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Screening of synergistic and antimicrobial effect of Cr (VI) and Ni (II) tolerant bacteria *Bacillus cereus*

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ABSTRACT

The East Kolkata Wetland is a combination of natural and human made area combining ponds, and agricultural lands, garbage disposal area containing pollutants such as heavy metal, oil, grease, and solid wastes. Nowadays, wetland assessment often lack of an in-depth analysis of soil parameters and factors affecting the agricultural development. Primarily this work considers the analysis of soil samples for key parameters such as temperature, water holding capacity, pH, measurement of electrical conductivity, determination of Organic Carbon, Nitrogen and Phosphorus, and presence of heavy metals chromium (VI) and nickel (II), whereas the main focus of this work is the reduction activity of the disposed of heavy metals (Cr and Ni) with bacterial source and to check the synergistic effect of bacterial resistance against both the heavy metals. Bacterial resistant capacity was checked against different concentrations of metals using agar plate and reduction was observed using different electron donor source. Further, the identified strains were also checked against different microbes for their antimicrobial activity. The isolates BRS 11 and BRS 17 showed a good reduction and accumulation capacity against chromium and nickel in the presence of electron donors. Furthermore, the statistical analysis showed positive significance between each other confirming the synergistic effects.

1. INTRODUCTION

Rapid development and release of untreated wastewater from various metal, electroplating, leather and fertilizer industries, leads to toxicity risk to human survival as well as disrupts ecological balance [1]. In general, chromate-reducing bacteria mostly isolated from chromium-contaminated soil and industrial wastes. Chromium is more toxic in nature and various standard methods have been used to reduce and remove Cr (VI) from the environment. One of the most recent is reduction of Cr (VI) to Cr (III) under aerobic and anaerobic or both conditions by bacteria with less cost and more convenience as trivalent chromium is less toxic than hexavalent chromium and forms insoluble oxides and hydroxides [2,3]. Nowadays, a diversity of bacteria having Cr (VI) reducing properties have been isolated from the environment and some proteins having chromate reducing properties were also purified and characterized [4,5] till now. Recent research is focused on some soluble enzymes such as cytoplasmic dimeric flavoproteins have been identified as chromate reductases (ChrR), which can fully reduce Cr (VI) – Cr (III) [6,7]. A new approach of chromium reduction can be *in situ* microbial bioreduction of Cr (VI) to Cr (III) because it may serve as a potential strategy for the detoxification and immobilization of chromate compared with more cost-prohibitive physical and chemical treatment methods [8-10]. Nickel also is the 24th most abundant element and high concentration of this can lead to oxidative stress in cells. Although biosorption, accumulation, precipitation, reduction, and chromate efflux are some of the known mechanisms involved in bioremediation of heavy metals, the best known example of nickel resistance is mediated by efflux pumps such as cnr CBA (cobalt-nickel resistance) from *Cupriavidus metallidurans* CH34 (formerly *Ralstonia metallidurans* CH34), NccCBA (Nickel-cobalt-cadmium), NreB (nickel resistance) from *Achromobacter xylosoxidans* 31A, and CznABC (Cadmium-zinc-nickel) from *Helicobacter pylori* [11,12]. In future microbes identified in this work can be used for the reduction of chromium and nickel and will be valuable for different bioremediation processes. In this work also the bacterial isolates which were resistant to both chromium and nickel independently was checked for the synergistic effect where it showed resistance to the metals together. This also can be due to some metal resistant genes or proteins [13,14].

2. MATERIALS AND METHODS

2.1. Sample Collection

Soil samples were collected from the East Kolkata Wetland area for three seasons, summer, monsoon, and winter from Brahmi root, chilparajhil area, mathpukurkhal area, metropolitan khal area, Five

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number jhil area, Seven number jhil area, Natarbheri area, Chingrighata area, Two number jhil area, and Nunebheri area [15,16].

2.2. Soil Sample Preparation

The soil samples were air dried first and then grinding was followed. A mortar or pestle was used for grinding to avoid contamination in the soil sample. After grinding soil samples were sieved using mesh sieve and stored in dry and clean screw cap jars with proper labeling [16,17].

2.3. Soil Parameter Tests

The soil parameter tests include determination of temperature [18], water holding capacity, pH, measurement of electrical conductivity, determination of organic carbon, nitrogen, phosphorous, and presence of heavy metals chromium and nickel [19].

2.4. Isolation of Bacteria from Root Soil

Soil sample collected from Brahmi root was used for the isolation process which falls under Metropolitan area. Samples were diluted in normal saline and inoculated (0.1 ml) on Luria-Bertani (LB) agar plate containing 500–2000 mg/l of potassium dichromate ($K_2Cr_2O_7$) concentration solutions as Cr(VI) by spread plate method. Plates were incubated at 37°C for 4 days. After 4 days single colonies were selected and preserved in nutrient broth for further studies. Gram staining was performed to check the morphological characteristics of the isolates [20]. For the screening of nickel(II) resistant bacteria, the same chromium-resistant single bacterial colonies were plated on nutrient agar medium with nickel chloride as Ni(II) concentration ranging from 200 to 800 g/L. Again Gram staining was performed to check the morphological characteristics [21].

