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Evaluation of the Bioremediation Potential of *Staphlococcus lentus* Inoculations of Plants as a Promising Strategy Used to Attenuate Chromium Toxicity

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Abstract: Current industrial developments, advanced farming techniques, and further anthropogenic activities are adding substantial amounts of heavy metals into the ecosystem and having dangerous effects on lifeforms, including plants and animals, and changing their biological activities. Decontamination following the heavy metal contamination is an important point deserving attention in the current scenario. Among all the other approaches used for this purpose, bioremediation is ecofriendly and green approach that can be used to remediate heavy metal toxicity. In plant cells, the regulation of ionic homeostasis is a primary physiological prerequisite for upholding plant development, growth, and production. To avoid the dreadful effects of toxic heavy metal exposure, plants manifest physiological, biochemical, and structural responses. In the present research, we reported on the isolation and molecular identification of an effective heavy-metal-tolerant bacterial strain, *Staphylococcus lentus* (E3), having a minimum inhibitory concentration of 300 µg/mL for chromium, Cr, taken from soil polluted with industrial effluents at Kasur, Pakistan. Bacterial inoculations enhanced all the growth parameters of *Triticum aestivum* and *Helianthus annus*. To observe the physiological strain, the proline content and peroxidase (POD) activities were estimated under Cr stress in the bacterial-inoculated plants. The chlorophyll content and Cr uptake in the aerial parts the of plants were also studied, along with the overexpression of proteins. The bacterial inoculations produced encouraging results. Bioremediation using PGPR is an efficient, convincing, and reliable approach to attenuating heavy metal toxicity.

Keywords: bacterial inoculants; bioremediation; heavy metal stress; chromium toxicity; *Triticum aestivum*; *Helianthus annus*; *Staphylococcus lentus*

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1. Introduction

Rapid urbanization and industrialization have resulted in the dramatic utilization of heavy metals. The unregulated disposal of industrial effluents, containing toxic heavy metals, in our surroundings causes devastation to the ecosystem. Chromium (Cr) metal, present in industrial discharges, is considered to be extremely toxic and is often found in the form of dichromate and chromate ions [1]. Chromium is found in the transition elements and is widely used in cement, electroplating, wood preservatives, textile, pigments, and paints, and in the dyeing, steel fabrication, canning, and leather-tanning industries [2]. The oxidation state of Cr determines its fate in ecosystems. Chromium exists in various

oxidation states, with a range from Cr^{2+} to Cr^{6+}, but the trivalent and hexavalent forms of Cr are commonly present and are highly stable, showing remarkable effects on organisms [3]. At all the pH levels, Cr^{6+} is soluble in water, as Cr^{3+} either precipitates in the form of Cr $(OH)_3$ or adsorbs on the surface of the soil. Thus, trivalent Cr contaminates the environment and depletes the surface water and aquifers [4]. The Environmental Protection Agency (EPA) lists Cr^{6+} as a Group 'A', which is a major pollutant and human carcinogen. Both the hexavalent and trivalent forms of Cr are causes of allergic contact dermatitis. Hexavalent chromium, Cr (VI), is highly soluble and readily bioavailable, and under neutral conditions, trivalent chromium has less solubility and toxicity and cannot cross the membranes of the cell [5]. Because of its carcinogenicity, mutagenicity, and teratogenicity in plants, humans, and animals, Cr (VI) is classified as a group 'A' human carcinogen [6]. Thus, it is necessary to treat this harmful pollutant before disposing of it. Some conventional methods, including adsorption, reduction, ion exchange, precipitation, and electrodialysis, have been used for the treatment of industrial waste containing Cr (VI), but they have some disadvantages, which include a higher consumption of reagents, production of toxic sludges, energy requirements, and incomplete metal removal, and they are not economical [7]. Additionally, a large number of these methods have limitations. They are economically viable at very high or moderate metal concentrations but are not viable at low concentrations. Due to these difficulties, the strategy of bioremediation for the detoxification of heavy metals has vast implications [8]. Therefore, a great interest in microbe-metal interactions has emerged in the past few decades, as investigators and industrialists learn to eliminate, stabilize, or recover heavy metals in effluents and soil. Microbial products and microorganisms are efficient in eliminating particulate and soluble forms of metals, particularly from dilute solutions, through bioaccumulation. Thus, microbe-based technologies provide an alternative [9]. Though the levels of Cr (VI) are toxic to many microbes, various resistant bacterial species have been identified, which could ultimately be employed in strategies of remediation. An initial site evaluation is required for the formulation of an in-situ method that can be used to remove Cr (VI) efficiently, followed by bacterial strain selection, which is based on the extent of contamination [10]. In a recent study, a strain of *Escherichia coli* isolated from heavily polluted soil was used to efficiently reduce Cr (VI) to the less toxic Cr (III) form [11]. Another study reported a similar strategy of reducing Cr (VI) to a less harmful form, trivalent chromium (Cr^{3+}), using *Bacillus* sp. [12]. Keeping in view the microbial potential of bioremediation, the present study was designed to isolate and identify a potential microbial agent displaying a strong potential to reduce the Cr metal present in industrial wastewater. The technology used for the removal of Cr (VI) should adhere to regulatory requirements and standards. In general, many heavy metals are constantly released by the industries into open environments, where these metals produce deleterious effects on our ecosystem. The identified bacterial strains could be adopted as an emerging technology that can be used to minimize the deleterious effects of other heavy-metal-related problems [13].

2. Materials and Methods

2.1. Industrial Effluent Sample Collection

Industrial effluent samples containing heavy metals were collected from leather industries in Kasur (31.1179° N, 74.4408° E), Pakistan. The samples were collected in clean screw-capped bottles, properly labeled, and transported to the laboratory within 8 h. All the collected samples were processed immediately for the isolation of bacterial strains.

