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RESEARCH ARTICLE

Acinetobacter schindleri SR-5–1 decipher morpho-physio-biochemical and nutritional improvements to *Pisum sativum* L. and *Linum usitatissimum* L. maintained under wastewater/cadmium stress

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Abstract

Metal retention in wastewater fertigated crops poses a potential hazard to food chain. Current work demonstrates the bioremediation and growth-promoting potential of *Acinetobacter schindleri* SR-5–1 by using nitrogen-fixing (pea) and non-nitrogen fixing (linseed) plants under cadmium (Cd) and wastewater irrigation regimes. Both plants were grown at 250 or 500 $CdCl_2$ and 75 or 100% wastewater, each separately with and without *A. schindleri* SR-5–1 inoculation. The results revealed that Cd and wastewater significantly decreased growth, biomass, antioxidants, and nutrient acquisition through increased malondialdehyde, H_2O_2, and Cd accumulation. However, application of *A. schindleri* SR-5–1 significantly promoted morpho-physio-biochemical attributes while diminishing MDA and H_2O_2 under applied Cd and wastewater stress levels in both pea and linseed. Further, PGPR inoculation positively influenced pea and linseed seedlings through a substantial decline in Cd accumulation in roots/shoots and retained the optimal level of essential nutrients. It was inferred that both pea and linseed, with *A. schindleri* SR-5–1 application, exhibited higher growth and metabolism under Cd and wastewater stress but substantial tolerance was acquired under wastewater stress. Studied plants exhibited tolerance in order of 75% WW ≥ 250 µM Cd ≥ 100%WW ≥ 500 µM Cd treatment under *A. schindleri* inoculation. Current findings revealed the potential of *A. schindleri* to be exploited both for bioremediation and bio-fertilization under Cd, and wastewater-polluted regimes to reduce metal contamination of edible plants. It was suggested that with inoculation of *A. schindleri* SR-5–1, 75% WW dilution can be applied for irrigation of both nitrogen-fixing and non-nitrogen-fixing crops.

Keywords *Acinetobacter schindleri* · Bioremediation · Bio-fertilization · Linseed · Nitrogen fixation · Pea · Wastewater

Introduction

Pakistan is included in the World's freshwater scariest countries in terms of per capita availability which is lower than 1000 m^3 (Akhtar et al. 2018). It is necessary to find appropriate wastewater recycling techniques in order to preserve available freshwater for sustainable agricultural utilization in Pakistan (Sleet 2019). Further, out of total annual household and industrial discharge of wastewater (6.8 billion m^3) in Pakistan, about 0.88 billion m^3 is utilized for crop and vegetable fertigation (Khan et al. 2020) due to unsuitability of ground water or unavailability of surface irrigation water in some areas. Twenty-six percent of the total vegetables produced in Pakistan are directly fertigated with wastewater (Naz et al. 2016). Wastewater released from industrial units and metropolitan disposals is a rich source of metallic and non-metallic salts including (Cd, Cu, As, Mn, Cr, Pb, Mo, Co, B, N, P), detergents, pesticides, PAHs, and dyes. Most of these materials are toxic for plant growth and human health when present in higher concentrations (Egbuikwem et al. 2020; Tanwir et al. 2021).

The plants' metabolic processes including photosynthesis, nutrient accumulation, water balance enzymatic activity, and respiration rate are retarded due to excess wastewater fertigation-based generation of oxidative stress mediated by the synthesis of ROS (Ain et al. 2019). Alteration of all these

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essential metabolic processes abridged crop production and plant growth (Hajihashemi et al. 2020).

Pisum sativum L. (pea) is a nitrogen-fixing legume and its seeds, due to the presence of essential protein contents, have been largely consumed as a vegetable. In Pakistan, pea was cultivated at an area of 27,000 ha with an average yield of 55 tons/ha (Hashmat et al. 2021). The other experimental plant used in the current study was a non-nitrogen-fixing flax (*Linum usitatissimum* L.), commonly known as linseed. It is cultivated worldwide on a large-scale for its fiber and seed oil. Furthermore, it is also utilized as a heavy metal hyperaccumulator for the phytoremediation of various heavy metals like Cd and Cu (Bjelkova et al. 2011; Saleem et al. 2020).

Various physical and biochemical techniques are in practice for wastewater treatment to make it suitable for crop/plant cultivation. Prior treatment of wastewater before irrigation is costly, difficult, and required a large treatment area. However, application of metal-tolerant microbes extracted from wastewater for coping the toxic effects of wastewater fertigation in nitrogen-fixing and non-nitrogen-fixing plants could be an eco-friendly and economical approach. Metal-tolerant plant growth-promoting rhizobacteria (PGPR) have been previously reported as seed inoculants for the eradication of heavy metal toxicity and improvement of crop yield under different soil conditions (Shahid et al. 2019; Abbas et al. 2020; Tanwir et al. 2021). These metal-tolerant PGPR, when applied to plants growing in metal-contaminated soils, ameliorate metal stress through plummeting toxic metal uptake utilizing various mechanisms such as biosorption, bioaccumulation, biotransformation, etc. (Du et al. 2016; Ojuederie and Babalola 2017) and enhanced root elongation and growth of plants (Shahid et al. 2018; Abbas et al. 2020). Metal-tolerant microbes also restrain heavy metals in the rhizosphere through the release of siderophores, phosphatases, exopolysaccharides, and/or through acidification of the root rhizosphere (Ali et al. 2017). Many PGPR strains, belonging to the genus *Acinetobacter*, exist in soil and water reservoirs and were known for the detoxification of heavy metals like Cu, Cd, and Cr in the plant root rhizosphere (Irawati et al. 2017; Yasin et al. 2018; Abbas et al. 2021).

Recent research is the continuation of our earlier works (Hashmat et al. 2021) where different wastewater dilutions (50, 75, and 100%) were applied to a pea plant together with canal water and biologically treated wastewater. The conclusion elaborated that the pea plant was able to tolerate wastewater fertigation up to 50% dilution without altering its physiology, ionomics, and yield. The current research aimed to assess the possible ameliorative potential of metal-tolerant PGPR inoculation on growth, physiological processes, antioxidant production, and nutrient uptake in both nitrogen and non-nitrogen-fixing crop plants cultivated under various regimes of wastewater and Cd stress. The main purposes of this study were (a) to find out the feasible wastewater dilution level for crops growing under metallotolerant PGPR inoculation and (b) also to find the physio-biochemical difference between two different nitrogen and non-nitrogen fixing crops under wastewater and heavy metal stress.

Materials and method

Experimental setup

A pot experiment was conducted under controlled environment in a growth chamber at Fluorescence Microscopy Laboratory, Government College University, Faisalabad, Pakistan. Small plastic pots (15 cm height, 8 cm from top and bottom with 10 cm circumferences) filled with sand were used as a growth medium. Two plant species were used, where one was nitrogen-fixing pea (*Pisum sativum* L.) and the other was non-nitrogen-fixing linseed (*Linum usitatissimum* L.). Seeds of both pea and linseed plants were obtained from a seed bank established at Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Seeds were first sieved for removing debris and other unwanted materials followed by sterilization with 5% (w/v) sodium hypochlorite solution for 15 min and rinsing several times with distilled water. A pure culture of *Acinetobecter schindleri* SR-5–1 was obtained from the Department of Biotechnology and Bioinformatics, Government College University Faisalabad, Pakistan. The fresh culture was grown overnight in nutrient broth, centrifuged at 8000 g for 5 min followed by washing twice with sterile water. These microbial cells were again suspended in an equal volume of saline solution (0.85% NaCl). The culture solution was diluted further with saline to maintain the cell density of 1×10^8 CFU ml^{-1}. Around 600 seeds (300 peas and 300 linseeds) were inoculated with Cd-tolerant PGPR (*Acinetobecter schindleri* SR-5–1) in flasks. The seeds of each plant species were dipped in separate flasks containing 300 ml of freshly prepared *A. schindleri* SR-5–1 inoculum for 60 min (Tanwir et al. 2021). At the same time, 600 non-inoculated seeds of both pea and linseed were dipped in dH_2O for 60 min. There was one control, two wastewater (WW; 100 and 75%) and two Cd (250 and 500 µM) treatment groups. The treatments were applied after seven days of seedling emergence and subsequently 1 week after the first application. The experiment was composed of CRD design with 144 pots comprising of two plant varieties, five different toxicity levels and 4 replicates for each toxicity level. Pots were placed in a growth chamber at a mean day/night length of 14/10 h and day/night temperature of 28/18 °C ± 2. Relative humidity was maintained at 70% during the course of the experiment. Pots were irrigated with 1/2 strength Hoagland solution (10 ml/pot) firstly after 4 days of germination and then on weekly basis. Plants were harvested after 20 days of emergence and three out of five

plants were preserved in the refrigerator after freezing with liquid nitrogen, while the other two were oven dried after measuring morphological characteristics to analyze nutrient content in roots and shoots.

Estimation of photosynthetic pigments

Photosynthetic pigments (Chlorophyll *a*, *b*, total chlorophylls, and carotenoids) were calculated after extracting them from fresh frozen plant sample with the help of 80% acetone. For this purpose, 0.5 g frozen plant material was ground and left for 24 h with 80% acetone (10 ml). The plant extract was then centrifuged at 10,000 g for 15 min. The supernatant was separated carefully and examined through a spectrophotometer (Hitachi U-2910, Tokyo Japan) at different wavelengths (480, 645, and 663 nm) for the quantification of photosynthetic pigments (Arnon 1949).

