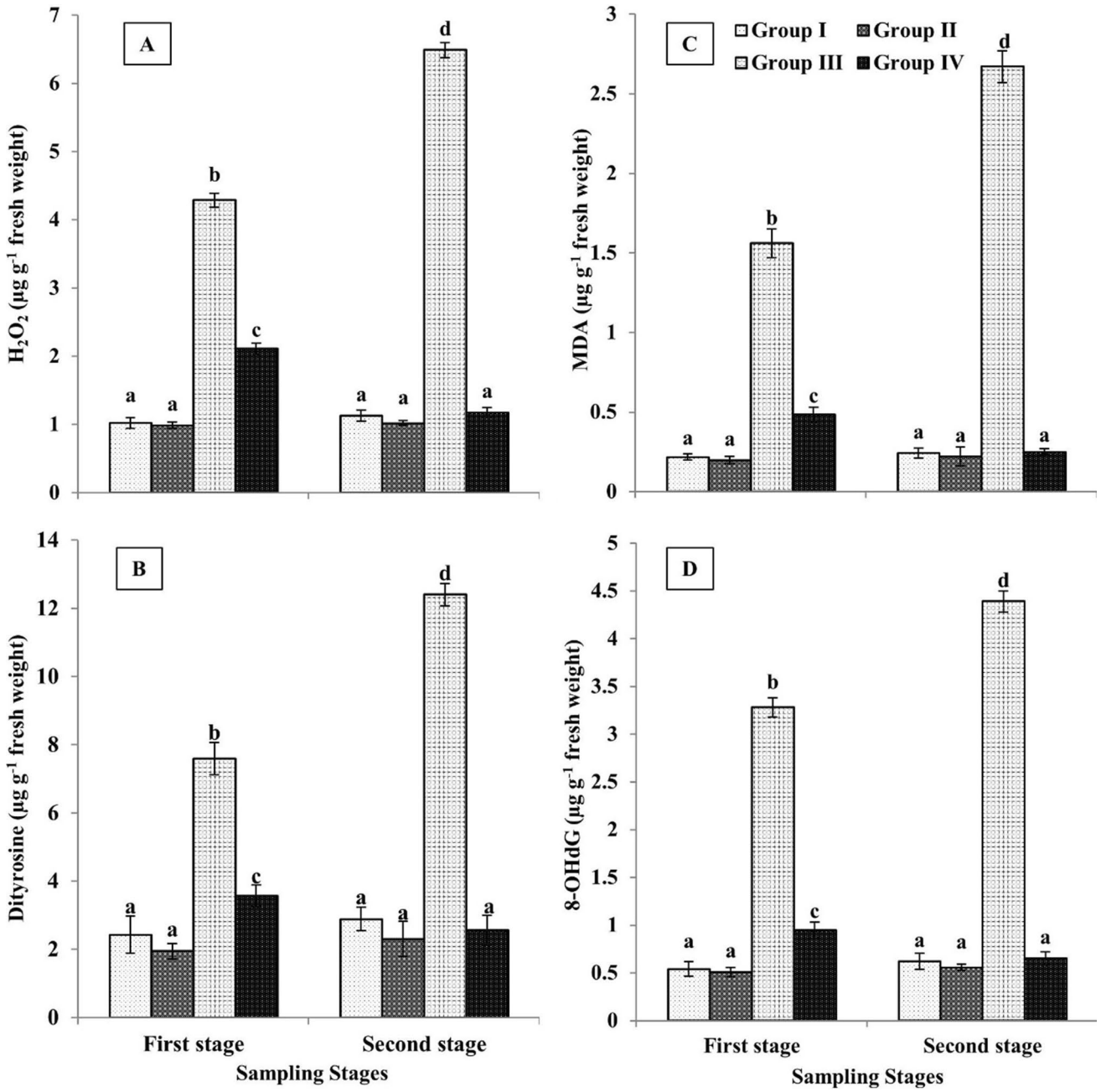

Fig. 4 Effect of nano-water hyacinth protein treatment on oxidative stress markers (hydrogen peroxide (H2O2) (**A**), protein oxidation marker, dityrosine (**B**), lipid peroxidation marker (MDA) (**C**), nucleic acid oxidation marker (8-OHdG) (**D**)) in common bean shoots after a week (first stage) and 3 weeks (second stage) after Cd treatment. Group I: Control plants (plants grown in non-treated soil without Cd treatment); Group II: Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment); Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment); and Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment). Columns followed by different letters are significantly different according to a two-way ANOVA test ($P = 0.05$). The bars represent the standard deviation

high surface area. Optimization the different conditions of the immobilization is an important point determines the immobilization efficiency [110]. The immobilization efficiency of water hyacinth protein on the nano-chitosan increased with the increase in incubation time until 48 h. Beyond this timeframe, no further increase in immobilization efficiency occurred. This indicates saturation of the nanoparticles with immobilized materials. This observation aligns with existing literature, as reported by Dwevedi [30].

