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Reduction of hexavalent chromium in wastewater and three varying agricultural soils using a bacterial strain *Bacillus* sp. SR18 isolated from a contaminated field

Saher Rubab[1], Faisal Mahmood[1], Tanvir Shahzad[1], Muhammad Shahid[2] and Sabir Hussain[1,*]

[1]Department of Environmental Sciences, Government College University, Faisalabad, Pakistan; [2]Department of Bioinformatics & Biotechnology, Government College University, Faisalabad, Pakistan
*Corresponding author e-mail: sabirghani@gmail.com

Soil and water resources are often contaminated with different types of pollutants including metal ions due to indiscriminate discharge of industrial effluents. Hexavalent chromium [Cr(VI)] is one of such pollutants which has a tendency to adversely affect the environment. Microbial bioremediation is one of the cost-effective methods which might be used to remediate such metals. In current study, a bacterial strain, *Basillus* sp. SR18, having the potential to tolerate and reduce Cr(VI), was isolated from a soil of district Kasur Pakistan which has been repeatedly irrigated with wastewaters containing the tannery effluents. This strain had a good potential not only to tolerate the presence of Cr(VI) in the medium with MIC value of 20 mM but also to completely (>90%) reduce Cr(VI) even at its initial concentration of 20 mg L^{-1}. *Basillus* sp. SR18 was also effective for the Cr(VI) reduction in the presence of different concentrations of NaCl while, maximum Cr(VI) reduction was detected at pH 8.5. Moreover, SR18 was observed to reduce the Cr(VI) in varying agricultural soils and in real tannery wastewater. The reduction rate was significantly ($p \leq 0.05$) different between different treatments of tannery wastewater. Bioaugmentation of three different agricultural soils with the strain SR18 resulted into a significant but varying decrease in the level of Cr(VI) with the maximum decrease in sandy soil. This study presents the significance of *Bacillus* sp. SR18 as a potential living source for remediation of Cr(VI) in the soils and water resources.

Keywords: Wastewater treatment, Hexavalent chromium, Soil bioaugmentation, Bioremediation, *Bacillus* sp.

INTRODUCTION

The metal ions are inorganic contaminants which cause serious hazards in soil as well as in water and require suitable remedial actions (Ackerley 2004; Iyer and Mastorakis, 2010; Yan *et al*., 2020). Industries and manufacturing units are the foremost sources for release of different heavy metals (Tokatli 2019; Kanwar *et al*., 2020). In developing countries, these contaminants are discharged into the environment without pretreatment which trigger the pollution of soil and water resources.

Leather industry is among the important industrial sectors in several developing countries which generates the revenue through conversion of animal hides into chemically and physically stable product known as leather. During the tanning and other finishing processes required for conversion of animal skins to stable and finished leather, a number of chemicals are used which are released as contaminant along with the effluents coming from the leather industries (China *et al*., 2020; Sawalha *et al*., 2020). Chromium is one of those chemicals which are extensively used during the tanning process and is excessively released along with the effluents originating from the leather industries (Junaid *et al*., 2017; Sawalha *et al*., 2020). Chromium is found in various oxidation states. Relatively more dominant and stable forms of chromium are trivalent and hexavalent forms. The hexavalent [Cr(VI)] form of chromium is more harmful as compared with trivalent form [Cr(III)] and is more frequently released through tanneries wastewaters (Maqbool *et al*., 2015; Hedberg, 2020). Existence of Cr(VI) in the environment is very harmful since it is not only a strong oxidizing agent but also stated to be teratogenic and mutagenic (Hedberg, 2020). Hexavalent form of chromium is enumerated as class-A carcinogen compound in human beings by the US-EPA (Rathore *et al*., 2021). Many human diseases including the diarrhea, ulcers, eye irritation, kidney failure, lungs cancer and various pulmonary disorders are linked with Cr(VI) exposure (Fu *et al*., 2020; Hedberg, 2020; Farooqi *et al*., 2021). Hence, it is needed to devise suitable strategies for Cr(VI) remediation from soil and water resources.

The Cr(VI) can be detoxified by several physicochemical approaches e.g. chemical reduction, ion exchange methods,

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adsorption, membrane filtration, electrochemical precipitation and solvent extraction etc. (Jamshidifard *et al*., 2019; Yao *et al*., 2020). Despite that some of the physicochemical techniques have good potential for detoxification of Cr(VI), there are many substantial disadvantages related to these techniques including the high cost and the production of secondary pollutants (He *et al*., 2018). However, numerous recent studies indicate that biological approaches might serve as sustainable approach to deal with Cr(VI) because they are considered not only safe and reliable but also environment friendly and cost effective (Barrera-Díaz *et al*., 2012; Maqbool *et al*., 2015; He *et al*., 2018; Xia *et al*., 2019). Microorganisms especially bacteria are preferred for this purpose because they have short life cycle and are relatively easier to culture in varying conditions. During the recent years, the researchers have reported different bacteria including *Pseudomonas aeruginosa* ZM130 (Maqbool *et al*., 2020), *Enterobacter cloacae* (Pattnaik *et al*., 2020), *Bacillus sp.* (Zeng *et al*., 2020), *Shewanella sp.* (Zinicovscaia *et al*., 2020), *E. coli* (Mohamed *et al*., 2020), *Lysinibacillus sp.* (Chen *et al*., 2021; Zhu *et al*., 2021), *Sphingopyxis macrogoltabida* SUK2c (Prabhakaran *et al*., 2019) for reduction and remediation of Cr(VI). Despite that several bacteria have been isolated for this purpose, it is the need of the hour to isolate and characterize novel bacterial isolates which might have better ability for reduction and remediation of Cr(VI) in diverse types of environments. The aim of current research was to determine the optimal conditions for the reduction of Cr(VI) as well to check out the potential of *Bacillus* sp. SR18 as a bioremediation agent for Cr(VI) from contaminated wastewater and agricultural soils.