2.2. Isolation and Identification of Cr-Tolerant Bacterial Strain

Heavy-metal-tolerant bacterial strains were isolated from the collected water samples following the spread plate procedure [11]. In brief, wastewater was serially diluted, and a 100 µL of appropriate dilution was spread over L-agar-containing plates, followed by incubation for 24 h at 37 °C. After incubation, the pure bacterial colonies with distinct characteristics were selected and streaked on the L-agar plates, freshly supplemented

with 150 µg/mL Cr. Successfully grown bacteria were further cultured on Petri plates containing Cr at a 500 µg/mL concentration until the bacterial proliferation was completely inhibited by the Cr. The chromium level that completely inhibited the bacterial growth was designated as the minimum inhibitory concentration (MIC). The bacterial strains that were unable to grow at any Cr level were discarded; only the Cr-tolerant strains were further exposed to a higher Cr concentration of 300 µg/mL. Four bacterial strains showing tolerance at up to 300 µg/mL were selected for further studies. After that, in the screening process, the further molecular identification of these bacterial strains was performed by amplifying the 16S rDNA region using universal primers: 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1462 R (5′-GGT GTT TGA TTG TTA CGA CTT-3′). The final sequences were submitted to the GenBank database in order to assign accession numbers to the nucleotides (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 3 March 2018).

2.3. Seed Sample Procurement and Sterilization

Certified seeds of *Triticum aestivum* (variety, FSD. 08) and *Helianthus annus* (NKS 278) were procured from Punjab Seed Corporation, Lahore. Healthy seeds were surface-sterilized by treating them with 0.1% $HgCl_2$ solution for 3–4 min and then repeatedly washed (5–6 times) with sterilized distilled water. All the seeds were placed on blotter paper to absorb the excessive moisture.

2.4. Bacterial Inoculations and Chromium Treatments

Bacterial strains were grown for 24 h at 37 °C using an L-broth medium. After 24 h of incubation, the pellet was re-suspended in an equal amount of autoclaved distilled water, and the optical density of the bacterial culture was recorded at 600 nm and was adjusted to the same value. The bacterial cultures adjusted to the same O.D. were used for the inoculations of the seeds for plant–microbe interaction growth experiment. The seeds of *T. aestivum* and *H. annus* were soaked in bacterial cultures of *S. lentus* for 40 min and sown in pots containing 275 g of dried and sieved soil. Seven inoculated seeds were sown at uniform distances in each pot for three sets of treatments, along with the respective untreated control. The experiment was performed with 7 replicates for each bacterial treatment and without bacterial treatment as a control. After the germination of the seeds, a 10 mL solution of 150 µg/mL and 300 µg/mL of K_2CrO_4 was poured into the 2nd and 3rd set of each bacterial treatment, respectively, and into the control (without bacterial treatment).

2.5. Study of the Growth Parameters and Stress Tolerance Index

The seedlings were removed from the pots after 20 days, and various parameters, including the shoot length, root length, fresh weight, and dry weight, were studied. Moreover, the stress tolerance index is an important tool used to determine the threshold potential of plants' tolerance against specific stress factors. The stress tolerance percentage was determined using the given formula [14]. The root length stress tolerance index (RLSTI) = (Root length of stress plant/Root length of control plant) × 100.

The shoot length stress tolerance index (SLSTI) = (Shoot length of stress plant/Shoot length of control plant) × 100.

Fresh biomass (FSTI) = (Root fresh weight of stress plant/Root fresh weight of control plant) × 100.

2.6. Protein Contents

The Lowery method for total protein estimation was used for the soluble protein estimation. The absorbance was taken at 750 nm using a spectrophotometer. The amount of soluble protein was calculated using a standard curve [15].

2.7. Proline Contents

The total proline content was measured using the modified method of Bates et al. (1973). Its absorbance was measured at 520 nm, and the amount of proline was determined in μg/g using a standard curve [16].

2.8. Chlorophyll Contents

The chlorophyll estimation was performed according to the modified method of Wellburn [17]. The absorbance of the solution was read at 663 and 645 nm using a spectrophotometer [18]. The total chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll } `a' \text{ (mg/g)} = (12.72A663 - 2.59A645)\ V/(m \times 1000)$$

$$\text{Chlorophyll } `b' \text{ (mg/g)} = (22.88A663 - 4.67A645)\ V/(m \times 1000)$$

$$\text{Total chlorophyll content} = \text{Chl } `a' + \text{Chl } `b'$$

2.9. Chromium Uptake

The chromium uptake in the aerial parts of the plants was measured using the method of [19] with Aqua Regia as a digesting mixture and 1,5-diphenylcarbazide as a color-developing reagent. The absorbance was measured at 540 nm using a spectrophotometer.

2.10. Peroxidase Contents

The peroxidase contents were determined by using the method of (Arnon, 1949). The absorbance was recorded at 420 nm to determine the amount of purpurogallin formed against the blank. One unit of enzyme activity denoted the quantity of the enzyme that inhibited 50% of the auto-oxidation rate of pyrogallol at 25 °C. The enzyme activity was expressed as the unit mg^{-1} of protein [20].

3. Results

The tolerance of the bacterial strain was evaluated through the relation of the minimum inhibitory concentration (MIC) to Cr. The results expressed that Cr stress greater than 300 μg/mL led to the inhibition of bacterial growth. Thus, this Cr concentration was considered as the MIC, and an efficient bacterial strain (E3) was selected for further studies. The bacterial E3 was molecularly characterized through 16S rDNA sequencing. After matching the partial sequences of 16S rDNA with sequences in GenBank, the BLAST results suggested that E3 expressed a 99% similarity with *Staphylococcus lentus*. These sequences were submitted to the GenBank database under the accession number MG988293.

3.1. Effects of Bacterial Inoculation on the Growth Attributes and Cr-Tolerance Index

The results regarding the effects of the bacterial inoculations on the growth parameters indicated that Cr stress significantly decreased the shoot length, root length, fresh weight, and dry weight as compared to the non-inoculated plants grown without stress treatments. However, the inoculations of plants with Cr-resistant *S. lentus* improved the growth of all these parts of the plant under Cr stress as compared to the non-inoculated *Triticum aestivum* (Figure 1) and *Helianthus annus* (Figure 2) plants grown in contaminated pots.

