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Influence of Metal-Resistant *Staphylococcus aureus* Strain K1 on the Alleviation of Chromium Stress in Wheat

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Abstract: Chromium (Cr) is recognized as a toxic metal that has detrimental effects on living organisms; notably, it is discharged into soil by various industries as a result of anthropogenic activities. Microbe-assisted phytoremediation is one of the most emergent and environmentally friendly methods used for the detoxification of pollutants. In this study, the alleviative role of *Staphylococcus aureus* strain K1 was evaluated in wheat (*Triticum aestivum* L.) under Cr stress. For this, various Cr concentrations (0, 25, 50 and 100 mg·kg^{-1}) with and without peat-moss-based bacterial inoculum were applied in the soil. Results depicted that Cr stress reduced the plants' growth by causing oxidative stress in the absence of *S. aureus* K1 inoculation. However, the application of *S. aureus* K1 regulated the plants' growth and antioxidant enzymatic activities by reducing oxidative stress and Cr toxicity through conversion of Cr^{6+} to Cr^{3+}. The Cr^{6+} uptake by wheat was significantly reduced in the *S. aureus* K1 inoculated plants. It can be concluded that the application of *S. aureus* K1 could be an effective approach to alleviate the Cr toxicity in wheat and probably in other cereals grown under Cr stress.

Keywords: chromium; *Staphylococcus aureus*; wheat; oxidative stress; antioxidants

1. Introduction

Environmental pollution by toxic metals has dramatically increased because of various man-made actions taken while revolutionizing industries and urban life. Although these activities have substantially improved the living standards of humans, they have, at same time, deteriorated the environment [1]. Direct or indirect discharge of sewage and industrial effluent into surface water bodies has resulted in augmentation of chromium (Cr) and other toxic metals in soils [2], causing toxicity to

plants [3], animals and humans [4]. In agricultural systems, Cr can easily move to different parts of crops and accumulate there to be later consumed by the animals and humans [5]. Soil contamination by Cr and other heavy metals impacts biodiversity negatively and badly disturbs the living entities in the soil [6].

Major origins of Cr contamination are the leather industry [7,8], mining [1], steel industry, paint industry, wood preservatives, volcanic eruption and weathering [9]. Chromium exerts negative effects on plants by reducing the plant height and root growth, interrupting the germination process, causing disproportion in nutrient levels, exerting harmful effects on photosynthesis, retarding soil microbial activities, inhibiting enzyme activity and stimulating the formation of reactive oxygen species (ROS) which result in induction of oxidative stress in plants [1,10,11]. Chromium can cause different malfunctions in human biological systems that may lead to the death of affected persons [12–14]. Wastewater effluents from the industries are discharged directly into water bodies that are utilized mostly for irrigation purposes. Farmers have to rely on this untreated contaminated water due to limited resources and inadequate sanitation facilities [15].

A major staple food across the world is wheat (*Triticum aestivum* L.), which fulfils food requirements of about 50% of the worldwide population [16]. Amongst wheat-producing countries, Pakistan comes ninth in the world. Wheat subsidizes necessary amino acids, vitamins and minerals, dietary fibers and phytochemicals in our diet [17]. Wheat can accumulate higher Cr concentration in stems followed by leaves and grains [18]. According to the literature, increased heavy metal accumulation in wheat tissues has become a potential source of food chain contamination that can cause serious abnormalities to human biological systems [19,20]. Crops may also have the ability to reduce the Cr from Cr^{6+} to Cr^{3}. This reduction process is likely to happen in roots as a detoxification mechanism [21]. There are a number of remediation methods used to treat sites contaminated with toxic metals. Presently, scientists have made rampant use of biologically centered techniques to deal with such toxic contaminants in order to remove them from environmental entities, including water, air and soil, or at least make them less damaging to the ecosystem [22]. The phytoremediation technique is a modernized method with a lower budget and environmentally sustainable system [23]; it destroys contaminants by using plants along with their rhizospheric microorganisms. Microbial-assisted phytoremediation helps to deal with toxic heavy metals by stabilizing or transforming them to less toxic forms in carrier materials such as soil, shallow water, sediments or groundwater [24]. Microbes have the capability to modify their genetic sequences in response to variation in environmental factors [25]. In soil polluted with heavy metals, microbes assist the plants by producing various growth-regulating substances, such as organic acids, hormones, siderophores and enzymes, that help in plant growth promotion by involving diverse mechanisms, namely acidification, precipitation, redox reactions and chelation [26]. Likewise, roots excrete beneficial nutrients to support the successful colonization and growth of microbes [26]. Chromium-reducing bacteria have the capability to remediate Cr toxicity by reducing Cr^{6+} into Cr^{3+} in the rhizosphere through bioaccumulation and biosorption mechanisms [27]. *Staphylococcus aureus* is a Gram-positive, ubiquitous and round-shaped facultative anaerobe that grows in clusters, forming a biofilm on surfaces. It can grow in a range of growth temperature from 7 to 48 °C, with 37 °C as the optimal temperature for growth [28]. It was isolated from tannery effluent and characterized as a chromium-reducing bacterium. The application of phytoremediation, along with Cr-resistant bacteria for detoxification of Cr^{6+}, has been considered a safe, effective and economical technique over customary techniques [29,30]. In this study, the alleviative role of *Staphylococcus aureus* strain K1 under Cr stress was evaluated in wheat plants. It was hypothesized that microbes (such as *Staphylococcus aureus* strain K1) may alleviate Cr toxicity in wheat by enhancing antioxidant enzymatic activities of wheat while reducing oxidative stress through biotransformation (Cr^{6+} into Cr^{3+}) and biosorption of Cr.

2. Materials and Methods

2.1. Soil Preparation

Sandy clay loam soil was brought from nursery and was air-dried without direct sunlight. After air-drying, soil sieving was done by a mesh with a pore size of 2 mm. Soil was then sterilized at a temperature of 121 °C for 20–30 min for the purpose of removing any kind of contaminant or bacteria that can cause hindrance in further findings [31]. Chromium solutions of different concentrations were prepared from stock solution of $K_2Cr_2O_7$ in the laboratory, and soil was spiked with final Cr concentrations of 0, 25, 50 and 100 $mg{\cdot}kg^{-1}$ of soil.

These different concentrations of Cr were taken to determine the maximum concentration of hexavalent Cr tolerable by strain K1. However, in case of Cr reduction, the lower concentration of Cr was used due to the fact that Cr is found in lower concentrations in the natural environment, especially in industrial effluents [31]. The concentrations of Cr used were similar to those used in the literature and were chosen considering the fact that, in field conditions, we had to establish the reduction ability of this particular strain rather than its maximum potential to survive in response to metal stress [15]. The soil was added in the pots (5 kg soil per pot) with proper mixing following the treatment plan. Electrical conductivity and pH from saturated soil were determined by making a soil-to-water ratio of 1:25. Soil was extracted with ammonium bicarbonate diethylenetriaminepentaacetic acid (AB-DTPA) solution for the measurement of bioavailable trace elements in the soil [32]. Soil organic matter was determined following the prescribed method [33]. Soil physicochemical characteristics are given in Table 1.

