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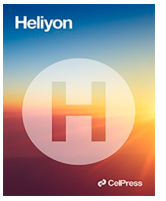

Research article

Isolation of chromium resistant bacteria from tannery waste and assessment of their chromium reducing capabilities – A Bioremediation Approach

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ABSTRACT

Every year different industries generate numerous toxic environmental polluting agents throughout the world. Among the polluting agents, chromium (Cr) toxicity is a great concern nowadays. It is continuously released in soil and water, causing environmental and health problems thereby raising several public health issues in developing countries like Bangladesh. The primary goal of this study was to provide a bioremediation option to reduce toxic hexavalent chromium to a less toxic trivalent form by isolating chromium resistant bacteria from Cr contaminated environments. Bacterial isolates were obtained from seven tannery waste samples collected from Hazaribag and Hemayetpur, Savar, Dhaka. Twenty morphologically distinct colonies were screened, of which six showed the highest resistance. These were designated as A1, A2, B1, F1, K1, and P1. Their maximum tolerance to Cr (VI) was determined through growth assays in varying chromium concentrations up to 8000 mg/L on LB agar media. Strains A2 and B1 exhibited the highest resistances to chromium at 7700 mg/L and 7200 mg/L respectively. Bacterial strains A2 and B1 were identified through several biochemical tests and after PCR analysis finally identified as Bacillus sp. and Micrococcus sp. respectively. Their Cr (VI) reduction capabilities were assessed quantitatively using the diphenylcarbazide colorimetric assay. Both strains exhibit approximately 100% reduction of chromium from 100 mg/L concentration to non-toxic form within 48 h using accurate analytical methods. This study demonstrates the isolation of highly chromium-resistant bacteria from tannery waste that can efficiently bioremediate Cr (VI) pollution, thus providing an eco-friendly and cost-effective bioremediation approach.

1. Introduction

Chromium (Cr) is widely used in a variety of industrial processes, including the production of steel, paper, automobiles, electroplating, tanning, and ore processing [1,2]. There are two stable oxidation states of chromium in the natural environment such as Cr (III) and Cr (VI). Cr (III) is considered as an essential micronutrient and is relatively nontoxic [3]. The appropriate levels of Cr (III) are essential for human health and are also required for lipid and glucose metabolism [4]. The Maximum Contaminant Level (MCL) for the total amount of chromium in drinking water, regulated by the USEPA, is 0.1 mg/L. According to World Health Organization (WHO)

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recommendations, the permissible level of chromium in drinking water is 0.05 mg/L [5].

In contrast, Cr (VI) is an oxyanionic species which is extremely hazardous [6]. Cr (VI) has detrimental impacts on the environment and, even in small amounts, can lead to several health problems in humans. As Cr (VI) is toxic and extremely soluble in water, it can pass quickly through cell membranes and ultimately interact with proteins and nucleic acids [7]. Accumulation of Cr (VI) in humans leads to mental retardation, renal dysfunction, carcinogenesis, and other abnormalities [8].

Tanneries of Bangladesh are considered as the most polluting sectors with the greatest environmental impact [9]. Although both chrome and vegetable tanning are widely used around the world, the majority of tanneries in Bangladesh produce leather that has been chrome-tanned, and as a result, they release a significant amount of chromium along with their untreated effluents into the nearby water bodies [10]. Previous research has shown that the Hazaribagh tannery area's soil and water were highly contaminated with Cr [11–15], leading to substantial human health hazards [16,17]. The incidence of several diseases, such as scabies (73.9%), gastrointestinal disorders (71.7%), diarrhea (71.7%), asthma (49.9%), eye problems (46.7%), and high blood pressure (52.2%), had been reported by Bangladeshi tannery workers [18].

To reduce environmental pollution and the disease burden, it is crucial to treat these effluents prior to their discharge into the

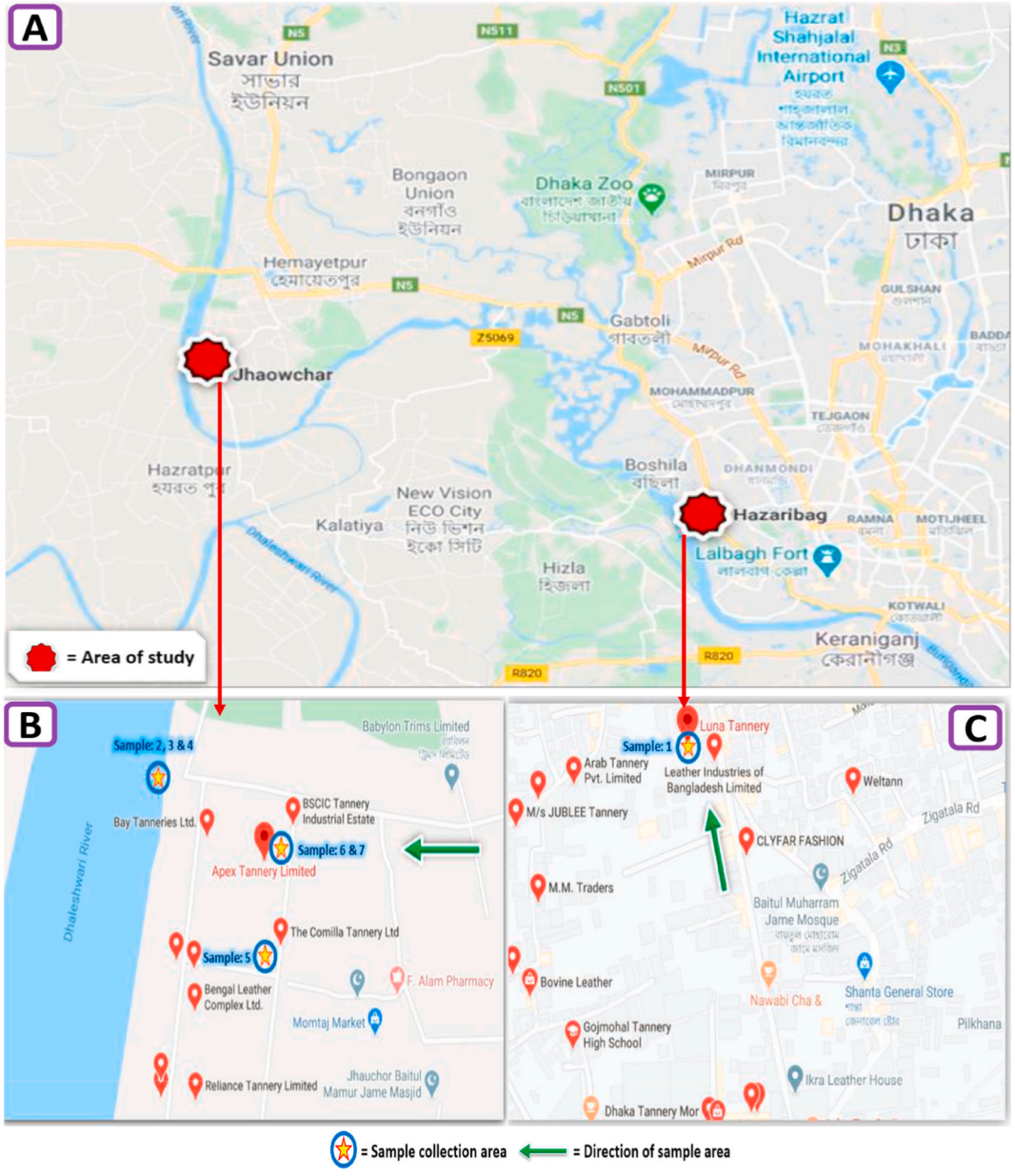

Fig. 1. Location of selected sampling areas in Dhaka, Bangladesh.

environment [19,20]. There are many technologies such as reverse osmosis, redox chemical reactions [21], coagulation, and precipitation [22,23] are available to mitigate Cr-polluted wastewater. These processes are expensive and energy consuming, and they have a chance for secondary chemical contamination [21,24].

