Ecotoxicology (2022) 31:565–580
https://doi.org/10.1007/s10646-021-02445-y

# Biosorption efficiency of nickel by various endophytic bacterial strains for removal of nickel from electroplating industry effluents: an operational study

Saket Kashyap[1] · Rachna Chandra[2] · Bikash Kumar[1] · Pradeep Verma[1]

Accepted: 8 June 2021 / Published online: 28 June 2021


## Abstract

Realising the hazardous effect of nickel on human health, microbes and plants are effectively used for bioremediation. The endophytic microorganisms have an important role in the phytoremediation of nickel using *Vigna radiata*. Therefore, in order to harness the potential of microbial strains, the present study was designed to examine the metal biosorption ability of endophytic bacterial strains isolated from plants growing in nickel-contaminated soil. A total of six endophytic nickel resistance bacteria were isolated from the plant *Vigna radiata*. The metal tolerant bacterial strains were identified following 16 S rRNA gene sequence analysis. Nickel biosorption estimation and plant growth-promoting (PGP) activities of isolated strains were performed and found high nickel biosorption efficiency of 91.3 ± 0.72% at 600 mg $L^{-1}$ using *Bacillus safensis* an isolated endophytic strain from *Vigna radiata*. Furthermore, high indole acetic acid (IAA) and exopolysaccharide (EPS) production were obtained in all the strains as compared to without nickel-containing medium used as control. Moreover, the production of high EPS suggests improved biosorption ability of isolated endophytic strains. In addition, a kinetic study was also performed to evaluate different adsorptions isotherms and support the nickel biosorption ability of endophytic strains. The treatment of nickel electroplating industrial effluent was also demonstrated by isolated endophytic strains. Among six (6) strains, *B. cereus* showed maximum 57.2 ± 0.62% biosorption efficiency of nickel which resulted in the removal of 1003.50 ± 0.90 mg $L^{-1}$ of nickel from the electroplating industry effluents containing initial 1791 ± 0.90 mg $L^{-1}$ of nickel. All other strains were also capable of significant nickel biosorption from electroplating industry effluents as well. Thus, isolated endophytic nickel tolerant strains can be further used at large-scale biosorption of nickel from electroplating industry effluent.

**Keywords** Endophytic · Phytoremediation · Biosorption · Decolourization · Heavy metals · Nickel

## Introduction

The sustainability of agriculture and civilization relies on natural resources like water and the land but they are the one which has gone through maximum exploitation and are severely degraded due to anthropogenic activities (Akhtar et al. 2018). The natural resources are polluted mainly by point sources like industrial solid discharge, effluents, emission, vehicles exhaustion, metals from mining and smelting, and non-point sources like utilization of pesticides and insecticides, excessive utilization of fertilizers in the agricultural farm, disposal of agricultural and municipal waste in the agriculture field, soluble salts (artificial or natural) (Nriagu and Pacyna 1988; McGrath et al. 2001; Masindi and Muedi 2018). The sources enhancing the level of heavy metal proportion into water and soil are more life-threatening because of their diligence and carcinogenic nature into the environment and human beings respectively (Wang et al. 2017). Some transition metals like nickel, at its low concentration the growth is not much affected but at high metal concentration, it increases stress. Thus, transition metal nickel is being considered as a heavy metal because it

✉ Pradeep Verma
vermaprad@yahoo.com

[1] Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, NH-8, Bandarsindri, Kishangarh, Ajmer 305817 Rajasthan, India

[2] Terrestrial Ecology Division, Gujarat Institute of Desert Ecology, Mundra Road, Bhuj 370001 Gujarat, India

is a biologically essential heavy metal and can be tolerated at a low level but become toxic at a high level (Singh et al. 2011; Engwa et al. 2019; Kaur et al. 2019).

Nickel is required in trace amount for several molecular and physiological roles in plants and animals however presence in excessive amount may have several hazardous effects (Ahmad and Ashraf 2012; Genchi et al. 2020). In plants, the presence of a high concentration of nickel in soil and nutrient solution negatively affects the metabolism (photosynthesis and transpiration), seed germinability, abnormal branching/flowering pattern, causes leaf spotting, chlorosis, and necrosis (Ahmad and Ashraf 2012). In animals, exposure to nickel can cause several negative health-associated effects i.e. allergy, cancer (lung, nasal), lung fibrosis, and cardiovascular and kidney diseases (Genchi et al. 2020). This toxic impact is due to mitochondrial dysfunctions and oxidative stress caused to biota in presence of nickel (Genchi et al. 2020).

Their destruction is almost impossible but their organic complex or oxidation state can be changed from one to another (Garbisu and Alkorta 2001; Egorova and Ananikov 2017). The contamination of water and soil by heavy metal has become a serious concern and threat worldwide. Many physical, chemical, and biological methods have been suggested for the removal of heavy metals (Shi et al. 2009; Govarthanan et al. 2014). Among all the methods, microorganisms-based bioremediation is deliberated as a viable and promising method because it is less expensive as well as eco-friendly. Several factors govern the microbial bioremediation process such as the viability of bacterial strains in contaminated water and soil, abiotic factors, metal detoxification process, the rate of expression of genes that are governing these metal detoxification, and the impact of the pollutant on the bacterial activity (Govarthanan et al. 2015a, 2015b; Loganathan et al. 2015).

In recent years, there is more focus on the bioremediation process of heavy metals using endophytic bacteria having plant growth-promoting activity (Ma et al. 2015). Singh et al. (2010) observed that the *Vigna radiata* plant is a hyperaccumulator for heavy metals. Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for phytoremediation as it increases the bioavailability and uptake of the metals in plants from the soil (Dipu et al. 2012; Evangelou et al. 2007). The endophytic bacteria are widely distributed in the leaves, stems, roots, fruits, and flowers of the plants. The endophytic bacteria plays a substantial role in the growth and development of plants by producing plant growth-promoting factors like indole-3-acetic acid (IAA), exopolysaccharide (EPS) (Sun et al. 2010; Compant et al. 2011; Tiwari et al. 2016). Some extra polymeric substances are also produced by them which either prevents or nullifies heavy metal toxicity (Weyens et al. 2011). The endophytic bacteria in the nickel hyperaccumulator plant adapted to survive in such specific conditions even after the accumulation of heavy metal in high amounts (Barzanti et al. 2007; Mosa et al. 2016). The bacteria associated with the nickel hyperaccumulator *Thlaspi goesingense*. were studied and the presence of specialized endophytic bacteria was reported (Idris et al. 2004).