Table 1. Soil characterization of pot experiment.

Soil Properties	Unit	Values
Texture Properties		**Sandy Clay Loam**
pH	-	7.71
Sand	%	63.7
Clay	%	21.9
Silt	%	14.4
Electrical Conductivity (EC)	dSm^{-1}	4.77
Soluble Ion Values		
Cl^-	$mmol_cL^{-1}$	7.15
$Ca^{2+} + Mg^{2+}$	$mmol_cL^{-1}$	14.92
CO_3^{2-}	$mmol_cL^{-1}$	0.85
OM	%	0.90
CEC	$cmol_ckg^{-1}$	13.2
HCO_3^-	$mmol_cL^{-1}$	3.84
Metal Concentration		
Available Cr^{6+}	$mg{\cdot}kg^{-1}$	0.04
Available Zn^{2+}	$mg{\cdot}kg^{-1}$	0.72
Available Cu^{2+}	$mg{\cdot}kg^{-1}$	0.23

2.2. Segregation of Cr-Resistant Bacteria

A modified method of serial dilution was adopted to isolate the Cr-tolerant bacteria from metal-contaminated industrial effluent [34]. For this, ten-fold serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were prepared from samples of collected wastewater using sterilized distilled water [34]. Then, 0.1 mL from each dilution was added to petri plates having Tryptic Soy Agar complemented with 0.5 mM Cr^{6+}. Morphologically different colonies were picked and transferred to petri plates supplemented with gradually elevated levels (0.0, 0.5, 2.5, 5.0, 10.0, 15.0, 20, 22 and 23 mM) of Cr^{6+} [35]. The bacteria

that showed maximum resistance to the highest concentration of hexavalent Cr were selected for use in further studies.

2.3. Bacterial Identification

Molecular characterization was carried out through the amplification of 16S rDNA gene via polymerase chain reaction (PCR) using the following universal primers: 27F (5′-AAACTCAAATGAATTGACGG-3′) and 1492R (5′-ACGGGCGGTGTGTAC-3′) [36]. For genomic DNA extraction, Favorgen DNA extraction kit was used following the manufacturer's guideline. The initial denaturation temperature was set at 94 °C for a period of 5 min, and this was followed by 40 recurring cycles of denaturizing DNA at 94 °C for 45 s, annealing at 53 °C for 45 s and elongation at 72 °C for 60 s. Final extension was set at 72 °C for 10 min, and this was followed by temperature being held at 4 °C [37]. PCR product (5 µL) was loaded in gel wells, and the reaction was allowed to complete; the product was then visualized using Gel Documentation System (Slite 200 W) under ultraviolet light [37]. After validation, 30 µL PCR product was delivered to Macrogen (Seoul, Korea) for the purpose of sequencing. ChormasPro (v1.7.1) software was used for correction of sequences that were submitted to GenBank for accession number. A phylogenetic tree was constructed by downloading similar partial 16S rDNA gene sequences from the NCBI BLAST database with the help of computer software MEGA (v7.0.) [38].

2.4. Bacterial Inoculum Preparation

In order to obtain pure inoculum of *S. aureus* strain K1, an individual isolated colony was inoculated in 250 mL sterilized nutrient broth and incubated at 150 rpm on orbital rotary shaker for 48 h (at 37 °C). The pure culture was harvested via centrifugation at 6000× *g* for 10 min, and the supernatant was discarded. The pellet was washed with sterilized distilled water and resuspended in 100 mL of normal saline (0.85% NaCl) solution. Overall, cell density for the inoculum was maintained at 1×10^8 CFU mL^{-1} [39].

2.5. Seed Coating and Pot Experiment

For this study, seeds of wheat variety Sehar were taken from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Seeds were first washed thoroughly with distilled water, and this was followed by surface sterilization using 10% hydrogen peroxide (H_2O_2) for 30 min [40]. The sterilized seeds were immersed in double volume of bacterial suspension (1×10^8 CFU mL^{-1}) and kept at 37 ± 2 °C on a rotary shaker (90 rpm) for 2 h. To facilitate the attachment of bacterial inoculum to the seeds, carboxymethyl cellulose (CMC) (2%) was added to the suspension as a sticking agent. Seeds were dried under shade after 2 h of inoculation for further experimental use. Uninoculated sterilized seeds were used as control. Clay and peat moss in equal parts (1:1) were mixed and the seeds were added to this mixture, which was shaken well for proper coating and incubated overnight in the dark. The completely randomized design had a total of eight treatments, with three replicates for each treatment. A total of eight seeds per pot were sown, and thinning was performed to result in four seedlings per pot after 3 weeks of seed germination.

2.6. Treatments

The experiment was conducted in plastic pots using different concentrations of Cr (0, 25, 50 and 100 mg·kg^{-1}) in the presence and absence of bacterial inoculation. Different treatments were as follows: T1 (Control), 0 mg·kg^{-1} Cr; T2, 25 mg·kg^{-1} Cr; T3, 50 mg·kg^{-1} Cr; T4, 100 mg·kg^{-1} Cr; T5, 0 mg·kg^{-1} Cr + *S. aureus* K1; T6, 25 mg·kg^{-1} Cr + *S. aureus* K1; T7, 50 mg·kg^{-1} Cr + *S. aureus* K1; T8, 100 mg·kg^{-1} Cr + *S. aureus* K1.

2.7. Plant Harvesting

At 135 days after seed sowing, plants were harvested at maturity. The height and spike lengths of plants were measured with a meter rod. Shoots, roots, spikes and grains were separated properly.

Then, 0.1 M HCl was used to remove the metals from the root surface, and the roots were washed with distilled water. Samples of roots and shoots were kept in a hot air oven (70 °C) for a period of 72 h. Afterwards, dry weight was recorded and samples were crushed to small pieces and processed for further analyses.

2.8. Determination of Chlorophyll Contents and Gas Exchange Parameters

At 8 weeks after seed germination, fresh leaf samples were taken to determine chlorophyll contents using acetone (85% *v*/*v*) for pigment extraction. These leaf samples were kept in the dark at 4 °C for 24 h. Centrifugation of samples was done to get the supernatant. Absorbance was recorded by spectrophotometer at three different wavelengths (470, 647 and 664.5 nm), and final chlorophyll contents were calculated by following the prescribed method [41]. Photosynthetic rate, transpiration rate and stomatal conductance of samples were recorded 8 weeks after seed germination on a fully sunny day using an infrared gas analyzer (IRGA, LCA-4, Analytical Development Company, Hoddesdon, UK).