Several new methods were conducted for waste management. Such as strategies for managing nutrient-rich organic soil in the context of climate change policy [25], hybrid inulin-TEOS bio-sorbent assay [26], and the impact of using cold plasma on the self-cleaning, antimicrobial, and structural characteristics of α-Fe_2O_3 (HEMATITE) thin films [27] etc. According to a recent study, using potential nano-adsorbents based on core-shell biomagnetic nanoparticles, magnetically assisted chemical separation methods may be used to extract Cr (VI) from aqueous solutions [28]. The main drawback of this separation method was that it could only conduct positive and negative selections, was not quantitative, and needed secondary quantification. Therefore, it is essential to create a novel, economical, and ecologically sound technique for removing harmful heavy metals from wastewater.

A wide variety of microorganisms such as bacteria, yeast, algae, protozoa, and fungi are found in waters receiving industrial effluents. These microbes can defend themselves against the harmful effects of heavy metals through a variety of methods, including adsorption, absorption, methylation, oxidation, and reduction. Numerous Cr-reducing bacteria have been identified from different contaminated areas including *Bacillus* [29,30], *Pseudomonas* [30,31], *Microbacterium* [32], *Desulfovibrio* [33], *Enterobacter* [34], *Escherichia* [35], and so on, indicating essential strategies for the bioremediation of Cr (VI) contamination [36].

The novel contribution of this study identifies highly chromium-reducing bacteria from diverse ecological and geographical locations in Bangladesh, differing from previous publications [37]. This research investigates the minimum inhibitory concentration (MIC) of bacteria against varying chromium concentrations and incubation times. It compares previous studies, using similar methods for isolation, screening, and characterization of bacterial strains. The study also evaluates the chromium reduction abilities of isolated bacteria spectrophotometrically [37]. So the previous studies have found chromium-resistant bacteria from contaminated sites in different countries rather than Bangladesh, suggesting a different geographic location and slightly different bacteria genera in these studies.

However, the use of microorganisms for the bioremediation of heavy metals and other harmful compounds has not yet been initiated in developing countries like Bangladesh, no relevant research has been conducted and no high chromium-reducing bacteria have been observed according to the previous studies. Therefore, the goal of this research was i) isolation and identification of high chromium reducing bacterial strain from tannery effluents, ii) assessment of their reduction capabilities from highly toxic Cr (VI) to less or non-toxic Cr (III).

2. Materials and methods

2.1. Chemicals & reagents

All chemicals and reagents used in this study were American Chemical Society (ACS) analytical grade unless stated otherwise. American Society for Testing and Materials (ASTM) type 1 deionized water was used for all experiments which was supplied from Ultrapure Water Purification Systems (Arium comfort 2, Sartorius, Germany). All the agar media (LB Agar, Nutrient Agar, Kligler's Iron agar, MIU medium base, and Simmon's citrate agar) used in the present study were procured from HiMedia Laboratories, India. 1, 5-diphenylcarbazide, Potassium dichromate ($K_2Cr_2O_7$), Sodium chloride (0.85% NaCl), Sodium hydroxide (2 mol/L NaOH), Sulfuric Acid (0.2 mol/L H_2SO_4), Acetone, and Hydrogen peroxide (H_2O_2) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 1 mol/L HNO_3 and NaOH solutions were prepared using analytical grade nitric acid (HNO_3) and sodium hydroxide (NaOH) and, were used to adjust the pH of the solution [38]. All stock solutions were kept in amber glass bottles in a dark place to prevent degradation.

2.2. Sample collection

The areas where this study was conducted were Hazaribag, Dhaka and Jhaowchar, Hemayetpur, Savar, Dhaka (Fig. 1). A total of

Table 1
List of different samples collected from study areas.

Sample No.	Sample Type	Sample location	Place	Condition of Sample Collection
1	Soil	Tannery Mor, Hazaribag, Dhaka	Luna Tannery	3 cm deep soil
2		Jhaowchar, Hemayetpur, Savar, Dhaka	Dhaleshwari Riverbank	5 cm deep soil
3		Jhaowchar, Hemayetpur, Savar, Dhaka	Apex Tannery Limited	5 cm deep soil
4	Effluent or/and Water	Jhaowchar, Hemayetpur, Savar, Dhaka	Apex Tannery Limited	Direct effluent from tank
5		Jhaowchar, Hemayetpur, Savar, Dhaka	The Comilla Tannery Ltd.	15 cm deep from salt-treated store house
6		Jhaowchar, Hemayetpur, Savar, Dhaka	Central drain Water	Effluent stream
7		Jhaowchar, Hemayetpur, Savar, Dhaka	Dhaleshwari River water	15 cm deep

seven samples (Table 1) were collected from different tannery industries and surrounding areas. The data in Table 1 was used to collect different samples for remediating different contaminated sites. Considering the analysis of samples from both soil and effluent provides a clear picture of the chemicals environmental impact in terms of persistence, mobility, and potential contamination pathways. Finally collected samples were immediately transported to the laboratory and stored in the refrigerator at −4 °C until further use [39].

2.3. Screening of chromium resistant bacteria

For the isolation of chromium resistant bacteria, about 1.0 g soil sample was dispersed in 100 mL deionized water. A serial dilution was made up to 10^{-5} of both effluent and soil samples with sterile physiological saline (0.85%). From different dilutions, 100 μl of the sample was spread on LB agar media and nutrient agar media supplemented with 500 mg/L of $K_2Cr_2O_7$ and incubated for 48–72 h at 37 °C to identify bacterial colonies [40].

2.4. Isolation of pure cultures

Out of several colonies obtained from serial dilution by spread plate techniques, unique colonies were selected based on the colony morphology. The selected colonies were separately cultured by streak plate method and incubated at 37 °C for 48–72 h. The colony shape, color, size, opacity, pigmentation, and texture of each isolate were examined. Then the pure colonies were enriched in fresh LB broth media for 18–24 h of incubation [41]. Finally, stocks were prepared with 40% glycerol and kept at −20 °C for future investigation [42].

2.5. Determination of minimum inhibitory concentration (MIC)

MIC of chromium was assessed by growing the desired isolates on different media (LB agar media and Nutrient agar media) until the isolates failed to grow on agar plates. The concentration of $K_2Cr_2O_7$ used for the determination of MIC ranged from 500 mg/L to 8000 mg/L on LB agar media and 500 mg/L to 800 mg/L on Nutrient agar media. After 48 h of incubation period, the growth of the bacterial isolates was observed [43].

2.6. Stock solution preparation for chromium reduction

Chromium stock solution was prepared by adding various concentrations of $K_2Cr_2O_7$ (50, 100, 200, 300, 500, and 1000 mg/L) on LB media. 0.5% of 1, 5-diphenylcarbazide solution was prepared in acetone and mixed readily with 0.2 mol/L H_2SO_4 for the acid digestion of the solution. The 24-h bacterial cultures were inoculated into the leveled test tubes containing chromium solution (50, 100, 200, 300, 500, 1000 mg/L of chromium). A reagent blank was used as a control to compare chromium reducing activity of bacterial isolates. Sulfuric acid (0.2 mol/L, 1 mL) and 0.5% 1, 5-diphenylcarbazide (1 mL) were also added to specific leveled test tubes. The mixture was left for at least 5 min for full-color development. The bacterial isolates were assessed for reduction if any Cr (VI) remained on the supernatant [44].