MATERIALS AND METHODS

Soil Sampling and its characterization: The soil for isolation of bacteria was collected from the district Kasur (Pakistan) from the surface of an agricultural land (0–15 cm) often irrigated with the wastewaters also containing the tanneries effluents. All the samples were stored in the polythene sacks. Samples were shifted to the laboratory, ground, cleaned and sieved by using 2 mm sieve. Five soil samples were assorted to get the homogeneous sample in the laboratory.

Isolation of bacteria: An enrichment procedure followed by dilution plating was adopted to isolate Cr(VI) tolerant bacteria. For this purpose, 50 mL mineral salt medium (MSM) spiked with 10 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$, added with five grams of contaminated soil. The enrichment cultures were subjected to incubation under shaking (150 rpm) condition for 5 days at 28°C. After 5 days, the quantity of the Cr(VI) remaining in the MS medium was estimated using diphenylcarbazide procedure as already explained by Maqbool *et al*. (2015) and reduction was assessed by the given equation:

$$Cr(VI)\ reduction\ \% = \frac{(a\ -b)}{a} \times 100$$

in above equation a denotes absorbance of control without inoculation and b stands for the samples inoculated with bacterial isolate. It was detected that primarily added Cr(VI) was removed more than 80% from the medium. 5 mL aliquot from the first enrichment flask was inoculated in another flask comprising 50 mL of same media spiked with Cr(VI) and incubated as well as analyzed in the similar way. The same process was repeated four time and the final enriched culture was serially diluted from 10^{-1} to 10^{-6} dilutions and 50 µL from each dilution was inoculated on MS agar media plates containing 10 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$. These plates were kept in dark at 28°C. After 5 days, morphologically different colonies growing on the plates were purified through repeated streaking and preserved for further screening experiments.

Cr(VI) tolerance by efficient Cr-reducing bacterial isolates: The bacterial isolates' potential to resist the Cr(VI) was studied by assessing the MIC (Minimum inhibitory concentration) of Cr(VI) for the bacteria. The bacteria were streaked on nutrient ager media plates with various concentrations (1.0 to 25 mM) of Cr(VI). These plates were kept in dark at 28°C. After 5-day incubation, bacterial growth was observed with the help of digital colony counter. The MIC was noted as the least concentrations which restrain the growth of bacterial isolates. The Cr(VI) reducing ability of the carefully chosen bacterial isolates was determined by inoculating them in MSM broth media containing 10 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$. The un-inoculated control and bacterial cultures in the form of three replicates were inoculated in shaking incubator (150 rpm) at 28°C. After 24h, 1 mL aliquot from each culture was subjected to centrifugation (10 min at 7000 rpm) and supernatants were analyzed for Cr(VI) as already described above. The growth of bacterial strains in the mineral salt media containing the Cr(VI) was assessed by the optical density (OD600) of the cultures as well as the controls. Based on the growth, resistance and Cr(VI) reduction, the isolate SR18 was selected for further studies and identified following the protocols described by Hussain *et al*. (2013). and its culture was also preserved at -20°C in glycerol.

Reduction of Cr(VI) at various pH levels: Reduction of Cr(VI) by SR18 was examined at different pH values (4.5, 5.5, 6.5, 7.5, 8.5 and 9.5) using mineral salt medium containing 10 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$. The pH of the sterilized medium was adjusted using standard solutions of HCl or NaOH. For each pH level three replicates were processed. The cultures were firmly wrapped under static conditions for 72h and incubated at 25°C. After the incubation of 24, 48 and 72h, aliquots from each culture were taken and centrifuged (7000 rpm, 10 min). Against un-inoculated medium as control, the supernatants were used to reveal the remaining concentration of Cr(VI) by using diphenylcarbazide method at λmax (540 nm). The remaining Cr(VI) concentration estimation for each pH level and un-

inoculated control samples were run. On the completion of the incubation period, the growth of SR18 as colony formation units (CFU) was estimated at various pH values. For this purpose, after completing the incubation period, the cell suspensions were subjected to serial dilution and 100 mL of each dilution ranging from 10^4 to 10^6 was inoculated onto nutrient agar (NA) media plates. These plates were then placed under incubation at 25 °C for a duration of five days to allow the growth of bacterial colonies. Subsequently, the number of colony-forming unit (CFU) was determined through enumeration as already described by Hussain *et al*. (2013).

Impact of NaCl on Cr(VI) reduction by SR18: To study the impact of NaCl on Cr(VI) reduction by SR18, different amounts (0, 10, 20 and 50 g L^{-1}) of NaCl were added in separated mineral salt along with 10 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$. After inoculation of SR18, triplicates of the test tubes were incubated at 28°C in dark. Aliquots were collected after regular intervals and centrifuged (7000 rpm, 10 min) and Cr(VI) reduction was determined by taking absorbance at 540 nm following diphenylcarbazide method illustrated by Maqbool *et al*. (2015).