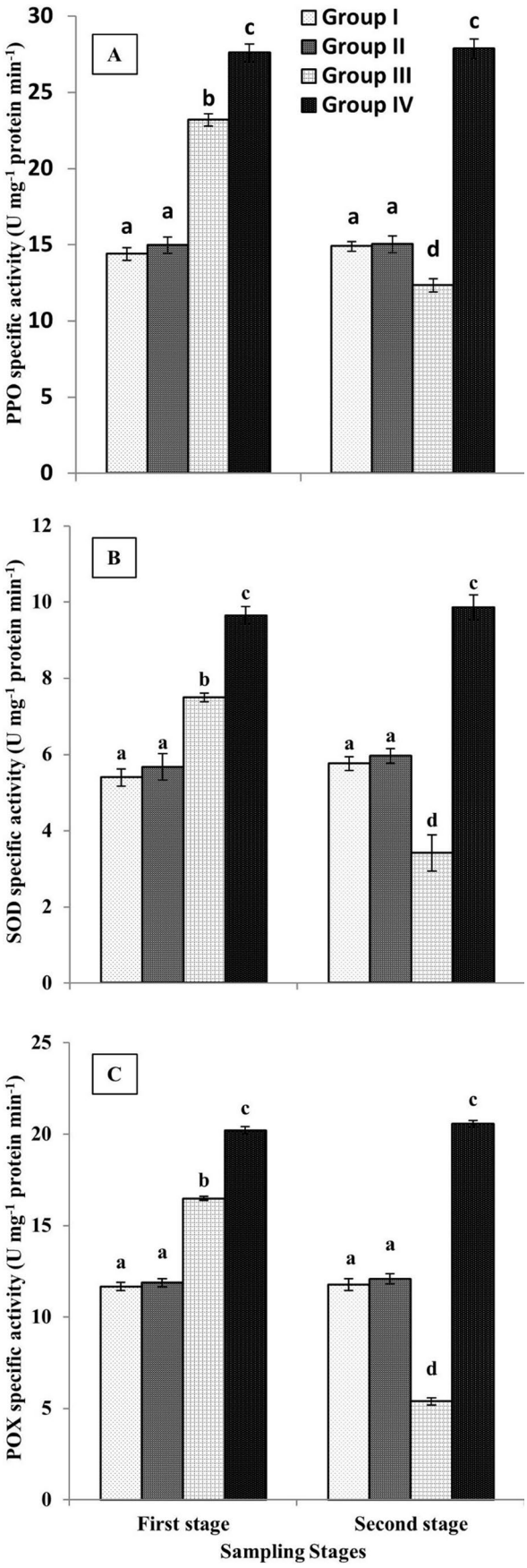

Fig. 5 Effect of nano-water hyacinth protein treatment on antioxidant enzymes (superoxide dismutase (SOD) (**A**), polyphenol oxidase (PPO) (**B**), and peroxidase (POX) (**C**) in common bean shoots after a week (first stage) and 3 weeks (second stage) after Cd treatment. Group I: Control plants (plants grown in non-treated soil without Cd treatment); Group II: Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment); Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment); and Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment). Columns followed by different letters are significantly different according to a two-way ANOVA test ($P=0.05$). The bars represent the standard deviation

One of the applications of the immobilized protein is its use as adsorbent to various environmental pollutants such as heavy metals. The amount of adsorbent used is a crucial factor influencing the effectiveness of the metal adsorption process [62]. Notably, nano-water hyacinth protein (nano-WHP) demonstrated a Cd removal efficiency of about 96% at 5 g/L, with no significant change in efficiency beyond this concentration. Higher adsorbent masses resulted in the saturation of active sites due to interference between these sites, as reported by Poonam et al. [76]. However, the adsorption capacity decreased with an increase in nano-WHP concentration, attributed to insufficient solutes to occupy all active adsorption sites. This reduction is consistent with findings by Masoumi et al. [66] and Poonam et al. [76], emphasizing the impact of the unsaturation of active sites.