2.7. Assessment of Cr (VI) reduction activity

The ability of the bacterial isolates to reduce Cr (VI) into a less toxic form was analyzed using the diphenylcarbazide (DPC) method by estimating the decrease in hexavalent chromium concentration [45]. The 24-h bacterial cultures were inoculated in LB broth medium containing (50, 100, 200, 300, 500, and 1000 mg/L) of Cr (VI) as $K_2Cr_2O_7$ at 30 °C to estimate the chromium reduction activity. The isolates were collected by centrifugation at 13,000 rpm for 10 min. The concentration of Cr (VI) in the supernatant was determined by UV–Visible spectrophotometer to measure the absorbance of Cr (VI)-DPC complex against reagent blank. When chromate and DPC combine, a purple complex is developed that absorbs light at 540 nm. The following formula was used to calculate the percentage of Cr (VI) reduction [46]:

$$\%\text{of Cr}\,(VI) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

The reduction activity for 100 mg/L of $K_2Cr_2O_7$ concentrations was also measured separately for selected isolates.

2.8. Phenotypic, biochemical, and molecular characterization of desired isolates

Different isolates were characterized based on colony morphology, size, shape, opacity, color, pigment secretion, etc. Gram staining was also performed to differentiate gram-negative bacteria from gram-positive. The biochemical tests were performed e.g. Citrate utilization test, nitrate reduction test, oxidase test, catalase test, indole test, urease test, CO_2 production test, H_2S production test, etc. [47].

For molecular identification, genomic DNA was extracted by an automated DNA extractor (Maxwel-16, Promega, USA), and Maxwell blood kit reagent was used. Then 300 μl of 24-h bacterial culture was taken and DNA was extracted. DNA can be quantified by a NanoDrop Spectrophotometer (Model: ND2000, Origin: Thermo Scientific, USA). 16S rRNA gene was amplified by PCR using 16S

rRNA primers such as forward primer 27F (5' GAGAGTTTGA TCCTGGCTCAG-3') and reverse primer U1492R (5'-CTACGGC-TACCTTGTTACGA-3'). For PCR, Astec Thermal Cycler (Model: G2, Origin: Astec, Japan) was used and Hot Stat Master Mix, (Cat: M7432, Origin: Promega, USA) reagent was used. The total reaction volume was 30 μl. Genomic DNA from strains A2, and B1 was taken as templates. The total amplicon size was 1465 bp. PCR was performed by pre-heating at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min followed by 35 cycles, and finally hold at 4 °C for overnight.

For gel electrophoresis, agarose (Cat: V3125, Origin: Promega, USA); 100 bp DNA Ladder (Cat: G2101, Origin: Promega, USA); ethidium bromide solution (Cat: H5041, Origin: Promega, USA); TAE Buffer (Cat: V4251, Origin: Promega, USA) reagents were used. Finally gel documentation was done by alpha imager (Model: mini, Origin: ProteinSimple, USA).

3. Results

3.1. Screening and isolation of chromium resistant bacteria

A total of 20 bacterial strains were isolated from selected tannery industries and it was found that all strains were able to grow in the presence of 500 mg/L $K_2Cr_2O_7$ (Fig. 2). However, six isolates showed chromium reducing ability designated as A1, A2, B1, F1, K1, and P1 in Fig. 2, and resistance was determined against varying concentrations of chromium (500-8000 mg/L). Among these six isolates, A2 and B1 were found to be the most promising strains that were selected for further study based on their highest resistance properties.

3.2. Determination of minimum inhibitory concentration (MIC)

The study assessed bacterial strain's tolerance to chromium Cr (VI) using growth responses under different concentrations (Figs. 3 and 4). Six strains (A1, A2, B1, F1, K1, and P1) were examined up to 8000 mg/L of $K_2Cr_2O_7$ on LB agar media and 800 mg/L on Nutrient agar medium. Among them, A2 and B1 showed the highest Cr (VI) tolerance (7700 mg/L & 7200 mg/L respectively), whereas development of the other strains was significantly inhibited by Cr (VI) at concentrations greater than 6500 mg/L of $K_2Cr_2O_7$ on LB media (Table 2). The resistance level was determined at 37 °C after 48 h in a shaking incubator.

Figs. 3 and 4 indicate the graphical representation of chromium tolerance of isolated bacteria at different concentrations on LB agar media and nutrient agar media. If the concentration increases thus the number of types of bacterial strains decreases and determines MIC. So, the highest tolerance capability was 7700 mg/L and 800 mg/L on LB agar media and nutrient agar media respectively.

Bacterial strain A2 can tolerate chromium concentration of about 7700 mg/L on LB agar media, represented in Fig. 5(A-F).

A2 can tolerate up to 800 mg/L of $K_2Cr_2O_7$on nutrient agar media, represented in Fig. 6(A-D).

Bacterial strain B1 can tolerate up to 7200 mg/L of $K_2Cr_2O_7$ on LB agar media, represented in Fig. 7(A-F).

B1 can tolerate up to 580 mg/L of $K_2Cr_2O_7$ on Nutrient agar media, represented in Fig. 8(A-C).

3.3. Assessment of chromium reduction activity

The chromium stock solution was used to prepare both the control and samples for the assessment of reduction activities. Chromium (VI)-DPC complex, also known as diphenylcarbazide complex, was formed, yielding the solution a light to dark pink color. The

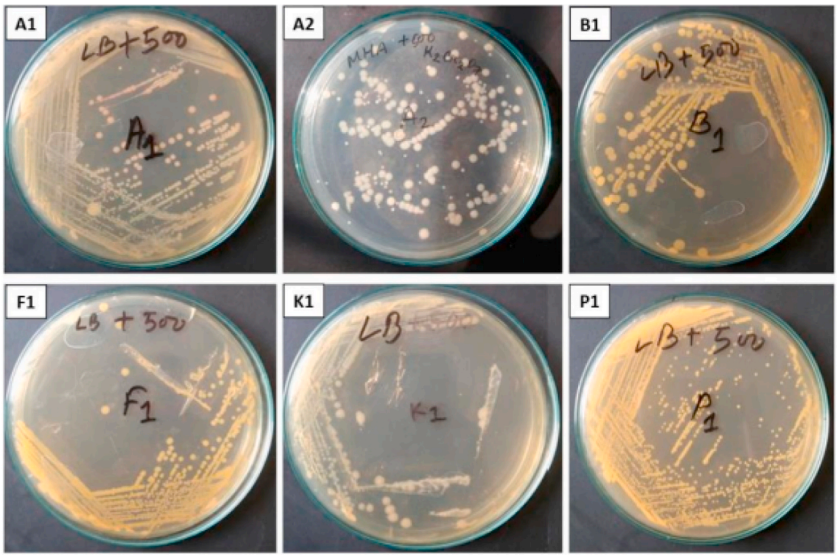

Fig. 2. Pure cultures of selected bacterial isolates.

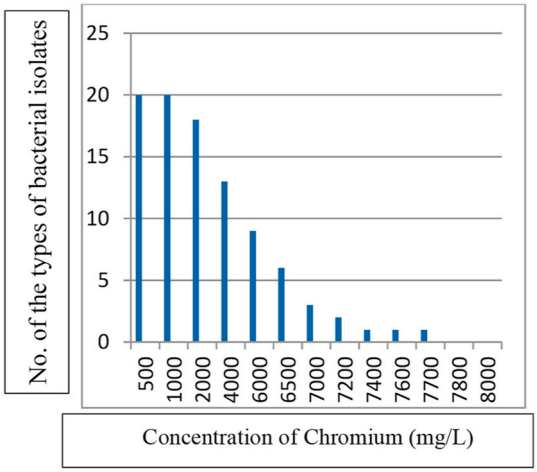

Fig. 3. Cr tolerance of bacteria at different concentration on LB agar media.