The strain *Pseudomonas* sp. can bind to some heavy metals from the environment and this ability may be due to phospholipids, protein, and lipopolysaccharides which are the components of the cell wall. The lipopolysaccharide of the cell has carboxyl, phosphate, and phospholipid. They are the main sites for metal ion binding and hence execute the metal biosorption process (Oyewole et al. 2019). Yeasts have nickel binding peptides which make them a suitable candidate for biosorbent. Biosorption with the help of cell surface is more influential because it does not need cell lysis to remove heavy metal whereas intracellular absorption needs lysis of cell (Li et al. 2019). There are many benefits of using dead biomass like they are not affected by toxicity, limited operation cost, act as an ion exchanger, reuse of biosorbent, can be stored for a long time, etc (Ahluwalia and Goyal 2007; Ahalya et al. 2014).

Therefore, the present work was focused to isolate endophytic strains from the plant *Vigna radiata* and examine the nickel biosorption efficiency of the isolated strains. Also, the effect on plant growth-promoting (PGP) activities, under high nickel stressed conditions were also determined at a very high concentration of synthetic nickel-metal salt solution and was also applied for the biosorption of nickel from the electroplating industry effluents (Fig. 1).

## Material and methods

### Materials

Nutrient agar media, nickel chloride, and ethylenediaminetetraacetic acid (EDTA) were obtained from Hi-Media, India. Ethanol was obtained from Fisher Scientific, UK. All chemicals used were of analytical grade. The molecular identification of isolated strain was done by Xcelris Labs Ltd. Gujarat Ahmedabad India. Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Analyst 400 was used at Gujarat Institute of Desert Ecology Bhuj, Gujarat, India. Nickel electroplating wastewater samples were collected from the local industry of Sitapura Industrial area, Jaipur, Rajasthan in pre-sterilized amber bottles and stored at 4 °C.

### Sampling and soil analysis

The surface layer of the garden soil was taken from the agricultural land of Bhuj, Gujarat, India. The

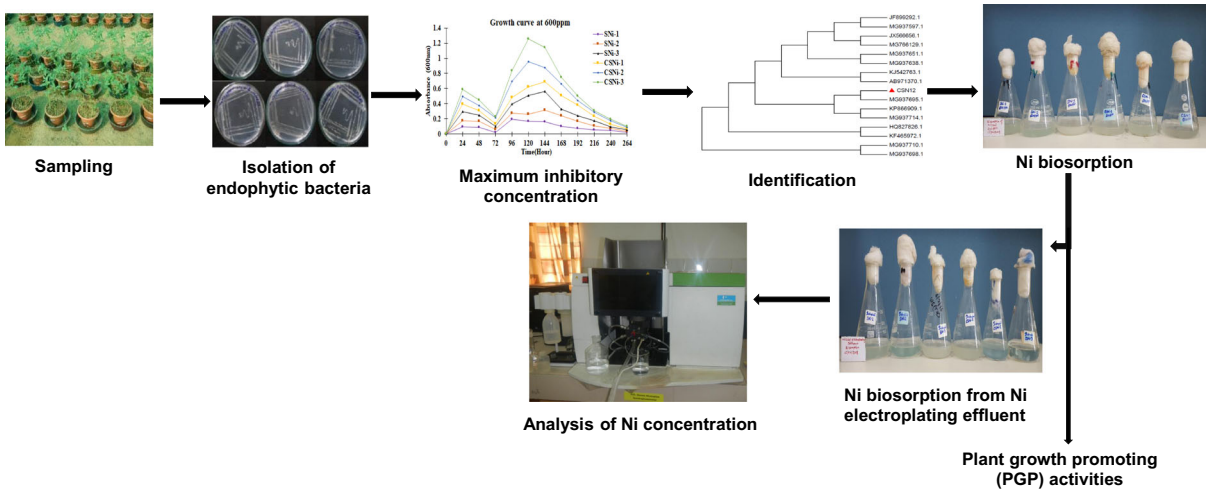


**Fig. 1** Schematic representation of overall work

physiochemical parameter (pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, soil organic carbon, total hardness, calcium, magnesium, sodium, potassium, lithium, phosphorous, sulphur, available nitrogen, cation exchange capacity) of soil was analyzed according to protocols suggested by Bulewicz et al. (1960), Haluschak (1981), and Corwin and Lesch (2005).

## Hyperaccumulation activity of nickel by *Vigna radiata* plants

The pot experiment was performed using garden soil spiked with nickel. Seeds of *Vigna radiata* were sown in each pot and an appropriate amount of water was given every day to maintain 70% moisture content. The chelator (EDTA) was added after 40 days of sowing seeds and after 50 days the plant was harvested (Liphadzi and Kirkham 2006; Dipu et al. 2012; Hasan et al. 2019). Metal hyper-accumulation activity of the plant was tested by harvesting the plant after the experiment. It was separated into the root, stem, and leaf later it was cleaned and washed into running tap water for 30 min. The sample was dried at 100 °C in an oven separately for 10 h. The dried samples were subjected to nitric acid digestion. One-gram (1.0 g) sample was taken into a beaker (100 mL) and concentrated $HNO_3$ (10 mL) was added. It was boiled for 45 min at 45 °C and then the temperature was increased to 150 °C for 3 h to obtain a clear solution. It was followed by the addition of concentrated $HNO_3$ (5.0 mL) and digested till the volume of solution was equal to 1.0 mL. The inner wall of the beaker was washed with distilled water to avoid sample loss. The sample was subjected to cooling and $HNO_3$ (1% of 5.0 mL) was added. The solution was filtered with the help of filter (Whatman 42) paper and volume was made up to 50 mL in the volumetric flask. The solution was analyzed using atomic absorption spectrophotometer (Hseu 2004).