The maximum reduction in the shoot length in non-inoculated plants under a Cr stress of 150 and 300 μg/mL ranged between 40 and 52% in *T. aestivum* and between 45 and 58% in *H. annus*, respectively, as compared to the non-inoculated plants without stress treatment. However, the inoculations with the bacterial isolate E3 caused maximal enhancements of up to 50, 36, and 34% under 0, 150, and 300 μg/mL Cr stress in *T. aestivum* and up to 46, 55, and 34% in *H. annus*, respectively, compared to non-inoculated control treatment. The root length was reduced by up to 19 and 29% under a Cr stress of 150 and 300 μg/mL as compared to the non-inoculated plants without stress treatment in the case of *T. aestivum*, but in *H. annus*, reductions of up to 22 and 37%, respectively, were observed. Plants inoculated with isolate E3 expressed the best results by increasing their root lengths by up

to 26, 21, and 21% under Cr concentrations of 0, 150, and 300 µg/mL in *T. aestivum* and up to 36, 31, and 28% in *H. annus*, respectively, as compared to the non-inoculated plants with the respective stress treatments. Cr concentrations of 150 and 300 µg/mL decreased the fresh weight of plants by up to 52 and 65% in *T. aestivum* and up to 32- and 43-fold in *H. annus*, respectively, compared to the non-inoculated controls without stress treatment. The inoculation of seeds with bacterial isolate E3 was the most effective and increased the fresh weight by up to 45, 53, and 34% under 0, 150, and 300 µg/mL of Cr stress in *T. aestivum* and up to 52, 76, and 49% in *H. annus*, respectively, compared to the non-inoculated controls with the respective stress treatments.

Figure 1. Effects of bacterial inoculation (E3) with and without Cr stress (K_2CrO_4 (0, 150, and 300 µg/mL)) on the growth of *Triticum aestivum*. A—control, B—non-inoculated treatment with Cr stress (150 µg/mL), C—non-inoculated treatment with Cr stress (300 µg/mL), D—inoculated treatment with E3, E—E3 + Cr stress (150 µg/mL), F—E3 + Cr stress (300 µg/mL).

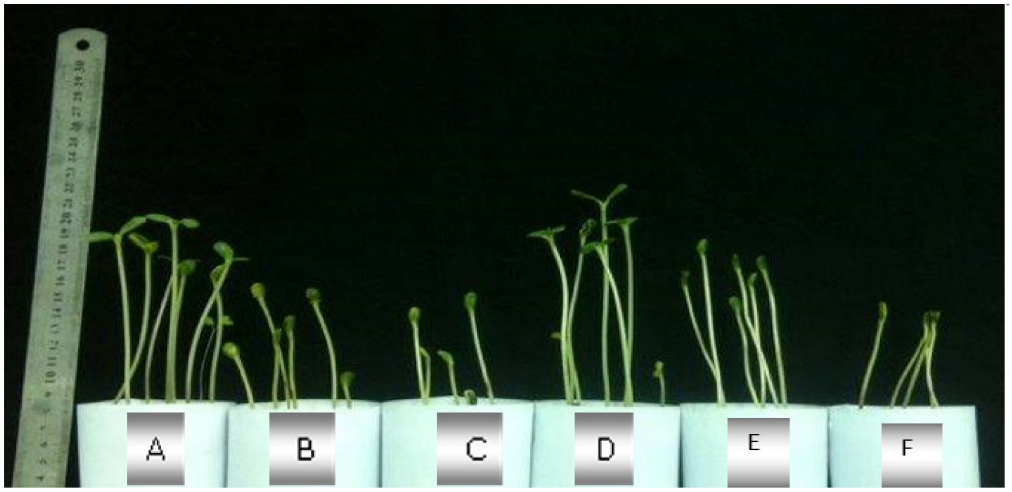

Figure 2. Effects of bacterial inoculation (E3) with and without Cr stress (K_2CrO_4 (0, 150 and 300 µg/mL)) on the growth of *Helianthus annuus*. A—control, B—non-inoculated treatment with Cr stress (150 µg/mL), C—non-inoculated treatment with Cr stress (300 µg/mL), D—inoculated treatment with E3, E—E3 + Cr stress (150 µg/mL), F—E3 + Cr stress (300 µg/mL).

Similarly, Cr stresses of 150 and 300 µg/mL decreased the dry weight of the plants by up to 22 and 64% in *T. aestivum* and led to increases of up to 60 and 81% in *H. annus*. Inoculations with bacterial isolate E3 increased the dry weight of the plants by up to

72, 47, and 27% in *T. aestivum* under Cr stress of 0, 150, and 300 µg/mL, respectively, and inoculations further increased the dry weight of *H. annus* by up to 141, 24, and 6%, respectively, compared to the non-inoculated controls with the respective stress treatments. Table 1.

Table 1. Effects of *Staphylococcus lentus* inoculations on the growth parameters of *Triticum aestivum* and *Helianthus annus* under Cr stress.

	Sr. #	Treatment	Shoot Length (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)
Wheat	1	Control	14.208 ± 0.515 f	6.888 ± 0.254 e	0.415 ± 0.0195 e	0.200 ± 0.012 cd
	2	E3	21.371 ± 0.378 i	8.800 ± 0.368 f	0.601 ± 0.020 i	0.343 ± 0.011 f
	3	C + Cr Stress (150 µg/mL)	8.500 ± 0.279 bc	5.657 ± 0.20 abc	0.198 ± 0.009 ab	0.156 ± 0.010 abc
	4	E3 + Cr Stress (150 µg/mL)	11.600 ± 0.272 e	6.857 ± 0.256 e	0.305 ± 0.014 d	0.20 ± 0.004 cd
	5	C + Cr Stress (300 µg/mL)	6.788 ± 0.248 a	4.857 ± 0.216 a	0.146 ± 0.006 a	0.073 ± 0.043 a
	6	E3 + Cr Stress (300 µg/mL)	9.114 ± 0.155 c	5.871 ± 0.18 abc	0.195 ± 0.012 ab	0.093 ± 0.002 ab
Sunflower	1	Control	9.22 ± 0.35 h	7.02 ± 0.29 e	0.59 ± 0.031 def	0.016 ± 0.003 a
	2	E3	13.51 ± 0.26 k	9.57 ± 0.26 g	0.90 ± 0.60 i	0.04 ± 0.005 abcde
	3	C + Cr Stress (150 µg/mL)	5.11 ± 0.19 c	5.48 ± 0.31 bc	0.40 ± 0.01 ab	0.026 ± 0.003 bcde
	4	E3 + Cr Stress (150 µg/mL)	7.91 ± 0.19 g	7.2 ± 0.27 e	0.71 ± 0.007 gh	0.033 ± 0.001 ef
	5	C + Cr Stress (300 µg/mL)	3.91 ± 0.29 a	4.45 ± 0.28 a	0.34 ± 0.01 a	0.03 ± 0.01 cde
	6	E3 + Cr Stress (300 µg/mL)	5.25 ± 0.22 cd	5.71 ± 0.15 bc	0.51 ± 0.03 cd	0.031 ± 0.0005 def

The presented values are the means of three replications. Letters represent the significant difference among the mean values and ± are standard error values of the means.