Determination of enzymatic antioxidants

Frozen leaves (0.5 g) were homogenized with frozen 50 mM phosphate buffer (10 ml) having 7.8 pH for antioxidative enzyme estimation. Plant leaf extract was centrifuged at 15,000 g for 20 min, and the aliquot was separated carefully. This plant extract after filtration was used for the estimation of antioxidants through spectrophotometry. The enzymatic antioxidant activities were presented in units/mg protein.

The activity of SOD (superoxide dismutase) was estimated on the basis of the photochemical reduction of NBT (nitroblue tetrazolium). The 50 µl plant extract was mixed with EDTA 75 nM, 50 mM phosphate buffer (pH 7.8), 13 mM methionine, and riboflavin for determination of SOD, and its absorbance was checked at 560 nm through spectrophotometer (Giannopolitis and Ries 1977).

POD activity was determined through absorbance of light at 470 nm through a spectrophotometer by following the standard protocol adopted by Chance and Maehly (1955). The 100 µl plant extract was mixed with phosphate buffer (50 mM), guaiacol (20 mM), and H_2O_2 (40 mM) for the measurement of POD.

Catalase enzyme was also estimated through spectrophotometry by adopting the methodology of Aebi (1984). The 0.1 ml plant extract was mixed with H_2O_2 (5.9 mM) and phosphate buffer (50 mM). The absorbance of light was checked through a spectrophotometer at 240 nm after every 20 s intervals up to 120 s.

Ascorbate peroxidase was examined by mixing 0.2 ml plant enzymatic extract with phosphate buffer (50 mM), EDTA (0.1 mM), ascorbate (0.5 mM), and H_2O_2 (1.0 mM). The absorbance of light was measured at 290 nm through a spectrometer (Nakano and Asada 1981).

Estimation of lipid peroxidation

To determine the oxidative stress in plant tissues, the frozen plant sample (0.5 g) was ground in 0.1% TCA (10 ml). This plant extract in TCA was centrifuged at 13,000 g for 20 min, and the aliquot was separated carefully. Then this plant extract (0.1 ml) was poured into a test tube containing 50 mM phosphate buffer (1 ml) and 1 M KI (2 ml). After gentle shaking, the absorbance of light was measured at 390 nm for the quantification of hydrogen peroxide (Velikova et al. 2000). The production of MDA content during oxidative stress was also quantified by dissolving 0.1 ml plant TCA extract into 5% thiobarbituric acid (4 ml) and 0.1% TCA (2 ml). The absorbance of light was measured for this plant extract by using a spectrophotometer at 440, 532, and 600 nm wavelengths (Dhindsa et al. 1981).

Estimation of the root, shoot Cd, and mineral content

The quantification of root and shoot nutrient uptake together with Cd accumulation was done by the wet digestion method (Wolf 1982). For this purpose, 0.1 g dry plant matter of root and shoots from each replicate was digested at high temperature by using a digestion mixture composed of $HClO_4$:HNO_3 (3:7 V/V). These digested plant samples were first diluted with 50 ml dH_2O. The root, shoot Cd, and nutrients (K, Mg, Fe, and Zn) were estimated by burning plant-digested sample in atomic absorption spectrophotometer (Hitachi Z-2000, Polarized Zeeman Atomic Absorption Spectrophotometer, Tokyo, Japan). The digested plant sample was examined for N content spectrophotometrically by using Barton's reagent (Bremner and Keeney 1965).

Statistical analysis and interpretation of data

Experimental data was first standardized by using logarithmic or inverse transformations wherever required. Data was subjected to a 3-way analysis of variance (ANOVA) by using statistical software SPSS-21. The means of treatment were equated by using Fisher's LSD at p value of ≤ 0.05 level. Correlations and principle component analysis were executed by using XLSTAT version 2016.1.

Results

Impression of A. schindleri inoculation on growth attributes of pea and linseed seedlings under Cd/wastewater stress

The effect of Cd and wastewater application on growth biomarkers such as (root and shoot lengths, fresh, and

dry biomass) of pea and linseed plants inoculated with *A. schindleri* are presented in (Fig. 1, 2). The root length (RL) and shoot length (SL) of pea and linseed plants were significantly decreased when plants were spiked with 250 and 500 µM Cd as well as irrigated with 75 and 100% wastewater. To this end, the pea plants supplemented with 500 µM Cd stress and 100% wastewater showed 76.46, 45.55% decrease in RL, whereas 63.90, 43.95% decrease in SL comparatively to non-inoculated control plants. Likewise, linseed plants subjected to 500 µM Cd and 100% wastewater had significant reduction of RL 84.55, 45.33% and SL upto 66.75, 55.09% as compared to their non-inoculated control. Nevertheless, inoculated pea and linseed plants exhibited distinctive growth responses when supplemented with 500 µM Cd stress and 100% wastewater (Fig. S1). The *A. schindleri* SR-5–1 had a positive impact on pea plants and significantly improved the RL up to 28.32, 35.50% and SL up to 19.29, 52.94% under 500 µM Cd and 100% WW stress, respectively. Similarly, SR-5–1 inoculated linseed exhibited a marked increase in RL 89.69, 37.62 and SL 33.57,76.21% under 500 µM Cd and 100% WW treatments, respectively.

Pea and linseed plants exposed to different levels of Cd, and wastewater stress showed diminished root and shoot fresh/dry biomasses respective to their control plants. Treatment of non-inoculated pea and linseed plants with 500 µM Cd and 100% WW significantly suppressed the root/shoot fresh and dry biomass in contrast to their controls, but the negative impact was more apparent at 500 µM Cd stress levels as compared to other stress treatments. Besides, inoculation of *A. schindleri* SR-5–1 significantly ameliorated the toxic effects of Cd and wastewater on the growth traits of both pea and linseed plants in contrast to their non-inoculated controls. Results demonstrated that *A. schindleri* SR-5–1 improved the root fresh biomass up to 18.65, 59.63% and root dry biomass up to 17.64, 58.94% whereas shoot fresh biomass was increased by 23.69, 59.54% and shoot dry weight increased by 16.16, 37.28% in pea plants under 500 µM and 100% WW stress, respectively. Likewise,

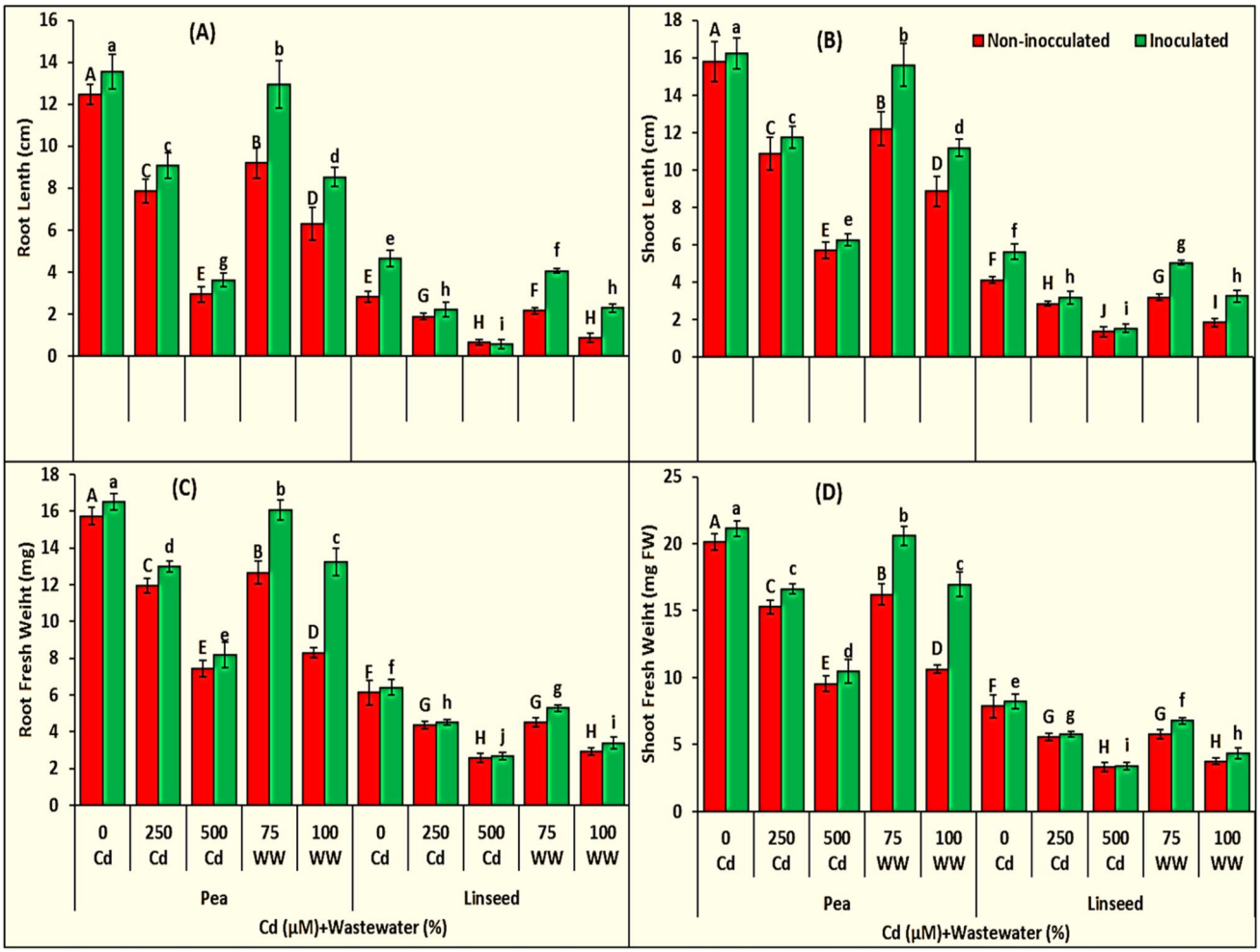

Fig. 1 Effect of *Acinetobacter schindleri* inoculation on **A** root length, **B** shoot length, **C** root fresh weight, and **D** shoot fresh weight of wastewater/Cd stressed *Pisum sativum* and *Linum usitatissimum*. $n = 4, \pm$ SE