Nano-WHP exhibited a remarkable adsorption capacity of approximately 150 mg Cd^{+2} g^{-1} (Fig. 1B). This is surpassing various adsorbent materials (Table 2). Also, nano-WHP showed high stability and reusability as it save more than 75% of its removal efficiency after 50 treatment cycle (Fig. 1C). This heightened adsorption capacity can be attributed to the synergistic effects of WHP and nano-chitosan, as reported by Sobral et al. [88]. The intrinsic heavy metal removal capabilities of water hyacinth, documented by Mahmood et al. [64], Zheng et al. [112], Cao et al. [17], Liu et al. [61], and Hemalatha et al. [44], further contribute to the impressive adsorption capacity of nano-WHP. The extensive surface area of nanoparticles enhances their adsorption effectiveness compared to bulk materials, as highlighted by Roberto et al. [79].

The heavy metal pollution is not only a danger in the aquatic environments but also, it represents a high danger in soil pollution, as heavy metals negatively affect the soil health and so plant growth [28]. Heavy metals render the soil enzymes activity [89]. Various soil enzymes have microbial origins and are intricately linked to essential cycles such as carbon, nitrogen, and phosphorus. Enzyme activity is widely measured in microbiology, biochemistry, and agricultural sciences, often serving as a crucial indicator of soil health [34, 48, 50, 92]. These enzymes are highly sensitive