Remediation of Cr(VI) in real tannery wastewater: For this purpose, centrifuged (6000 rpm for 10 min) tannery wastewater samples at different dilution levels (100, 75, 50 25, 0% wastewater) were spiked with Cr(VI) and then inoculated with the selected bacterial strain SR18. Samples were taken and centrifugated (10 mins at 7000 rpm) intermittently. The filtrates were used for the assessment of Cr(VI) contents by using the method of diphenylcarbazide as already narrated by Maqbool *et al*. (2015).

Bioremediation of Cr(VI) in three varying agricultural soils: Soils sample from different agro-ecological zones of Punjab were collected to study the bioremediation potential of selected chromium tolerant strain in soils with different physicochemical characteristics. The collected samples were immediately kept in sterilized polythene bags. Air-dried samples were ground and sieved to separate the roots, stones, and other coarse particles. Three soils having different texture i.e., clay soil, loam soil and sandy soil were selected from the sampled soils. Soils were double autoclaved for the purpose of sterilization. Sterilized and non-sterilized soils (250 g) were added into the vertical glass columns with dimensions of 12 cm in length and 8 cm in diameter after spiking with 25 mg kg^{-1} of Cr(VI) as $K_2Cr_2O_7$ salt. Experiment was run in triplicate. To homogenize the chromium in the soil, distilled water was added, and glass columns were incubated for two weeks. 25 mL of fresh bacterial cells having 0.5 optical density (10^{6-8} CFU mL^{-1}) were directly added in glass columns and the distilled water was added in control glass columns. After 15 days of incubation at 28°C, soil samples were taken and remaining Cr(VI) content in the soils were investigated by the following the procedure describes by Maqbool *et al*. (2015).

Statistical analysis: To reveal the significance of treatments, one way analysis of variance (ANOVA) was carried out. Multiple means comparisons were done by using Tukey's HSD test. The tables and figures comprise on the mean and standard error of the means of three repetitions.

RESULTS

In the present study, 25 bacterial isolates having varying colony formation on the agar media plates containing Cr(VI) had different tolerance for Cr(VI) because varying MIC values and growth values were observed (Table 1). The MIC of Cr(VI) for the tested 25 isolates were found to be ranging between 1 mM to 20 mM. The highest value (20 mM) of MIC was observed for the isolates SR18 and SR16 followed by MIC value of 15 mM for SR19 and MIC value of 10 mM for SR3 (Table 1). 25 tested isolates exhibited the Cr(VI) reduction potential but with varying extents. After 24 h, Cr(VI) reduction values were observed to range from 5.5% to 61% with the highest value of Cr(VI) reduction (61.0±1.8%) achieved by the isolate SR18 followed by the value of 54.9±3.5% achieved by the isolate SR19 and 45.2±1.9% achieved by the isolate SR22 (Table 1). Over the incubation period of 48 h, the values of Cr(VI) reduction were observed to be ranging from 24.0% to 96.6% with the highest value of Cr(VI) reduction (96.6±3.1%) achieved by the isolate SR22 followed by the value of 93.1±3.2% achieved by the isolate SR19 and 89.3±4.3% achieved by the isolate SR18 (Table 1). After 72 h, the values of Cr(VI) reduction were observed to be ranging from 46.0% to 97.2% with the isolates SR3, SR6, SR17, SR18, SR19, SR20, SR21 and SR22 showing the value of Cr(VI) reduction more than 90% (Table 1). All the tested isolates were also found having varying levels of growth (OD600) ranging from 0.8 to 1.9 in the media containing Cr(VI). The highest value (OD600 = 1.9) of growth was observed for the isolates SR6 and SR18 followed by the value of 1.8 for the isolates SR19 and SR21 (Table 1). In these experiments, the isolate SR18 was found to be more efficient in all three parameters as compared with other isolates. Therefore, SR18 strain picked for further investigations.

Identification and polygenetic study of SR18 Strain: The blastn analysis of 16S rRNA of SR18 showed highest similarity (> 97%) with genus *Bacillus*. Moreover, in silico analysis of bacterial isolate SR18 was performed by creating the phylogenetic tree using MEGA7 software performing by neighbor joining method. The isolate SR18 was found phylogenetically similar and placed in bacterial strains group which belong to the genus *Bacillus* (Fig. 1). On the bases of genetic association of SR18 with other *Bacillus* sp., it was designated as *Bacillus* sp. SR18 (GenBank Accession Number OM530527).

Table 1. Tolerance of the bacterial isolates against the presence of hexavalent chromium [Cr(VI)] in the medium and their potential to reduce Cr(VI)