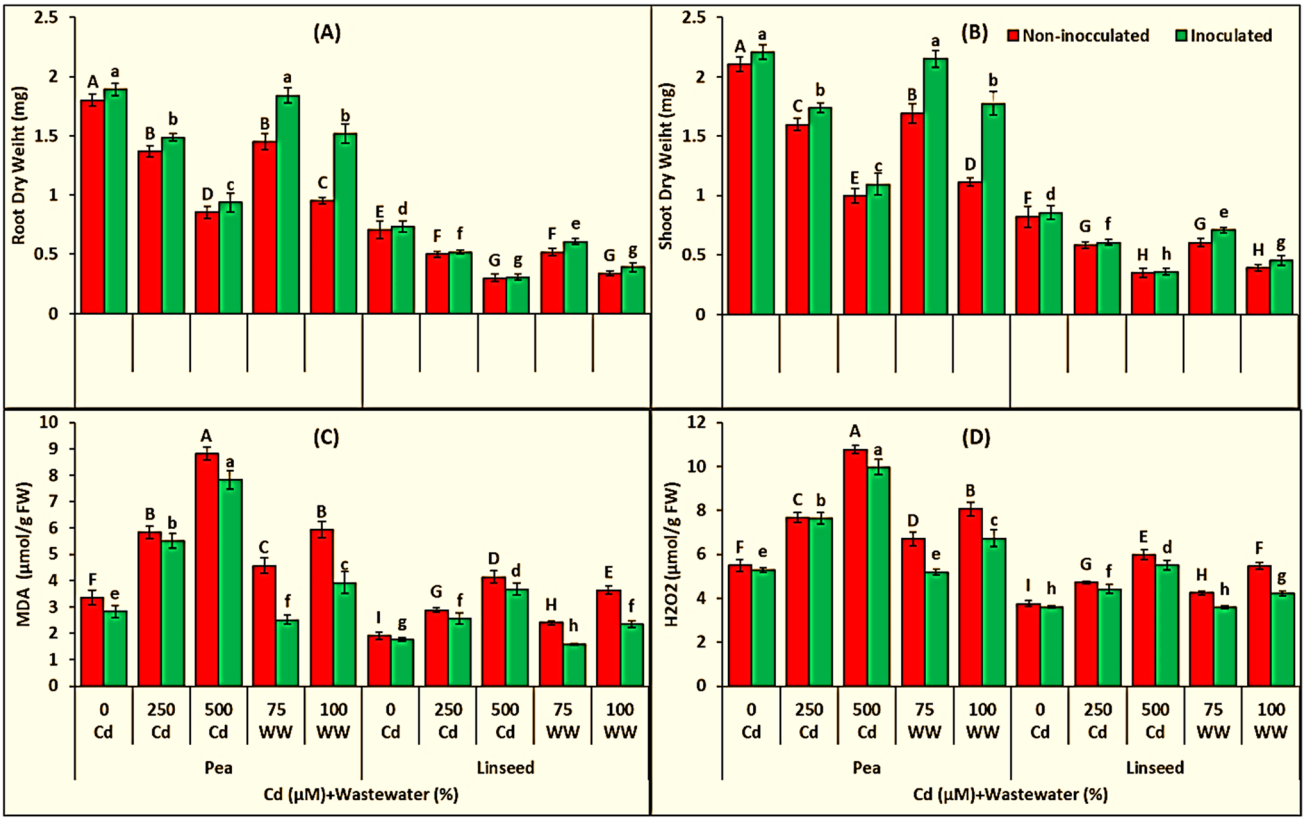

Fig. 2 Effect of *Acinetobacter schindleri* inoculation on **A** root dry weight, **B** shoot dry weight, **C** MDA, and **D** H_2O_2 of wastewater/Cd stressed *Pisum sativum* and *Linum usitatissimum*. $n = 4, \pm SE$

inoculated linseed showed improved root fresh biomass 15.89, 59.63% and root dry biomass 23.69, 16.08%) whereas shoot fresh biomass 13.34, 16.08% and shoot dry biomass 52.94, 41.02% at 500 µM and 100% WW respectively as compared to their non-inoculated control.

Consequence of A. schindleri application on photosynthetic pigments of stressed pea and linseed seedlings

The non-inoculated pea and linseed plants when exposed to Cd and wastewater stress significantly inhibited the plant photosynthetic pigments such as chlorophyll *a*, *b*, total chlorophyll, and carotenoids as presented in Fig. 3. The reduction in chlorophyll *a*, *b*, total chlorophyll, and carotenoids was 62.53, 69.10, 64.43, and 62.55% in pea, whereas in linseed seedlings the decline was 42.52, 64.28, 65.45, and 62.66%, respectively in non-inoculated seedlings at 500 µM Cd stress in comparison to their respective control. Likewise, at 100% WW, the decrease in chlorophyll *a*, *b*, total chlorophyll, and carotenoids was 29.57, 13.82, 21.95, and 40.56% in pea as well as in linseed the diminution was 42.52, 5.10, 31.35, and 36% as compared to control plants. Nevertheless, the inoculation of *A. schindleri* SR-5–1 significantly enhanced the photosynthetic indices in both plants at all applied Cd and wastewater stress treatments as compared to non-inoculated controls. The addition of *A. schindleri* SR-5–1 strain had a positive effect and significantly improved the chlorophyll *a*, *b*, total chlorophyll, and carotenoids up to 32.21, 28.94, 68.45, and 33.33% in pea, while in linseed the increment in photosynthetic related parameters was observed giving 25.84, 45.71, 64.71, and 39.28% increase at 500 µM Cd treatment. Similarly, in pea plants, upon exposure to 100% WW the chlorophyll *a*, *b*, total chlorophyll, and carotenoids contents were augmented up to 31.95, 34.74, 19.09, and 63.49%, whereas in linseed seedlings the up-regulation of photosynthetic indices was recorded as 60, 27.95, 26.74, and 72.91%, respectively in comparison to their non-inoculated controls under similar stress conditions.

Oxidative stress markers portraying A. schindleri-based reduction

Results depicted that Cd and wastewater treatment of the pea and linseed seedlings stimulated the lipid peroxidation and hydrogen peroxide accumulation which are indicators of oxidative stress. Further, MDA levels were drastically enhanced in the order 500 µM Cd > 100%WW > 250 µM Cd > 75%