2.5. Biochemical Characterization of Isolated Bacteria

After obtaining pure culture, biochemical tests were performed for the preliminary characterization purposes. These tests were used to identify the isolate according to the Bergey's manual of systematic bacteriology [21].

2.6. DNA, Protein Extraction, and 16S rRNA Sequencing

The isolated colonies were subjected to Agarose gel electrophoresis and SDS-Page electrophoresis for the extraction of DNA and protein from the samples. For the identification of positive isolates, 16SrRNA sequencing was performed [22].

2.7. Chromium (Vi) Assay

Hexavalent chromium was determined with a spectrophotometer using the S-diphenylcarbazide (DPC) method. The DPC reagent was prepared by adding 24 ml of 85% H_3PO_4 to 56 ml distilled water. This solution was mixed with 0.076 g DPC previously dissolved in 20 ml of 95% ethanol. The reagent was stored in dark at 4°C. Cr(VI) in the sample was assayed by adding 125 µl of the DPC reagent to 1 ml of chromium samples, mixed gently and kept at room temperature for 20 min. The absorbance of the color produced was measured at 540 nm using a spectrophotometer. Cr(VI) concentration in the sample was calculated from a standard curve using $K_2Cr_2O_7$ as standard [23].

2.8. Nickel (ii) Assay

The sample broth was transferred to a clean test tube. 10 ml of citrate ammonia solution, 5 ml of iodine solution, and 20 ml of dimethylglyoxime solution were added to nickel ions solutions. The sample was mixed thoroughly with the prepared solutions. Sample mixed with the chemical solution was transferred to the cuvette and measured absorption at the wavelength of 530 nm. The concentrations of Ni ions were observed and calculated from a standard curve [11].

2.9. Reduction of Chromium and Nickel

Cells from overnight grown culture were harvested by centrifugation at 10,000 RPM for 10 min, washed and suspended in sterile phosphate buffer (0.2 M; pH 7.0). Reduction was carried out in sterile medium (20 ml/100 ml flask) containing 20 mg/l Cr(VI) and Ni (II). The flasks were incubated at 30°C under continuous shaking (120 rpm) with different electron donors such as glucose, glycine, peptone, and Na-acetate and the reduction was estimated following usual method [23,24].

2.10. Screening of Synergistic and Antagonistic Effect of Chromium and Nickel Resistant Bacteria

The bacterial isolates were checked for the resistance to both the metals together. Different concentrations of chromium and nickel (500 mg/l–2000 mg/l) were prepared using potassium dichromate and nickel chloride. Then, both the concentrations were plated and cultures were spread onto the plates. The plates were incubated at 37°C for 3–4 days. Then, the colonies were checked for the chromium and nickel assay as mentioned above [25].

2.11. Antimicrobial Activity of *Bacillus cereus*

2.11.1. Microorganisms

Staphylococcus hominis (MTCC 10220), *Staphylococcus cohnii* (MTCC 10219), *B. cereus* (MTCC 430), *E. coli* (MTCC 443), and *Pseudomonas aeruginosa* (MTCC 8076) were used for antimicrobial study. All the stock cultures were collected from CSIR – Institute of microbial technology, Chandigarh, India. All of the bacterial strains were grown and maintained on their specific medium. The bacteria were subcultured overnight for further use.

2.11.2. Antimicrobial activity test

The antimicrobial activity of the isolated *B. cereus* was determined by disk diffusion Technique. Cotton swab was used to inoculate the test tube suspension onto the surface of nutrient agar plate and the plate was allowed to dry. Using a sterilized forcep, sterilized Whatman paper disks were transferred onto the agar surface. Each sterile disk was impregnated with test organism. Amoxicillin was used as control. The experiment was conducted in triplicates. The plates were incubated at 37°C for 24 h. At the end of the period, the inhibition zone against each microorganism by test organism was measured and analyzed [26,27].

2.12. Statistical Analysis

Triplicate measurements were done in all the cases during the observation and assessment of bacterial growth incorporated with different levels of heavy metals. Data were captured into Microsoft Excel Software, version 2010 which was used to calculate means, standard deviations and standard errors [16]. Pearson's correlation was also performed for the heavy metal accumulation and reductions using IBM SPSS statistics 22 software.

3. RESULTS AND DISCUSSION

3.1. Soil Parameter Test

The Kolkata Municipal Corporation area generates more than 2500 metric tons of garbage daily, making the soil much more polluted every

passing year. This ecosystem having different structural components (water, soil, macrophytes, and plants) acts as ecological features in east Kolkata wetland area. The comparative results obtained from different collected samples show that the temperature is slightly higher in metropolitan khal area, pH and organic carbon content of soil is high in 7 numberjhil area and nunebheriarea. Water holding capacity, Phosphorus, Nitrogen content, and Electrical Conductivity are also higher in nunebheri area, borodhaljhil, chilparajhil, and five numberjhil. Chromium content is higher in chilparajhil area and also Nickel content is high in five numberjhil. pH of the samples collected is in neutral condition, between 7 and 8. On the other hand, nitrogen was found to be similar for each area and the range is between 500 and 800kg/ha. The range of chromium present in all the collected sample is between 2 and 3 mg/kg, whereas nickel content is high in five numberjhil, borodhaljhil, and chilparajhil area, the range is 30–35 mg/kg [Table 1].