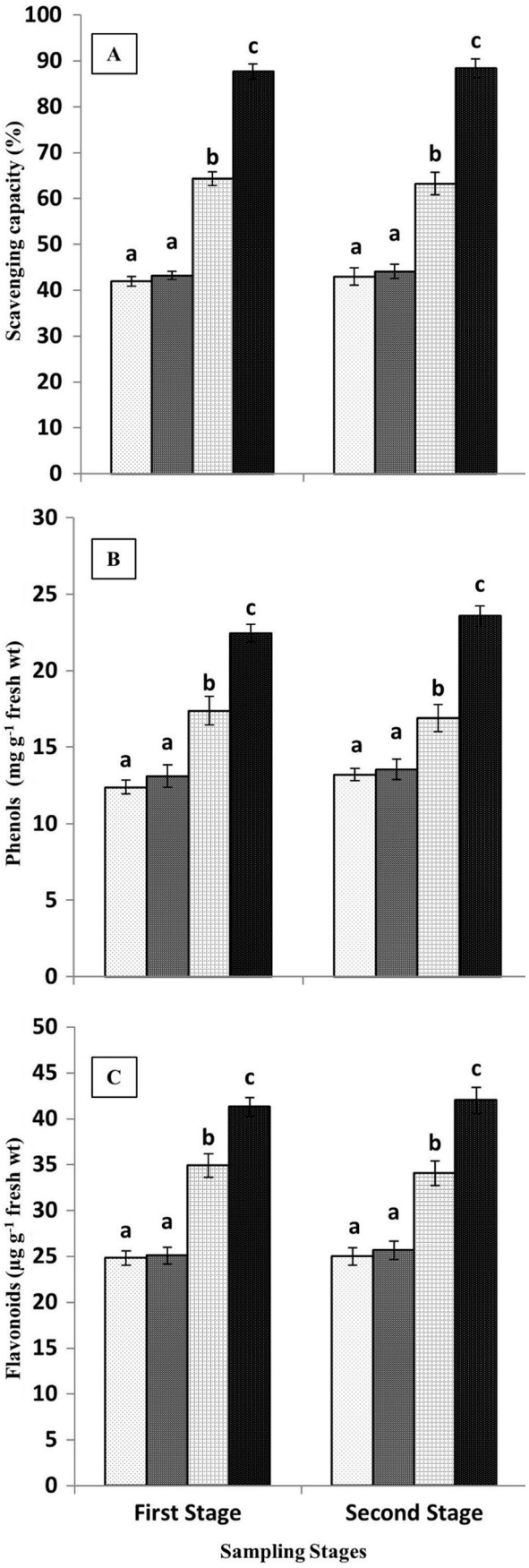

Fig. 6 Effect of nano-water hyacinth protein treatment on non-enzymatic antioxidants (DPPH radical scavenging% (**A**), total phenols (**B**), and total flavonoids (**C**)) in common bean shoots after a week (first stage) and 3 weeks (second stage) after Cd treatment. Group I: Control plants (plants grown in non-treated soil without Cd treatment); Group II: Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment); Group III: Cd-Plants (plants grown in non-treated soil with Cd treatment); and Group IV: Cd-Nano-WHP plants (plants grown in nano-WHP-treated soil without Cd treatment). Columns followed by different letters are significantly different according to a two-way ANOVA test ($P=0.05$). The bars represent the standard deviation

to changes in soil conditions, whether natural or anthropogenic. Notably, soil pollution with heavy metals, including Cu, Pb, Zn, Cd, V, and Ni, represents a significant alteration to soil ecosystems, with Cd posing the highest ecological risk among these metals [93].

The present study results ensured the negative effect of cadmium on soil enzymes activity (Fig. 2). This aligns with the findings by Lin et al. [60], who reported enzyme reduction under heavy metal pollution. Sharma et al. [86] similarly documented negative impacts on soil enzyme activity due to heavy metal pollution in the soil near the Yamuna River in Delhi. Tang et al. [94] demonstrated a significant decrease in soil enzymes with elevated Cd and Zn levels in heavy metal-polluted soil. Subsequently, Tang and colleagues associated the decline in soil enzyme activity with increased heavy metal concentrations in lead–zinc tailing pond soils [93].

The observed decline in soil enzyme activity can be attributed to the adverse effects of heavy metals on the growth and metabolism of soil microorganisms, which serve as the primary source of soil enzymes [12]. Tang et al. [94] proposed that the decrease in soil enzyme activity is linked to heavy metals interacting with enzyme proteins, leading to denaturation, chelation with enzyme substrates, or interfering with the formation of enzyme reaction products. Additionally, the accumulation of heavy metals in the soil can reduce available nutrients, further contributing to a decline in soil enzyme activity [24, 25].

On the other hand, with soil treatment with nano-Water Hyacinth Protein (nano-WHP), the soil enzymes activity significantly increased under either the normal conditions or Cd contamination condition (Fig. 2). This heightened enzyme activity is attributed to the activation of soil microbiota stimulated by the presence of nano-WHP. Similar findings were reported by Chaudhary et al. [19], who observed the activation of microbial diversity and improved soil health with the application of water hyacinth biochar. Additionally, Hammam et al. [41] demonstrated the positive impact of water hyacinth biochar on soil enzymes through the activation of the soil microbiota, further supporting the beneficial effects on soil health.

Table 1 Decrease percent in the oxidative stress markers in Nano-WHP-treated common bean shoots after a week (first stage) and thee weeks (second stage) after Cd treatment

Oxidative stress markers	Stage	Decrease percent
Lipid peroxidation	**1st Stage**	69.04
	2nd Stage	90.63
DNA oxidation	**1st Stage**	71.10
	2nd Stage	85.13
Protein oxidation	**1st Stage**	52.99
	2nd Stage	79.35
H_2O_2	**1st Stage**	50.84
	2nd Stage	81.85

The enhancement in soil enzyme activity with nano-WHP application can also be linked to another component, namely nano-chitosan. Khati et al. [55] documented the positive influence of nano-chitosan application on soil health. Furthermore, the increase in soil enzyme activity with nano-WHP treatment under Cd pollution can be attributed to the adsorption of Cd on nano-WHP, as illustrated in Fig. 1, where 5g of nano-WHP removed 96% of Cd in a 100 mL solution. This adsorption renders Cd less available in the soil, minimizing or eliminating its negative effects. Tang et al. [94] highlighted that the adverse impact of heavy metals on soil enzymes depends primarily on their availability in the soil. The more available the heavy metal, the greater its negative effect [36].

Furthermore, improving soil enzyme activity in the presence of nano-WHP can be related to the ability of nano-WHP to provide a suitable environment for the stability and activity of soil enzymes. Similarly, Yasin and his colleagues recently reported that the application of biochar-modified nanoparticles improved soil enzyme activity by providing suitable conditions for stability and activity [103].