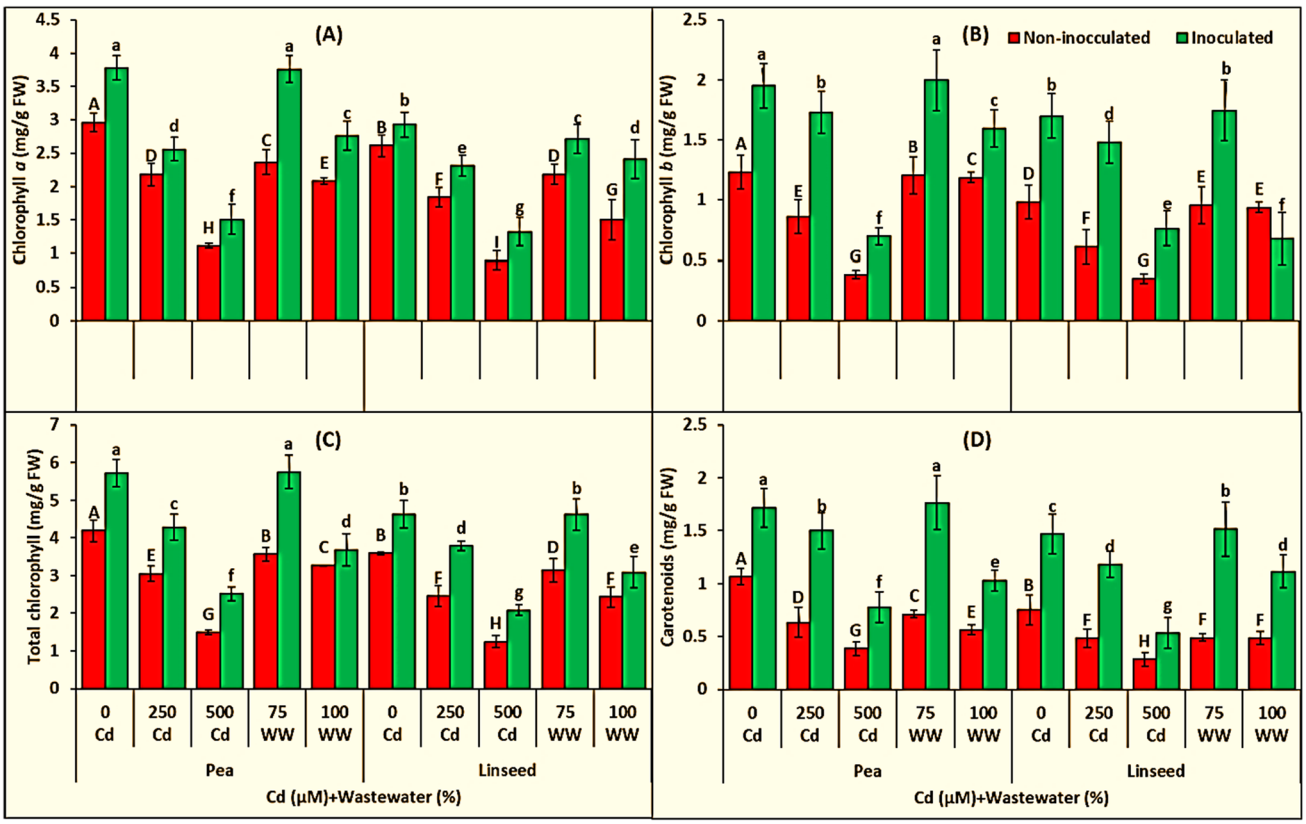

Fig. 3 Impact of *Acinetobacter schindleri* inoculation on **A** chlorophyll *a*, **B** chlorophyll *b*, **C** total chlorophyll, and **D** carotenoids of wastewater/Cd stressed *Pisum sativum* and *Linum usitatissimum*. $n = 4, \pm$ SE

WW stress in pea and linseed seedlings without any microbial treatment in comparison to their respective control. The inoculation of *A. schindleri* SR-5–1 significantly decreased the oxidative stress factors in pea and linseed seedlings at all applied Cd and wastewater stress treatments (Fig. 2). The interaction of Cd stress level 500 µM x bacterial inoculation did not yield a significant change in the MDA and H_2O_2 levels; however, interaction of 100% WW x bacterial inoculation found to be more effective for significant reduction in MDA and H_2O_2 activities. The results demonstrated that application of *A. schindleri* SR-5–1 significantly decreased MDA and H_2O_2 production in pea (28.90, 27.18%) and linseed (48.16, 39.77%) plants at 100% WW stress in contrast to their non-inoculated controls.

Inoculation of A. schindleri enhanced the antioxidants activity in stressed pea and linseed seedlings

Both Cd and wastewater stress modulated the activities of antioxidant enzymes comprising of SOD, POD, CAT, and APX in both non-inoculated pea and linseed seedlings. Evidently, at Cd stress level of 500 µM, significant decline in SOD, POD, CAT, and APX was observed in both non-inoculated pea and linseed seedlings as compared to 100% WW and other stress treatments (Fig. 4). However, *A. schindleri* significantly relieved the Cd and wastewater stress in pea and linseed plants. An increase of 21.93, 14.94, 25, and 19.26% in pea was observed, while an increase of 24.04, 19.31, 28.39, and 72.06% in linseed seedlings was recorded in comparison to their non-inoculated controls at 500 µM Cd treatment. The interaction of 100% WW and SR-5–1 found more influential and resulted in significant increase in antioxidant enzyme activity. At 100% WW stress, an increase of 52.27, 42.06, 30.04, and 68.58% in SOD, POD, CAT, and APX was observed in inoculated pea seedlings in contrast to their non-inoculated controls. Likewise, in linseed seedlings at 100% WW stress, *A. schindleri* SR-5–1 caused significant increase of 43.06, 43.01, 30.56, and 72.06% in SOD, POD, CAT, and APX activities in comparison to non-inoculated control plants subjected to similar stress conditions.

Effect of A. schindleri on Cd accumulation in pea and linseed seedlings

Results demonstrated that Cd bioaccumulation in roots and shoots of non-inoculated pea and linseed seedlings was significantly increased when plants were exposed to different Cd

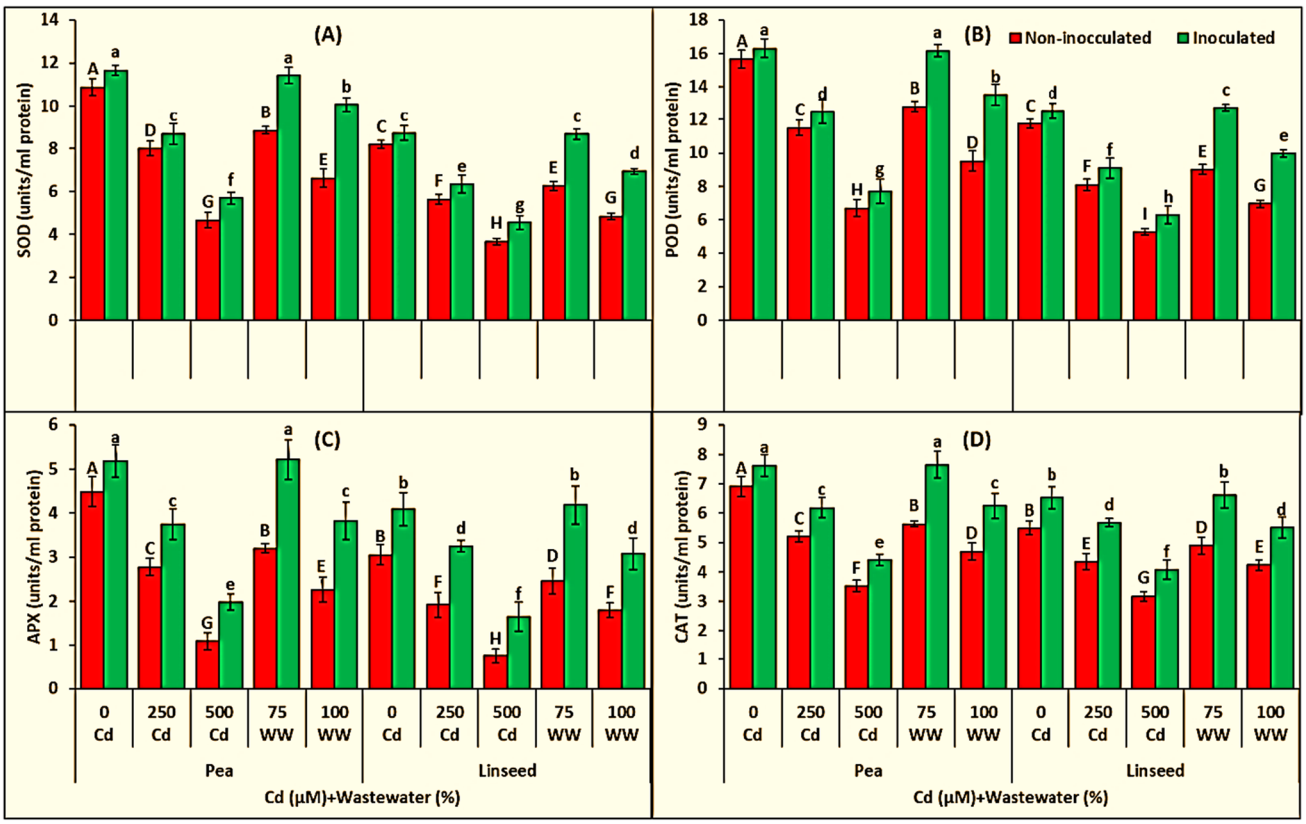

Fig. 4 Effect of *Acinetobacter schindleri* inoculation on **A** SOD, **B** POD, **C** APX, and **D** CAT of wastewater/Cd stressed *Pisum sativum* and *Linum usitatissimum*. $n = 4, \pm SE$

concentrations (250 and 500 µM) and waste water treatments (75% WW and 100% WW) (Table 1). To this end, Cd treatment of 500 µM caused a prominent rise in Cd content in root and shoot tissues of pea and linseed seedlings in comparison to their controls which antagonistically effected the uptake of other essential nutrients. However, SR-5–1 inoculated pea and linseed seedlings exhibited a marked reduction in Cd accumulation in the root and shoot of pea and linseed seedlings. A decrease of 9.79, 9.78% in root and shoot Cd accumulation in inoculated pea seedlings was observed, while in linseed inoculated plants, a decrease of 9.35, 9.36% root and shoot Cd accumulation was recorded at 500 µM Cd stress treatment in contrast to their controls. Interaction of 100% WW and *A. schindleri* SR-5–1 inoculation was found more efficient and a marked reduction of 28.09, 33.07% in root and shoot Cd concentration was found at 100% WW in pea plants, while in linseed seedlings a decline of 30.93, 33.16% root and shoot Cd was reported in comparison to non-inoculated controls.