2.9. Determination of Reactive Oxygen Species and Antioxidant Enzyme Activities

At 2 months after seed sowing, fresh leaves of plants were sampled for the estimation of reactive oxygen species (ROS) through the assessment of electrolyte leakage (EL) and the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Additionally, the activities of enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) were assessed. For the EL estimation, distilled water tubes were used to place leaf samples. Samples were autoclaved at 32 °C for period of 2 h, and the observed EC of the solution was termed as EC_1. Afterwards, this solution was autoclaved at 121 °C for 20 min to measure EC_2, and finally EL was calculated using the following equation as described by Dionisio-Sese and Tobita [42]:

$$EL = (EC_1/EC_2) \times 100$$

The concentration of MDA was measured using the method of Heath and Packer (1968) as modified by Dhindsa et al. [43] and Zhang and Kirham [44]. Hydrogen peroxide was recorded through homogenization of samples in phosphate buffer 50 mM (pH 6.5) and centrifugation followed by addition of 20% H_2SO_4 (*v*/*v*). Samples were centrifuged once more for 15 min, and readings were taken by spectrophotometer at 410 nm absorbance [45]. A spectrophotometer was utilized to record the activities of antioxidant enzymes such as SOD, POD, CAT and APX. Fresh leaf samples were crushed in liquid nitrogen (N_2), and 0.05 M phosphate buffer (pH 7.8) was utilized for the purpose of standardization. This was followed by centrifugation at 4 °C on 12,000× *g* for a period of 10 min. Supernatant was collected for the sake of antioxidant enzyme activity measurements. The method of Zhang [46] was employed to measure SOD and POD activities, while the Aebi method [47] was used for CAT activity. APX contents were estimated using the method of Nakano and Asada [48].

2.10. Estimation of Cr Contents in Plants

Digestion of dry shoot and root samples was performed for 1 g of each sample in 4:1 (*v*/*v*) ratio of HNO_3:$HClO_4$ as described by Rehman et al. [49]. Finally, the digested samples were run on an atomic absorption spectrophotometer for the estimation of Cr concentrations in the processed samples.

2.11. Statistical Analysis

The IBM SPSS Statistics for Windows, Version 21.0, was used for the data analyses, using the analysis of variance (ANOVA) tool at a 5% probability level. Tukey's HSD post hoc test was performed for multiple comparison of triplicates.

3. Results

The current study was envisaged to assess the capability of metal-resistant *Staphylococcus aureus* strain K1 to ameliorate the Cr stress in wheat plants.

3.1. Growth Characteristics of Isolate K1

The bacterial strain K1, capable of tolerating a Cr concentration of up to 22 mM, was selected for further studies. Morphologically, it is characterized by Gram-positive cocci (≈1 µm) with yellowish golden color. Chemically, it is oxidase- and coagulase-negative and catalase-positive (Table 2). The BLASTn investigation showed that it has a close resemblance (99%) to *Staphylococcus aureus* strain ATCC 12600 (NR_115606.1) and *Staphylococcus aureus* strain NBRC 100910 (MG971399.1). The similar 16S rDNA gene sequences from GenBank were used to carry out phylogenetic analysis, which also confirmed that the isolate K1 belongs to *Staphylococcus aureus*; therefore, it was named *Staphylococcus aureus* strain K1 (KX685332). This was done in order to remain confident that the bacterial strain used in this study was *Staphylococcus aureus* strain K1, as culture media can sometimes be contaminated with other bacteria.

Table 2. Biochemical and morphological characteristics of *S. aureus* strain K1.

Sr. No.	Characteristic	*Staphylococcus aureus* K1
1	Morphology	Convex, round
2	Color	Yellowish, golden
3	Gram-reaction	+ve
4	Catalase	+ve
5	Coagulase plasma reaction	−ve

3.2. Effect of S. aureus K1 Contact Time on Chromium (Cr^{6+}) Reduction

Staphylococcus aureus K1 exhibited optimum growth at pH 8 and 35 °C. Under optimum growth conditions, the effect of contact time on bacterial ability to reduce the hexavalent Cr in the medium was observed. It was observed that the Cr reduction of *S. aureus* K1 increased with increasing contact time (Figure 1). It was found that 26%, 45%, 71%, 80% and 99% Cr^{6+} (initial metal concentration = 1 mM) was removed from the medium by *Staphylococcus aureus* K1 after 2, 4, 8, 16 and 24 h of incubation, respectively (Figure 1).

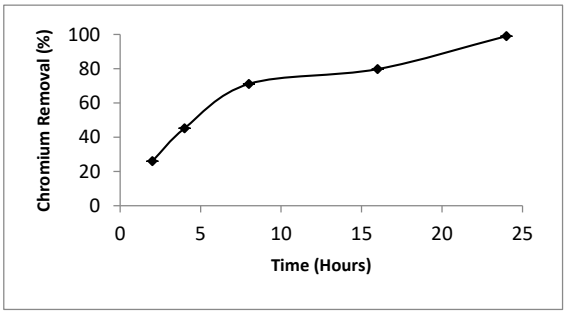

Figure 1. The impact of contact time (hours) impact on the Cr removal ability of *Staphylococcus aureus* K1.

3.3. Effect of S. aureus K1 on Plant Growth Promotion

Chromium stress substantially decreased the growth of wheat plants. A significant reduction in the length of shoots (31.18%), roots (32.02%) and spikes (40.70%) and the dry weight of shoots (34.29), roots (44.17) and grains (31.06%) of the plant was observed at 100 mg·kg^{-1} Cr concentration alone as compared to *S. aureus* K1 inoculated seeds + 100 mg·kg^{-1} Cr concentration (Figure 2). A significant change in shoot and root length was observed in inoculated plants as compared to uninoculated plants at all levels of Cr. Wheat plants stressed with 50 mg·kg^{-1} of Cr showed an observable reduction in growth attributes; however, this decrease was minimized in inoculated plants compared to uninoculated plants, as shown in Figure 2. The growth was gradually decreased when the Cr concentration in the growth medium increased from 25 to 100 mg·kg^{-1} (Figure 2A–D). Moreover, the maximum growth reduction was noticed with 100 mg·kg^{-1} of Cr stress. The data regarding plant growth attributes

indicated that inoculation with *S. aureus* K1 significantly improved the wheat growth and dry biomass under Cr stress conditions.