## Isolation of nickel resistant endophytic bacterial strains from *Vigna radiata* plant

A mature plant of *Vigna radiata* was collected and it was grown in artificially induced nickel soil and chelator EDTA. The plant sample was washed gently in running tap water for 30 min to remove dust and soil particles. It was then cleaned with alcohol (70%) for 1 min followed by cleaning with sodium hypochlorite (2.5%) for 4 min. The clean plant parts were rinsed with ethanol (90%) for 30 s and finally three-time rinse with sterile distilled water. The disinfection protocol was confirmed using aliquots of the sterile water of the final rinse was poured on a nutrient agar plate and incubated at 37 °C for 3 days and the plates were examined for microorganism growth. A section (5.0 mm) from plant parts like root, stem, and leaves of plant growing in the presence or absence of chelate and inoculated on the nutrient agar plate containing 50 mg $L^{-1}$ nickel chloride hexa-hydrated salt (Khan et al. 2017) The plates were incubated at 37 °C for 24 h (de Oliveira Costa et al. 2012). The pure colonies were obtained by inoculating a single colony on the separate nutrient agar plate containing 50 mg $L^{-1}$ of nickel chloride hexa-hydrated salt and incubated at 37 °C for 24 h (Rani et al. 2010).

## Minimum inhibitory concentration (MIC) analysis

The slice was done from all parts like root, stem, and leaves in the presence or absence of chelate, and based on a morphological study the different strains were further studied. The minimum inhibitory concentration (MIC) of the

isolated endophytic bacterial strains was examined to determine nickel resistance with an initial nickel concentration of 50 mg $L^{-1}$ and later increased to 1000 mg $L^{-1}$. Nickel-resistant bacteria were inoculated in nutrient broth having nickel chloride in a range of 50–1000 mg $L^{-1}$ and incubated at 37 °C for 11 days at 100 rpm in an orbital incubator shaker. The optical density (O.D.) was taken at 600 nm at a regular interval of 24 h using UV-Vis Spectrophotometer, Dynamica Halo DB (Marzan et al. 2017).

## Molecular identification of isolated strains using 16 S rRNA

The partial sequencing of 16 S rRNA of isolated strains Strain nickel-1 (SNi-1), Strain nickel-1 (SNi-2), Strain nickel-1 (SNi-3), Chelated strain nickel-1 (CSNi-1), Chelated Strain nickel-2 (CSNi-2) & Chelated Strain nickel-3 (CSNi-3) were analyzed commercially by DNA Sequencing Service, Xcelris Labs Ltd. Premchand Nagar Road, Bodakdev, Ahmedabad 380054, India. Isolated DNA was amplified with *16 S rRNA* specific primer (8 Forward (8F) and 1492 Reverse (1492R) using Veriti® 96 well Thermal Cycler (Model No. 9902). The PCR amplicon was enzymatically purified and further subjected to Sanger Sequencing. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with 8 F and 1492 R primers using the BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyser. The consensus sequence of 1369 bp. *16 S rDNA* was generated from forward and reverse sequence data using aligner software. The 16 S rDNA sequence was used to carry out the BLAST alignment search tool of the NCBI Genbank database. Based on the maximum identity score first fifteen sequences were selected and aligned using the multiple alignment software program ClustalW. The distance matrix was generated using the RDP database and the phylogenetic tree was constructed using MEGA 7 software.

## Nickel biosorption analysis using isolated endophytic strains

Nickel biosorption analysis of isolated bacterial strain was performed using dry bacterial biomass for biosorption of nickel (100–600 mg $L^{-1}$) from the metal solution because at studied range isolated strains had shown efficient growth and also demonstrated the positive effect of biosorption on increasing metal ion concentration. Biomass of endophytic bacterial strains was produced by growing in 500 mL of the conical flask containing 250 mL of nutrient broth (pH 7) at 37 °C and 150 rpm in an orbital incubator shaker for 7 days. The cells from the broth were harvested using centrifugation at 5724 g for 20 min. The obtained cell pellets were washed three times with distilled water. The dry biomass was obtained by drying at 90 °C in the oven and obtained constant dry biomass was used for heavy metal absorption studies (Oves et al. 2012). The analysis of biosorption of heavy metal was done at a range of 100–600 mg $L^{-1}$ from the stock solution (10,000 mg $L^{-1}$) of nickel. The biosorption of nickel using endophytic strains was determined by batch equilibrium methods (Khodaverdiloo and Samadi 2011). The experiment was performed in 250 mL of the conical flask containing 100 mL of 100, 300, & 600 mg $L^{-1}$ of metal ion solution. The constant dry bacterial biomass was exposed to the metal solution and incubated at 160 rpm in an orbital incubator shaker for 72 h. After completion of the incubation period, the biomass was filtered using filter paper (Whatman 42). Both filter paper with and without filtration was analyzed in AAS and the filtered solution was analyzed to cross-check the overall result. The filtered solution and biomass were analyzed by atomic absorption spectrophotometer (AAS) Perkin Elmer Analyst 400 (Rani et al. 2010). The measurement of the amount of metal-bound by the biosorbent (bacterial biomass) was calculated by:

$$Q = V(Ci - Cf)/M \quad (1)$$

Where, Q - Metal ion uptake capacity (mg $g^{-1}$); $C_i$ – initial concentration of metal in solution before the sorption analysis (mg $g^{-1}$); $C_f$ - final concentration of metal in solution after the sorption analysis (mg $g^{-1}$); M - Dry weight of bio sorbent (g); V - Solution volume (L).

Metal bound to the biosorbent is equal to the difference between initial and final metal ion concentration in the solution (Oves et al. 2012). The adsorption of metal by biosorbent (dry bacterial biomass) was calculated according to Burrell (1975), Rahman et al. (2019), and Torabia and Kardel (2019).