Consequence of A. schindleri application on nutrient acquisition in pea and linseed

Variation in root and shoot N, K, Mg, Fe, and Zn contents in non-inoculated and inoculated pea and linseed seedlings were presented in Table. 1. The impact of Cd and wastewater treatments was apparent which caused a reduction in essential nutrient in order of 500 µM Cd > 100%WW > 250 µM Cd > 75% WW when compared with their controls. Results depicted that Cd stress level of 500 µM significantly diminished the levels of N, K, Mg, Fe, and Zn up to 58.61, 57.48, 54.01, 44.34, and 48.42% in roots and 58.59, 57.51, 54.0, 48.45, and 48.42% in shoot of non-inoculated pea seedlings as compared to their controls. Similarly, at 500 µM Cd, significant reduction in N, K, Mg, Fe, and Zn contents was noticed giving a decrease of 56.46, 68.60, 49.57, 53.06, and 41.27% in root and 55.52, 68.61, 49.35, 54.56, and 41.29% in shoot of non-inoculated linseed seedlings in comparison to their controls. Meanwhile, at 100% WW, maximum reduction in N, K, Mg, Fe, and Zn was recorded by 48.04, 47.61, 45.06, 33.52, and 38.65% in roots and 50.98, 47.43, 47.38, 35.52, and 38.67% in shoots of non-inoculated pea seedlings as compared to their controls. Likewise, in non-inoculated linseed seedlings maximum decline in N, K, Mg, Fe, and Zn was observed by 41.85, 55.30, 26.06, 39.52, and 35.30% in root and 43.2, 55.29, 25.96, 39.54, and 35.28% in shoot as compared to their respective controls. However, the interaction between bacterial and 500 µM Cd treatment did not significantly persuade the nutrient acquisition in pea and

Table 1 Effect of *Acinetobacter schindleri* inoculation on root, shoot Cd, and plant nutrients content of wastewater/Cd stressed *Pisum sativum* and *Linum usitatissimum*. $n = 4, \pm SE$

Plants	Microbial treatment	Cd (µM) /Wastewater (%)	Root						Shoot					
			Cd	N	K	Mg	Fe	Zn	Cd	N	K	Mg	Fe	Zn
			µg/g DW	(mg/Kg DW)					µg/g DW	(mg/Kg DW)				
Pea	Non-inoculated	0 Cd	14.48 ± 0.77^{H}	230.3 ± 9.90^{C}	23.1 ± 1.86^{C}	3.24 ± 0.12^{B}	85.7 ± 0.76^{B}	34.51 ± 1.26^{A}	10.2 ± 0.54^{G}	157.8 ± 6.78^{B}	17.1 ± 1.39^{C}	2.8 ± 0.11^{B}	66.4 ± 0.59^{A}	30.5 ± 1.12^{B}
		250 Cd	34.57 ± 1.23^{D}	175.6 ± 6.36^{G}	15.28 ± 1.17^{F}	2.76 ± 0.05^{E}	65.2 ± 1.30^{E}	27.57 ± 0.78^{C}	24.3 ± 0.86^{D}	120.3 ± 4.38^{F}	11.3 ± 0.87^{F}	2.4 ± 0.04^{D}	50.5 ± 1.01^{D}	24.4 ± 0.69^{C}
		500 Cd	64.65 ± 2.09^{A}	95.3 ± 10.48^{I}	9.82 ± 0.61^{H}	1.49 ± 0.09^{H}	47.7 ± 1.03^{G}	17.8 ± 0.52^{E}	45.5 ± 1.47^{A}	65.29 ± 7.17^{I}	7.27 ± 0.45^{I}	1.3 ± 0.09^{H}	34.3 ± 2.20^{G}	15.7 ± 0.46^{E}
		75% WW	30.96 ± 1.10^{F}	200.6 ± 6.64^{D}	16.84 ± 1.35^{F}	2.77 ± 0.06^{E}	69.33 ± 0.96^{E}	29.33 ± 0.49^{B}	21.8 ± 0.77^{E}	130.7 ± 7.97^{C}	11.8 ± 0.72^{F}	2.4 ± 0.03^{E}	52.7 ± 1.32^{C}	25.3 ± 1.60^{C}
		100% WW	47.48 ± 1.88^{C}	119.6 ± 4.37^{H}	12.1 ± 0.87^{G}	1.78 ± 0.06^{G}	56.97 ± 1.62^{F}	21.17 ± 0.49^{D}	33.4 ± 1.32^{C}	77.29 ± 3.70^{H}	8.96 ± 0.65^{H}	1.5 ± 0.04^{G}	42.83 ± 1.55^{F}	18.7 ± 0.43^{D}
	Inoculated	0 Cd	11.82 ± 1.47^{I}	250.3 ± 9.38^{B}	24.77 ± 1.21^{B}	3.31 ± 0.05^{A}	87.37 ± 0.93^{A}	35.51 ± 0.64^{A}	8.3 ± 1.04^{H}	168.3 ± 4.85^{B}	18.3 ± 0.90^{B}	2.9 ± 0.03^{A}	67.7 ± 0.72^{A}	31.4 ± 0.57^{A}
		250 Cd	32.23 ± 2.25^{E}	184.3 ± 7.51^{E}	18.28 ± 0.56^{D}	2.83 ± 0.03^{D}	67.2 ± 1.35^{E}	28.23 ± 1.04^{C}	22.7 ± 1.59^{E}	126.2 ± 5.15^{D}	13.5 ± 0.41^{E}	2.5 ± 0.03^{C}	52.1 ± 1.05^{C}	24.9 ± 0.92^{C}
		500 Cd	58.32 ± 1.06^{B}	15.3 ± 4.41^{J}	11.32 ± 0.74^{G}	1.86 ± 0.06^{F}	54.7 ± 2.50^{F}	21.8 ± 0.61^{D}	40 ± 0.75^{B}	81.6 ± 3.02^{G}	9.51 ± 0.55^{G}	1.6 ± 0.06^{F}	44.53 ± 1.94^{E}	18.6 ± 0.54^{D}
		75% WW	19.29 ± 1.37^{G}	252 ± 5.51^{A}	25.17 ± 1.22^{A}	3.23 ± 0.05^{B}	82.67 ± 2.63^{C}	35 ± 0.93^{A}	10.6 ± 1.47^{G}	172.6 ± 3.77^{A}	19.6 ± 0.56^{A}	2.8 ± 0.05^{B}	65.42 ± 1.50^{A}	31.0 ± 0.82^{B}
		100% WW	32.14 ± 1.83^{E}	179.6 ± 7.69^{F}	20.07 ± 0.93^{E}	2.98 ± 0.06^{C}	70.3 ± 1.79^{D}	28.17 ± 1.18^{C}	19.4 ± 1.61^{F}	124.1 ± 4.32^{E}	14.9 ± 0.69^{D}	2.5 ± 0.05^{D}	54.5 ± 1.39^{B}	24.9 ± 1.05^{C}
Linseed	Non-inoculated	0 Cd	9.56 ± 0.53i	107.2 ± 3.01c	36.69 ± 1.18^{b}	3.53 ± 0.13^{c}	57.38 ± 0.84^{c}	24.47 ± 0.46^{b}	6.7 ± 0.37^{h}	73.42 ± 2.05^{c}	27.2 ± 0.88^{a}	3.1 ± 0.11^{b}	44.48 ± 0.65^{c}	21.6 ± 0.41^{b}
		250 Cd	27.05 ± 1.92^{e}	76.8 ± 6.93^{f}	22.7 ± 1.07^{d}	2.86 ± 0.04^{g}	45.37 ± 1.16^{e}	19.37 ± 0.59^{f}	19.0 ± 1.35^{d}	52.62 ± 4.74^{f}	16.8 ± 0.79^{e}	2.5 ± 0.03^{e}	35.17 ± 0.90^{f}	17.1 ± 0.52^{e}
		500 Cd	49.9 ± 0.33^{a}	47.7 ± 3.71^{i}	11.52 ± 1.20^{g}	1.78 ± 0.06^{i}	26.93 ± 0.99^{g}	14.37 ± 0.59^{h}	35.1 ± 0.23^{a}	32.65 ± 2.54^{h}	8.53 ± 0.89^{h}	1.6 ± 0.05^{h}	20.21 ± 1.29^{i}	12.7 ± 0.52^{g}
		75% WW	24.75 ± 1.70^{g}	94 ± 4.36^{d}	26.07 ± 0.96^{c}	2.92 ± 0.02^{e}	45.37 ± 1.20^{e}	19.63 ± 0.26^{f}	17.4 ± 1.20^{f}	57.72 ± 4.10^{e}	19.3 ± 0.71^{d}	2.6 ± 0.02^{d}	33.5 ± 1.13^{g}	17.4 ± 0.23^{e}
		100% WW	43.09 ± 2.11^{c}	62.3 ± 3.71^{g}	16.4 ± 0.94^{e}	2.61 ± 0.25^{g}	34.7 ± 0.90^{f}	15.83 ± 0.37^{h}	30.3 ± 1.49^{b}	41.69 ± 2.37^{g}	12.1 ± 0.70^{f}	2.3 ± 0.02^{f}	26.89 ± 0.69^{h}	14.1 ± 0.33^{f}
	Inoculated	0 Cd	8.89 ± 0.80^{j}	119.2 ± 7.1^{b}	37.02 ± 0.92^{a}	3.66 ± 0.07^{a}	59.05 ± 0.92^{b}	25.47 ± 0.59^{a}	6.3 ± 0.56^{h}	81.64 ± 4.87^{a}	27.4 ± 0.68^{a}	3.2 ± 0.06^{a}	45.77 ± 0.71^{b}	22.5 ± 0.53^{a}
		250 Cd	25.72 ± 1.19^{f}	84.2 ± 5.26^{e}	23.03 ± 0.79^{d}	2.89 ± 0.06^{f}	47.7 ± 1.61^{d}	20.03 ± 0.19^{e}	17.1 ± 0.83^{f}	57.71 ± 3.60^{e}	17.1 ± 0.58^{e}	2.6 ± 0.05^{d}	36.98 ± 1.24^{e}	17.7 ± 0.11^{e}
		500 Cd	45.23 ± 2.88^{b}	59.3 ± 4.05^{h}	13.93 ± 0.72^{f}	1.98 ± 0.06^{h}	33.93 ± 0.88^{f}	18.03 ± 0.12^{g}	29.8 ± 2.03^{c}	42.1 ± 2.77^{g}	11.1 ± 0.53^{g}	2.0 ± 0.05^{g}	27.20 ± 0.68^{h}	15.3 ± 0.11^{f}
		75% WW	12.42 ± 1.10^{h}	124.3 ± 3.93^{a}	36.07 ± 0.97^{b}	3.63 ± 0.10^{b}	65.37 ± 1.20^{a}	23.3 ± 1.41^{c}	8.41.46 ^{g}	77.16 ± 7.1^{b}	26.7 ± 0.71^{b}	3.2 ± 0.08^{a}	51.01 ± 0.63^{a}	20.6 ± 1.24^{c}
		100% WW	29.76 ± 2.64^{d}	95.7 ± 4.63^{d}	26.4 ± 0.94^{c}	3.04 ± 0.26^{d}	47.7 ± 1.82^{d}	22.03 ± 0.39^{d}	18.3 ± 1.26^{e}	65.52 ± 3.17^{d}	20.2 ± 0.30^{c}	3.0 ± 0.23^{c}	37.43 ± 1.40^{d}	18.7 ± 0.34^{d}