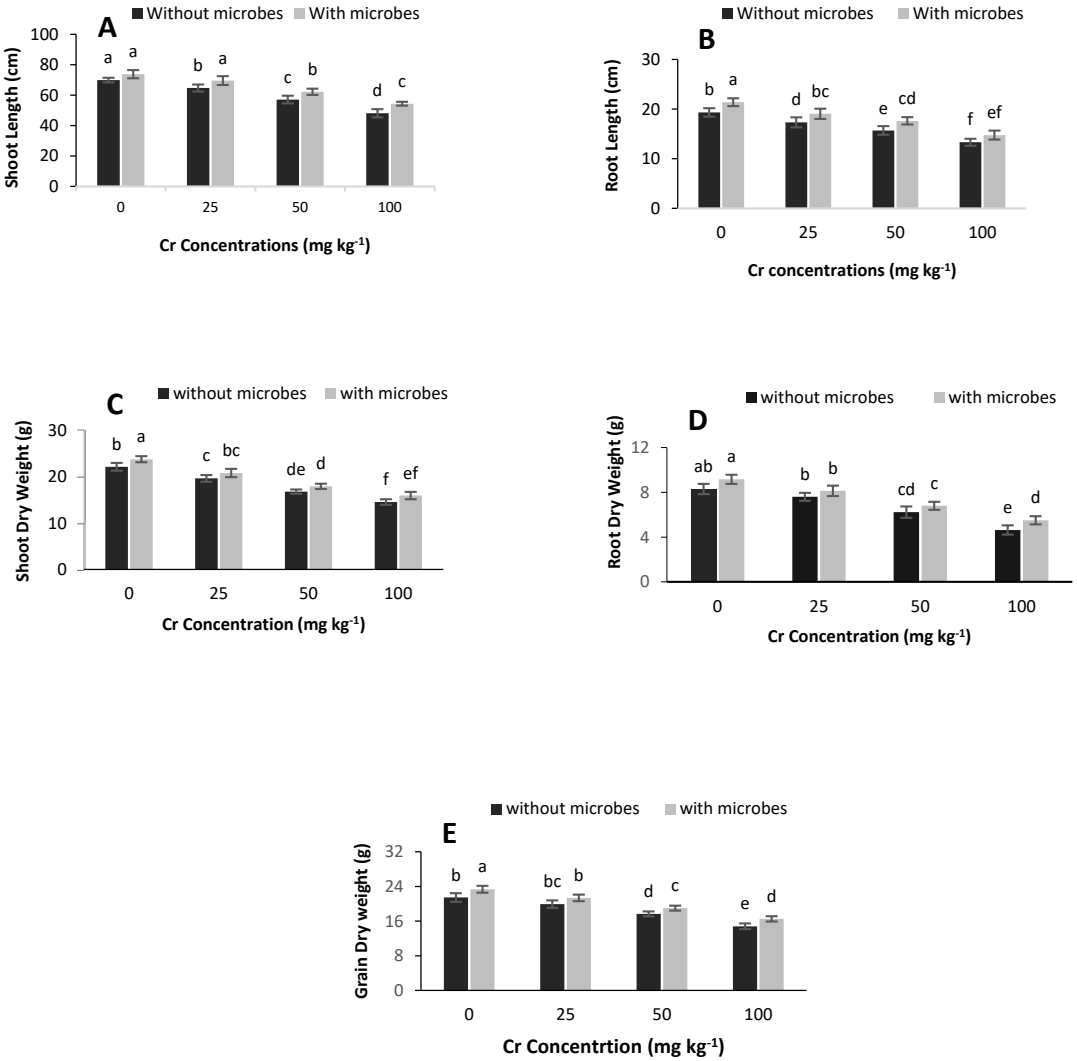

Figure 2. Influence of the different Cr levels (0, 25, 50, 100 mg·kg^{-1}), with and without peat-moss-based microbial inoculation, on length of shoot (**A**) and root (**B**), dry weight of shoot (**C**) and root (**D**) and grain dry weight (**E**) of wheat. Bars indicate the mean of three replicates with standard deviation (SD). Different bars with lowercase letters show noteworthy changes among various treatments at $p < 0.05$.

3.4. IRGA Parameters and Chlorophyll Contents

IRGA parameters such as transpiration rate, stomatal conductance and photosynthetic rate gradually reduced under increased Cr concentrations alone. The transpiration rate was greater at 25 mg·kg^{-1} of Cr stress and decreased with increasing Cr stress levels at concentrations from 50 to 100 mg·kg^{-1}. Without microbial inoculation, transpiration rate decreased by 12%, 21% and 32% under 25, 50 and 100 mg·kg^{-1} Cr stress, respectively, as compared to control (Figure 3A). Similarly, stomatal conductance and photosynthetic rate in uninoculated plants also reduced with increasing Cr concentrations. Stomal conductance decreased by 9%, 25%, 45% and photosynthetic rate decreased by 12%, 25% and 46% under 25, 50 and 100 mg·kg^{-1} Cr stress, respectively, as shown in Figure 3B,C. These results explain the effective role of bacterial inoculation in improving gas exchange attributes in Cr-stressed wheat plants by comparing uninoculated plants. Similarly, chlorophyll a contents decreased by 9.40%, 26.21% and 40.08% in inoculated plants and by 10.66%, 28.02% and 41.87% in uninoculated plants under 25, 50 and 100 mg·kg^{-1} Cr stress, respectively, as shown in Figure 3D.

On the other hand, as compared to untreated control, chlorophyll b was reduced by 15.36%, 27.27% and 40.80% in uninoculated wheat plants and by 14.44%, 27.24% and 40.63% in inoculated wheat plants under 25, 50 and 100 mg·kg^{-1} Cr stress, respectively, as shown in Figure 3E. A gradual decrease in carotenoid contents was also observed in inoculated and uninoculated plants with increasing level of Cr stress, where inoculated plants showed 6%, 19% and 27% reduction in carotenoid contents while uninoculated plants showed 9%, 19% and 28% reduction under 25, 50 and 100 mg·kg^{-1} Cr stress, respectively (Figure 3F)