## Adsorption isotherms

It describes the uptake of metal in per unit adsorbent mass ($q_e$) to the adsorbate equilibrium concentration ($C_e$) at a fixed temperature. In this work, Freundlich, Langmuir, and Temkin isotherms model were used to study adsorption mechanism.

### Langmuir isotherm

Langmuir isotherm model explains maximum adsorption capacity on the adsorbent surface as monolayer coverage. The Langmuir isotherm equation:

$$1/q_e = 1/q_m + 1/(q_m * K_L) + 1/C_e \quad (2)$$

Where, $q_m$ - maximum adsorption capacity constant (mg $g^{-1}$); $K_L$ - adsorption/desorption energy constant(L $mg^{-1}$); $q_e$ - metal adsorbed per unit mass (mg $g^{-1}$); $C_e$ - equilibrium concentration (mg $L^{-1}$) (Hameed et al. 2007; Senthil Kumar et al. 2010).

### Freundlich isotherm

Freundlich isotherm model describes the relation between equilibrium concentration ($C_e$) and absorbed metal per unit of mass ($q_e$). It assumes that in the adsorption process there are varying sites for adsorption energies (Agyei et al. 2000; Febrianto et al. 2009). The Freundlich isotherm equation:

$$Logq_e = \log K_f + 1/n \log C_e \quad (3)$$

Where n and $K_f$ - Freundlich coefficients/constants.

#### 2.7.3 Temkin isotherm

Temkin isotherm corresponds to the relations between the adsorbent of the biosorption system and adsorbate. It explains the interaction of heat of adsorption and the metal ions adsorbed (A.O 2012; Rajappa et al. 2015) The Temkin isotherm equation is as mentioned below:

$$q_e = RT/b(\ln A) + RT/b\,(C_e) \quad (4)$$

$$q_e = B \ln A + B \ln C_e \quad (5)$$

Where, A & B - Temkin isotherm constant (L $g^{-1}$); B = RT/b; T - temperature (°C); R - gas constant (8.314 J $mol^{-1}$ $K^{-1}$) (Mishra et al. 2017).

### PGP activities of isolated endophytic strains

The PGP activities of endophytic bacterial isolates were analyzed by the production of IAA and EPS in vitro. The Indole acetic acid produced by symbiotic bacteria is an important phytohormone signaling microbe-plant interaction, being therefore essential for rhizoremediation. The IAA production studies were performed by growing the isolates in Luria broth (LB) supplemented with L-tryptophan (0.0005 mg $L^{-1}$). The cells were harvested after 5 days of incubation at 37 °C using centrifugation at 3220 g for 5 min. An inoculum supernatant (2 mL) was mixed with 100 µL of 10 mM orthophosphoric acid and 4 mL of Salkowski's reagent (2% of 0.5 M $FeCl_3$ in 35% $HClO_4$). The absorbance of the pink colour developed after 30 min was recorded at 530 nm. The concentration of IAA was determined from the standard curve of pure IAA as standard following regression analysis (Das et al. 2014, 2016).

The EPS production ability of the endophytic bacterial strains was also monitored. The bacterial strains were inoculated in basal salt medium containing (g $L^{-1}$) 1.0 yeast extract, 0.14 $MgSO_4$, $7H_2O$, 0.3 $(NH_4)_2SO_4$, 0.1 NaCl, 0.2 $CaCl_{2\cdot2}H_2O$, 10.0 glucose and 0.6 mg $L^{-1}$ $H_3PO_3$ containing 5% sucrose. The endophytic bacterial strains were incubated at 37 °C on a rotary shake at 130 rpm for 72 h. The cells were harvested by centrifugation at 15,000 × *g* for 30 min, the supernatant was collected and a double volume of ice-cold ethanol (95%) was added and mixed uniformly. The reaction mixture was kept static at 4 °C for 24 h. The precipitated EPS was aggregated by centrifugation at 18,000 × *g* for 30 min at 4 °C and repeatedly cleaned with 95% ethanol. The dry weight of the washed EPS was taken after drying overnight at room temperature (Kazy et al. 2002).

### Nickel removal from electroplating industrial effluent

The experiment was performed in 250 mL of the conical flask containing 100 mL of effluent. The characterization (BOD, COD, pH, electrical conductivity, and TDS) of effluent was also performed (Table 1). The constant dry biomass was exposed to the nickel electroplating industrial effluent and incubated at 160 rpm in an orbital shaker incubator for 72 h. After incubation, biomass was filtered using filter (Whattman 42) paper. The metal content in the filtered solution adsorbed by biomass was analyzed using atomic absorption spectrophotometer (AAS) (Rani et al. 2010). The adsorption of metal bound to biosorbent (dry bacterial biomass) was calculated according to Burrell (1975), Rahman et al. (2019), and Torabia and Kardel (2019).

## Results & discussion

### Soil sample analysis

The soil used for growing the plants was basic with containing 560.4 ± 1.3 mg $kg^{-1}$ nitrogen and 796.1 ± 6.3 mg $kg^{-1}$ calcium. Various physiochemical properties such as pH, electrical conductivity, total dissolved solids (TDS) indicate that the soil quality is good enough for agriculture purposes (Table 2).