The soil sample used for the isolation of heavy metal-resistant bacteria when checked for different parameters have given a satisfactory result indicating a helpful material for plant growth [18,25,28]. The samples collected also consist of a good amount of nutrients such as nitrogen and phosphorus for plant nutrition [29]. However, because of the presence of heavy metals such as chromium and nickel the electrical conductivity value is higher than that of normal [16]. Thus, the microorganisms present in this soil have come up as metal resistant as well as metal reducing agents. The ability of microbial strains to grow in the presence of heavy metals is helpful in many terms including waste water treatment by decomposition of organic matter [30]. Primary study of the collected soil samples for heavy metal resistance ability showed that all samples were positively grown utilizing heavy metals present in their culture media [31-34]. The bacterial isolates were then characterized by morphological, biochemical tests according to Bergey's manual, DNA and protein extraction, and 16srRNA sequencing [35].

3.2. Isolation of Chromium and Nickel Resistant Bacteria

Chromium and nickel resistance bacteria were isolated using different concentrations of chromium (VI) and nickel (II) by spread plate method. The Brahmi root soil was spread in 500 mg/l–2000 mg/l concentrations and growth was observed till 1500 mg/l whereas no growth was observed in 2000 mg/l [Figure 1a]. The same sample was spread for different concentrations of nickel, from 200 mg/l to 1000 mg/l and growth was observed till 800 mg/l and no growth was observed in 1000 mg/l. Thus, it indicates that bacteria present in this sample can resist chromium and nickel up to 1500 mg/l and 800 mg/l concentrations, respectively [Figure 1b]. From the sample, a total of twenty single colonies were isolated and checked under microscope for morphological and biochemical characterization. The selected potential bacterial isolates resistant to chromium and nickel were subjected to identification by determining its biochemical characteristics as per Bergey's manual of systemic bacteriology. The isolate was found to be gram positive, catalase positive, rod shaped, spore forming, etc. [Table 2].

Genomic DNA was also extracted from the overnight grown culture of four stressed bacterial sample BRS 5, BRS 11, BRS 17, BRS 19, and one unstressed bacteria *B. cereus* (MTCC-430). The samples were electrophoresed on 1% agarose gel against 1kb DNA ladder [Figure 2a]. Bacterial samples when exposed to stress condition showed some pigment releasing activity where the color of broth culture changed from yellow to green. Before the molecular weight determination using SDS-PAGE protein concentration of the isolates were checked as per BSA standard curve. As per the result, protein concentration of all the samples increased after the color change [Table 3]. Overnight grown culture of four stressed bacterial sample and one control unstressed sample *B. cereus* (MTCC-430) electrophoresed using SDS-PAGE electrophoresis for estimation of protein. The protein is separated on the basis of it is molecular weight using protein ladder [Figure 3]. From the biochemical observation, the bacterial isolate was identified as *Bacillus sp*. Further the result of 16srRNA sequencing was performed which confirms it as *B. cereus* and the phylogenetic tree is given Figure 2b.

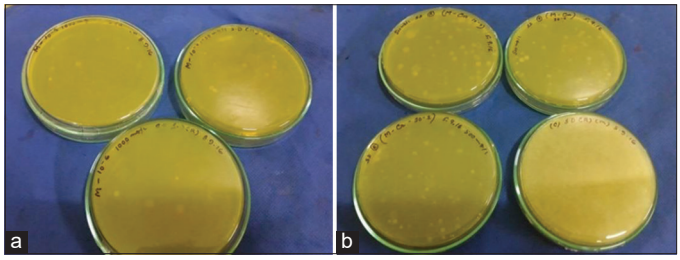

Figure-1: Metal-resistant bacterial plates (a = Chromium and Nickel plate with 1500 mg/l and 1000 mg/l Cr (VI) and Ni (II) concentration, b = Chromium and Nickel plate with 500 mg/l and 800 mg/l Cr(VI) and Ni(II) concentration).

Table 1: Different soil parameters tests of collected soil from some regions of East Kolkata Wetland area.

Area	Parameters								
	pH	Temperature (°C)	WHC (%)	EC (µs)	OC (%)	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Chromium (mg/kg)	Nickel (mg/kg)
Control	7.03	22.1	48.61	736.3	2.84	834.7	22	0.00	0.00
Borodhal Jhil area	7.86	21.7	48.67	865.3	5.72	533.3	35	2.48	34.36
Chilpara Jhil area	6.83	21.2	45.41	1167	4.79	725.3	22	2.74	32.58
Mathpukur area	7.54	21.7	46.39	2241.7	5.46	665	28	2.44	24.38
Metropolitan area	6.73	22.4	43.80	1718	5.53	658.3	24	2.63	25.53
5 numberjhil area	6.86	21.4	43.30	2445.7	4.46	637.7	15	2.24	35.73
7 numberjhilarea	7.92	20.3	42.78	923.3	5.68	596	15	2.35	29.43
Natarbheri area	6.76	20.1	46.34	1372	5.44	641	26	2.26	24.67
Chingrighata area	7.42	20.4	45.53	1285	5.11	619	31	2.36	25.33
2 numberjhil area	6.86	20.8	46.27	1245	4.67	675	20	2.47	25.14
Nunebheri area	7.58	20.4	48.75	1357	5.86	665	25	2.18	25.46

Table 2: Biochemical characterization of bacterial strain isolated from East Kolkata Wetland area.