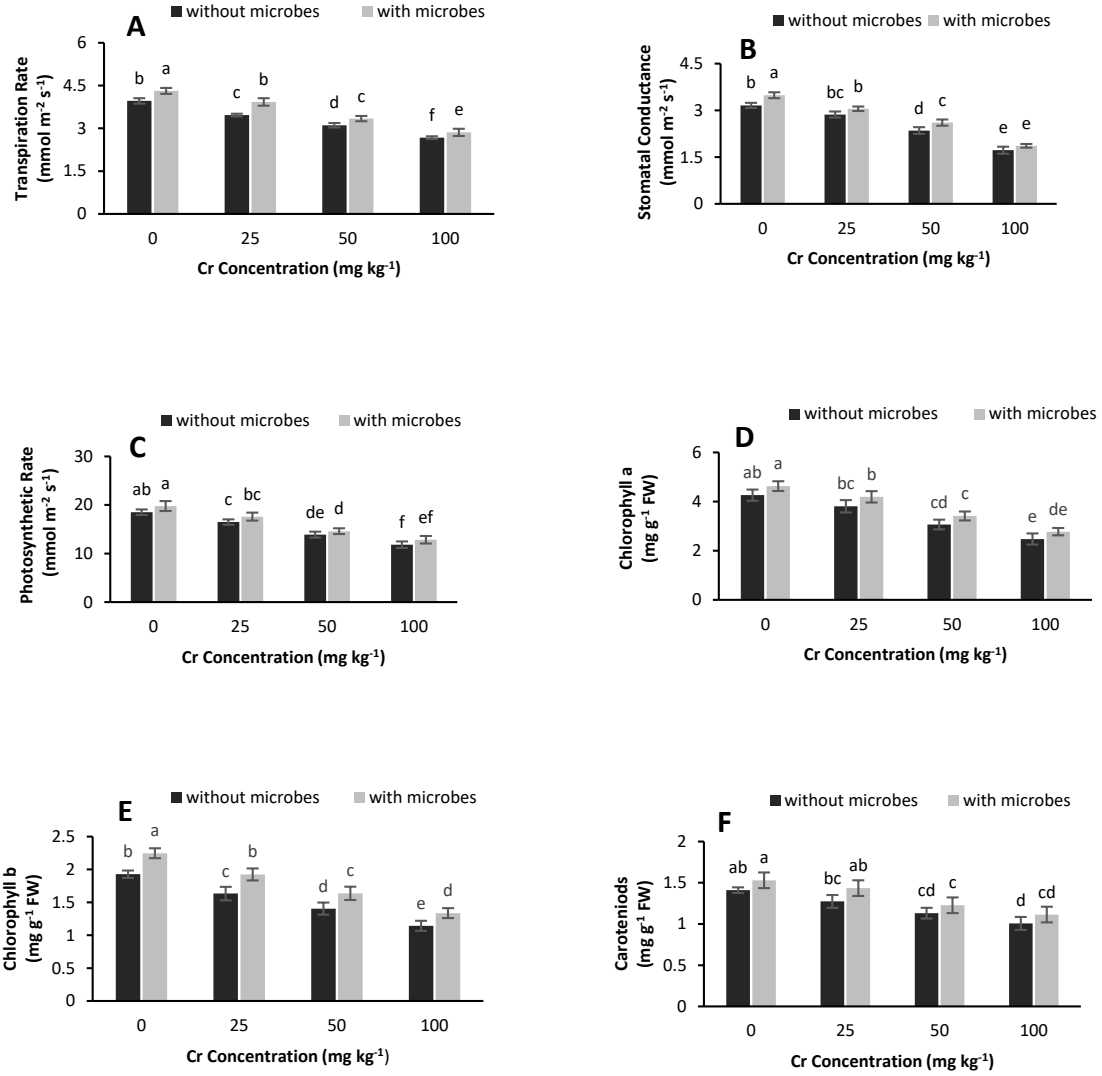

Figure 3. Influence of the different Cr levels (0, 25, 50, 100 mg·kg^{-1}), with and without peat-moss-based microbial inoculation, on transpiration rate (**A**), stomatal conductance (**B**), photosynthetic rate (**C**), chlorophyll a (**D**), chlorophyll b (**E**) and carotenoids (**F**) of wheat plants. Bars indicate the mean values and standard deviation of three replicates. Different bar letters show significant changes among various treatments at $p < 0.05$.

3.5. Estimation of EL, MDA and H_2O_2

A substantial increase in EL was noted in both roots and shoots of wheat plants under Cr stress, as shown in Figure 4A,B. Uninoculated wheat plants showed more EL in leaves and roots under all Cr levels (0, 25, 50 and 100 mg·kg^{-1}) as compared to inoculated plants. EL in uninoculated leaves was increased by 17.98%, 36.40% and 56.52% and EL in uninoculated roots increased by 9%, 32% and 53% under 25, 50 and 100 mg·kg^{-1} Cr, respectively. On the other hand, inoculation with *S. aureus* K1

increased EL in leaves by 15.83%, 33.26% and 55.90% and in roots by 13%, 33% and 56%, under 25, 50 and 100 mg·kg^{-1} Cr, respectively (Figure 4A,B).