**Table 1** Physicochemical parameters of the Ni electroplating effluent

| Sample | BOD (mg $L^{-1}$) | COD (mg $L^{-1}$) | pH | Electrical conductivity (ms $cm^{-1}$) | Total dissolved solids (ppt) |
|---|---|---|---|---|---|
| Nickel electroplating effluent | 643 | 3000 | 3.8 | 2.5 | 1.3 |

**Table 2** Physiochemical analysis of garden soil

| Parameters | Data |
|---|---|
| pH | 7.8 ± 0.06 |
| EC (μs $cm^{-1}$) | 850.1 ± 3.2 |
| TDS (mg $L^{-1}$) | 414.9 ± 2.9 |
| Chloride (mg $g^{-1}$) | 2.5 ± 0.014 |
| Salinity (g $kg^{-1}$) | 4.6 ± 0.07 |
| Organic carbon (%) | 0.2 ± 0.001 |
| Total hardness (mg $L^{-1}$) | 500.0 ± 4.2 |
| Calcium (mg $kg^{-1}$) | 796.1 ± 6.3 |
| Magnesium (mg $kg^{-1}$) | 243.4 ± 2.2 |
| Sodium (mg $kg^{-1}$) | 274.1 ± 1.8 |
| Potassium (mg $kg^{-1}$) | 73.3 ± 0.42 |
| Lithium (mg $kg^{-1}$) | 2.9 ± 0.016 |
| Phosphorous (mg $kg^{-1}$) | 9.5 ± 0.063 |
| Sulphur (mg $kg^{-1}$) | 194.7 ± 1 |
| Available nitrogen (mg $kg^{-1}$) | 560.4 ± 1.3 |
| Cation exchange capacity (meq100$g^{-1}$) | 3.2 ± 0.029 |

## Hyperaccumulation of nickel by *Vigna radiata* plant

The plant *Vigna radiata* was reported to be a hyperaccumulator for nickel (Samantaray et al. 1998; Ahmad et al. 2007). As the plant attains its maturity it was observed that the nickel is transferred to leaf (31.528 ± 0.16 mg $kg^{-1}$) from soil through root and stem. A low amount of nickel was also found in the stem part (14.247 ± 0.057 mg $kg^{-1}$) of the plant, which indicates its transfer route. Ishtiaq and Mahmood (2011) also observed nickel was transported from soil to plant tissue and a steady increase in nickel content was observed in root and shoot tissue. Similar observations were also made by Jagetiya et al. (2013), where nickel contents in the plant organs increased linearly with different concentrations of nickel supply. Nickel was accumulated primarily in roots, showing up to 40-time high concentrations than upper parts of the plant.

## Minimum inhibitory concentration (MIC) analysis of isolated endophytic strains

The microbes were grown in a medium supplemented with different concentrations of nickel (50–1000 mg $L^{-1}$). With an increase in concentration, a sharp decrease in the growth of isolated strains was observed from 48 to 72 h whereas after 72 h, increases in the microorganism growth was observed in all the cases and further increases may be due to the adaptation of the isolates to the metal present in the media. Similarly, Arifiyanto et al. (2017), observed that during the initial hours there was a sharp decline in the growth curve and considered as adaptation phase in the presence of lead. Under all nickel concentrations, CSNi-3 showed maximum growth compared to other strains. The strains CSNi-1, CSNi-2, CSNi-3 which were isolated from the plant grown in soil containing EDTA chelator had better growth as compared to the strains SNi-1, SNi-2, and SNi-3 i.e., isolated from the non-chelator plant. All isolated strains exhibited growth in the presence of a nickel concentration of 200–600 mg $L^{-1}$. A slight decline in the growth after 72 h was observed, however, between 96–144 h the growth increased sharply (Fig. 2). Figure 2(a–f) shows a growth curve for all six strains at 800 and 1000 mg $L^{-1}$ respectively, all the six strains showed growth at high nickel concentration. However, the growth obtained was not as comparable to the 600 mg $L^{-1}$ nickel concentration. Thus, it can be suggested that all strains were tolerant to high nickel concentration. The two strains SNi-1 and CSNi-2 showed maximum growth up to 1000 mg $L^{-1}$ nickel concentration. Devika (2014) observed that *Pseudomonas aeruginosa* was able to grow at 1000 mg $L^{-1}$ nickel concentration and was also able in nickel removal from the solution effectively by 90%. This may be explained by some alternative pathway may have become functioning to efficiently regulate growth in presence of a high concentration of nickel (Tognacchini et al. 2020). Thus, the isolated strains can be effectively used for bioremediation of heavy metal contamination.

## Molecular Identification of the isolated strains by 16 S rRNA

Total six (6) different types of endophytic nickel tolerant bacterial strains were isolated from the *Vigna radiata* (green gram) plant. Three (SNi-1, SNi-2, SNi-3) from nickel supplemented plant and three (CSNi-1, CSNi-2, and CSNi-3) from nickel and chelator EDTA supplemented plant. The bacterial isolates were subjected to the molecular identification and based on the phylogenetic analysis of the obtained sequence, it was suggested that SNi-1, SNi-2, SNi-3 showed 99% similarity with *Pseudomonas* sp. strain PrPy123 (Accession no- MF948922.1), *Bacillus cereus* strain L90 (KC428751.1), and *Paraburkholderia fungorum* strain OX1403 (Accession no- MG576013.1.) respectively (Fig. 3a, b, c). Similarly, CSNi-1 CSNi-2 CSNi-3 showed maximum similarity (99%) with *Burkholderia fungorum* strain TN (Accession no- KJ933410.1.), *Bacillus safensis* strain SML_M178 (Accession no- MG937695.1), and *Bacillus subtilis* strain 22 (Accession no- FJ435215.1) respectively (Fig. 3d, e, f). Li et al. (2012) also suggested that a wide range of metal-resistant endophytic microbes has been isolated from a wide variety of heavy metal hyperaccumulators and non–hyperaccumulators plants. Similarly, Barzanti et al. (2007) demonstrated that nickel tolerant endophytic *Bacillus* and *Pseudomonas* were isolated from the hyperaccumulator plant *Alyssum bertolonii*. Also, endophytic metal resistance (Cu, Cd, Cr) *Bacillus*