Basic characteristics	Properties of BRS 5 strain	Properties of BRS 11 strain	Properties of BRS 17 strain	Properties of BRS 19 strain
Catalase	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Citrate	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Gram staining	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Indole test	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Motility test	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Methyl red test	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Oxidase test	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Shape	Rod	Rod	Rod	Rod
Spore	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
VP test	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Arabinose	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Fructose	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Glucose	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Starch	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Mannose	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Lactose	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Manitol	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Acetate utilization	Positive (+ve)	Positive (+ve)	Positive (+ve)	Positive (+ve)
Lysine	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Phenylalanine deaminase	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)

Table 3: Protein concentration of different bacterial isolates using BSA standard curve.

Bacterial isolates	Protein conc. of isolates (mg/l)
BRS5	0.81
BRS11	0.83
BRS17	0.79
BRS19	0.87

Depending on the tests, the isolates were identified as gram positive and the sequencing proved that the strain is *B. cereus* (BF2) accession number KU955350.1. Bacterial growth was also observed in the presence of heavy metals chromium and nickel using different concentrations that were studied with bacterial growth [24,36,37]. For determination of bacterial genetic information, DNA profiling is important to locate the resistant genes in chromosomes. In our study, the positive strain was used for DNA extraction and observed against 1 kb ladder. The positive isolates were also checked for their protein profiling to understand the molecular weight of the genetic material to confirm the metal resistant genes [38]. According to some reports, ChrA and NerB genes are responsible for chromium and nickel resistance and the molecular weight varies from 43kDa to 66kDa. As per our results, the strains also showed bands between 48 kDa and 65 kDa which confirms the presence of the genes in bacterial sample also the concentration of protein was estimated according to the BSA standard curve [39].

3.3. Accumulation and Reduction of Cr(VI) and Ni(II) in BRS 5, BRS 11, BRS 17, and BRS 19

The current status of the bioremediation of heavy metal is much promising for metal biosorption and detoxification using genetically modified microbes. Biofilm-mediated techniques, microbial gene transfer, and microbial fuel cells-based techniques can be considered as strong approaches in coming years. The peptidoglycan and polysaccharides component presents in the microbial cell wall act as a great binding site for metals help in metal uptake as well as biosorbent [40]. However, some research focuses on the role of bacterium in reducing the heavy metal chromium and nickel from different soil samples and this bacterium also acts as chelating agents. According to some reports, *P. aeruginosa* and *Lactobacillus plantarum* MF042018 is also a potent bacterial strain involved in removal of heavy metals. *P. aeruginosa* was also recorded high removal percentage of different heavy metals at optimum condition for growth such as cadmium, lead, mercury, copper, and zinc other than chromium and nickel [41,42].

Here, in twenty isolated colonies the concentration of nickel is higher than that of chromium [Figure 4]. Influence of different Electron donor (glucose, glycine, Na-acetate, and peptone) on chromium (VI) concentration (20 mg/l) by different time intervals of BRS 5, BRS 11, BRS 17, and BRS 19 bacterial strains [Figure 5a-d]. Influence of different electron donor (glucose, glycine, Na-acetate, and peptone) on nickel (II) concentration (20 mg/l) by different time intervals of BRS 5, BRS 11, BRS 17, and BRS 19 bacterial strains [Figure 6a-d].

Chromium-resistant bacteria that can also reduce the concentrations have been reported earlier in different regions of India and also outside India of different oil contaminated soils but nickel reduction was not so common. The present study clearly indicates the presence of chromium and nickel resistant as well as reducing bacteria in soil of East Kolkata Wetland area. The most potent strain has been identified as *B. cereus* reduced chromate using different electron sources such as glucose, glycine, Na-acetate, and peptone. As per the result, all the sources have shown a good reduction rate;