Bacterial Isolates	MIC of Cr(VI) (mM)	Cr(VI) Reduction (%) 24 h	48 h	72 h	Growth (OD600)
SR1	2.0	10.0±1.7	24.0±2.2	69.7±2.5	1.4
SR2	1.5	8.6±1.4	30.1±1.8	74.6±2.4	1.2
SR3	10.0	20.7±2.1	41.5±3.6	93.3±4.1	1.3
SR4	1.0	24.0±1.8	28.3±1.7	56.6±3.2	0.8
SR5	1.5	5.5±1.1	57.4±2.9	58.1±1.8	1.1
SR6	20.0	44.3±3.5	63.2±2.4	92.2±2.4	1.9
SR7	1.5	17.1±2.1	51.4±3.2	73.3±3.4	0.9
SR8	2.0	5.9±1.6	27.0±1.8	60.2±1.5	0.8
SR9	1.0	27.0±2.2	23.1±1.2	67.7±2.7	1.1
SR10	1.0	33.8±2.4	29.2±2.1	73.2±1.9	1.3
SR11	2.5	17.0±1.9	30.0±2.3	58.5±3.0	1.0
SR12	1.0	22.0±2.0	32.0±1.9	46.6±2.3	1.2
SR13	1.5	61.6±2.5	35.3±1.8	55.2±3.3	1.1
SR14	1.0	21.9±1.8	38.7±1.1	59.7±2.4	0.9
SR15	1.0	28.3±1.5	28.7±1.7	64.0±1.9	1.2
SR16	1.0	6.5±0.8	25.9±1.4	53.0±0.9	1.0
SR17	2.0	38.1±2.4	48.3±2.6	93.0±2.7	1.6
SR18	20.0	21.0±1.8	89.3±4.3	91.2±3.6	1.9
SR19	15.0	54.9±3.5	93.1±3.2	87.6±3.1	1.8
SR20	1.0	32.7±3.1	62.3±3.4	91.6±4.0	1.7
SR21	1.0	38.0±2.3	96.6±3.1	91.1±2.5	1.8
SR22	1.0	45.0±1.9	65.2±2.8	90.9±3.8	1.5
SR23	1.0	37.2±1.8	65.5±1.7	88.2±2.4	1.6
SR24	2.0	31.1±2.0	78.9±2.5	87.4±2.1	1.4
SR25	1.0	45.0±1.6	74.9±3.1	89.7±3.2	1.8

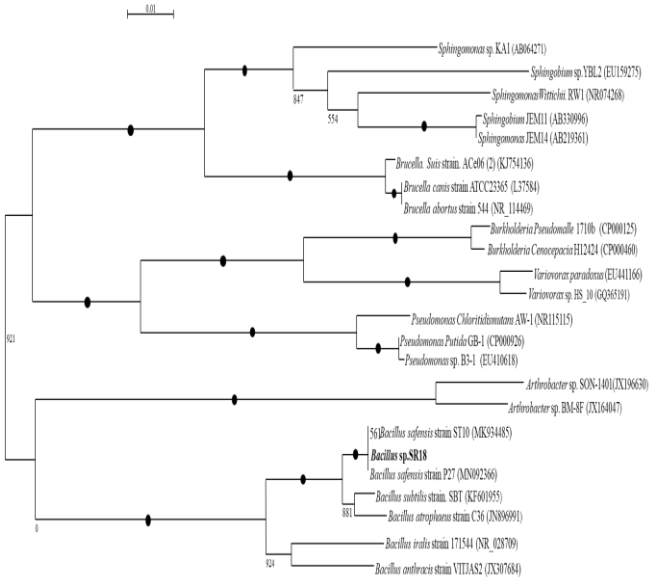

Figure 1. Neighbor-joining phylogenetic analysis of *Bacillus* sp.SR18 with other bacterial strains found in the database of GenBank. The accession number of the strains are given in brackets. Bootstrap values greater than 900% are marked as black circles.

Effect of initial chromium concentrations on Cr(VI) reduction: The growth of SR18 was found to increase with increase in time but it decreased when the level of Cr(VI) was increased in the medium (Fig. 2a). Despite that a slight decrease in SR18 growth was detected in the presence of 10 and 20 mg L^{-1} of Cr(VI) over all three incubation periods (24h, 48h, 72h), however, the growth was significantly decreased in the media containing higher concentrations 50 mg L^{-1} and 100 mg L^{-1} of Cr(VI). The strain SR18 exhibited a good potential for reduction of Cr(VI) at its varying initial concentrations (Fig. 2b). Cr(VI) reduction (%) by SR18 was decreased when the preliminary concentration of Cr(VI) was increased in the medium (Fig. 2b). Over 24 h incubation, 35.3(±5.5)%, 24.3(±3.4)%, 12.9(±2.2)% and 13.2(±1.4)% Cr (VI) reduction was observed by SR18 in the media containing 10 mg L^{-1}, 20 mg L^{-1}, 50 mg L^{-1} and 100 mg $L^{-1}$of Cr (VI), respectively. At the end of incubation (72h), almost complete (>90%) reduction of Cr(VI) was detected in the media having 10 and 20 mg $L^{-1}$of Cr (VI). Over the same incubation period, 57.2(±6.4)% and 25.0(±5.3)% of Cr (VI) reduction was detected in the Cr (VI) (50 and 100 mg L^{-1}) containing media.