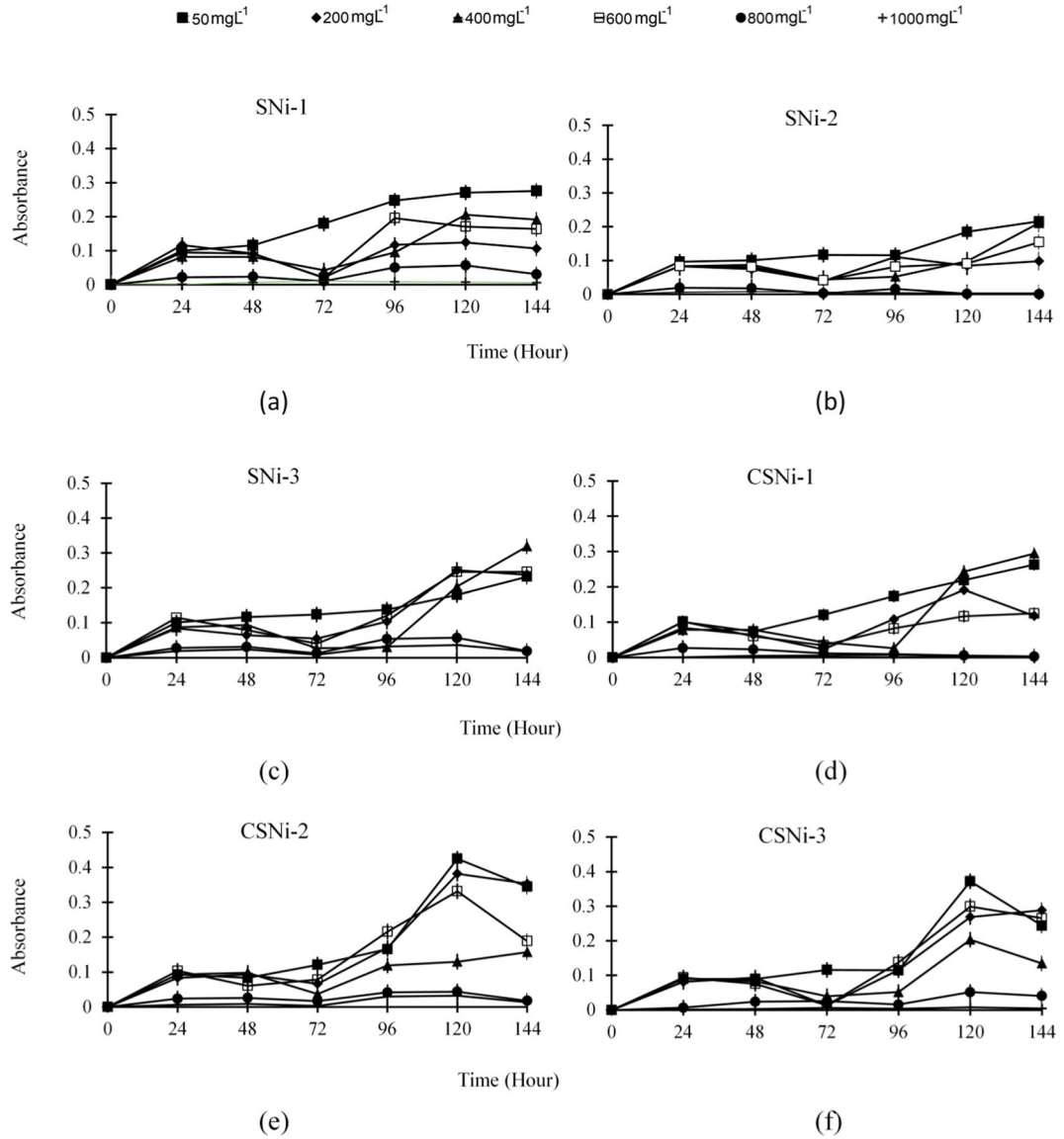


**Fig. 2** The graphical representation of the growth of strains in term of absorbance at different concentration (100–1000 mg $L^{-1}$) of nickel in the medium (**a**) SNi-1, (**b**) SNi-2, (**c**) SNi-3, (**d**) CSNi-1, (**e**) CSNi-2, (**f**) CSNi-3

strains were isolated from leaf, stem, and roots of metal hyperaccumulator plant *Solanum nigrum* plant (Guo et al. 2010).

## Nickel biosorption of synthetic nickel solution using isolated endophytic strains

The isolated endophytic strains were subjected to the biosorption using synthetic nickel (Ni) solution of different concentrations (100, 300, 600 mg $L^{-1}$). All the strains were capable of biosorption of nickel from solution with different strains showing maximum potential at different nickel concentrations. For e. g. *Bacillus subtilis* (68 ± 0.19 mg $L^{-1}$) at 100 mg $L^{-1}$ concentration, *Bacillus safensis* (251 ± 0.32 mg $L^{-1}$) at 300 mg $L^{-1}$, and *Bacillus safensis* (548.0 ± 2.4 mg $L^{-1}$) at 600 mg $L^{-1}$. Similarly, *B. cereus, Bacillus* spp. and *B. altitudinis* (Abd Elhady et al. 2020) used for the treatment of sterile industrial wastewater resulted in biosorption potential for the six different heavy metals i.e. $Zn^{+2}$, $Fe^{+2}$, $Co^{+2}$, $Cd^{+2}$, $Cu^{+2}$ and $Pb^{+2}$. *Microbacterium oxydans* have a bioremediation potential of copper and nickel as demonstrated by Heidari et al. (2020). They also suggested that microbial assisted bioremediation of heavy metal can be a natural way for metal removal with high potential. In the present study, the percentage of biosorption increased gradually with the increase in metal concentration from 100 to 600 mg $L^{-1}$. Oves et al. (2012) reported that *Bacillus thuringiensis* at a high concentration of metal showed lower absorption yield but in the present work, the biosorption percentage increased as the metal concentration