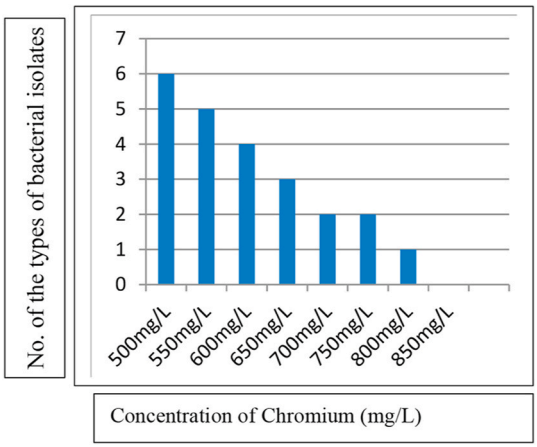

Fig. 4. Cr tolerance of bacteria at different concentration on Nutrient agar media.

Table 2
Minimum Inhibitory Concentration of selected isolates.

Minimum Inhibitory Concentration (mg/L)		
Isolates	**Media**	**Concentration**
A1	LB agar	7100 mg/L
	Nutrient agar	650 mg/L
A2	LB agar	7700 mg/L
	Nutrient agar	800 mg/L
B1	LB agar	7200 mg/L
	Nutrient agar	580 mg/L
F1	LB agar	6800 mg/L
	Nutrient agar	550 mg/L
K1	LB agar	6900 mg/L
	Nutrient agar	700 mg/L
P1	LB agar	6500 mg/L
	Nutrient agar	500 mg/L

reduction of chromium for six bacterial isolates was assessed (A1, A2, B1, F1, K1, and P1) at the concentrations of 50, 100, 200, 300, 500, and 1000 mg/L after 48 h of incubation using LB broth and all bacterial isolates were successfully reduced 100 mg/L of chromium (shown in Table 3).

The reduction activity of Cr (VI) was assessed for the two most promising isolates (A2 and B1) and both were successfully reduced

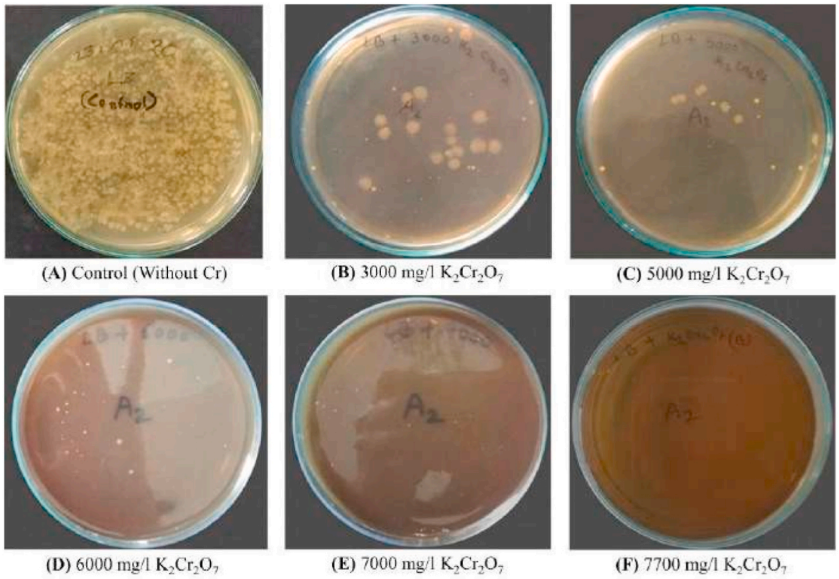

Fig. 5. Assessment of Minimum inhibitory concentration (MIC) of A2 on LB agar media (after 48 h of incubation). (A) Control that has huge bacterial growth; (B–E) bacterial growth decreases gradually, and (F) no bacterial growth. That means that the bacterial growth on the plate (F) was inhibited completely by 7700 mg/L of chromium concentration.

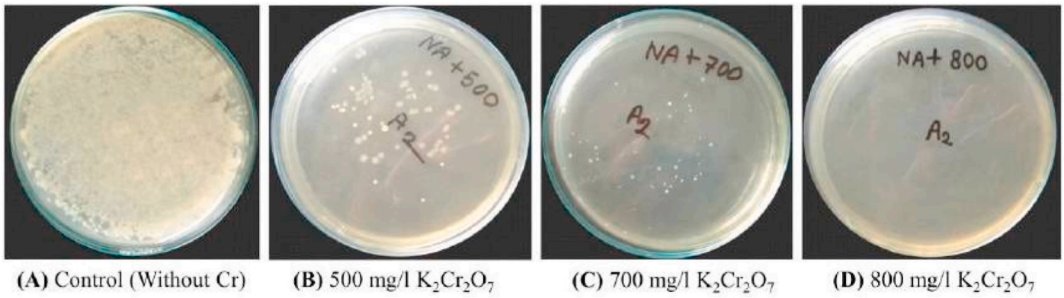

Fig. 6. Assessment of Minimum inhibitory concentration (MIC) of A2 on Nutrient agar media (after 48 h of incubation). (A) Control has huge bacterial growth; (B and C) bacterial growth decreases gradually, and (D) no bacterial growth. That means that the bacterial growth on plate (D) was inhibited completely by 800 mg/L of chromium concentration.

100 mg/L of chromium (Fig. 9 & Fig. 10). Fig. 9 shows the percentages of Cr (VI) reduction by isolate A2 where the concentration of Cr (VI) was ranging from 0 mg/L to 1000 mg/L, at the concentration of 50 mg/L, the optical density (OD) was 0.00259 and the reduction of Cr (VI) was 97.99%, after increasing the conc. at 100 mg/L, 200 mg/L, 300 mg/L, 500 mg/L and 1000 mg/L, the chromium reduction was 97.68%, 84.21%, 66.40%, 44.33%, and 26.30% respectively. On the other hand, Fig. 10 shows the percentage of Cr (VI) reduction by isolate B1, where at the concentration of 50 mg/L, the optical density (OD) was 0.00019 and the reduction of Cr (VI) was 99.81%, after increasing the conc. at 100 mg/L, 200 mg/L, 300 mg/L, 500 mg/L and 1000 mg/L, the chromium reduction was 99.48%, 91.30%, 88.22%, 27.69%, and 11.35% respectively.

3.4. Characterization and identification of desired isolates

Six potential bacterial isolates were characterized, and promising two were further evaluated. Gram staining confirmed that only one (K1) showed negative results, while all five (A1, A2, B1, F1, P1) showed positive results. The KIA test showed A2 and K1 were gas-producing isolates, while P1 was motility-positive. A2 and P1 were indole-positive, and all isolates were urease-negative. Strain A1 and A2 were citrate test positive. Based on this biochemical test, two isolates (A2 and B1) were presumed to be *Bacillus* sp. and *Micrococcus* sp.