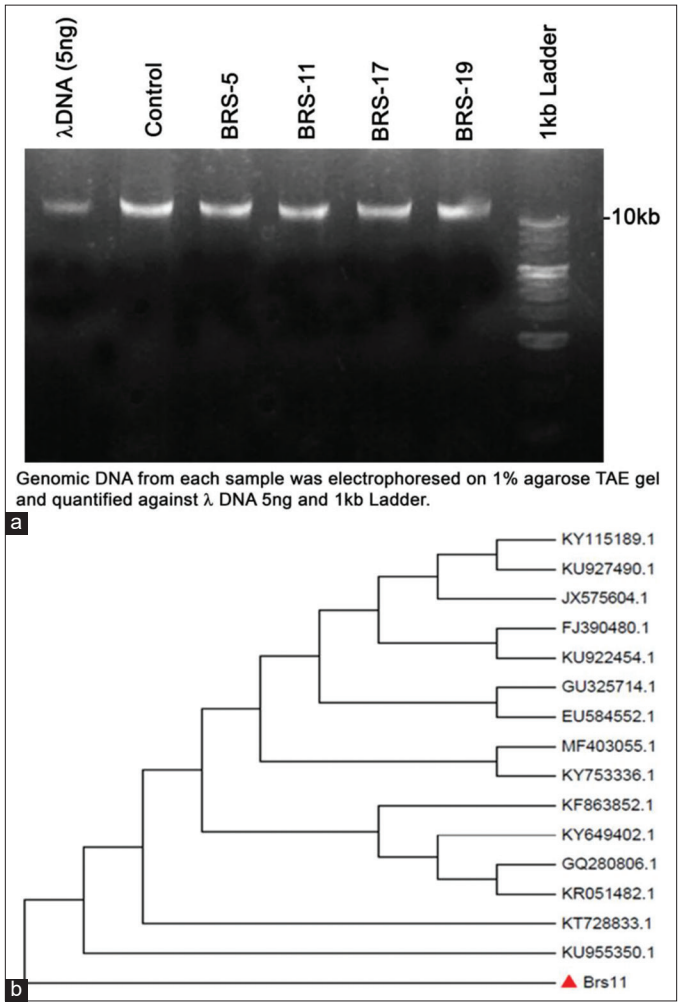

Figure 2: Identification of bacterial strain (a) genomic DNA of different bacterial isolates on 1% agarose TAE gel. (b) Phylogenetic tree of the bacterial isolate by 16SrRNA sequencing.

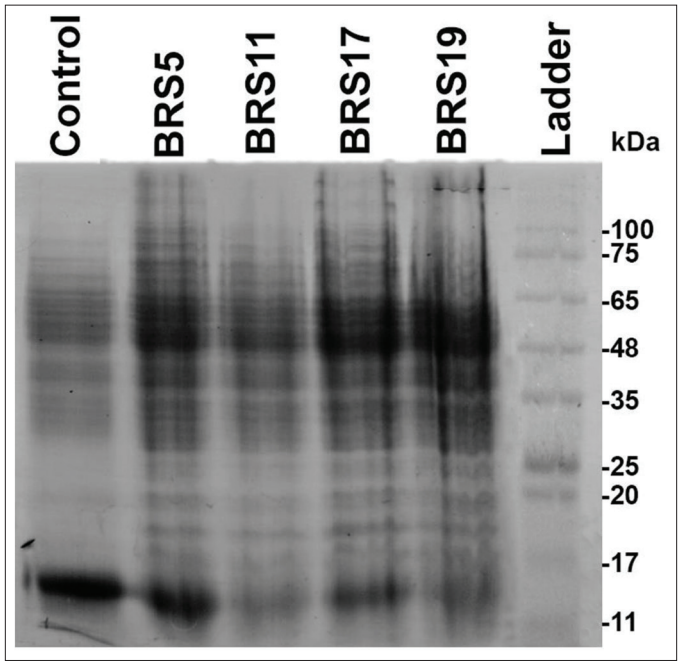

Figure 3: SDS-PAGE (10%) separation of bacterial isolates.

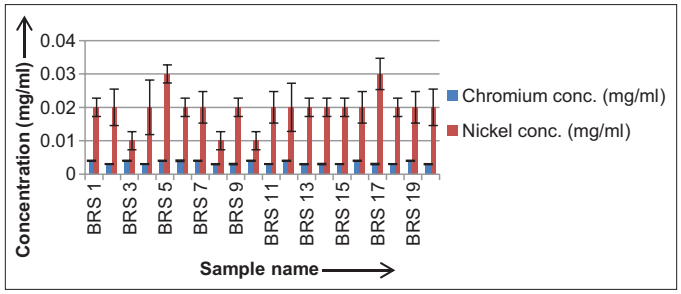

Figure 4: Cr(VI) and Ni(II) concentration in bacterial isolates – Standard deviation graph of chromium and nickel concentration in bacterial species.

however, Na-acetate and peptone have shown a remarkable change in reduction of chromium (VI) [43], whereas in nickel glycine along with Na-acetate and peptone has shown a significant reduction rate. The positive isolates when checked with both chromium and nickel concentrations together they have interestingly shown some synergistic and antagonistic effect as well. Previously, the isolates were able to resist chromium and nickel up to 1500 mg/l and 800 mg/l, respectively. However, when the metals were used together the resistant capacity for chromium was reduced to 1000 mg/l whereas the resistant capacity of nickel was increased to 1000 mg/l indicating antagonistic and synergistic effects [44]. For the removal of heavy metals such as chromium and nickel, microbial remediation and reduction is one of the most viable and sustainable methods. For attenuation of excess Cr(VI) accumulation in the environment. Based on the current study, it can be said that for the removal of heavy metal and specifically Cr(VI) much more work at bigger scale is needed to develop reliable and cost-effective technologies for this problem [45].

3.4. Antimicrobial Activity of Isolates

The antimicrobial activity of isolated BRS 5, BRS 11, BRS 17, and BRS 19 was determined by the disk diffusion method against five different bacterial strains. A standard amoxicillin antibiotic was used in this study as control. None of the samples showed activity against *P. aeruginosa* MTCC 8076, whereas only BRS 17 showed activity against *E. coli* MTCC 10220 which indicates it as a potent antimicrobial organism. All four samples showed antimicrobial activity against *B. cereus*, *S. cohnii,* and *S. hominis* [Figure 7].

The isolates were checked for their antimicrobial activity against different microorganisms collected from CSIR-Institute of microbial technology, Chandigarh, India, where all four samples showed antimicrobial activity against *B. cereus*, *S. cohnii* and *S. hominis* and only BRS 17, which also showed morphological and biochemical characters same as *B. cereus*, showed activity against *E. coli* MTCC 10220 indicating it as a potent antimicrobial organism [27,46-48].