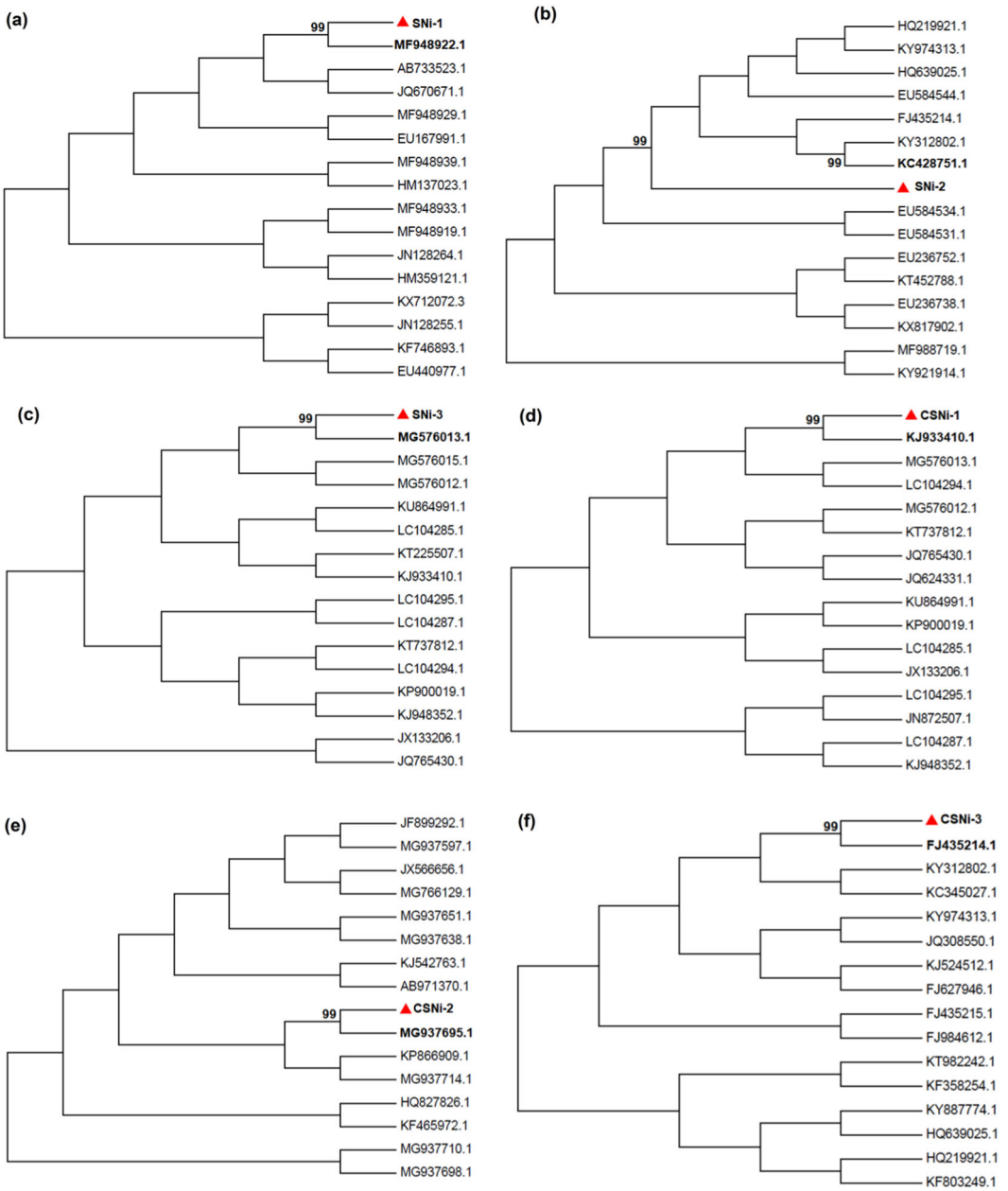


**Fig. 3** Phylogenetic tree of identified bacterial strains; (**a**) SNi-1, (**b**) SNi-2, (**c**) SNi-3, (**d**) CSNi-1, (**e**) CSNi-2, (**f**) CSNi-3

increased from 100 to 600 mg $L^{-1}$. Ni concentration (100 mg $L^{-1}$) maximum biosorption percentage was 68.5 ± 0.52% by *B. subtilis*, whereas in the case of *Bacillus safensis* the percentage was 83.7 ± 0.44 and 91.3 ± 0.72% at 300 and 600 mg $L^{-1}$ respectively (Table 3). In the study by Wang et al. (2020), *Bacillus* sp. shown a high (74.11 ± 3.01%) adsorption percentage of nickel from synthetic nickel solution. Thus, suggesting *Bacillus* sp. can be efficiently used toward nickel removal from the pollutants. Also, in the present study other isolated strains were capable of nickel absorption with comparable efficiency from the synthetic metal solutions, therefore, were further checked for the biosorption of nickel from the nickel electroplating effluents. The adsorption isotherms were also evaluated for the adsorption potential of nickel from the synthetic nickel salt solution.

## Adsorption isotherms for nickel biosorption

### Langmuir isotherm for nickel biosorption

The Langmuir isotherm data were plotted as $1/C_e$ as X-axis and $1/q_e$ as Y-axis according to the obtained data from all isolated strains (Fig.4 a–f). $R^2$, $K_L$ (L $mg^{-1}$), and