3.4.1. Agarose gel analysis for genomic DNA

PCR product of isolated DNA of two chromium resistant bacteria (obtained from contaminated tannery waste), such as *Bacillus* sp. and *Micrococcus* sp., and a 100bp DNA ladder have been used for agarose gel analysis. It was observed that the DNA (amplicon) of these

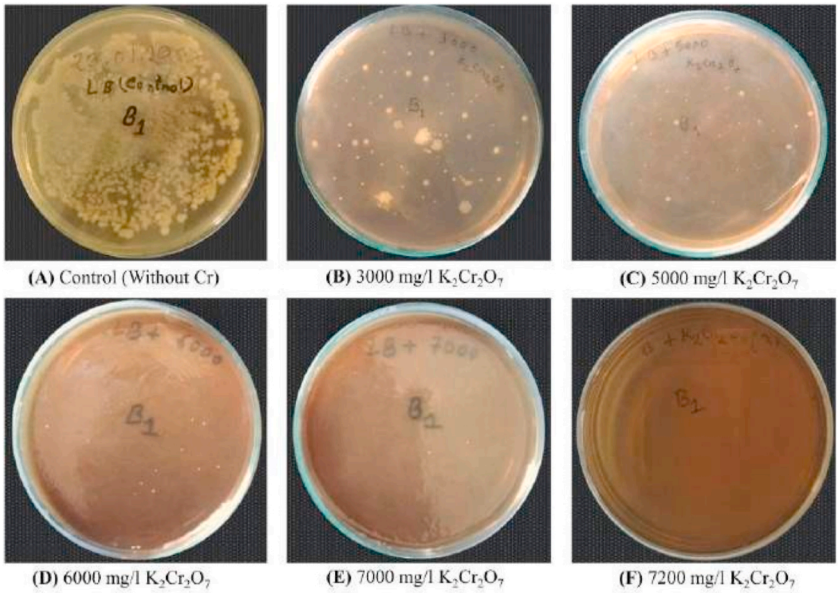

Fig. 7. Assessment of Minimum inhibitory concentration (MIC) of B1 on LB agar media (after 48 h of incubation). (A) Control that has huge bacterial growth; (B–E) bacterial growth decreases gradually, and (F) no bacterial growth. That means that the bacterial growth on the plate (F) was inhibited completely by 7200 mg/L of chromium concentration.

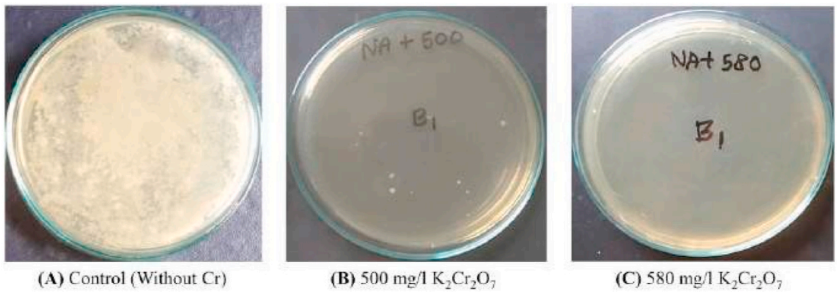

Fig. 8. Assessment of Minimum inhibitory concentration (MIC) of B1 on Nutrient agar media (after 48 h of incubation). (A) Control has huge bacterial growth; (B) little bacterial growth and, (C) no bacterial growth. That means that the bacterial growth on the plate (C) was inhibited completely by 580 mg/L of chromium concentration.

Table 3
Chromium reduction capabilities of six selected bacterial isolates using LB broth.

Isolates	Media	Concentration of Cr (VI)	% of Cr (VI) Reduction	Time
A1	Luria broth	100 mg/L	~100%	48 h
A2	Luria broth	100 mg/L	~100%	48 h
B1	Luria broth	100 mg/L	~100%	48 h
F1	Luria broth	100 mg/L	~100%	48 h
K1	Luria broth	100 mg/L	~100%	48 h
P1	Luria broth	100 mg/L	~100%	48 h

2 isolates falls within the size range of approximately 1500bp when compared with the 100bp DNA ladder (marker DNA) (Fig. 11).

Table 4 summarized the morphological and biochemical characteristics of A2 and B1 bacterial strains and their presumptive identification was *Bacillus* sp. and *Micrococcus* sp. They can tolerate chromium up to 7700 mg/L (for A2) and 7200 mg/L (for B1) on LB agar media. They can also grow on Nutrient agar media. Both strains showed ~100% reduction capability for 100 mg/L of chromium.

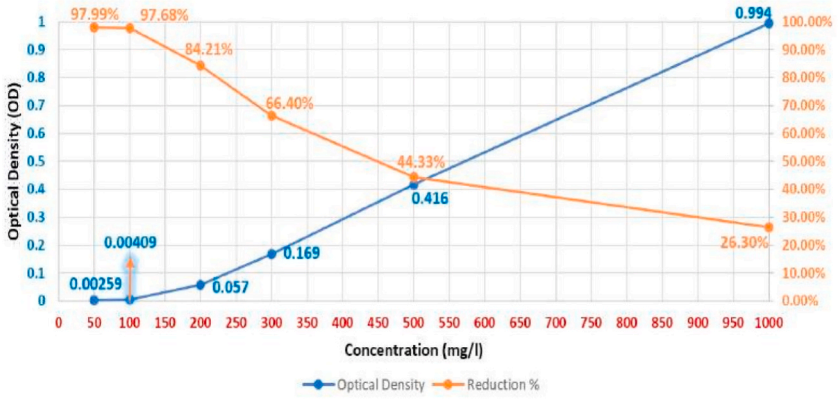

Fig. 9. Percentages of Cr (VI) reduction by isolate A2. **X-axis:** represents the concentration of Cr (VI) in mg/L. **Y-axis**: represents the optical density. **Blue Line:** shows that as the concentration of Cr (VI) increases, the optical density also increases. **Orange Line:** represents that as the concentration of Cr (VI) increases, the percentage of Cr (VI) reduction decreases. This suggests that isolate A2 was effective in reducing approximately 100% Cr (VI) at 100 mg/L concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

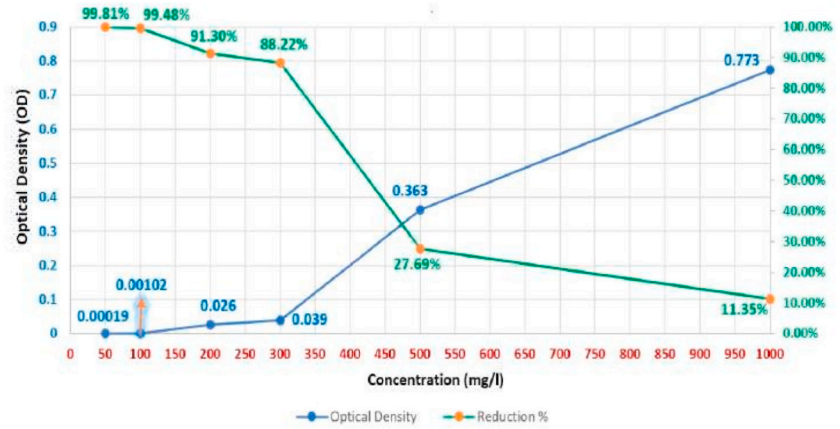

Fig. 10. Percentages of Cr (VI) reduction by isolate B1. **X-axis**: shows the concentration of Cr (VI) in mg/L. **Y-axis**: represents the optical density. **Blue Line:** represents that as the concentration of Cr (VI) increases, the optical density also increases. **Green Line:** shows that as the concentration of Cr (VI) increases, the percentage of Cr (VI) reduction decreases. This suggests that isolate B1 was effective in reducing approximately 100% Cr (VI) at 100 mg/L concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Chromium and other toxic heavy metal pollution is a major issue in developing countries like Bangladesh, Pakistan, and India, posing threats to the natural fauna and flora, cropland, water sources, and food chains [48–52]. Several physical or chemical processes, such as adsorption, electrodialysis, ion exchange, precipitation, reduction, reverse osmosis, etc. are used to remove chromium from industrial effluent. Physical or chemical methods for heavy metal removal are expensive, have drawbacks like insufficient removal, excessive reagent consumption, high energy requirements, and toxic sludge production, and are only viable at moderate to high metal concentrations [53]. In contrast to all other attempts to address chromium contamination in the environment, bioremediation is the most eco-friendly, cost-effective, and safe method to perform [54].