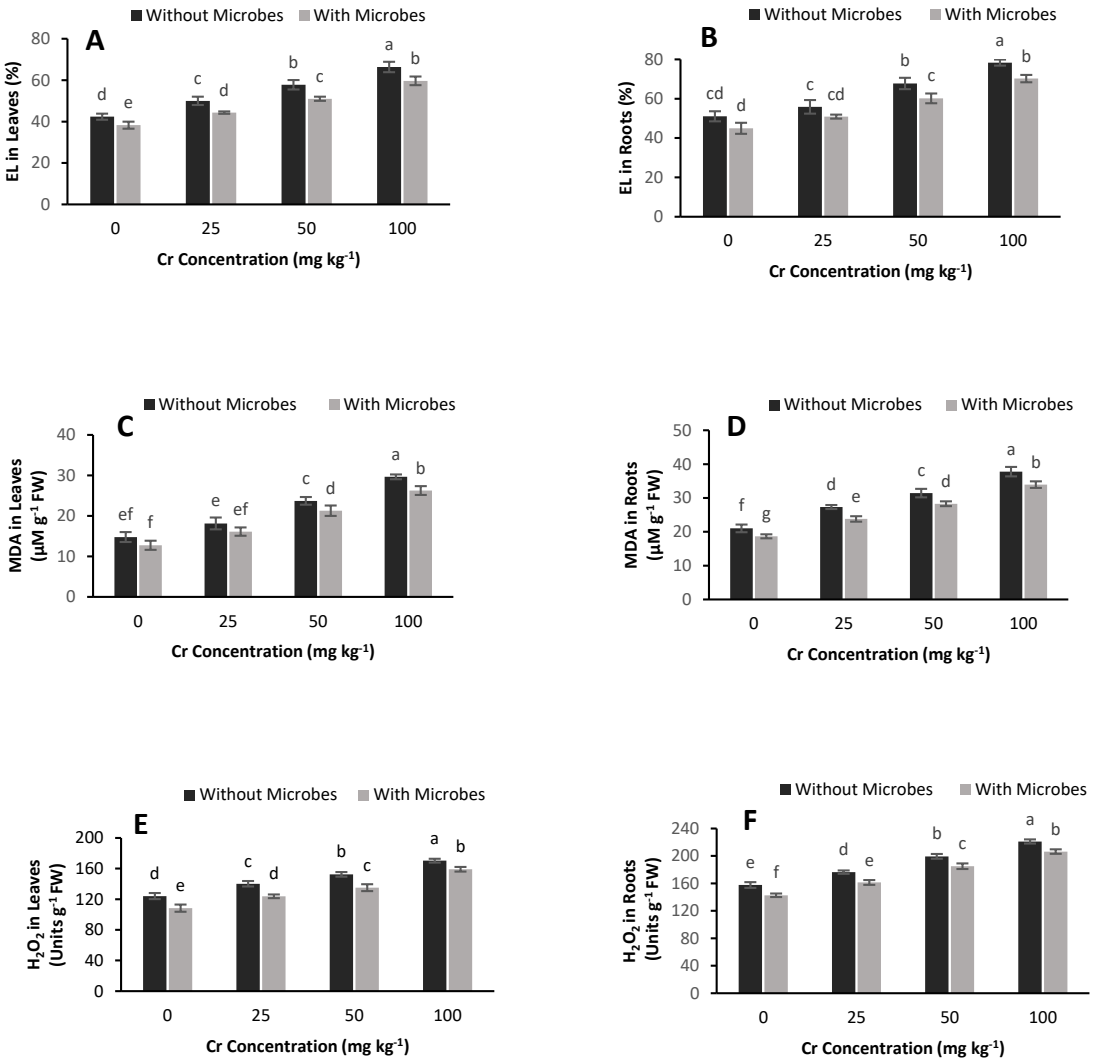

Figure 4. Influence of the different Cr levels (0, 25, 50, 100 mg·kg^{-1}), with and without peat-moss-based microbial inoculation, on EL in leaves (**A**), EL in roots (**B**), MDA in leaves (**C**), MDA in roots (**D**), H_2O_2 in leaves (**E**) and H_2O_2 in roots (**F**) of wheat plants. Bars indicate the mean values and standard deviation of three replicates. Different bar letters show significant changes among various treatments at $p < 0.05$.

There was a noticeable increase in MDA content of leaves, showing lipid peroxidation due to high level of Cr stress, as shown in Figure 4C,D. Maximum MDA contents were observed in leaves and roots of uninoculated plants under 100 mg·kg^{-1} Cr stress as compared to their respective controls. However, inoculation with *S. aureus* K1 reduced MDA content in all the plants of varying level of Cr stress compared to uninoculated plants. Likewise, a gradual rise in H_2O_2 of wheat leaves was observed with increasing levels of Cr (Figure 4E,F). Furthermore, a noteworthy decrease in H_2O_2 content was observed in *S. aureus* K1 inoculated plants, both Cr-stressed and control.

3.6. Effect of S. aureus on Antioxidant Enzyme Activities

The findings revealed that SOD activity in leaves and roots was significantly higher at the 25 mg·kg^{-1} Cr level but gradually decreased with increasing Cr levels, both in uninoculated and inoculated plants. SOD activity increased by 19.59%, 5.22% and 6.98% in uninoculated plant leaves and

by 17.58%, 5.22% and 3.08% in uninoculated plant roots under 25, 50 and 100 mg·kg^{-1} Cr treatments, respectively. However, inoculation with *S. aureus* K1 enhanced the SOD activity by 24.71%, 9.64% and 3.51% in leaves and 20.83%, 9.49%, and 4.34% in roots under 25, 50 and 100 mg·kg^{-1} Cr, respectively (Figure 5A,B). As compared to noncontaminated treatments (control), a decline in the CAT activity was observed under Cr contamination (Figure 5C,D). Inoculation with *S. aureus* K1 provoked a substantial increase in the activity of the CAT enzyme in wheat leaves (Figure 5C). CAT activity in roots also improved (114.31 Units·g^{-1} FW) under bacterial inoculation as compared to uninoculated plants (102.66 Units g^{-1} FW) at 25 mg·kg^{-1} Cr (Figure 5D). Moreover, abridged CAT activity was noticed at the highest level of Cr stress (100 mg·kg^{-1}); activity at this level was increased by 5.52% in leaves and 3.63% in roots for uninoculated plants, while inoculated plants showed increase of 5.06% in leaves and 1.37% in roots, as shown in Figure 5C,D. The POD activity substantially ($p < 0.05$) increased due to addition of Cr as compared to control (Figure 5E,F). There was a noticeable reduction in POD activity in leaves under bacterial inoculation with *S. aureus* strain K1 (22.27%, 11.99% and 0.21%) as compared to uninoculated treatments (21.63%, 10.12% and 2.92%) (Figure 5E). There was a substantial increase in the activity of the APX enzyme observed under Cr stress in wheat plants, as shown in Figure 5G,H. There was increase in APX activity in plant shoots and roots, with the maximum production occurring at the Cr concentration of 25 mg·kg^{-1}, and the APX activity decreased at the highest Cr level in the growth medium (Figure 5G,H). Furthermore, the maximum APX activity was observed in roots without inoculation at Cr concentration of 25 mg·kg^{-1}, as shown in Figure 5H.