linseed seedlings and no significant change in the uptake of essential nutrients (N, K, Mg, Fe, and Zn) was observed in comparison to their non-inoculated controls. Nevertheless, at 100% WW, *A. schindleri* SR-5–1 significantly improved the uptake of N, K, Mg, Fe, and Zn by 50.16, 65.88, 56.7, 23.41, and 33.06% in roots and 60.43, 81.15, 2.91, 27.24, and 39.36% in shoots of pea seedlings in contrast to non-inoculated controls. Likewise, in *A. schindleri* SR-5–1 inoculated linseed seedlings, a maximum increase in N, K, Mg, Fe, and Zn was recorded by 53.48, 60.97, 16.47, 31.70, and 26.55% in roots and 57.15, 66.41, 16.45, 31.75, and 26.55% in shoots as compared to their non-inoculated controls.

Correlation between growth, photosynthetic efficiency with Cd uptake

A Pearson correlation analysis was conducted to explore different morpho-physiological traits in linseed and pea plants (Fig. 5). In pea plant, Cd concentration in the roots was positively correlated with Cd concentration in shoots, malondialdehyde and hydrogen peroxide contents while negatively correlated with carotenoid content, chlorophyll *a*. *b* content, catalase activity, total chlorophyll content, potassium concentration in roots and shoots, ascorbate peroxidase activity, root/shoot length, superoxidase activity, root/shoot fresh weight, nitrogen in the roots/shoots, peroxidase activity, root/shoot dry weight, zinc concentration in the roots/shoots, magnesium concentration in the root/shoots, and iron concentration in the roots/shoots. Similarly, the Cd concentration in the shoots was positively correlated Cd concentration in the roots, malondialdehyde content, and hydrogen peroxide content, while negatively correlated with carotenoid content, chlorophyll *a*, *b* content, catalase activity, total chlorophyll content, potassium concentration in the roots/shoots, ascorbate peroxidase activity, root/shoot length, superoxidase activity, root/shoot fresh weight, iron concentration in the roots/shoots, peroxidase activity, root/shoot dry weight, zinc concentration in the roots/shoots, magnesium concentration in the roots/shoots, and nitrogen concentration in the roots/shoots.

Similarly, for linseed plant, the Cd concentration in the roots was positively correlated Cd concentration in the shoots, malondialdehyde content, and hydrogen peroxide content, while negatively correlated with carotenoid content, chlorophyll *a*, *b* content, catalase activity, total chlorophyll content, potassium concentration in the roots/

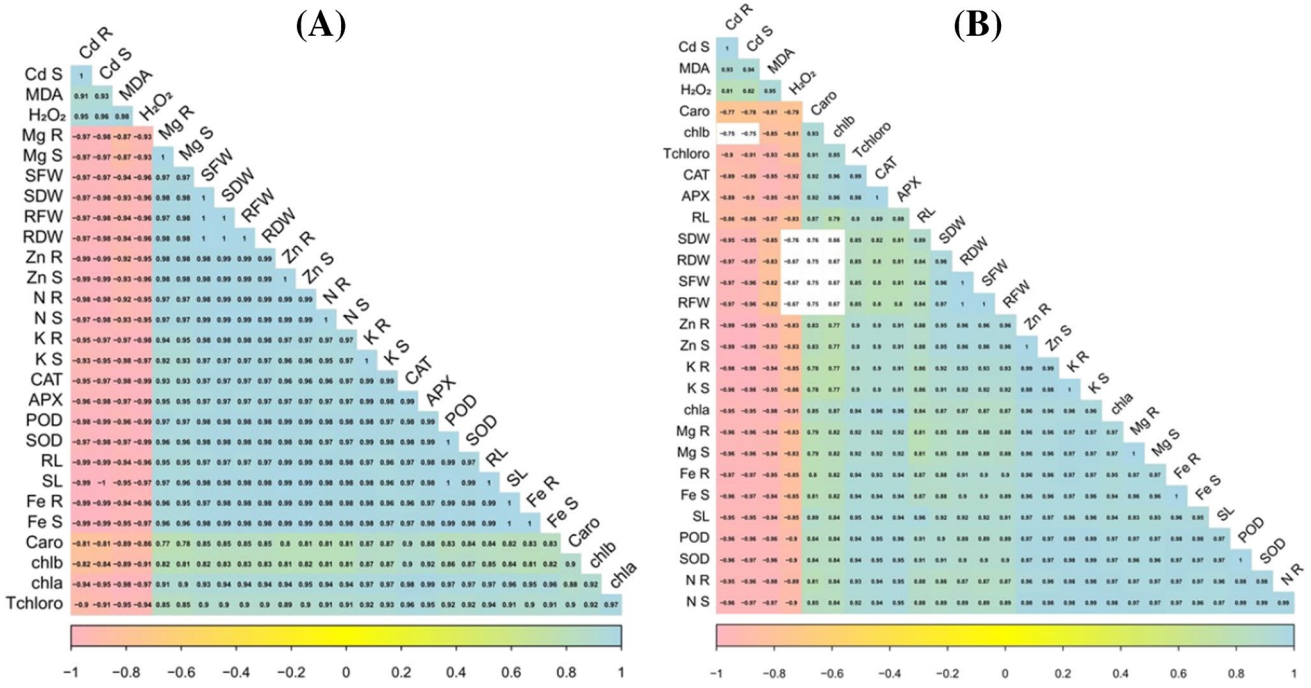

Fig. 5 Correlation between different morphological traits, physiological attributes, and Cd accumulation in pea (**A**) and linseed (**B**) plants grown with and without SR-5–1 inoculation. Different abbreviations used in this figure are as follows: Cd S (Cd concentration in the shoots), Cd R (Cd concentration in the roots), MDA (malondialdehyde content), H_2O_2 (hydrogen peroxide content), Caro (carotenoid content), chlb (chlorophyll *b* content), CAT (catalase activity), Tchoro (total chlorophyll content), KS (potassium concentration in the shoots), KR (potassium concentration in the roots), APX (ascorbate peroxidase activity), chla (chlorophyll *a* content), RL (root length), SOD (superoxidase activity), SFW (shoot fresh weight), FeS (iron concentration in the shoots), RFW (root fresh weight), SL (shoot length), NS (nitrogen in the shoots), POD (peroxidase activity), SDW (shoot dry weight), RDW (root dry weight), ZnR (zinc concentration in the roots), ZnS (zinc concentration in the shoots), MgS (magnesium concentration in the shoots), MgR (magnesium concentration in the roots), FeR (iron concentration in the roots), and NR (nitrogen concentration in the roots)

shoots, ascorbate peroxidase activity, root/shoot length, superoxidase activity, root/shoot fresh weight, iron concentration in the roots/shoots, peroxidase activity, root/shoot dry weight, zinc concentration in the roots/shoots, magnesium concentration in the roots/shoots, and nitrogen concentration in the roots/shoots. Similarly, the Cd concentration in the shoots was positively correlated with Cd concentration in the roots, malondialdehyde content, and hydrogen peroxide content, while negatively correlated with carotenoid content, chlorophyll *a*, *b* content, catalase activity, total chlorophyll content, potassium concentration in the roots/shoots, ascorbate peroxidase activity, root/shoot length, superoxidase activity, root/shoot fresh weight, peroxidase activity, root/shoot dry weight, zinc concentration in the roots/shoots, magnesium concentration in the roots/shoots, iron concentration in the roots/shoots, and nitrogen concentration in the roots/shoots. This relationship depicted a close concentration in Cd uptake with eco-physiology of both plants, i.e., linseed and pea.