The decrease in soil enzymes activity results in the decrease in the available nutrients in the soil, which negatively affect the plant growth [33]. The deleterious impact of cadmium on plant growth is extensively documented, prompting a comprehensive exploration of strategies to enhance plant tolerance to cadmium stress [117]. This study provides evidence for the efficacy of nano-Water Hyacinth Protein (nano-WHP) in alleviating the adverse effects of cadmium on common bean growth. Cadmium significantly decreased the growth of common bean seedlings (Fig. 3D). On the other, plants treated with nano-WHP showed enhanced growth under normal conditions, or under cadmium stress. These findings underscore the potential of nano-WHP in promoting common bean growth and mitigating the inhibitory effects of cadmium stress, highlighting its application as a promising strategy for enhancing plant tolerance to heavy metal-induced stress.

In addition to assessing growth changes, we investigated shoot live fine fuel moisture (LFFM), a reliable indicator of plant health and combustibility that reflects shoot water content relative to its dry mass [29]. The results showed a significant enhancement in shoot LFFM with nano-WHP treatment under both normal and cadmium stress conditions. This indicates that nano-WHP treatment contributes to increased shoot moisture, thereby aiding in biomass formation. Moreover, nano-WHP treatment significantly improved leaf growth parameters, including leaf relative water content (RWC), leaf moisture (LM), and leaf dry matter content (LDMC), under both normal conditions and cadmium contamination (Fig. 3A-C). This growth increase is attributed to the positive impact of nano-WHP on soil enzyme activity, consistent with findings by Hammam et al. [41], who observed enhanced corn growth with water hyacinth biochar application due to its positive effects on soil enzymes. Increased soil enzyme activity enhances nutrient availability, benefiting plant growth parameters [113]. Similarly, Attaran Dowom et al. [8] reported that chitosan enhanced plant growth by improving soil health, leading to increased nutrients and improved physiological and biochemical status under stress. The significant growth increase observed with nano-WHP treatment suggests a synergistic effect between nano-chitosan and water hyacinth protein.

One of the most perilous consequences of heavy metal stress is the initiation of oxidative stress, characterized by the production of reactive oxygen species (ROS) that oxidize crucial cellular components such as lipids, proteins, and nucleic acids, causing oxidative stress. The products of the lipids, proteins, and nucleic acids oxidation are considered as oxidative stress markers which content directly proportion with ROS content in the cells [38, 52, 115]. The study finds that exposure to cadmium stress increases oxidative stress markers in plants. Initially, both nano-Water Hyacinth Protein (nano-WHP)-treated and untreated plants show elevated levels of these markers. However, in the second stage, plants treated with nano-WHP demonstrate a significant decrease in oxidative stress markers, while levels continue to rise in untreated plants. The initial surge in oxidative stress markers plays a crucial role as signaling molecules, inducing plant defense mechanisms against various stresses [85,

Table 2 Maximum adsorption capacity of some different materials to Cd ions

Material	Adsorption capacity (mg cd^{+2} /g)	Reference
Water Hyacinth Protein Immobilized on Nano-Chitosan	150	Present study
composite sponge combining of metal–organic framework and chitosan	0.193	[69]
α-amino nitrile modified magnetic	65	[108]
Biomass-derived carbon/iron composite (FexOy-BC (RM))	13	[77]
Magnetic biochar from Leb Mu Nang banana peel	21.82	[59]
Earth-abundant serpentine	0.112	[98]
Fe–Mn binary oxide biochar	87.58	[106]
Polycarboxylated sugarcane bagasse	65	[31]
Cassia fistula seed carbon	68.02	[83]
Magnetic nanoparticles coated zirconia	24.19	[47]
Cerium oxide nanoparticles	34.2	[65]
Magnetic nanomaterials (Mg1 – xCaxFe2O4	100	[116]
Sodium tripolyphosphate and vanillin modified chitosan-based magnetic nanosorbents	91.75	[18]
Mesoporous silica and chitosan-coated magnetite nanoparticles	126.26	[6]
Cerium oxide nanoparticles	89.33	[9]
Green synthesized iron oxide nanoparticles	78	[68]
Surface-loaded phosphorus-modified lignite	55	[22]
Coated magnetic nanoparticles in activated carbon derived from corncob waste	100	[7]
Red mud modified bean-worm skin biochars	73.52	[101]