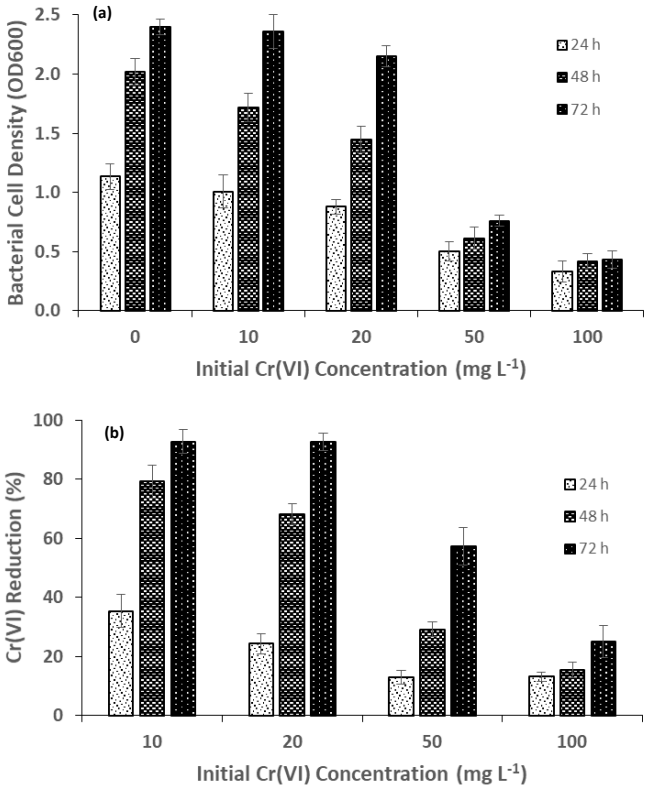

Figure 2. Effect of varying initial concentrations of Cr(VI) (0, 10, 20, 50, 100 mg L^{-1}) on the growth of the strain SR18 (a) and on Cr(VI) reduction (b) (10, 20, 50, 100 mg L^{-1}) by the strain SR18 at different time intervals.

Cr(VI) Reduction at varying pH values: The pH was observed to affect the Cr(VI) reduction by the bacterial isolate SR18 (Fig. 3a). The highest rate of Cr(VI) reduction (3.38 % h^{-1}) by *Bacillus* sp. SR18 was detected at pH 8.5 followed by at pH 7.5 (2.98 % h^{-1}) and the lowest rate of Cr(VI) reduction (1.25% h^{-1}) was examined at pH 4.5 (Fig. 3a). The bacterial growth in terms of colony formation unit was noticeably different at various pH levels. Interestingly, the bacterial growth (CFU) at various pH values was observed to be interrelated with the rate of reduction (% h^{-1}) of Cr(VI) at varying pH values (Fig. 3b).

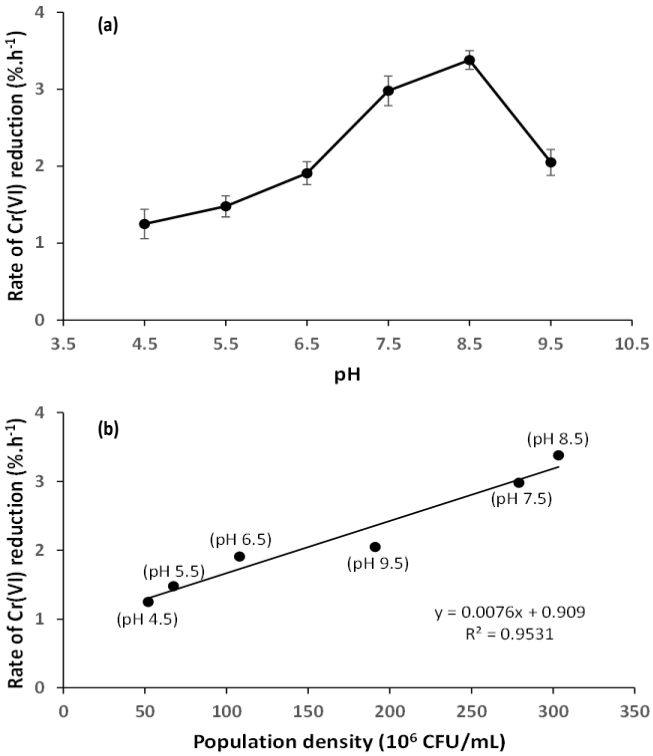

Figure 3. Impact of different pH levels (3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5) on rate of Cr(VI) reduction by the strain SR18 (a), Correlation between the rate of Cr(VI) reduction (%. h^{-1}) and the population density (growth) of the strain SR18 (b).

Cr(VI) reduction at various levels of NaCl: The potential of *Bacillus* sp. SR18 to eliminate the Cr(VI) at different NaCl concentrations (0 g L^{-1} to 50 g L^{-1}) in MS medium has been presented in Fig. 4a. The bacterial strain SR18 removed the Cr(VI) at different concentrations of NaCl but with different ability and rate. Over 36 h incubation, 81.4(±2.3)%, 70.1(±5.0)%, 53.7(±3.5)% and 23.9(±2.2)% Cr (VI) reduction was done by SR18 in the media comprising on NaCl 0, 10, 20 and 50 g L^{-1} correspondingly. It is noteworthy that, at the end of incubation (72h), nearly complete (>90%) reduction of Cr(VI) was observed at 0 g L^{-1}, 10 g L^{-1} and 20 g L^{-1} of NaCl in the media. Over the same incubation period, 40.8(±3.8)% of Cr (VI) reduction was carried out in the medium containing 50 g L^{-1} of NaCl. Similarly, the maximum bacterial cell density was observed at 0 g L^{-1} of salt concentration which was decreased over an increase in the concentration of NaCl in the medium. Bacterial cell growth increased with the passage of time but it decreased when NaCl level was increased in the medium. Growth of bacterial strain SR18 is comparable with Cr(VI) reduction at the incubation period of 36 h and 72 h (Fig. 4b)