Principal component analysis

A PCA was also conducted for both plants, i.e., pea and linseed to study different morpho-physiological traits, ions accumulation, and Cd uptake by the plant (Fig. 6). In pea plants genotype, Dim1 (PCA-1) comprised 95.3% and Dim2 (PCA-2) comprised 2.5%, while in linseed plants Dim2 (PCA-2) comprised 31.5% and Dim2 (PCA-2) comprised 4.2% from the whole database. In both plants, i.e., pea and linseed, all the variables dispersed successfully in the whole database. This gave a clear indication that Cd toxicity significantly affected the morpho-physiological attributes of both plant species. For both plant species, Cd concentration in the shoots, Cd concentration in the roots, malondialdehyde content, and hydrogen peroxide content were positively correlated with all other studied variables in this experiment. While a significant negative relationship was observed in carotenoid content, chlorophyll *a*, *b* content, catalase activity, total chlorophyll content, potassium concentration in the roots/shoots, ascorbate peroxidase activity, root/shoot length, superoxidase activity, root/shoot fresh weight, peroxidase activity, root/shoot dry weight, zinc concentration in the roots/shoots, magnesium concentration in the roots/

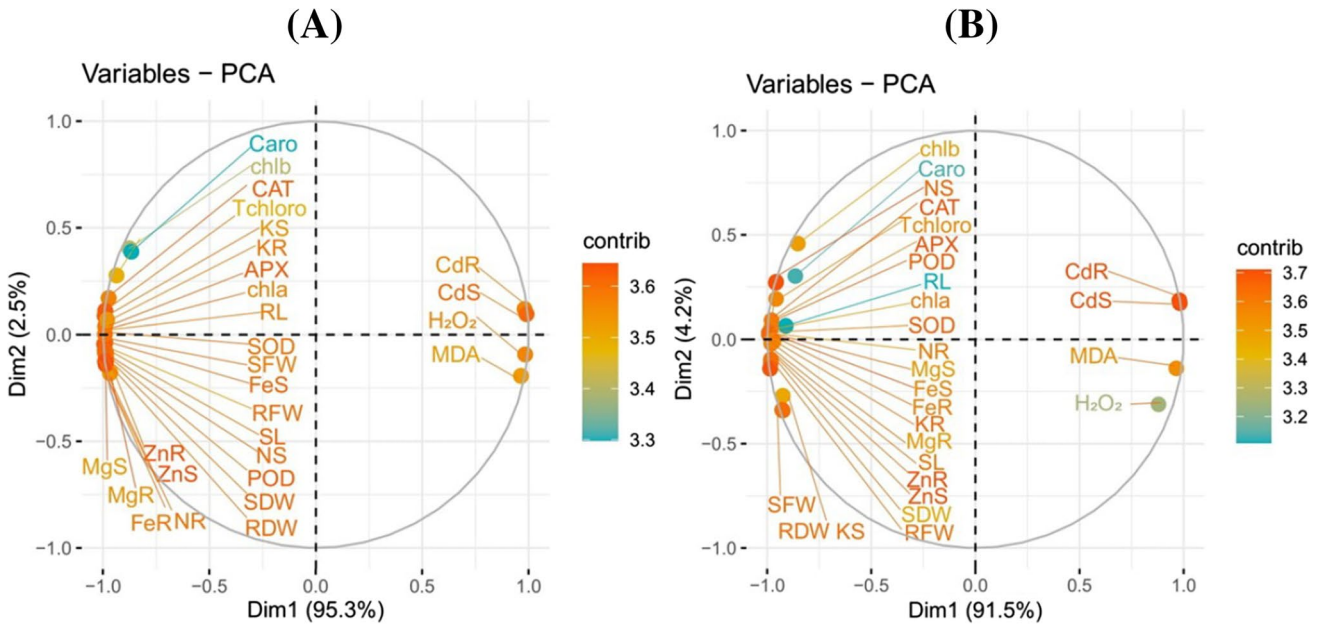

Fig. 6 Loading plots of principal component analysis on different morpho-physiological traits and Cd accumulation in pea (**A**) and linseed (**B**) plants grown with and without SR-5–1 inoculation. Different abbreviations used in this figure are as follow: Different abbreviations used in this figure are as follows: Cd S (Cd concentration in the shoots), Cd R (Cd concentration in the roots), MDA (malondialdehyde content), H_2O_2 (hydrogen peroxide content), Caro (carotenoid content), chlb (chlorophyll *b* content), CAT (catalase activity), Tchoro (total chlorophyll content), KS (potassium concentration in the shoots), KR (potassium concentration in the roots), APX (ascorbate peroxidase activity), chla (chlorophyll *a* content), RL (root length), SOD (superoxidase activity), SFW (shoot fresh weight), FeS (iron concentration in the shoots), RFW (root fresh weight), SL (shoot length), NS (nitrogen in the shoots), POD (peroxidase activity), SDW (shoot dry weight), RDW (root dry weight), ZnR (zinc concentration in the roots), ZnS (zinc concentration in the shoots), MgS (magnesium concentration in the shoots), MgR (magnesium concentration in the roots), FeR (iron concentration in the roots), and NR (nitrogen concentration in the roots)

shoots, iron concentration in the roots/shoots, and nitrogen concentration in the roots/shoots.

Discussion

Toxic metals and wastewater negatively affect the bioactivity of microorganisms in the soil; however, metal-tolerant bacteria with PGPR characteristics could survive the toxic habitats and could be isolated and characterized for the potential use in implicating plant growth promotion in contaminated environments. In the conception of the current study, we selected metal-tolerant bacteria which could resist Cd toxicity regimes in vitro conditions. A pot experiment was executed to monitor the outputs of plant–microbe association for the amelioration of Cd and wastewater stress in a nitrogen-fixing pea and non-nitrogen-fixing linseed plants.

Upgraded plant growth through inoculation of A. schindleri

Outcomes of the current study depicted that application of different Cd and wastewater treatments inhibited the growth (root and shoot lengths) as well as fresh and dry biomass in both non-inoculated pea and linseed seedlings (Fig. 1, 2). Our results corroborated with the previous studies which indicated that Cd stress decreased pea (Shamshad et al. 2018), and linseed growth attributes (Shahid et al. 2019) due to the overproduction of reactive oxygen species (ROS). Furthermore, wastewater fertigation significantly diminished *H. esculentus* growth due to the increased accumulation of salts and heavy metals in root and shoot tissues (Kumar et al. 2016). It was inferred that higher reduction in root and shoot lengths as well as fresh and dry biomasses in a Cd dose-dependent manner without any bacterial inoculation pointed out that Cd stress exerted more negative effect on photosynthetic efficiency and water relations in both plants than the wastewater which might be due to increased levels of lipid peroxidation and disruption in nutrient acquisition. On the other hand, plant growth and biomass accumulation were significantly increased under Cd and wastewater stress in SR-5–1 inoculated pea and linseed plants in comparison to their non-inoculated controls (Fig. 1, 2). In the current study, increased plant growth and biomass traits in both inoculated plants at higher Cd and wastewater stress levels were envisioned owing to the Cd-tolerance, ACC deaminase activity, and phosphate solubilizing activity of SR-5–1 which corroborates with earlier reports that *Acinetobacter* sp. inoculation increased plant growth and biomass by ameliorating the toxic effects of heavy metals (Abbas et al. 2020). Likewise, inoculation of *Acinetobacter* sp. exhibited IAA production in symbiosis with host plants which enhanced the root elongation and development and thereby improved the water uptake, nutrient transport, and conferred tolerance to chickpea plants (Srivastava and Singh 2014). The reduction in growth attributes of pea plants under Cd and wastewater regimes also indicated the non-occurrence or weak population of nodulating bacteria in the root zone of pea plants.

Improved photosynthesis through inoculation of Acinetobacter schindleri

Current findings exhibited that Cd and wastewater stress significantly decreased the photosynthetic efficiency of non-inoculated pea and linseed plants in a dose-dependent manner by deteriorating the photosynthetic pigments which indicate the imposition of oxidative stress damage (Fig. 3). Higher reduction in chlorophyll and carotenoid contents may have occurred due to Cd-triggered diminution of protochlorophyllide reductase enzymes, suppression of thylakoid membrane, boost of plastoglobuli and reduction in Mg uptake in non-inoculated plants under Cd stress (Hussain et al. 2019; Tanwir et al. 2021). Intriguingly, wastewater dilutions, i.e., 75% and 100% WW have less negative impact on both non-inoculated pea and linseed seedlings. Different studies reported the stimulatory effects of wastewater on plants (Hashmat et al. 2021) while some indicate the antagonistic effects of wastewater on photosynthetic traits in plants due to the presence of heavy metals (Slima and Ahmed 2020). Conversely, in our study bacterial inoculation enhanced the photosynthetic attributes in both pea and linseed resulting in a higher photosynthetic rate suggesting that SR-5–1 strengthens the synthesis of light-harvesting pigments under Cd and wastewater stress (Fig. 3). Likewise, SR-5–1 inoculation markedly enhanced the chlorophyll contents in wastewater irrigated pea and linseed plants than Cd treated plants resulting in pronounced growth under stress. Previously, it has been reported that *Acinetobacter* sp. inoculation improved photosynthetic parameters, i.e., chlorophyll synthesis, photo chlorophyllide, and chloroplast development owing to IAA biosynthesis and phosphate solubilization activity of the selected strain depicting its role in stress management (Yang et al. 2018; Yasin et al. 2019). Evidently, application of *Acinetobacter* sp. enhanced photosynthetic attributes and these outcomes are concurrent to the findings in maize under Cd stress (Abbas et al. 2020), chickpea under arsenic stress (Srivastava and Singh 2014), and maize under wastewater application (Khan and Bano 2016).