3.5. Statistical Analysis using SPSS

The IBM SPSS statistics 22 software was used to understand the following correlation matrix between different electron donors used to accumulate and reduce the metal concentrations of different soil samples using bacterial culture. This test actually is performed to measure the statistical relationship or association between two or more continuous variables as well as the direction of the relationship. As the metals containing

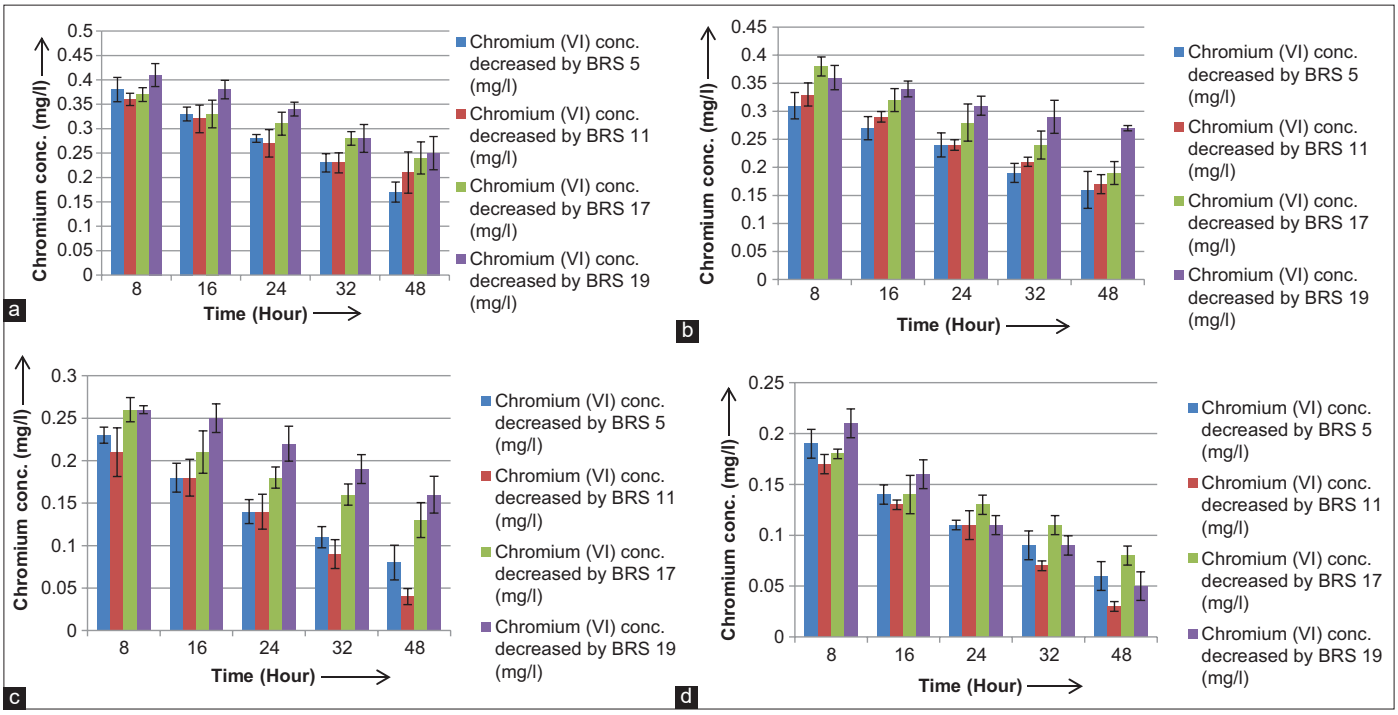

Figure 5: Reduction of Cr(VI) by different electron donor sources. (a) Reduction of Cr (VI) influence by glucose source as electron donor. (b) Reduction of Cr (VI) influence by Glycine source as electron donor. (c) Reduction of Cr (VI) influence by Na-acetate source as electron donor. (d) Reduction of Cr (VI) influence by peptone source as electron donor.

Table 4: Pearson's correlation matrix of BRS 11 (*Bacillus cereus*) culture by IBM SPSS 22.