The shoot length stress tolerance index (SLSTI) of *T. aestivum* and *H. annus* was decreased by (59.8%, 55.4%) at the 150 µg/mL Cr level and (47.7%, 42.4%) at the 300 µg/mL Cr level, respectively. On the other hand, the bacterial inoculations increased the SLSTI by (136.4%, 154.7%) and (134.2%, 134%) in *T. aestivum* and *H. annus*, respectively, as compared to the non-inoculated treatments. Similarly, the bacterial inoculants displayed significant effects on the root length stress tolerance index (RLSTI) percentage and fresh biomass stress tolerance index (FSTI) percentage of both the *T. aestivum* and *H. annus* plants. At the 150 µg/mL and 300 µg/mL Cr doses, the RLSTI percentages of *T. aestivum* and *H. annus* were recorded as (82.3%, 78%) and (70.5%, 63.3%), respectively, while the bacterial inoculations enhanced the RLSTI to (121.5%, 131%) and (120.8%, 128.3%) at the 150 µg/mL and 300 µg/mL doses, as presented in Table 2. Similar trends were observed in the fresh biomass stress tolerance index (FSTI) percentage of both the plants. The FSTI percentage was observed as (47.7%, 67.7%) and (35.1%, 57.6%) at 150 µg/mL and 300 µg/mL Cr doses in *T. aestivum* and *H. annus*. In contrast, the bacterial inoculation enhanced the tolerance index percentage, reaching (154%, 177.5%) and (133.5%, 150%), as compared to respective stress treatments of both non-inoculated plants.

Table 2. Effects of *Staphylococcus lentus* inoculations on the tolerance index (TI) of *Triticum aestivum* and *Helianthus annus* under Cr stress.

	Treatments	SLSTI %	RLSTI %	FSTI %
Wheat	C + Cr (150 µg/mL)	59.8	82.3	47.7
	E3 + Cr (150 µg/mL)	136.4	121.5	154
	C + Cr (300 µg/mL)	47.7	70.5	35.1
	E3 + Cr (300 µg/mL)	134.2	120.8	133.5
Sunflower	C + Cr (150 µg/mL)	55.4	78	67.7
	E3 + Cr (150 µg/mL)	154.7	131	177.5
	C + Cr (300 µg/mL)	42.4	63.3	57.6
	E3 + Cr (300 µg/mL)	134	128.3	150

SLSTI = shoot length stress tolerance index; RLSTI = root length stress tolerance index; FSTI = fresh biomass stress tolerance index.

3.2. Effect of Bacterial Inoculations on the Protein Contents

Generally, an increase in the protein content was observed following bacterial treatment, but the inoculations with isolate E3 caused maximal enhancements in the protein content by up to 69 and 63% in *T. aesivum* and *H. annus* in comparison to the non-inoculated controls without Cr stress treatment. Cr stresses of 150 and 300 µg/mL increased the protein content by up to 41 and 75% in *T. aestivum* and 38 and 82% in *H. annus*, respectively, as compared to the non-inoculated controls without stress treatment. However, inoculations with bacterial isolate E3 further caused a maximal increase in the protein content by up to 34 and 108% under Cr stresses of 150 and 300 µg/mL in *T. aestivum* and up to 24 and 111% in *H. annus*, respectively, as compared to the non-inoculated controls with the respective stress treatments.

3.3. Effect of Bacterial Inoculation on the Proline Content

The bacterial inoculations expressed significant effects on the proline content by increases of up to 67 and 272% in *T. aestivum* and *H. annus*, respectively, after inoculations with bacterial isolate E3, as compared to the non-inoculated plants without stress control. Cr stresses of 150 and 300 µg/mL increased the proline content by up to 124 and 152% in *T. aestivum* and up to 255 and 283% in *H. annus*, respectively, as compared to the non-inoculated controls without stress treatment, but inoculations with bacterial isolate E3 decreased the proline content by up to 46 and 37% in *T. aestivum* and up to 41 and 29% in *H. annus* as compared to the non-inoculated plants with the respective stress control treatments.

3.4. Effect of Bacterial Inoculation on the Chlorophyll Contents

Bacterial inoculations exerted significant effects on the chlorophyll content of wheat and sunflower. Inoculations with bacterial isolate E3 enhanced the chlorophyll 'a', 'b', and 'a+b' by up to 365, 235, and 277%, respectively, in the absence of Cr stress as compared to the non-inoculated controls without stress treatment. Cr stresses of 150 and 300 µg/mL reduced the chlorophyll content of ('a', 'b' and 'a+b') by up to (30, 39, and 36%) and (34, 51, and 46%) in *T. aestivum* and up to (15, 46, and 34%) and (83, 74, and 79%), respectively, compared to the non-inoculated controls without stress treatment. However, inoculations with isolate E3 increased the chlorophyll content of ('a', 'b', and 'a+b') by up to (529, 241 and 343%) in *T. aestivum* and up to (419, 435, and 437%) in *H. annus*, respectively, under a Cr stress of 150 µg/mL, and inoculations with isolate E3 caused maximal enhancements in the chlorophyll content of ('a', 'b', and 'a+b') by up to (506, 230, and 341%) in *T. aestivum* and up to (866, 789, and 809%), respectively, under a Cr stress of 300 µg/mL compared to the non-inoculated controls with the respective stress treatments.

3.5. Effect of Bacterial Inoculation on Chromium Uptake in the Aerial Parts

Bacterial inoculations had significant effects on the Cr content in the aerial parts of the plants by decreasing translocation from the root to the upper parts. Inoculations of seeds with bacterial isolate E3 caused maximal decreases in the Cr content in the aerial parts of the plants by up to 81 and 85% under Cr stresses of 150 and 300 µg/mL in *T. aestivum* and up to 82 and 49% in *H. annus*, respectively, as compared to the non-inoculated controls with the respective stress treatments. A significant increase in the peroxidase content in wheat plants was observed after increasing the Cr stress, with increases of up to 18 and 73% under Cr concentrations of 150 and 300 µg/mL in *T. aestivum* and up 51 and 112% in *H. annus*, respectively, compared to the non-inoculated controls without stress treatment. However, inoculations of seeds with bacterial isolate E3 increased the peroxidase content by up to 187, 224, and 150% under Cr stresses of 0, 150, and 300 µg/mL in *T. aestivum* and up to 60, 60, and 40% in *H. annus*, respectively, compared to the non-inoculated controls with the respective stress treatments (Table 3).