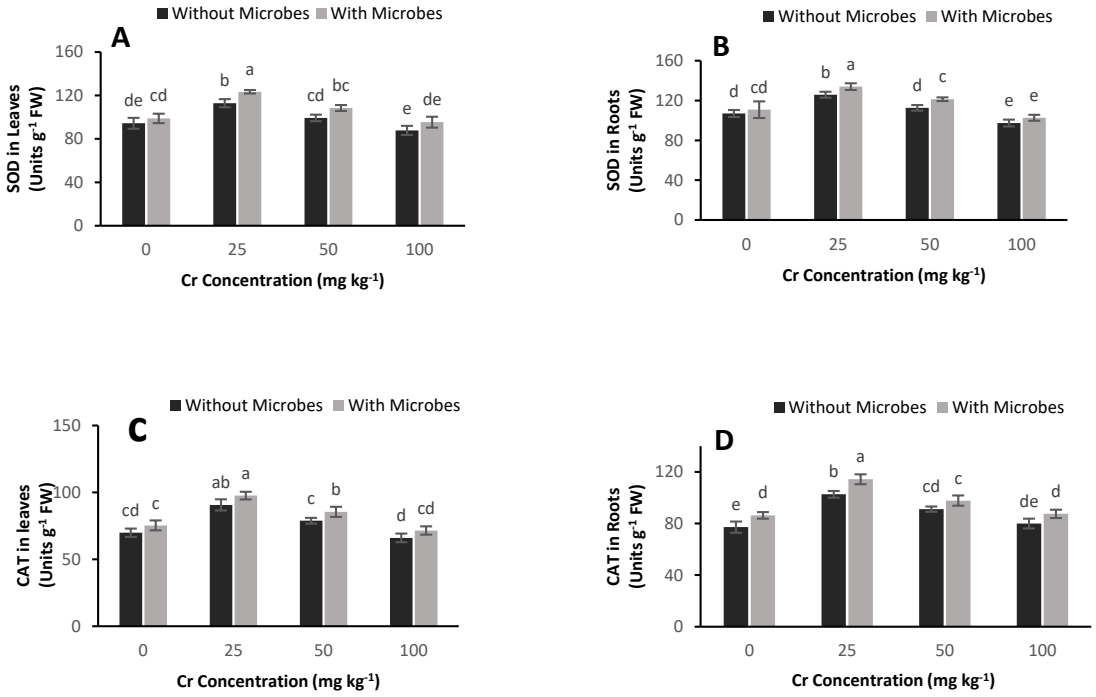

Figure 5. *Cont.*

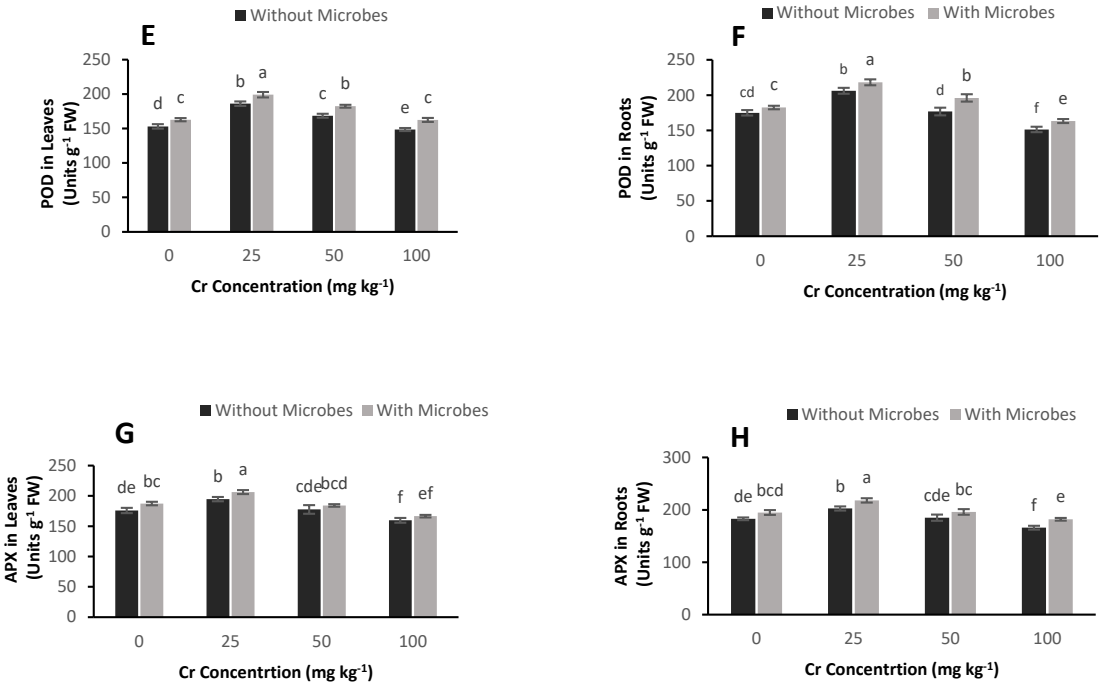

Figure 5. Influence of the different Cr levels (0, 25, 50, 100 mg·kg^{-1}), with and without peat-moss-based microbial inoculation, on SOD in leaves (**A**), SOD in roots (**B**), CAT in leaves (**C**), CAT in roots (**D**), POD in leaves (**E**), POD in roots (**F**), APX in leaves (**G**) and APX in roots (**H**) of wheat plants. Bars indicate the mean values with standard SD of three replicates. Different bar letters show significant changes among various treatments at $p < 0.05$.

3.7. Cr Accumulation in Plants

The data regarding Cr accumulation in shoots and roots of the wheat plants are shown in Figure 6A,B. With increasing concentration of applied Cr, a gradual increase in Cr concentrations was observed in roots and shoots in a dose-additive manner. In addition, inoculation of *S. aureus* K1 significantly decreased the Cr concentrations both in shoots and roots as compared to uninoculated plants.

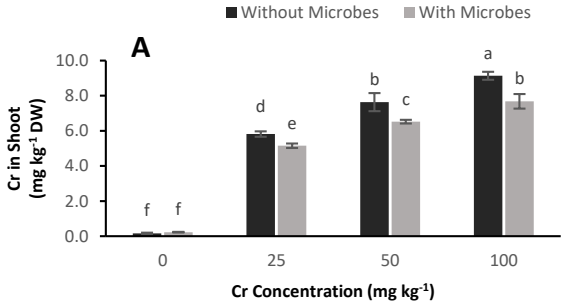

Figure 6. *Cont.*

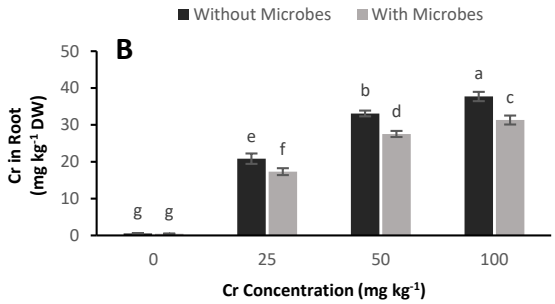

Figure 6. Influence of the different Cr levels (0, 25, 50, 100 mg·kg^{-1}), with and without peat-moss-based microbial inoculation, on Cr concentrations in shoots (**A**) and roots (**B**) of wheat plants. Bars indicate the mean values with standard SD of three replicates. Different bar letters show significant changes among various treatments at $p < 0.05$.

4. Discussion

The major objective of our research was to appraise the effectiveness of *Staphylococcus aureus* K1 treatment in reducing the toxic effects of Cr stress in wheat plants. An indigenous bacterial strain, *Staphylococcus aureus* K1 (GenBank accession no. KX685332), capable of tolerating up to 22 mM of Cr^{6+} was isolated from a metal-polluted environment. Numerous research studies with similar metal-tolerant bacterial isolations from metal-contaminated sites have been reported [35,50,51]. Our results also supported the findings of Mustapha and Halimoon [52], who isolated a total of 21 isolates from electroplating industries and reported that merely 5 of them were Cr-tolerant (up to 50 mg·L^{-1}). The results of the current study show that *S. aureus* K1 increased plant growth parameters under Cr metal stress (Figure 2).