		Correlations							
		Chromium glucose	**Chromium glycine**	**Chromium Na-acetate**	**Chromium peptone**	**Nickel glucose**	**Nickel glycine**	**Nickel Na-acetate**	**Nickel peptone**
Chromium glucose	Pearson correlation	1	0.994**	0.977**	0.976**	0.987**	0.991**	0.991**	0.993**
	Sig. (two-tailed)		0.001	0.004	0.005	0.002	0.001	0.001	0.001
	N	5	5	5	5	5	5	5	5
Chromium glycine	Pearson correlation	0.994**	1	0.988**	0.987**	0.997**	0.996**	0.991**	0.983**
	Sig. (2-tailed)	0.001		0.002	0.002	0.000	0.000	0.001	0.003
	N	5	5	5	5	5	5	5	5
Chromium Na-acetate	Pearson correlation	0.977**	0.988**	1	0.994**	0.990**	0.983**	0.995**	0.983**
	Sig. (two-tailed)	0.004	0.002		0.001	0.001	0.003	0.000	0.003
	N	5	5	5	5	5	5	5	5
Chromium peptone	Pearson correlation	0.976**	0.987**	0.994**	1	0.994**	0.972**	0.990**	0.976**
	Sig. (two-tailed)	0.005	0.002	0.001		0.001	0.006	0.001	0.005
	N	5	5	5	5	5	5	5	5
Nickel glucose	Pearson correlation	0.987**	0.997**	0.990**	0.994**	1	0.988**	0.989**	0.976**
	Sig. (two-tailed)	0.002	0.000	0.001	0.001		0.002	0.001	0.004
	N	5	5	5	5	5	5	5	5
Nickel glycine	Pearson correlation	0.991**	0.996**	0.983**	0.972**	0.988**	1	0.988**	0.981**
	Sig. (two-tailed)	0.001	0.000	0.003	0.006	0.002		0.002	0.003
	N	5	5	5	5	5	5	5	5
Nickel Na-acetate	Pearson correlation	0.991**	0.991**	0.995**	0.990**	0.989**	0.988**	1	0.996**
	Sig. (two-tailed)	0.001	0.001	0.000	0.001	0.001	0.002		0.000
	N	5	5	5	5	5	5	5	5
Nickel peptone	Pearson correlation	0.993**	0.983**	0.983**	0.976**	0.976**	0.981**	0.996**	1
	Sig. (two-tailed)	0.001	0.003	0.003	0.005	0.004	0.003	0.000	
	N	5	5	5	5	5	5	5	5

**Correlation is significant at the 0.01 level (2-tailed)

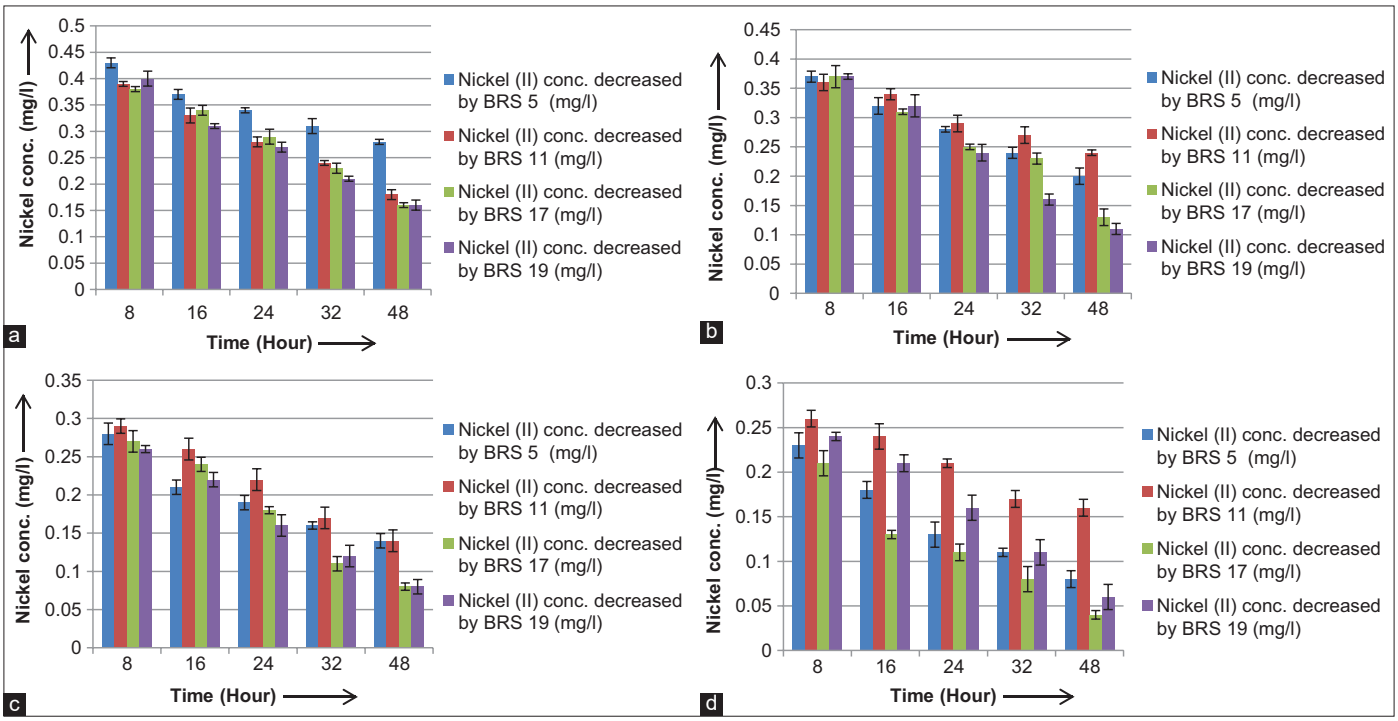

Figure 6: Reduction of Ni (II) by different electron donor sources. (a) Reduction of Ni (II) influence by glucose source as electron donor. (b) Reduction of Ni (II) influence by Glycine source as electron donor. (c) Reduction of Ni (II) influence by Na-acetate source as electron donor. (d) Reduction of Ni (II) influence by Peptone source as electron donor.

Table 5: Pearson's correlation matrix of BRS 17 culture by IBM SPSS 22.