Table 3. Effects of *Staphyllococcus lentus* inoculations on the biochemical and physiological attributes of *Triticum aestivum* and *Helianthus annus* under Cr stress.

Sr. #	Treatment	Protein Content (µg/g)	Proline Content (µg/g)	Ch. 'a' (µg/g)	Ch. 'b' (µg/g)	Ch. 'a+b' (µg/g)	Chromium Uptake (mg/kg)	Peroxidase Content (µg/g)
1	Control (wheat)	121.6 ± 8.33 a	33.0 ± 6.08 a	0.02 ± 0.0 ab	0.04 ± 0.0 ab	0.06 ± 0.0 a	0	20.5 ± 3.82 g
2	E3	205 ± 14.4 cd	54.6 ± 4.97 de	0.1 ± 0.04 f	0.15 ± 0.0 e	0.26 ± 0.0 e	0	30.3 ± 3.07 cd
3	C + Cr (150 µg/mL)	171.6 ± 8.3 b	74.0 ± 5.50 f	0.01 ± 0.0 a	0.02 ± 0.0 ab	0.04 ± 0.0 a	72.2 ± 0.84 e	33.1 ± 3.02 a
4	E3 + Cr (150 µg/mL)	205 ± 14.4 cd	39.6 ± 2.0 abc	0.09 ± 0.0 f	0.09 ± 0.0 e	0.19 ± 0.0 d	13.5 ± 0.08 a	46.5 ± 3.08 fg
5	C + Cr (300 µg/mL)	213.0 ± 8.3 d	82.66 ± 3.8 f	0.01 ± 0.0 a	0.02 ± 0.0 a	0.03 ± 0.0 a	98.0 ± 0.57 f	40.4 ± 4.04 b
6	E3 + Cr (300 µg/mL)	443.0 ± 8.3 f	51.6 ± 5.81 cd	0.08 ± 0.0 ef	0.07 ± 0.0 bcde	0.16 ± 0.0 cd	14.4 ± 0.23 a	56.0 ± 0.94 a
1	Control (sunflower)	211.6 ± 8.3 a	29 ± 8.71 a	0.021 ± 0.03 abc	0.05 + 0.006 bc	0.002 ± 0.008 bc	0.00	23.2 ± 2.10 a
2	E3	468 ± 8.3 cde	108.3 ± 9.7 e	0113 ± 0.19 f	0.305 ± 0.008 h	0.013 ± 0.017 h	0.00	46.66 ± 1.47 cd
3	C + Cr (150 µg/mL)	168.3 ± 8.3 b	103.3 ± 3.3 cf	0.018 ± 0.017 ab	0.035 ± 0.006 ab	0.0013 ± 0.016 ab	75.81 ± 1.195 g	53.4 ± 1.38 b
4	E3 + Cr (150 µg/mL)	221.6 ± 8.3 e	61 ± 5.03 b	0.095 ± 0.092 f	0.188 ± 0.001 g	0.0048 ± 0.0057 g	13.86 ± 0.44 c	56.06 ± 1.68 ef
5	C + Cr (300 µg/mL)	121.6 ± 8.3 e	111 ± 6.96 e	0.003 ± 0.008 a	0.0117 ± 0.0008 a	0.0006 ± 0.001 a	87.06 ± 1.43 h	55.23 ± 1.70 cd
6	E3 + Cr (300 µg/mL)	198.3 ± 6.6 h	79.33 ± 5.23 bc	0.034 ± 0.072 bcd	0.142 ± 0.002 f	0.0041 ± 0.0048 e	44.36 ± 1.59 f	73.20 ± 2.22 g

The presented values are the means of three replications. Letters represent the significant difference among the mean values and ± are standard error values of the means.

3.6. Effect of Chromium on Peroxidase Content

A significant decreasing trend in the peroxidase content was observed in the wheat plants growing under Cr stress with increased concentrations ranging from 150 to 300 µg/mL. Under Cr stresses of 150 and 300 µg/mL, the peroxidase content decreased by up to 62 and 97%, respectively, as compared to the plants grown without Cr stress. Microbial inoculations also exerted significant effects on the peroxidase content. The inoculation of *Triticum aestivum* seed with microbial isolate E3 increased the peroxidase content by up to 48% compared to the non-inoculated control without stress. Similarly, under a Cr stress of 150 µg/mL, inoculations with these E3 bacterial isolates increased the peroxidase content by up to 41% compared to the non-inoculated control with the respective Cr stress. Under a Cr stress of 300 µg/mL, inoculations of the seeds with the Cr-resistant microbial isolate E3 increased the peroxidase content by up to 39% as compared to non-inoculated control with the respective stress.

A significant increasing trend in the peroxidase content was observed in sunflower plants growing under Cr stress with increasing concentrations ranging from 150 to 300 µg/mL. Under Cr stresses of 150 and 300 µg/mL, the peroxidase content was increased by up to 131 and 138% as compared to plants grown without Cr stress. Microbial inoculations also exerted significant effects on the peroxidase content. The inoculation of sunflower seeds with microbial isolate E3 increased the peroxidase content by up to 101% compared to the non-inoculated control without stress. Similarly, under a Cr stress of 150 µg/mL, the inoculations of these bacterial isolates E3 also increased the peroxidase content by up to 5% compared to the non-inoculated control with the respective Cr stress. Under a Cr stress of 300 µg/mL, the inoculations of seeds with the Cr-resistant microbial isolate E3 increased the peroxidase content by up to 33% as compared to the non-inoculated control with the respective stress.

4. Discussion

The microbes present in heavy metal (HM)-contaminated soils and industrial effluents show varying tendencies to survive under conditions of HM contamination. In our study, bacterial strains isolated from industrial wastewater were screened for the selection of Cr-tolerant bacterial strains. The bacterial strain E3 showed the highest tolerance against Cr at concentrations of up to 300 µg/mL. These findings are supported by a re study where isolates of Pseudomonas, Micrococcus, and Aeromonas reduced 70% of the Cr (VI) under anoxic conditions, and nearly 38% of the bacterial isolates displayed tolerance against Cr (VI) at a concentration level of more than 400 µg/mL [21].