The current study conducted with the primary screening of all bacterial isolates from different soil and effluent samples through culture on different bacterial growth media containing 500 mg/L of $K_2Cr_2O_7$ revealed that these isolates can tolerate a high level of chromium. Based on these tolerance capabilities, 20 different strains were isolated. High levels of resistance have been reported in bacteria isolated from Cr (VI) contaminated sites [55]. Six different isolates were sorted out from all other isolates by measuring MIC and they were leveled as A1, A2, B1, F1, K1, and P1. Different concentrations of Cr (VI) from 500 mg/L to 8000 mg/L were used to determine the MIC of all isolates. For further study A2 and B1 were selected for the assessment of chromium reduction activity by culturing them on LB agar and Nutrient agar media. For strain A2, the MIC was 7700 mg/L on LB agar, and 800 mg/L on Nutrient agar media. On the other hand, for strain B1, the MIC was 7200 mg/L, and 580 mg/L on LB agar and Nutrient agar media respectively.

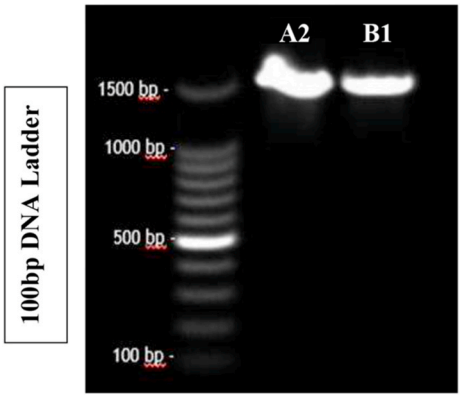

Fig. 11. Agarose gel analysis of DNA (PCR product) of two chromium resistant bacteria. Lane 1: A2, Lane 2: B1, and comparison of different base pairs (bp) with marker DNA (100 bp DNA ladder).

Table 4
Overview of the study: Identification of the two most promising isolates (A2 & B1).

Bacterial isolates	A2	B1
Morphological characteristics		
Colony color	Grey	Golden yellow
Colony size	Large	Medium
Colony shape	Rough, circular	Smooth, circular
Opacity	Transparent	Light transparent
Capsule	Capsulated	No
Pigment secretion	No	No
Biochemical characteristics		
Gram nature	Positive	Positive
Gas production	CO_2	No
H_2S production	No	No
Motility	Non-motile	Non-motile
Indole	Positive	Negative
Urease	Negative	Negative
Citrate	Positive	Negative
Catalase	Positive	Positive
Chromium tolerate capability		
Luria-Bertani agar	7700mg/L	7200mg/L
Nutrient agar	800mg/L	580mg/L
Chromium reducing capability		
Concentration: 50 mg/L	97.99%	99.81%
Concentration: 100 mg/L	97.68%	99.48%
Concentration: 200 mg/L	84.21%	91.30%
Concentration: 300 mg/L	66.40%	88.22%
Concentration: 500 mg/L	44.33%	27.69%
Concentration: 1000mg/L	26.30%	11.35%
Identification of isolates		
Presumptive identification	*Bacillus* sp.	*Micrococcus* sp.

Several biochemical tests, including gram staining, catalase, citrate, KIA, and MIU were used to identify desired isolates A2 and B1. Based on morphological and biochemical characteristics, these isolates are assumed to be *Bacillus* sp. and *Micrococcus* sp. but phenotypic characterization may not confirm species identification, requiring 16S rRNA gene sequencing for stronger evidence.

The resistance properties of *Bacillus* sp. and *Micrococcus* sp. on different media vary depending on media compositions and the collected sources. Many different *Bacillus* sp. collected from different sources can tolerate $K_2Cr_2O_7$ between 104 mg/L and 8834 mg/L on different media [56]. *Ochrobactrum intermedium* and *Brevibacterium* spp. could tolerate up to 40 mg/mL K_2CrO_4 on nutrient agar, 25 mg/mL in nutrient broth, and up to 10 mg/mL in acetate-minimal media [57]. In our study, *Bacillus* sp. (A2) was cultured on both LB agar, and nutrient agar media, and their tolerance capability was 7700 mg/L, and 800 mg/L respectively.

On the other hand, different *Micrococcus* sp. collected from different sources can tolerate up to 7067 mg/L of $K_2Cr_2O_7$ on LB agar media [57]. Our isolated strain *Micrococcus* sp. (B1) was cultured on LB agar, and nutrient agar media, and their tolerance capability was 7200 mg/L, and 580 mg/L respectively. This means the resistance properties of different bacteria are dynamic, and not stable depending on used media and collected sources [58]. Our present research confirms the validity of bacterial Cr (VI) tolerance capability, with *Bacillus* sp. and *Micrococcus* sp. able to tolerate up to 7700 mg/L and 7200 mg/L of Cr (VI) respectively on LB agar media.

Bacteria are highly resistant which doesn't mean they can reduce the high or the same amount but it might be the most important criteria to judge reduction capabilities [58]. In our present study, the reduction activity of *Bacillus* sp. (A2) and *Micrococcus* sp. (B1) was measured by using a UV–Visible Spectrophotometer using LB broth medium. The measurement was taken for both two isolates after 48 h of incubation at concentrations of 50, 100, 200, 300, 500, and 1000 mg/L of Cr (VI) respectively. *Micrococcus* sp. (B1) has the highest chromium reduction capability (99.48%) at 100 mg/L chromium concentrations than *Bacillus* sp. (A2) (97.68%). All six selected isolates (A1, A2, B1, F1, K1, and P1) showed approximately 100% reduction capability at 100 mg/L of Cr (VI) concentration. According to Masood and Malik [59], *Bacillus* sp. can reduce 50 mg/L and 100 mg/L of chromium 100% within 12 h and 48 h respectively. We have observed the reduction capabilities for both 50 mg/L and 100 mg/L at 48 h and found almost 100% reductions from both of these observations, a result consistent with *Micrococcus* sp. as well.

This research was so challenging due to the highly toxic nature of chromium, its screening capabilities, and the accuracy of quantitative chromium reduction assessment by isolated strains using analytical methods. So, the original achievement was the identification of *Bacillus* sp. (A2) and *Micrococcus* sp. (B1) with high chromium tolerance, demonstrating nearly 100% reduction of chromium at 100 mg/L concentration to non-toxic form using accurate optimized analytical methods. Finally, this study may provide some valuable information about the hazardous consequences of Cr (VI) and may be useful to achieve an environment that is free of Cr (VI) toxicity.