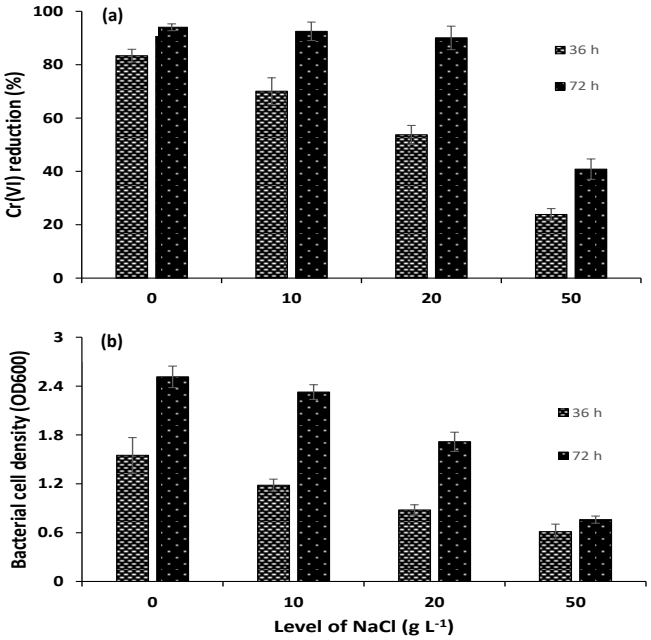

Figure 4. Effect of different NaCl (0, 10, 20, 50 g L^{-1}) levels on Cr(VI) reduction by the bacterial strain SR18 (a) and on the bacterial cell density (OD600) of the strain SR18 (b) at various time intervals.

Bioremediation of Cr(VI) in real tannery wastewater: Results concerning the removal of Cr(VI) by the strain SR18 in four concentrations of tannery wastewater after 24 and 48 h of incubation is depicted in the Fig. 5. Over 24 h of incubation, maximum Cr(VI) removal (95.5%) by the bacterial strain SR18 was observed at 50% of tannery wastewater which was statistically similar to Cr(VI) reduction at 75% of wastewater with 94.8% reduction in Cr(VI) followed by the Cr(VI) reduction (51.4%) at 25% of wastewater. The Cr(VI) reduction at 100% and 0% wastewater were 20.5 and 13.4%, respectively. Similarly, after 48 h of incubation, maximum Cr(VI) reduction by SR18 was 98.5% at 75% of wastewater followed by Cr(VI) reduction (96.2%) at 25% wastewater. The Cr(VI) reduction at 100, 50 and 0% wastewater were recorded as 52.4, 90.4 and 71.9%, respectively.

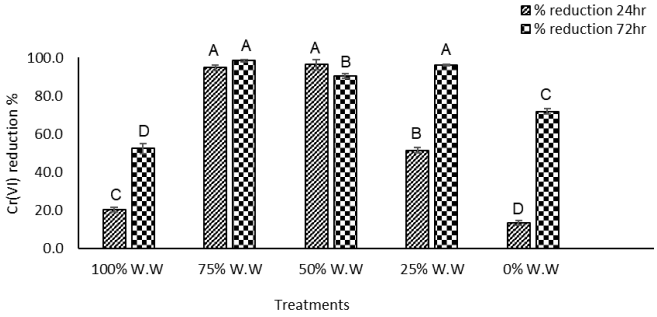

Figure 5. Cr(VI) by SR18 reduction at different concentration (100, 75, 50, 25, 0%) of real tannery wastewater.

Bioremediation of Cr(VI) in three varying soils: The data regarding Cr(VI) removal in inoculated and uninoculated three soils are shown in Fig. 6. The end result revealed that bio augmentation in both soil columns (non-sterilized and sterilized) with the strain SR18 leads to the subtraction of Cr(VI) efficiently. However better results regarding the removal of Cr(VI) were observed in sandy soil than loam and clay soils irrespective of the sterilized or non-sterilized soil. Higher reductions in Cr(VI) content were observed in sterilized soils as compared to non-sterilized soils. Results revealed that minimum remaining Cr(VI) content after inoculation with strain SR18 was observed in sterilized sandy soil that was 8.32 mg kg^{-1}, while the remaining Cr(VI) content in the uninoculated control of sandy soil was 19.82 mg kg^{-1}. The value of the remaining Cr(VI) in inoculated sterilized clay soil was 13.85 mg kg^{-1} as compared to 16.72 mg kg^{-1} in the respective uninoculated control of sterilized clay soil. The Cr(VI) content in sterilized loam soil due to inoculation with SR18 was 14.65 mg kg^{-1} and 15.54 mg kg^{-1} in respective uninoculated control. Similarly, in non-sterilized soils., the remaining Cr(VI) content in sandy, clayey and loam control soils were 21.29, 16.86 and 22.89 mg kg^{-1}, respectively. While the inoculation with SR18 resulted in lower Cr(VI) content which were 15.53, 16.12 and 20.27 mg kg^{-1}, respectively.

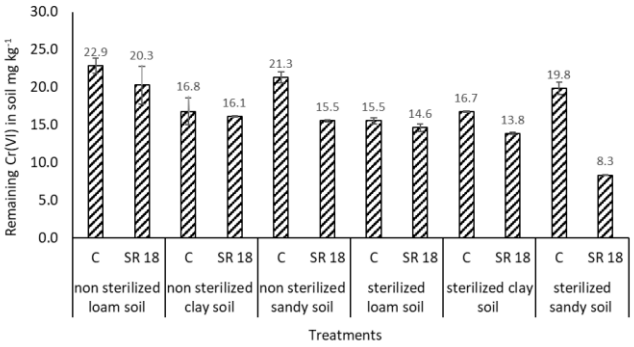

Figure 6. Effect of bacterial inoculation SR18 on Cr(VI) concentration (mg kg^{-1}) in non-sterilized and sterilized loam, clay and sandy soils.

