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

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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 enr 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 Brahi root, chilparajhil area, mathpukurkhali area, metropolitan khal area, Five...
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 (K₂Cr₂O₇) 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% H₃PO₄ 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 K₂Cr₂O₇ 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
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 1500 mg/l and growth was observed till 1500 mg/l whereas no growth was observed in 2000 mg/l [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 its 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.
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) |
|-------------------|----------------------------------|
| BR51              | 0.81                             |
| BR511             | 0.83                             |
| BR17              | 0.79                             |
| BR19              | 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 chrome 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;
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].

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.

|                        | 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            | 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          | 0.000            | 0.003          |
| N                      | 5                | 5                | 5                   | 5                | 5             | 5              | 5                | 5              |
| Chromium Na-acetate    | Pearson correlation 0.977** | 0.988** | 1 | 0.994** | 0.972** | 0.990** | 0.995** | 0.983** |
| Sig. (two-tailed)      | 0.004            | 0.002            | 0.001               | 0.001            | 0.003         | 0.000          | 0.003            | 0.003          |
| N                      | 5                | 5                | 5                   | 5                | 5             | 5              | 5                | 5              |
| Chromium peptone       | Pearson correlation 0.976** | 0.987** | 0.994** | 1 | 0.994** | 0.988** | 0.998** | 0.976** |
| Sig. (two-tailed)      | 0.005            | 0.002            | 0.001               | 0.001            | 0.006         | 0.002          | 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          | 0.001            | 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          | 0.002            | 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** | 1 | 0.988** | 0.996** |
| Sig. (two-tailed)      | 0.001            | 0.001            | 0.000               | 0.001            | 0.001         | 0.002          | 0.001            | 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          | 0.003            | 0.000          |
| N                      | 5                | 5                | 5                   | 5                | 5             | 5              | 5                | 5              |

**Correlation is significant at the 0.01 level (2-tailed)
Table 5: Pearson’s correlation matrix of BRS 17 culture by IBM SPSS 22.

|                          | Chromium glucose | 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             | 0.005         | 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             | 0.004         | 0.002         |
|                          | N                 5      | 5                 | 5               | 5           | 5           | 5                 | 5             | 5             |
| Chromium Na-acetate      | Pearson correlation 0.986** | 0.994**       | 1               | 0.980**     | 0.966**      | 0.976**           | 0.961**       | 0.994**       |
|                          | Sig. (two-tailed) 0.002 | 0.000          | 0.001           | 0.007       | 0.005       | 0.009             | 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             | 0.013         | 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             | 0.002         | 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             | 0.012         | 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             | 0.012         | 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)**
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 bioaugmentation, biosorption, accumulation, and bioremediation of heavy metals in polluted sites.

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 bioaugmentation, biosorption, accumulation, and bioremediation of heavy metals in polluted sites.

5. AUTHORSHIP AND ACKNOWLEDGEMENTS

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8. CONFLICTS OF INTEREST

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