97]. Cadmium, being a non-redox metal, rapidly induces the production of ROS, leading to oxidative stress in plants exposed to cadmium pollution. This rapid ROS production occurs indirectly by disrupting the electron transport chain and cellular metabolism. Additionally, cadmium can displace redox-active metals, such as ferrous and copper, triggering the Fenton and Haber–Weiss reactions—common mechanisms for ROS production in living cells, thus contributing to oxidative stress [26]. The oxidative stress markers were significantly higher in the non-treated plants than in those treated with nano-WHP. This difference can be attributed to the adsorption capacity of nano-WHP, which reduces the availability of Cd in the soil. Consequently, the toxic effects on the treated plants' proteins are diminished. This aligns with findings from Haider et al. [39, 40], who observed that increased Cd availability in the soil led to heightened oxidative stress, causing peroxidation of proteins and lipids as well as DNA damage. Additionally, Shaari et al. [84] reported that the extent of oxidative stress damage resulting from Cd toxicity is contingent upon its availability in the soil.

Plants exhibit tolerance to increased reactive oxygen species (ROS) and the consequent oxidative stress by employing highly effective antioxidant machinery, which is induced to detoxify excess ROS and maintain cellular oxidative homeostasis. This antioxidant machinery includes various molecules, such as enzymes (peroxidases, polyphenol oxidases, catalase, and superoxide dismutase), and non-enzymatic compounds like phenols and flavonoids [15]. Nano-WHP treatment significantly increased both antioxidant enzymes (Fig. 5) and non-enzymatic antioxidant compounds (radical scavenging capacity, phenols, and flavonoids) (Fig. 6) in both the first and second stages. Consequently, nano-WHP-treated plants demonstrated enhanced tolerance to oxidative stress under cadmium exposure, leading to a significant decrease in oxidative stress markers comparable to control plants. In contrast, non-treated plants failed to sustain antioxidant machinery activity, resulting in a significant decrease in the second stage and a subsequent increase in oxidative stress markers. This observation aligns with the findings of Cuypers et al. [26], emphasizing the importance of balanced redox biology for plant adaptation to oxidative stress induced by cadmium pollution. Additionally, reports by Paithankar et al. [73] highlight the role of signaling molecules in activating the antioxidant machinery to suppress oxidative stress induced by heavy metal stress. Many studies have underscored the activation of the antioxidant machinery as a crucial biochemical change necessary for inducing heavy metal tolerance. For instance, melatonin has been shown to induce cadmium tolerance in soybean and strawberry plants through the activation of antioxidant signaling cascades [46, 81]. Nano-selenium has also been reported to counteract Cd, Pb, and Hg toxicity in *Brassica chinensis* by improving its antioxidant system [114].

4.1 Conclusion and future prospective

In conclusion, our study represents a pioneering effort to evaluate the efficacy of nano-water hyacinth protein as a soil amendment for cadmium (Cd) phytoremediation and its impact on plant growth under Cd stress. We highlight the economic feasibility and effectiveness of nano-Water Hyacinth Protein (nano-WHP) when applied as soil amendment at a minimal rate of 5 g per 1 kg of soil. The application of nano-WHP activates soil enzymes, thereby improving soil

health and enhancing common bean plant growth under both normal and Cd stress conditions. Nano-WHP not only promotes growth but also induces Cd tolerance in plants by activating the antioxidant machinery, mitigating oxidative stress.

This research opens avenues for further exploration into the potential of nano-water hyacinth protein as soil amendment to mitigate Cd stress. Future studies could delve into understanding various factors influencing the Cd adsorption and desorption capacity, providing valuable insights for optimizing its application. Additionally, assessing the Cd content in soil and plants, as well as investigating different factors influencing the protein's efficiency would contribute substantially to the existing knowledge on its role in Cd removal and improving plant tolerance. Our findings pave the way for the development of sustainable and economically viable strategies for addressing Cd contamination in agricultural settings.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10450-024-00511-5.

Author contributions Abdulrahman A Alzandi, Layla Yousif Abdullah Al Hijab, Zarah I. Alzahrani, and Deyala M. Naguib contributed to the study conception and design, Material preparation, data collection and analysis. Deyala M. Naguib wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This paper is self-funding and we did not take any funds from any organization or person.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval This research article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication Not applicable.

Consent for participation Not applicable.

Competing interests The authors declare no competing interests.

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