DISCUSSION

Tanneries effluents loaded with different pollutants including hexavalent chromium [Cr(VI)] are considered as a serious threat (Banerjee *et al*., 2019). This study was conducted to isolate a Cr(VI) tolerant bacterium capable of reduction of Cr(VI) in the soil and aqueous media. This study exhibited the potential of Cr(VI) resistance and reduction of various bacteria isolated from the soil collected from district Kasur Pakistan. The results revealed that maximum isolated bacteria had the variable ability to resist and to carry out reduction of Cr(VI) with variable efficiency. This variation in the competence of isolated bacteria to resist and reduce the Cr(VI) depends upon their adaptation to the presence of Cr(VI) (Upadhyay *et al*., 2017). It has been previously reported that different bacterial isolates have varying properties to reduce and tolerate the chromate under laboratory as well as in field (Ibrahim *et al*., 2011; Mishra *et al*., 2012; Maqbool *et al*., 2020). In the current research work, the bacterium SR18 exhibited the maximum potential to carry out reduction of Cr(VI). Phylogenetic analysis as well as Blastn analyses on the bases of 16S rRNA of SR18 strain indicated its maximum similarity with bacteria belonging to genus *Bacillus*. Potential of *Bacillus* spp. to carry out Cr(VI) reduction under different environmental conditions has also been previously reported in different studies (Rajkumar *et al*., 2006; Rehman and Faisal, 2015; Banerjee *et al*., 2019; Din *et al*., 2020). So, the isolation and characterization of *Bacillus* sp. SR18 from district Kasur (Pakistan) is a novel contribution in the potential Cr(VI) reducer bacteria.

In current study, increasing the initial level of Cr(VI) resulted into a decrease in the growth of SR18 strain (Fig. 2a). However, the strain SR18 showed a good potential for reduction of Cr(VI) at its varying initial concentrations which was significantly decreased at its higher levels. Relatively lower Cr(VI) reduction at highest concentration might be due to the impact of Cr(VI) on the growth of SR18 as observed in Fig 2b. It has already been reported that higher concentrations of chromium can decrease the chromium reduction potential of bacteria which can cause the toxic and mutagenic effects of Cr (VI) on bacterial cell metabolism (Thacker *et al*., 2007; Bharagava and Mishra, 2018; Mishra *et al*., 2021). At elevated Cr(VI) concentrations the decrease in Cr(VI) reduction indicates that Cr(VI) toxicity destroy some active Cr(VI)-reducing contributors (McLean *et al*., 2000; Zhu *et al*., 2019).The Cr(VI) reduction extent and mechanisms are variable. It depends on numerous factors e.g., isolation resource, growth of microorganism, enzymatic systems of microorganisms, etc. (Chen and Tian, 2021). For example, the reduction rate of Cr(VI) depends upon the initial cell density. Before the beginning of Cr(VI) reduction, acclimatization is required for cells to adopt the toxicity of Cr(VI) (Chen and Hao, 1996; Zhu *et al*., 2019). The bacterial growth and the function of Cr(VI) reduction were also found to have almost

similar pattern at different levels of Cr(VI) in this study. However, there is need to explore these mechanisms of Cr(VI) reduction in detail by studying the impacts of the Cr(VI) on the growth as well as the enzymes involved in this reduction.

pH is a considerable factor which affects the growth and activity of the microorganisms including the reduction reactions (Hussain *et al*., 2013; Maqbool *et al*., 2015; Chen and Tian, 2021). In this study, Cr(VI) reduction by the strain SR18 was highest at pH 8.5 followed by at pH 7.5 and even lower values of Cr(VI) reduction were detected at other pH values. However, it is noteworthy that a considerable Cr(VI) reduction by the strain SR18 was observed even at acidic and alkaline conditions which shows a wide range of pH tolerance of this strain. The neutral conditions to slightly alkaline conditions have been previously reported to be optimal for various bacteria to carry out different reduction reactions including the reduction of Cr(VI) (Maqbool *et al*., 2015; Chen *et al*., 2020). Similar to this study, different bacterial strains including *Cellulosimicrobium cellulans*, *Bacillus pumilis*, and *Staphylococcus capitis* have also been described to show an optimal Cr (VI) reduction at pH values near the neutral point to slightly alkaline conditions (Zahoor and Rehman 2009; Rehman and Faisal 2015; Maqbool *et al*., 2015; Wani *et al*., 2019). The impact of pH on the Cr(VI) reduction might be either due to its effect on growth or enzymatic activities of the reducing bacteria (Wani *et al*., 2019; Chen and Tian, 2021). For example, changes in pH have been reported to alter the active sites of chromium reducing (Wani *et al*., 2018; Wani *et al*., 2019). Nevertheless, the rate of Cr(VI) reduction at different pH levels in this study was having a correlation with the growth at respective pH values. Hence, it might be concluded that the growth of the strain SR18 was affected by pH which eventually affected the Cr(VI) reduction. However, there is need to understand the underlying processes for this Cr(VI) reduction regulation with pH by studying the growth and enzymes together.