Chromium has toxic effects on plant growth and development. It has been observed that it can alter the germination process, including the growth of roots, stems, and leaves, because of which total dry matter production and yield are also affected [22]. Along with this, plant physiological processes such as photosynthesis, water relation, and mineral nutrition are adversely affected by Cr contaminants [23]. In the current study, Cr-resistant

S. lentus (E3) was isolated, and its impacts in reducing the Cr toxicity and improving the plant growth were studied.

Triticum aestivum (wheat) and *Helianthus annus* (sunflower) seeds were treated with the selected Cr-tolerant bacterial strains and were grown in Cr-contaminated soil to evaluate their microbial-assisted plant growth promotion abilities under Cr stress. In our experiment, the Cr stress showed negative effects on all the growth parameters of the plants grown at all the Cr concentration levels. On the other hand, the seeds bacterized with *S. lentus* showed significant enhancements in all the plant growth parameters, including the shoot and root length, along with the fresh and dry weight and chlorophyll pigments. Overall, E3 showed a significant increase in the shoot length of *T. aestivum* and *H. annus* under 0, 150, and 300 µg/mL Cr stress in comparison to the respective non-inoculated stress treatments. In a recent study, *Providencia* sp. and *Proteus mirabilis*, two potential bacterial agents, were reported to increase the plant growth parameters and plant photosynthetic efficiency under drought and Cr stress conditions [24].

With increasing concentrations of Cr stress, the toxic effects of Cr on the water and nutrient uptake from the roots to the shoots and on the physiological mechanisms increase, which results in reductions in the shoot and root lengths of plants. Cr-resistant bacterial isolates can convert Cr (VI) into Cr (III) in the roots of the plants, and it becomes unavailable to the plants for absorption. Multiple possible mechanisms of action are involved in the development and growth patterns of *T. aestivum* in the context of the inoculation of Cr-tolerant rhizobacterial isolates [25]. In our study, the inoculation of *T. aestivum* and *H. annus* with Cr-resistant *S. lentus* (E3) showed significant increase in the plant growth parameters. Similar findings were reported in other studies [26,27]. Various bacterial attributes, including phosphate solubilization and phytohormones production, guarantee growth promotion in plants. Bacterial inoculants produce phytohormones, auxin, ethylene, gibberellins, and cytokinins, which can result in plant growth promotion. Moreover, auxins are very important hormones that regulate the development of plants, including the processes of organogenesis, differentiation, and cell expansion [28].

In our study, the non-inoculated plants grown under various Cr stress levels showed a decreased tolerance index, while the plants bacterized with E3 showed a significant increase in the tolerance index. A similar study reported that bacterial seed inoculation significantly increased the seed germination and metal tolerance capacity by minimizing the passage of HMs to the plants [29]. A more recent study showed that a multi-HM-resistant bacterial strain of *Streptomyces* sp. significantly improved the plant growth attributes in Maize and also increased the metal tolerance index under As^{3+} and Cr^{6+} stress [30].

Chromium toxicity decreases the chlorophyll contents, which has direct effect on the photosynthetic activity and, ultimately, results in less biomass production [31]. The chlorophyll content decreases because of the degradation and deterioration of antenna complex proteins. Heavy metals rigorously restrict plant growth and cause the death of plants by upsetting the movement of nutrients, which causes deterioration of chlorophyll, ultimately resulting in decreased photosynthetic activity [32]. In contrast, bacterial inoculation improves the pigment content of plants. Our study reported an increase in the protein contents of *T. aestivum* and *H. annus* under Cr stress conditions when inoculated with *S. lentus*. Similarly, the seeds' inoculation with bacterial isolate E3 showed a maximum increase in the protein content compared to the non-inoculated plants. Heavy metal stress is responsible for the additional proteins formation and restriction of the synthesis of cellular proteins. The proteins produced under conditions of heavy metal toxicity may be involved in the synthesis of specialized proteins which stabilize the membranes [33].

In this study, the proline contents of *T. aestivum* and *H. annus* were affected by bacterial inoculation, as well as Cr stress. Previous research demonstrated that the plants could withstand water deficit stress conditions by osmoregulation, which is a function of proline. Proline can be included among the antioxidants (non-enzymatic) which are utilized by animals, plants, and microbes to cope with abiotic stress [33,34]. A recent study reported that seed inoculation with *Providencia* sp. and *Proteus mirabilis* significantly increased the

plant growth parameters, pigments, proteins, phenolics, and relative water contents and decreased the lipid peroxidation, proline, and superoxide dismutase activity (SOD) under drought and Cr stress [24].

Our analysis of the Cr uptake showed that there is always a high level of Cr in the roots in comparison to the shoots of plants growing under a Cr stress environment. There might be a restricted uptake of Cr from the roots to the shoots [35]. Treatments of *T. aestivum* seeds with Cr-tolerant bacterial isolates significantly decreased the translocation of Cr (VI) from the soil to the roots and then, finally, to the shoots. When Cr stress is increased in plants, it results in the formation of reactive oxygen species, which cause oxidative damage and disturbance in the functions of the cell. There are some defense mechanisms, such as peroxidase and catalase enzymes, that provide protection against the damage caused by these reactive oxygen species [36]. In this study, the inoculations of bacterial isolates showed significant effects on the peroxidase content as compared to the non-inoculated treatments under Cr stress of 0, 150, and 300 µg/mL. The inoculations of *T. aestivum* and *H. annus* seeds with the Cr-resistant bacterial isolate E3 caused a maximal enhancement in the peroxidase content in wheat plants as compared to non-inoculated controls without Cr stress. A recent study supported the notion that the bacterial inoculation of *Klebsiella* sp. and *Enterobacter* sp. significantly enhanced the superoxide dismutase, catalase, peroxidase, total phenolic, and ascorbic acid levels in tomato plants grown under Cr stress conditions [37].

In the current study, the *S. lentus* strain showed a significant ability to reduce Cr (VI) to Cr (III) and to induce plant growth promotion under Cr stress conditions. Hence, this bacterium can act as a promising inoculant for bioremediating the toxic Cr species and could be tested against other HMs. The results of this study are important, particularly in regard to future research, with respect to the water crisis and climate change [38–43], which will increase the need to use groundwater and wastewater treatments.