4.1. Detoxification of Metals by S. aureus K1

Microbes have a number of metal resistance mechanisms involving chromosomes, transposon-encoded genes or plasmids. These mechanisms are mostly plasmid-facilitated and show resistance to some particular anion or cation [53]. Metals can have different impacts inside cells depending upon their concentration [53]; once a certain level is exceeded, bacteria respond with the initiation of a number of resistance mechanisms, including metallothioneins, P-type ATPases, CDF transporters and RND efflux pumps [54]. The genes located on plasmids, chromosomes or transposons that are responsible for resistance can easily be transferred to new community members from their point of location [53,55].

The genotype of bacteria, the nature and type of the metal and the pH of the culturing media are among the factors responsible for showing the degree of tolerance of microbes to various metals (Hg, Co, Pb, Ag, Zn, Mn, Cu, Cr) [56]. This kind of resistance against toxic heavy metals might be recognized by employing a number of potential methods like bioaccumulation of heavy metals by microbes, ion exclusion and low-molecular-weight binding protein production [57,58]. Elevated levels of metal resistance systems in bacterial cells are an indication of environmental heavy metal bioavailability [59]. The results of Chudobova et al. [60] showed a maximum resistance and capability of *S. aureus* strains under Cd^{2+} and Zn^{2+} ions. This resistance observed in *S. aureus* might be due to the efflux system containing a P-type ATPase transport system acting against Cd^{2+} ions [53,61].

4.2. Effect of S. aureus on Plant Growth Promotion under Cr Metal Stress

Different wheat varieties may differ in their response to different concentration of Cr in the soil. This could be attributed to various biological aspects of wheat varieties, as different wheat varieties show differences in growth parameters (e.g., leaf size). A heavy metal like Cr can easily make its way to aerial portions of plants, where it will affect their shoot metabolism at the cellular level and cause severe damage to minerals, water and nutrients, consequently retarding plant growth [10,62].

However, bacterial inoculation may improve the nutritional requirements of both micro- (Mn, Zn, Cu and Fe) and macronutrients (N, P and K) by modifying host physiology, which results in changed uptake pattern of roots. Similarly, a recent investigation done by Islam et al. [63] showed an increase in Fe and K concentrations in maize plants under Cr stress due to bacterial inoculations. According to an observation, plants with bacterial inoculation showed a reduction in metal accumulation in their aerial parts, which might be due to delayed translocation of metals from roots to upper parts [64]. Similar observations were recorded in this current research. Moreover, we isolated *S. aureus* K1 from wastewater that was contaminated with Cr, so the microbes may have the capability of performing metal detoxification as a part of their metabolic system. There was substantial improvement in plant growth and leaf pigments due to inoculation of specific microbes [63].

4.3. Chlorophyll Contents

Higher chlorophyll contents were observed in plants with bacterial inoculation compared to uninoculated plants (Figure 3). However, with further increasing metal concentrations, a reduction in chlorophyll contents was noted. This is in agreement with the findings of another research study, where chlorophyll a and chlorophyll b in wheat plants decreased with increasing concentrations of Pb in the growth medium [65].

4.4. ROS Species and Antioxidant Enzyme Production

Reactive oxygen species can be produced in plants when exposed to Cr^{6+}, which may damage the photosynthetic apparatus and protein complex of thylakoid membranes and result in inhibition of chlorophyll production [66]. In adverse conditions, plants release MDA contents; this reveals the level of lipid peroxidation, as MDA is the last decomposition product of membrane lipid peroxidation [67]. The increase in MDA contents found in the present study is indicative of imbalance between the generation and removal of free radicals in the cells [68]. The decreased lipid peroxidation with *S. aureus* K1 inoculation under Cr stress could be due to the increase in ROS-scavenging enzyme production in plants. This may be supported by a previously published study which revealed that the gene profile of metal detoxifying enzymes was activated by bacterial inoculation to deal with metal stress [69]. Reactive oxygen species are generated in response to stress caused by heavy metals like hexavalent Cr, and plants have a detoxifying antioxidant enzyme system for their maintenance. These enzymes are POD, SOD, APX and CAT, and they work alongside other non-enzymatic antioxidants. The activities performed by antioxidant enzymes in plants under metal stress are extremely variable and dependent on plant species, metal concentration, metal ions and exposure time period [70]. At low metal concentration, SOD activity may increase, but it becomes constant with increased metal concentration [71]. The enhancement in CAT activity was also noted in a number of plants under metal stress [72]. An increase in CAT activity was also observed as an adaptive trait of isolate CPSB21 [73]. Increased antioxidant enzyme activities in plants with inoculation of CPSB21 may be due to increases in mRNA/gene expression of antioxidant enzymes as compared to uninoculated plants [74].

4.5. Reduction of Cr Concentration in Plants by Bacterial Inoculation

A significant difference was found between uninoculated and *S. aureus* K1 inoculated plants in terms of Cr concentration. In contaminated soil, the results showed that the level of Cr was higher in the roots of wheat plants than it was in the shoots, which may be due to decreased translocation of Cr from roots to shoots of plants [75,76]. Immobilization of Cr in root cell vacuoles may lead to higher Cr accumulation in roots, which can cause toxicity in plants [77]. In the present study, inoculation of wheat plants with Cr-resistant microbes decreased the Cr concentration and its translocation from soil to roots and upper parts of wheat plants. The reduction of hexavalent Cr (Cr^{6+}) to trivalent Cr (Cr^{3+}) by bacterial isolates may be the reason for the improved growth of wheat plants [78] and hence the decreased level of the Cr contents in soil. Hasnain and Sabri [79] also reported a pattern of decreased Cr uptake and accumulation in roots and shoots of wheat plants inoculated with *Pseudomonas* sp.

A decrease in Cr concentration in soil was observed after wheat plant harvesting. This decrease was recorded in uninoculated Cr-contaminated wheat plants as a result of increased accumulation and uptake of Cr in roots and shoots [80]. Such decrease may also be due to Cr^{6+} reduction into Cr^{3+} under the influence of bacterial inoculation [78,81]. Scientists are also considering the use genetically engineered microorganisms (GEM), which may be well adjusted to their local environment (both climatic and soil) for effective elimination of heavy metals from contaminated soils [58,82,83].

5. Conclusions

The outcomes of this study indicate that the application of the peat-moss-based microbial inoculum improved plant growth and yield parameters and comparatively decreased metal accumulation by the plants. Overall, gas exchange attributes and chlorophyll contents increased with *S. aureus* K1 inoculation. This research study concluded that *S. aureus* K1 reduced the toxicity of Cr in wheat plants. The Cr-resistant *S. aureus* K1 supported the plant growth, decreased and detoxified Cr in plants and allowed better production of wheat in a Cr-contaminated environment. However, in-depth exploration (i.e., at the molecular level) of the alleviative mechanisms in plants should be conducted in future studies.

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