In this study, the bacterium SR18 was found to remove the Cr(VI) at various tested concentrations (0 to 50 g L^{-1}) of NaCl but with varying efficacy. The same results have also been described by different other scientists (Zhu *et al*., 2019; Su *et al*., 2021). Zhu *et al*. (2019) carried out an experiment to find out the Cr(VI) reduction by a bacterial strain *Bacillus* sp. CRB-1 under saline conditions. After 48 h, almost 100% of Cr(VI) was reduced in the presence of 0.5% and 1% salt at 100 mg L^{-1} concentration of Cr(VI). The salt concentration declined (0.5% to 0%) the removal of Cr(VI) ($25.5 \pm 0.20\%$) was dropped as well. At the salt concentration of 5% Cr(VI) was reduced ($15.1 \pm 0.79\%$) only. The strain SR18 in the present study carried out almost complete removal of Cr(VI) up to 2% (20 g L^{-1}) of salt in the medium and more than 40% removal of Cr(VI) even in the presence of 5% (50 g L^{-1}) of NaCl which shows a batter NaCl tolerance capacity of the strain. The lethal result of NaCl concentrations on the Cr(VI) reduction due to the imbalance of ions, because metabolic activity depends upon the homeostasis of vital ions (Wang *et al*., 1997), or ion toxicity harmful to the reduction of chromate (Zhu *et al*., 2019). ROS accumulation increase due to salt stress, it was hypothesized that ROS associated toxicity might be contributive to this negative effect (Nxele *et al*., 2017). A lesser amount of salt concentration means decline in Cr(VI) removal efficiency because suitable salinity required to maintain cell growth and internal homeostasis. In this study, higher concentration of NaCl salt in the medium resulted in a reduced growth of the isolate as well as the Cr(VI) removal efficiency.

The strain SR18 potential was also studied for bioremediation of Cr(VI) in the real wastewater and also in three soils having varying textures. While studying the strain SR18 potential for elimination of Cr(VI) in the varying dilutions (0, 25, 50, 75 and 100%) of the wastewater, variable levels of Cr(VI) removal were observed in different dilutions with maximum (>90%) Cr(VI) removal in 25%, 50% and 75% dilutions of the wastewater. It is noteworthy that the strain SR18 exhibited a good level of Cr(VI) removal even in 100% wastewater. Despite that Cr(VI) removal by the bacterial strains has been extensively studied in synthetic media (Maqbool *et al*., 2015; Zhu *et al*., 2019; Chen and Tian, 2021; Su *et al*., 2021), though, only few studies reporting the remediation of Cr(VI) by the bacterial strain in real wastewaters (Zahoor and Rehman., 2009; Chatterjee *et al*., 2011; Elahi *et al*., 2018; Chen and Tian, 2021). For example, Zahoor and Rehman (2009) tested the Cr(VI) reduction in tannery wastewater. It was observed that the up to 86% and 89% Cr(VI) was reduced by *Bacillus* sp. JDM-2-1 and *Staphylococcus capitis*, respectively, after an incubation of 144 h. Similarly, Elahi *et al*. (2018) determined that *Basillus arerius* S1 and *Basillus iodinum* S2 were capable to reduce Cr(VI) up to 99% at 2 mM Cr(VI) concentration in the wastewater when incubated for 6 days. Cr(VI) reduction by the strain SR18 in the varying dilutions of the real tannery wastewater is a unique feature of this strain which makes it prominent in the Cr(VI) reducing bacteria.

Bioremediation of Cr(VI) by the strain SR18 has also been studied in three varying soils spiked with Cr(VI). The results revealed that bioaugmentation equally in non-sterilized and sterilized soil columns with the *Bacillus* sp. SR18 strain caused into the removal of Cr(VI) efficiently. However, higher reduction in Cr(VI) content were observed in sandy soils regardless of sterilized and non-sterilized soils. Under sterilized conditions, the lowest Cr(VI) content was observed in sandy soil bioaugmented with bacterial strain SR18. Despite that bacterial Cr(VI) removal has been extensively studied in aqueous cultures, there are only few studies indicating the Cr(VI) reduction in the soils through bioaugmentation with Cr(VI) reducing bacterial strains (Nie, *et al*., 2018; Yang *et al*., 2021). The variability in Cr(VI) reduction in three varying soils might linked with varying

physicochemical properties of three soils. For example, the presence of the organic matter and carbon sources such as biochar in the soil can enhance the microbial growth and resultantly the microbial reduction of Cr(VI) (Nie *et al*., 2018; Yang *et al*., 2021). Similarly, the texture of the soil also affects the bioavailability and transformations of the contaminants Ellis *et al*. (2002) said that particles of clay can be deliberated as Cr Fe exhibiting phase in the optimum fraction, advocating the reaction among Fe(II) and Cr(VI) in the clay fraction. It is evidenced that there is strong combination of clay fractions with Fe(II). They similarly observed that there is a correlation among Cr(VI) reduction and clay partials. In the experiment they further explained that clay particles bearing the functional groups which enhance the soil diversity in terms of microbial community, so endorsing the Cr(VI) reduction. Similarly, Xia *et al*. (2019) observed that the maximum bioremediation potential of a combined bacterial consortium of *Bacillus* sp. and *Geotrichum* sp. might accredited the alternative growth patterns. It can boost up the Cr(VI) bioremediation in contaminated sites.

Conclusion: On the basis of current study, it is concluded that Cr(VI) resistant bacterial strain *Bacillus* sp. SR18 has the potential to reduce the Cr(VI) in the chromium contaminated soils, as well as it can reduce more than 90% chromium in tannery effluents obtained from the industry. Therefore, the selected strain can perform as an excellent bioresource for the treatment of tannery effluent. However, evaluation on large scale is needed before its use on commercial scale.

Declarations

***Conflicts of interest*:** All authors declare that there is no conflict of interest

***Authors' Contribution Statement*:** Saher Rubab performed the experiments, collected the data and wrote the first draft of the manuscript. Sabir Hussain, Tanvir Shahzad, Faisal Mahmood and Muhammad Shahid designed the research methodology, analyzed data and carried out proof reading of the manuscript.

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