5. Conclusions

The current study concludes that inoculations with heavy-metal-resistant *Staphylococcus lentus* enhanced the growth parameters of plants subjected to growth under Cr stressed conditions in terms of the root and shoot lengths and fresh and dry weights. A considerable increment in the levels of photosynthetic pigment was also observed in *Triticum aestivum* and *Helianthus annus* plants exposed to Cr stress. It was also observed that microbial inoculation also reduced the Cr uptake and accumulation in the aerial parts of the plants, because inoculated bacteria have the capacity to alleviate Cr toxicity by converting hexavalent chromium into the trivalent form, which becomes unavailable for absorption and reduces translocation to the shoots. Increments in the peroxidase and proline contents, which are involved in the defense mechanisms of plants growing under stress conditions, were also observed. The current study therefore envisions the influence of microbes as bioremediating agents that can be used to alleviate Cr toxicity in plants, providing better health, along with a better performance of all the biological activities, even in HM stress environments.

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References

1. DesMarias, T.L.; Costa, M. Mechanisms of chromium-induced toxicity. *Curr. Opin. Toxicol.* **2019**, *14*, 1–7. [CrossRef]
2. Vaiopoulou, E.; Gikas, P. Regulations for chromium emissions to the aquatic environment in Europe and elsewhere. *Chemosphere* **2020**, *254*, 126876. [CrossRef] [PubMed]
3. Antuzevics, A.; Krieke, G.; Ozols, H.; Fedotovs, A.; Sarakovskis, A.; Kuzmin, A. Oxidation State and Local Structure of Chromium Ions in LaOCl. *Materials* **2021**, *14*, 3539. [CrossRef]
4. GracePavithra, K.; Jaikumar, V.; Kumar, P.S.; SundarRajan, P. A review on cleaner strategies for chromium industrial wastewater: Present research and future perspective. *J. Clean. Prod.* **2019**, *228*, 580–593. [CrossRef]
5. Singh, P.; Itankar, N.; Patil, Y. Biomanagement of hexavalent chromium: Current trends and promising perspectives. *J. Environ. Manag.* **2021**, *279*, 111547. [CrossRef]
6. Alvarez, C.C.; Gómez, M.E.B.; Zavala, A.H. Hexavalent chromium: Regulation and health effects. *J. Trace Elem. Med. Biol.* **2021**, *65*, 126729. [CrossRef]
7. Devi, P.; Kumar, P. Effect of bioremediation on internodal length and leaf area of maize plant cultivated in contaminated soil with chromium metal. *J. Pharmacogn. Phytochem.* **2020**, *9*, 1408–1413.
8. Ayele, A.; Godeto, Y.G. Bioremediation of chromium by microorganisms and its mechanisms related to functional groups. *J. Chem.* **2021**, *2021*, 7694157. [CrossRef]
9. Diaconu, M.; Roșca, M.; Cozma, P.; Minuț, M.; Smaranda, C.; Hlihor, R.-M.; Gavrilescu, M. Toxicity and Microbial Bioremediation of Chromium Contaminated Effluents. In Proceedings of the 2020 International Conference on e-Health and Bioengineering (EHB), Iasi, Romania, 29–30 October 2020; pp. 1–4.
10. Guo, S.; Xiao, C.; Zhou, N.; Chi, R. Speciation, toxicity, microbial remediation and phytoremediation of soil chromium contamination. *Environ. Chem. Lett.* **2021**, *19*, 1413–1431. [CrossRef]
11. Mohamed, M.S.; El-Arabi, N.I.; El-Hussein, A.; El-Maaty, S.A.; Abdelhadi, A.A. Reduction of chromium-VI by chromium-resistant Escherichia coli FACU: A prospective bacterium for bioremediation. *Folia Microbiol.* **2020**, *65*, 687–696. [CrossRef] [PubMed]
12. Seragadam, P.; Rai, A.; Ghanta, K.C.; Srinivas, B.; Lahiri, S.K.; Dutta, S. Bioremediation of hexavalent chromium from wastewater using bacteria-a green technology. *Biodegradation* **2021**, *32*, 449–466. [CrossRef] [PubMed]
13. Murthy, M.K.; Khandayataray, P.; Padhiary, S.; Samal, D. A review on chromium health hazards and molecular mechanism of chromium bioremediation. *Rev. Environ. Health* **2022**. [CrossRef] [PubMed]
14. Kausar, A.; Ashraf, M.Y.; Ali, I.; Niaz, M.; Abbass, Q. Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as screening tool. *Pak. J. Bot.* **2012**, *44*, 47–52.
15. Waterborg, J.; Matthews, H. The Lowry method for protein quantitation. Basic protein and peptide protocols. *Humana* **1994**, *1*, 1–4.
16. Carillo, P.; Gibon, Y. Protocol: Extraction and determination of proline. *PrometheusWiki* **2011**, *2011*, 1–5.
17. Kumari, S.; Vaishnav, A.; Jain, S.; Varma, A.; Choudhary, D.K. Bacterial-mediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (Glycine max L. Merrill). *J. Plant Growth Regul.* **2015**, *34*, 558–573. [CrossRef]
18. Lalelou, F.S.; Fateh, M. Effects of different concentrations of zinc on chlorophyll, starch, soluble sugars and proline content in Cucurbita pepo. *Int. J. Biosci.* **2014**, *4*, 6–12.
19. Gheju, M.; Balcu, I.; Ciopec, M. Analysis of hexavalent chromium uptake by plants in polluted soils. *Ovidius Univ. Ann. Chem.* **2009**, *20*, 127–131.
20. Misra, A.; Dwivedi, S.; Srivastava, A.; Tewari, D.; Khan, A.; Kumar, R. Low iron stress nutrition for evaluation of Fe-efficient genotype physiology, photosynthesis, and essential monoterpene oil (s) yield of *Ocimum sanctum*. *Photosynthetica* **2006**, *44*, 474–477. [CrossRef]
21. Sinha, S.N.; Biswas, M.; Paul, D.; Rahaman, S. Biodegradation potential of bacterial isolates from tannery effluent with special reference to hexavalent chromium. *Biotechnol. Bioinform. Bioeng.* **2011**, *1*, 381–386.
22. Mathur, S.; Kalaji, H.; Jajoo, A. Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. *Photosynthetica* **2016**, *54*, 185–192. [CrossRef]
23. Hayat, S.; Khalique, G.; Irfan, M.; Wani, A.S.; Tripathi, B.N.; Ahmad, A. Physiological changes induced by chromium stress in plants: An overview. *Protoplasma* **2012**, *249*, 599–611. [CrossRef] [PubMed]
24. Vishnupradeep, R.; Bruno, L.B.; Taj, Z.; Karthik, C.; Challabathula, D.; Kumar, A.; Freitas, H.; Rajkumar, M. Plant growth promoting bacteria improve growth and phytostabilization potential of *Zea mays* under chromium and drought stress by altering photosynthetic and antioxidant responses. *Environ. Technol. Innov.* **2022**, *25*, 102154. [CrossRef]
25. Regan, J.; Dushaj, N.; Stinchfield, G. Reducing hexavalent chromium to trivalent chromium with zero chemical footprint: Borohydride exchange resin and a polymer-supported base. *ACS Omega* **2019**, *4*, 11554–11557. [CrossRef]
26. Karthik, C.; Arulselvi, P.I. Biotoxic effect of chromium (VI) on plant growth-promoting traits of novel *Cellulosimicrobium funkei* strain AR8 isolated from *Phaseolus vulgaris* rhizosphere. *Geomicrobiol. J.* **2017**, *34*, 434–442. [CrossRef]
27. Tirry, N.; Kouchou, A.; El Omari, B.; Ferioun, M.; El Ghachtouli, N. Improved chromium tolerance of *Medicago sativa* by plant growth-promoting rhizobacteria (PGPR). *J. Genet. Eng. Biotechnol.* **2021**, *19*, 149. [CrossRef]