5. Conclusion

Urban areas frequently suffer from land pollution due to industrial and municipal wastes, which pose a threat to humans, aquatic life, plants, and other organisms. Tannery industries, which produce leather from hides and skin, are responsible for most of this pollution. This study, therefore, aims to identify the bacteria that can reduce chromium (VI) to (III) using a spread plate technique on Luria Bertani agar medium and Nutrient agar media amended with $K_2Cr_2O_7$. Using these media Bacillus sp. named as A2 and *Micrococcus* sp. named as B1 were identified from tannery effluent in the Hazaribag and Hemayetpur industrial area of Dhaka, Bangladesh. These strains can effectively reduce hexavalent chromium under a wide range of environmental parameters. The isolated bacterial strains showed excellent activity towards the reduction of chromium. So, the bacterial strains *Bacillus* sp. and *Micrococcus* sp. can be applied in the bioremediation of chromium-containing areas, as it found to have the potential to reduce the toxic hexavalent chromium to its less toxic trivalent form that could safely be released into the environment.

Data availability statement

All data included within the study will be available for everyone as per journal policy.

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CRediT authorship contribution statement

Roksana Khanam: Writing – review & editing, Supervision, Conceptualization. **Sheikh Abdullah Al Ashik:** Writing – original draft, Investigation, Formal analysis, Data curation. **Umme Suriea:** Writing – original draft, Validation, Investigation, Formal analysis. **Shahin Mahmud:** Writing – review & editing, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

All the authors declared no conflict of interest.

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Appendix A. Supplementary data

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References

[1] E. Leghouchi, E. Laib, M. Guerbet, Evaluation of chromium contamination in water, sediment and vegetation caused by the tannery of Jijel (Algeria): a case study, Environ. Monit. Assess. 153 (2009) 111–117.
[2] G. Yang, J. Xia, Comment on "Chromium contamination accident in China: viewing environment policy of China.", Environ. Sci. Technol. 45 (2011) 8605–8606.
[3] J.M. Besser, W.G. Brumbaugh, N.E. Kemble, W. Thomas, C.G. Ingersoll, Effects of sediment characteristics on the toxicity of chromium (III) and chromium (VI) to the amphipod, Hyalella azteca. Environ. Sci. Technol. 38 (2004) 6210–6216.
[4] Agency for toxic substances and disease registry, Toxicological Profile for Chromium, U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, 2012.
[5] M. Owlad, M.K. Aroua, W.A.W. Daud, S. Baroutian, Removal of hexavalent chromium-contaminated water and wastewater: a review, Water Air Soil Pollut. 200 (2009) 59–77.
[6] B. Wielinga, M.M. Mizuba, C.M. Hansel, S. Fendorf, Iron promoted reduction of chromate by dissimilatory iron-reducing bacteria, Environ. Sci. Technol. 35 (2001) 522–527.
[7] B. Markiewicz, I. Komorowicz, A. Sajnóg, M. Belter, D. Barałkiewicz, Chromium and its speciation in water samples by HPLC/ICP-MS–technique establishing metrological traceability: a review since 2000, Talanta 132 (2015) 814–828.
[8] B.P. Rosen, Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes, Comp. Biochem. Physiol. Part. Mol. Integr. Physiol. 133 (2002) 689–693.
[9] A. Mamun, S. Howlader, H.B. Yeahyea, M.R. Uddin, Leather Industry of Bangladesh: a New Hope for Export Diversification Editorial Overview, Emerging Credit Rating Limited, Dhaka, 2016.
[10] M. Nur-E-Alam, M. Abu Sayid Mia, F. Ahmad, M.M. Rahman, Adsorption of chromium (Cr) from tannery wastewater using low-cost spent tea leaves adsorbent, Appl. Water Sci. 8 (5) (2018) 129.
[11] M.A.H. Bhuiyan, N.I. Suruvi, S.B. Dampare, M.A. Islam, S.B. Quraishi, S. Ganyaglo, S. Suzuki, Investigation of the possible sources of heavy metal contamination in lagoon and canal water in the tannery industrial area in Dhaka, Bangladesh, Environ. Monit. Assess. 175 (1–4) (2011) 633–649.
[12] M.R. Karim, S. Manshoven, M.R. Islam, J.A. Gascon, M. Ibarra, L. Diels, M.M. Rahman, Assessment of an urban contaminated site from tannery industries in Dhaka city, Bangladesh, J. Hazard Toxic Radioact. Waste 17 (1) (2013) 52–61.
[13] K.M. Shams, G. Tichy, M. Sager, T. Peer, A. Bashar, M. Jozic, Soil contamination from tannery wastes with emphasis on the fate and distribution of tri- and hexavalent chromium, Water Air Soil Pollut. 199 (1–4) (2009) 123–137.
[14] J. Uddin, A. Abdul, Heavy metal contamination in water, soil and vegetables of the industrial areas in Dhaka, Bangladesh, Environ. Monit. Assess. 166 (2010) 347–357.
[15] A. Zahid, K.D. Balke, M.Q. Hassan, M. Flegr, Evaluation of aquifer environment under Hazaribagh leather processing zone of Dhaka city, Environ. Geol. 50 (4) (2006) 495–504.
[16] H.M. Anwar, S. Safullah, T. Yoshioka, Environmental exposure assessment of Chromium and other tannery pollutants at Hazaribagh area, Dhaka, Bangladesh, and health risk, J. Environ. Chem. 10 (3) (2000) 549–556.
[17] M.M. Hasan, S. Hosain, P. Poddar, A.A. Chowdhury, E.W. Katengeza, U.K. Roy, Heavy metal toxicity from the leather industry in Bangladesh: a case study of human exposure in Dhaka industrial area, Environ. Monit. Assess. 191 (2019) 530.
[18] M. Hasan, S. Hosain, A.M. Asaduzzaman, M.A. Haque, U.K. Roy, Prevalence of health diseases among Bangladeshi tannery workers and associated risk factors with workplace investigation, J. Pollut. Eff. Control 4 (2016) 1–3.
[19] K. Grace Pavithra, V. Jaikumar, P.S. Kumar, P. Sundar Rajan, A review on cleaner strategies for chromium industrial wastewater: present research and future perspective, J. Clean. Prod. 228 (2019) 580–593.
[20] G. Lofrano, S. Meriç, G.E. Zengin, D. Orhon, Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: a review, Sci. Total Environ. 461 (2013) 265–281.
[21] D.V. Franco, L.M. Da Silva, W.F. Jardim, Reduction of hexavalent chromium in soil and ground water using zero-valent iron under batch and semi-batch conditions, Water. Air Soil Pollut 197 (2009) 49–60.
[22] S.S. Ahluwalia, D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, Bioresour. Technol. 98 (2007) 2243–2257.
[23] W. Xu, G. Duan, Y. Liu, G. Zeng, X. Li, J. Liang, W. Zhang, Simultaneous removal of hexavalent chromium and o-dichlorobenzene by isolated *Serratia marcescens* ZD-9, Biodegradation 29 (2018) 605–616.
[24] Z. Liu, Y. Wu, C. Lei, P. Liu, M. Gao, Chromate reduction by a chromate-resistant bacterium, *Microbacterium* sp, World J. Microbiol. Biotechnol. 28 (2012) 1585–1592.
[25] I. Līcīte, D. Popluga, P. Rivža, A. Lazdiņš, R. Meļņiks, Nutrient-rich organic soil management patterns in light of climate change policy, Civil Engineering Journal 8 (10) (2022) 2290–2304.
[26] W.N. Wan Ismail, M.I.A.I. Syah, N.H.A. Muhet, N.H.A. Bakar, H.M. Yusop, N.A. Samah, Adsorption behavior of heavy metal ions by hybrid inulin-TEOS for water treatment, Civil Engineering Journal 8 (9) (2022) 1787–1796.
[27] A.A. Khadayeir, A.H. Wannas, F.H. Yousif, Effect of applying cold plasma on structural, antibacterial and self cleaning properties of α-Fe_2O_3 (HEMATITE) thin film, Emerging Science Journal 6 (1) (2022) 75–85.
[28] A.F.C. Campos, H.A.L. de Oliveira, F.N. da Silva, F.G. da Silva, P. Coppola, R. Aquino, A. Mezzi, J. Depeyrot, Core-shell bimagnetic nanoadsorbents for hexavalent chromium removal from aqueous solutions, J. Hazard Mater. 362 (2019) 82–91.
[29] M.N. Kathiravan, R. Karthick, K. Muthukumar, Ex situ bioremediation of Cr (VI) contaminated soil by *Bacillus* sp.: batch and continuous studies, Chem. Eng. J. 169 (2011) 107–115.
[30] X. Pan, Z. Liu, Z. Chen, Y. Cheng, D. Pan, J. Shao, Z. Lin, X. X. Guan, Investigation of Cr (VI) reduction and Cr (III) immobilization mechanism by planktonic cells and biofilms of *Bacillus subtilis* ATCC-6633, Water Res. 55 (2014) 21–29.
[31] M. Faisal, Detoxification of carcinogenic Cr (VI) by combined action of *Bacillus pumilus*-S4 and *Pseudomonas doudoroffii*-S5 in associated with hydrophytes, J. Pure Appl. Microbiol. 8 (2014) 4289–4295.