		Correlations							
		Chromium glucose	**Chromium glycine**	**Chromium Na-acetate**	**Chromium peptone**	**Nickel glucose**	**Nickel glycine**	**Nickel Na-acetate**	**Nickel peptone**
Chromium glucose	Pearson correlation	1	0.997**	0.986**	0.995**	0.992**	0.988**	0.973**	0.981**
	Sig. (two-tailed)		0.000	0.002	0.000	0.001	0.002	0.005	0.003
	N	5	5	5	5	5	5	5	5
Chromium glycine	Pearson correlation	0.997**	1	0.994**	0.993**	0.988**	0.986**	0.978**	0.986**
	Sig. (two-tailed)	0.000		0.000	0.001	0.002	0.002	0.004	0.002
	N	5	5	5	5	5	5	5	5
Chromium Na-acetate	Pearson correlation	0.986**	0.994**	1	0.989**	0.966**	0.976**	0.961**	0.994**
	Sig. (two-tailed)	0.002	0.000		0.001	0.007	0.005	0.009	0.001
	N	5	5	5	5	5	5	5	5
Chromium peptone	Pearson correlation	0.995**	0.993**	0.989**	1	0.973**	0.980**	0.951*	0.994**
	Sig. (2-tailed)	0.000	0.001	0.001		0.005	0.003	0.013	0.001
	N	5	5	5	5	5	5	5	5
Nickel glucose	Pearson correlation	0.992**	0.988**	0.966**	0.973**	1	0.983**	0.986**	0.950*
	Sig. (2-tailed)	0.001	0.002	0.007	0.005		0.003	0.002	0.013
	N	5	5	5	5	5	5	5	5
Nickel glycine	Pearson correlation	0.988**	0.986**	0.976**	0.980**	0.983**	1	0.953*	0.963**
	Sig. (two-tailed)	0.002	0.002	0.005	0.003	0.003		0.012	0.008
	N	5	5	5	5	5	5	5	5
Nickel Na-acetate	Pearson correlation	0.973**	0.978**	0.961**	0.951*	0.986**	0.953*	1	0.937*
	Sig. (two-tailed)	0.005	0.004	0.009	0.013	0.002	0.012		0.019
	N	5	5	5	5	5	5	5	5
Nickel peptone	Pearson Correlation	0.981**	0.986**	0.994**	0.994**	0.950*	0.963**	0.937*	1
	Sig. (two-tailed)	0.003	0.002	0.001	0.001	0.013	0.008	0.019	
	N	5	5	5	5	5	5	5	5

**Correlation is significant at the 0.01 level (two-tailed). *Correlation is significant at the 0.05 level (two-tailed)

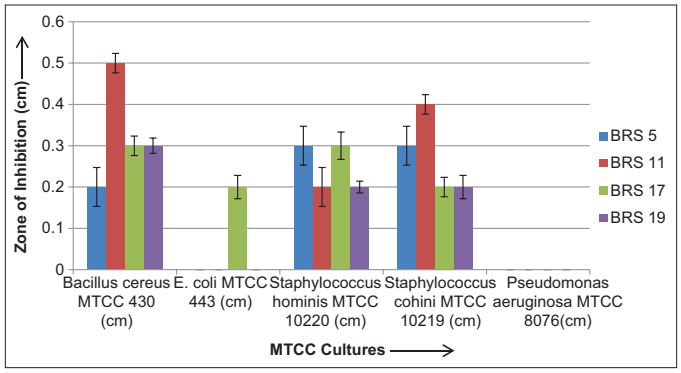

Figure 7: Antimicrobial activity of different bacterial isolates against some specific Gram (+ve) and Gram (−ve) bacteria such as *Pseudomonas aeruginosa* (MTCC 8076), *Staphylococcus cohnii* (MTCC 10219), *Staphylococcus hominis* (MTCC 10220), *E. coli* (MTCC 443) and *Bacillus cereus* (MTCC 430).

different electron sources shown a good result to accumulate and also some synergistic characteristics also came up this correlation was done to check if there is any significant relationship present between them or not. According to correlation matrix, $P \leq 0.05$ for the result to become significant and the test is not significant if $P \geq 0.05$. As per the table shown below, there is a positive significance present between all the sources used for the accumulation and reduction proving its synergistic effect. Here, two different cultures were used for the analysis of correlation, that is, *B. cereus* (BRS 11) and also BRS 17, which is morphologically same as BRS 11 and also showed a good reduction and accumulation of metals. As per the following result in BRS 11, all the donors showed significant relationship between each other at 0.01 levels [Table 4], whereas in BRS 17 the relationship was significant at 0.01 and 0.05 levels [Table 5].

4. CONCLUSION

The ability of microbial stains to grow in the presence of heavy metals would be helpful in the contaminated soil treatment. The isolated strain *B. cereus* is characterized with remarkable tolerance against heavy metals chromium and nickel and when both were mixed together it showed some synergistic as well as antagonistic effects. It can be used as potential agents for the development of a soil inoculant applicable in bio augmentation, biosorption, accumulation, and bioremediation of heavy metals in polluted sites.

5. AUTHORSHIP AND ACKNOWLEDGEMENTS

S.D.; performed the experiments, analysis of data for the work and co-wrote the paper. K.F.; interpretation and analysis of the data and co-wrote the paper. S.C.; supervised the research.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

Not applicable.

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