28. Haouas, A.; El Modafar, C.; Douira, A.; Ibnsouda-Koraichi, S.; Filali-Maltouf, A.; Moukhli, A.; Amir, S. Alcaligenes aquatilis GTE53: Phosphate solubilising and bioremediation bacterium isolated from new biotope "phosphate sludge enriched-compost". *Saudi J. Biol. Sci.* **2021**, *28*, 371–379. [CrossRef]
29. Wu, L.; Li, Z.; Han, C.; Liu, L.; Teng, Y.; Sun, X.; Pan, C.; Huang, Y.; Luo, Y.; Christie, P. Phytoremediation of soil contaminated with cadmium, copper and polychlorinated biphenyls. *Int. J. Phytoremediat.* **2012**, *14*, 570–584. [CrossRef]
30. Al-Huqail, A.; El-Bondkly, A. Improvement of *Zea mays* L. growth parameters under chromium and arsenic stress by the heavy metal-resistant *Streptomyces* sp. NRC21696. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 5301–5322. [CrossRef]
31. Sharma, A.; Kapoor, D.; Wang, J.; Shahzad, B.; Kumar, V.; Bali, A.S.; Jasrotia, S.; Zheng, B.; Yuan, H.; Yan, D. Chromium bioaccumulation and its impacts on plants: An overview. *Plants* **2020**, *9*, 100. [CrossRef]
32. Amin, H.; Ahmed Arain, B.; Abbasi, M.S.; Amin, F.; Jahangir, T.M.; Soomro, N.-U.-A. Evaluation of chromium phyto-toxicity, phyto-tolerance, and phyto-accumulation using biofuel plants for effective phytoremediation. *Int. J. Phytoremediat.* **2019**, *21*, 352–363. [CrossRef]
33. Rasheed, A.; Hassan, M.U.; Fahad, S.; Aamer, M.; Batool, M.; Ilyas, M.; Shang, F.; Wu, Z.; Li, H. Heavy metals stress and plants defense responses. In *Sustainable Soil and Land Management and Climate Change*; CRC Press: Boca Raton, FL, USA, 2021; pp. 57–82.
34. Bhagyawant, S.S.; Narvekar, D.T.; Gupta, N.; Bhadkaria, A.; Koul, K.K.; Srivastava, N. Variations in the antioxidant and free radical scavenging under induced heavy metal stress expressed as proline content in chickpea. *Physiol. Mol. Biol. Plants* **2019**, *25*, 683–696. [CrossRef] [PubMed]
35. Diaconu, M.; Pavel, L.V.; Hlihor, R.-M.; Rosca, M.; Fertu, D.I.; Lenz, M.; Corvini, P.X.; Gavrilescu, M. Characterization of heavy metal toxicity in some plants and microorganisms—A preliminary approach for environmental bioremediation. *New Biotechnol.* **2020**, *56*, 130–139. [CrossRef] [PubMed]
36. Qadir, M.; Hussain, A.; Hamayun, M.; Shah, M.; Iqbal, A.; Murad, W. Phytohormones producing rhizobacterium alleviates chromium toxicity in *Helianthus annuus* L. by reducing chromate uptake and strengthening antioxidant system. *Chemosphere* **2020**, *258*, 127386. [CrossRef] [PubMed]
37. Gupta, P.; Kumar, V.; Usmani, Z.; Rani, R.; Chandra, A.; Gupta, V.K. Implications of plant growth promoting *Klebsiella* sp. CPSB4 and *Enterobacter* sp. CPSB49 in luxuriant growth of tomato plants under chromium stress. *Chemosphere* **2020**, *240*, 124944. [CrossRef] [PubMed]
38. Valipour, M. Calibration of mass transfer-based models to predict reference crop evapotranspiration. *Appl. Water Sci.* **2017**, *7*, 625–635. [CrossRef]
39. Valipour, M.; Dietrich, J. Developing ensemble mean models of satellite remote sensing, climate reanalysis, and land surface models. *Theor. Appl. Climatol.* **2022**. [CrossRef]
40. Valipour, M.; Dietrich, J. Correction to: Developing ensemble mean models of satellite remote sensing, climate reanalysis, and land surface models. *Theor. Appl. Climatol.* **2022**. [CrossRef]
41. Preet, M.S.; Kumar, R.; Valipour, M.; Singh, V.P.; Neha; Singh, A.K.; Iqbal, R.; Zafar, M.U.; Sharma, R.; Singh, S.V.; et al. Soil Nutrient Status and Morphometric Responses of Guava under Drip Irrigation and High-Tech Horticultural Techniques for Sustainable Farming. *Hydrology* **2022**, *9*, 151. [CrossRef]
42. Usman, F.; Zeb, B.; Alam, K.; Valipour, M.; Ditta, A.; Sorooshian, A.; Roy, R.; Ahmad, I.; Iqbal, R. Exploring the Mass Concentration of Particulate Matter and Its Relationship with Meteorological Parameters in the Hindu-Kush Range. *Atmosphere* **2022**, *13*, 1628. [CrossRef]
43. Efon, E.; Ngongang, R.D.; Meukaleuni, C.; Wandjie, B.; Zebaze, S.; Lenouo, A.; Valipour, M. Monthly, Seasonal, and Annual Variations of Precipitation and Runoff Over West and Central Africa Using Remote Sensing and Climate Reanalysis. *Earth Syst. Environ.* **2022**. [CrossRef]