[32] F. Xu, T. Ma, L. Shi, J. Zhang, Bioreduction of Cr (VI) by *Bacillus* sp. QH-1 isolated from soil under chromium-containing slag heap in high altitude area, Ann. Microbiol. 64 (2014) 1073–1080.
[33] Z. Liu, Y. Wu, C. Lei, P. Liu, M. Gao, Chromate reduction by a chromate-resistant bacterium, *Microbacterium* sp, World J. Microb. Biotechnol. 28 (2012) 1585–1592.
[34] C. Michel, M. Brugna, C. Aubert, A. Bernadac, M. Bruschi, Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria, Appl. Microbiol. Biotechnol. 55 (2001) 95–100.
[35] J. Panda, P. Sarkar, Biosorption of Cr (VI) by calcium alginate-encapsulated *Enterobacter aerogenes* T2, in a semibatch plug flow process, Water Air Soil Pollut. 226 (2015) e2157.
[36] Y.H. Lin, Modeling chromium (VI) reduction by *Escherichia coli* 33456 using ceramic pearl as a supporting medium, Int. J. Environ. Sci. Technol. 11 (2014) 1887–1896.
[37] M.S. Sanjay, D. Sudarsanam, G.A. Raj, K. Baskar, Isolation and identification of chromium reducing bacteria from tannery effluent, J. King Saud Univ. Sci. 32 (2020) 265–271.
[38] F. Sharifan, A. Shahidi, S.A. Khashei, Assessing the efficiency of continuous flow column of pine fruit powder in removal of chromium (VI) from aqueous solution, Journal of Environ. Water Eng 3 (2) (2017) 181–189.
[39] M.R. Carter, E.G. Gregorich, Soil Sampling and Methods of Analysis, CRC press, 2007.
[40] A. Smrithi, K. Usha, Isolation and characterization of chromium removing bacteria from tannery effluent disposal site, Int. J. Adv. Biotechnol. Res. 3 (3) (2012) 644–652.
[41] M.I. Hossain, M.N. Anwar, Isolation and identification of heavy metal tolerant Bacteria from tannery effluents, Bangladesh J. Microbiol. 29 (2012) 23–26.
[42] R. Khanam, R. Moni, M.Z. Islam, M.M. Billah, U.S. Zohora, F. Sabrin, M.S. Rahman, Study of an arsenic metabolizing bacteria from arsenic contaminated soil of Chandpur district, Bangladesh, Jahangirnagar University J. Biol. Sci. 8 (1) (2019) 57–65.
[43] Kalsooma, A. Batoola, G. Dina, S.U. Dina, J. Jamila, F. Hasana, S. Khana, M. Badshaha, A.A. Shaha, Isolation and screening of chromium resistant bacteria from industrial waste for bioremediation purposes, Braz. J. Biol. 83 (2023) 1–8.
[44] A. Lace, D. Ryan, M. Bowkett, J. Cleary, Chromium monitoring in water by colorimetry using optimized 1,5-diphenylcarbazide method, Int J Environ Res Public Health 16 (2019) 1803.
[45] A. Zahoor, A. Rehman, Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater, J. Environ. Sci. 21 (2009) 814–820.
[46] N. Upadhyay, K. Vishwakarma, J. Singh, et al., Tolerance and reduction of Chromium (VI) by *Bacillus* sp. MNU16 isolated from contaminated coal mining soil, Front. Plant Sci. 8 (2017) 778.
[47] P. Vos, G. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, et al., Bergey's Manual of Systematic Bacteriology, in: The Firmicutes, ume 3, Springer Science & Business Media, 2011.
[48] R. Robin, P.R. Muduli, K.V. Vardhan, D. Ganguly, K.R. Abhilash, T. Balasubramanian, Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the Southwest coast of India, J. Am. Chem. Soc. 2 (2012) 191–208.
[49] D.F. Ackerley, C.F. Gonzalez, M. Keyhan, R. Blake, A. Matin, Mechanism of chromate reduction by the E. coli. Protein, Nfs A, and the role of different chromate reductases in minimizing oxidative stress dur*ing chr*omate reduction, Environ. Microbiol. 6 (2004) 851–860.
[50] D.R. Mount, J.R. Hockett, Use of toxicity identification evaluation methods to characterize identify, and confirm hexavalent chromium toxicity in an industrial effluent, Water Res. 34 (2000) 1379–1385.
[51] Z. Feng, W. Hu, W.N. Rom, M. Costa, M.S. Tang, Chromium (VI) exposure enhances polycyclic aromatic hydrocarbon-DNA binding at the p53 gene in human lung cells, Carcinogenesis 24 (2003) 771–778.
[52] R. Turpeinen, T. Kairesalo, M.M. Haggblom, Microbial community structure and activity in arsenic, chromium and copper contaminated soils, FEMS Microbiol. Ecol. 47 (2004) 39–50.
[53] E.S. Cossich, C.R.G. Tavares, T.M.K. Ravagnani, Biosorption of chromium (III) by *Sargassum* sp biomass, Electron. J. Biotechnol. 5 (2002) 133–140.
[54] S. Yadav, O.P. Shukla, U.N. Rai, Chromium pollution and bioremediation, Environews Newsletter of ISEB India 11 (1) (2005).
[55] H.C. Kasan, A.A.W. Baecker, An assessment of toxic metal biosorption by activated sludge from the treatment of coal-gasification effluent of a petrochemical plant *Water Sci*, Technol. 21 (1989) 297–303.
[56] S. Mahmood, A. Ansar, J. Durham, Heavy metals in poultry products in Bangladesh: a possible death threat to future generations, Journal of Social and Political Sciences 2 (1) (2019) 98–105.
[57] M. Faisal, S. Hasnain, Comparative study of Cr (VI) uptake and reduction in industrial effluent by *Ochrobactrum intermedium* and *Brevibacterium* sp, Biotechnol. Lett. 26 (2004) 1623–1628.
[58] M. Narayani, K.V. Shetty, Chromium-Resistant Bacteria and their environmental condition for hexavalent chromium removal: a Review, Crit. Rev. Environ. Sci. Technol. 43 (9) (2013) 955–1009.
[59] F. Masood, A. Malik, Hexavalent chromium reduction by *Bacillus* sp. strain FM1 isolated from heavy-metal contaminated soil, The B. Environ. Contam. Tox. 86 (2011) 114–119.