Acinetobacter schindleri-based reduction in oxidative stress markers

Results depicted that imposed Cd and wastewater stress triggered MDA formation which indicates cellular membrane damages associated with enhanced H_2O_2. This may lead plants towards oxidative stress and increased ion leakage in

non-inoculated plants (Fig. 2). Wastewater/Cd stress collectively generates oxidative stress through ROS genesis which enhanced lipid peroxidation and disrupts cellular structures simultaneously with impaired enzymatic activities (Kumar et al. 2017; Din et al. 2020; Hashmat et al. 2021). Besides, application of *A. schindleri* significantly ameliorated the Cd and wastewater-induced oxidative stress in both pea and linseed plants through reduced MDA accumulation and H_2O_2 scavenging via enhanced antioxidant activities (Fig. 2). Seed inoculation with *A. schindleri* SR-5–1 resulted in significant decrease of MDA and H_2O_2 predominantly under wastewater application as compared to Cd stress. Our results corroborated with previous findings of Khan and Bano (2016) that *Pseudomonas* sp., and *Bacillus cerus* application amplified the maize growth under heavy metal-contaminated wastewater. Further, *Acinetobacter* sp. application relieved the Cd stress and caused a notable increase in plant growth through decreased lipid peroxidation levels (Abbas et al. 2020). Nevertheless, many PGPRs modify the metal availability via glutathione-derived peptides secretion associated with defense against heavy metal-triggered oxidative stress damage (Tanwir et al. 2021).

Influence of Acinetobacter schindleri on antioxidant activities

Plants under metal stress exhibit various antioxidant markers as the first line of defense to protect themselves from oxidative stress triggered by ROS accumulation (Ahmad et al. 2022). In the present work, different Cd stress levels and wastewater treatments decreased the antioxidative machinery (SOD, POD, CAT, and APX) in both non-inoculated pea and linseed seedlings (Fig. 4). Significant reduction in antioxidant activity was observed in non-inoculated plants which indicate that plants encountered higher lipid peroxidation and their derivatives ROS which originated from diminished enzymatic biosynthesis and protein degradation due to Cd and wastewater toxicity (Hajihashemi et al. 2020; Hashmat et al. 2021). Inoculation with SR-5–1 significantly enhanced the activities of SOD, POD, CAT, and APX in both pea and linseed plants in comparison to their non-inoculated control plants. Bacterial inoculation reflected higher antioxidant levels to control the oxidative stress injury and initiated defense system predominantly under wastewater as compared to Cd (Fig. 4). Recently, research reports described the upregulation of antioxidant activities with *Acinetobacter* sp. strain CS9 application in *Catharanthus longifolius* plants (Yasin et al. 2018). In another study, the inoculation of *Acinetobacter* sp. substantially augmented the mRNA expression for the antioxidant system enabling plants to cope with ROS-induced oxidative damage (Desoky et al. 2020). Besides, PGPR-induced improved antioxidant system protects membrane degradation by transforming reactive oxygen species into non-toxic compounds (Gupta et al. 2020; Sharma et al. 2021).

Effect of A. schindleri inoculation on root shoot accumulation of Cd, N, and K in pea and linseed plants

In the present study, Cd and wastewater application in pea and linseed caused higher Cd retention in root apoplast which triggered enhanced Cd accumulation in non-inoculated plants (Sardar et al. 2022). Higher Cd accumulation could be associated with Cd competition with other nutrients at the root uptake site whereas wastewater application increase the concentration of trace metals that may restrict the uptake of essential nutrients (Slima and Ahmed 2020). The Cd stress may hinder the uptake of essential nutrients due to its harmful effects on root growth, negatively charged sites in the cell walls, membrane transporters, and plasma membranes permeability thereby causing mineral shortage in plants (Zhang et al. 2019). Cadmium stress depolarizes the root cell plasma membrane thereby disrupting the driving potential for nutrient uptake. It also deteriorates the rate of photosynthesis by terminating the DNA and plasma membrane of plastids (Abbas et al. 2018; Sardar et al. 2022). Contrarily, Cd and wastewater stress significantly diminished the uptake of N and K in both non-inoculated pea and linseed plants as compared to controls (Table 1). In accordance with our outcomes, previous studies have reported Cd stress hindered the nutrient uptake in *Medicago sativa* plants (Zhang et al. 2019), and wastewater irrigation diminished the content of N and K in pea plants (Galal et al. 2018; Slima and Ahmed 2020, Hashmat et al. 2021). However, application of SR-5–1 significantly decreased the Cd uptake and accumulation in plant tissues predominantly in wastewater stress in both pea and linseed plants. Our results demonstrated that *A. schindleri* ameliorated Cd toxicity either by Cd sequestration in nearby rhizospheric zone or through binding with soil colloids in both inoculated plants (Table 1). The ability of SR-5–1 to decrease Cd uptake might be related to the biosynthesis of bacterial exopolysaccharide carrying high affinity towards metallic cations like Cd ions thereby playing an important role in plant resistance against metal-induced stress (Etesami and Maheshwari 2018). It was anticipated that *A. schindleri* SR-5–1 significantly improved the uptake of essential nutrients which had a positive effect on both plants and reduced ROS overproduction and abridged the NADPH oxidase activity (Ahmed et al. 2022).

Influence of A. schindleri inoculation on uptake and translocation of Mg, Fe, and Zn

Results depicted that Cd and wastewater irrigation curtailed the uptake and accumulation of essential nutrients (Mg, Fe,

and Zn) and the malnutrition of these nutrients subsequently caused the leaf chlorosis (Farhat et al. 2021; Sardar et al. 2022). Various studies have demonstrated that Cd stress negatively impacts the uptake of essential nutrients (Zhao et al. 2020; Abbas et al. 2021). The trace metals present in wastewater can substitute the necessary cations from specific binding's site and appeared to disrupt the radical migration of Mg over the root section and enhanced the binding of Cd to the chlorophyll owing to decreased levels of Mg in the chloroplast (Khaliq et al. 2019). Previous studies elaborated that metal-induced stress down-regulates the Fe transporter *IRT1* in wheat linked with inhibition of Fe uptake (Greger et al. 2016). Since Cd uses the same transport channels for influx into root tissue, therefore, it was suggested that Cd utilized the *ZNT1* transport channel resulted in antagonistic effects on mineral uptake (Khaliq et al. 2019). Inoculation with SR-5–1 enhanced the accumulation of Mg, Fe, and Zn in both pea and linseed plants under Cd and wastewater stress as compared to non-inoculated controls (Table 1). Our results coincided with the study of Abbas et al. (2020) who concluded that nutrient uptake was augmented in maize plants due to the phosphate solubilization activity of *Acinetobacter* SG-5. Likewise, *Serratia* sp. CP-13 evidently increased the nutrient contents of linseed crop through enhanced root surface area which offer more uptake site for nutrient binding (Shahid et al. 2019). Plant growth-promoting bacteria increased plant growth by improved cell division and cell elongation, and altering the expression of certain genes through biosynthesis of IAA and ACC deaminase activity (Etesami and Maheshwari 2018). Moreover, interaction of *A. schindleri* SR-5–1 with pea (nitrogen fixing) confers higher growth through increased biomass, photosynthetic pigments, antioxidant system, nutrient uptake, and by reducing the transport of Cd into the root and shoot tissues as compared to non-nitrogen fixing linseed. These results corroborated the findings of Bianucci et al. (2013), who showed that the PGPR and nitrogen-fixing plant synergy triggered the plant growth through improved physio-biochemical processes and nutrient uptake in *Arachis hypogaea* L. plant**.**

Conclusion

Our results demonstrated that heavy metal-tolerant bacteria can thrive in contaminated vicinities which may retain PGP activity even under metal and wastewater stress. The potential of a newly isolated strain, *Acinetobacter schindleri* SR-5–1 from wastewater was assessed and its ability to promote plant growth in nitrogen-fixing pea and non-nitrogen-fixing linseed plants under Cd and wastewater stress was monitored. The Cd and wastewater stress diminished the physio-biochemical and nutrient uptake in both plants without bacterial inoculation and had significant effect on Cd absorption. At the same time, *A. schindleri* SR-5–1 relieved the pea and linseed plants from harmful impacts of Cd and wastewater through exhibition of PGR traits together with plant symbiosis. *A. schindleri* SR-5–1 interaction with pea and linseed plants promoted the plant growth through increased biomasses, photosynthetic pigments, antioxidant system, nutrient uptake and reducing the transport of Cd into the root and shoot tissues. The interaction between wastewater and plant growth was positively correlated which indicated the potential of beneficial PGPB to be used as bioinoculant in crops irrigated with wastewater. It was also suggested that with inoculation of SR-5–1, wastewater dilution of 75% WW for both nitrogen-fixing and non-nitrogen-fixing crops can be applied. Moreover, the presented results revealed that synergistic association of SR-5–1 and nitrogen-fixing macrophytes further improved the plant growth and nutrient uptake. Study outcomes further suggest that the field trials should be executed to draw parallels among *Acinetobacter schindleri* SR-5–1 inoculation, arable crops, and physio-biochemical modulations to validate the PGPB as bioremediation and biofertilizer tool under metal and wastewater contamination.

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Declarations

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