**Table 3** Biosorption of nickel in the synthetic nickel salt solution at different concentration

| Isolated strains | Biosorption/biomass (mg $L^{-1}$) | Metal ion uptake capacity (mg $g^{-1}$) | Biosorption | % |
|---|---|---|---|---|
| **100 mg $L^{-1}$** | | | | |
| *Pseudomonas sp.* | 62.3 ± 0.09 | 3.114 ± 0.016 | 3.0 ± 0.012 | 62.3 ± 0.23 |
| *B. cereus* | 65.1 ± 0.12 | 3.254 ± 0.021 | 2.8 ± 0.008 | 65.1 ± 0.37 |
| *P. fungorum* | 66.8 ± 0.26 | 3.342 ± 0.017 | 2.6 ± 0.005 | 66.8 ± 0.45 |
| *B. fungorum* | 63.3 ± 0.33 | 3.165 ± 0.024 | 2.9 ± 0.015 | 63.3 ± 0.66 |
| *B. safensis* | 65.5 ± 0.42 | 3.275 ± 0.009 | 2.8 ± 0.022 | 65.5 ± 0.49 |
| *B. subtilis* | 68.5 ± 0.19 | 3.425 ± 0.038 | 2.5 ± 0.009 | 68.5 ± 0.52 |
| **300 mg $L^{-1}$** | | | | |
| *Pseudomonas sp.* | 249.6 ± 1.6 | 12.482 ± 0.032 | 4.0 ± 0.039 | 83.2 ± 0.39 |
| *B. cereus* | 251.0 ± 1.9 | 12.549 ± 0.068 | 3.9 ± 0.018 | 83.7 ± 0.21 |
| *P. fungorum* | 249.6 ± 0.07 | 12.480 ± 0.097 | 4.0 ± 0.012 | 83.2 ± 0.68 |
| *B. fungorum* | 250.4 ± 0.11 | 12.520 ± 0.042 | 4.0 ± 0.024 | 83.5 ± 0.61 |
| *B. safensis* | 251.2 ± 0.32 | 12.561 ± 0.062 | 3.9 ± 0.011 | 83.7 ± 0.44 |
| *B. subtilis* | 250.1 ± 2.2 | 12.504 ± 0.026 | 4.0 ± 0.033 | 83.4 ± 0.38 |
| **600 mg $L^{-1}$** | | | | |
| *Pseudomonas sp.* | 544.8 ± 2.2 | 27.2 ± 0.11 | 4.4 ± 0.034 | 90.8 ± 0.17 |
| *B. cereus* | 547.0 ± 3.6 | 27.4 ± 0.06 | 4.2 ± 0.040 | 91.2 ± 0.62 |
| *P. fungorum* | 546.7 ± 0.7 | 27.3 ± 0.18 | 4.3 ± 0.025 | 91.1 ± 0.59 |
| *B. fungorum* | 547.4 ± 4.4 | 27.37 ± 0.091 | 4.2 ± 0.037 | 91.2 ± 0.28 |
| *B. safensis* | 548.0 ± 2.4 | 27.40 ± 0.31 | 4.2 ± 0.026 | 91.3 ± 0.72 |
| *B. subtilis* | 546.6 ± 5.6 | 27.33 ± 0.22 | 4.3 ± 0.044 | 91.1 ± 0.64 |

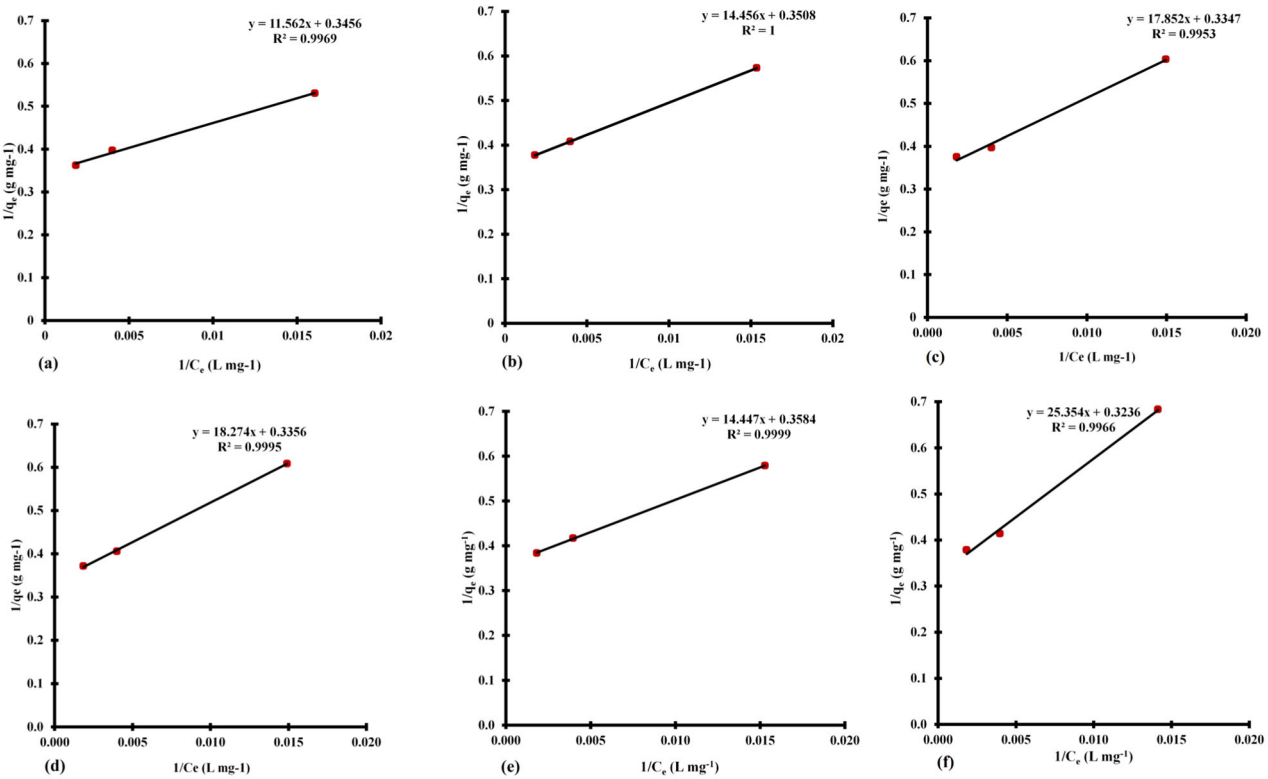


**Fig. 4** Langmuir isotherm of nickel adsorption for isolated strains (**a**) *Pseudomonas* sp. (**b**) *B. cereus* (**c**) *P. fungorum* (**d**) *B. fungorum* (**e**) *B. safensis* (**f**) *B. subtilis*

**Table 4** Adsorption isotherms of nickel adsorption for isolated strains

| Langmuir isotherm | | | |
|---|---|---|---|
| Isolated strains | $R^2$ | $K_L$ (L $mg^{-1}$) | $q_m$ (mg $g^{-1}$) |
| *Pseudomonas sp.* | 0.997 | 2.894 | 0.029 |
| *B. cereus* | 0.999 | 2.851 | 0.024 |
| *P. fungorum* | 0.995 | 2.988 | 0.019 |
| *B. fungorum* | 0.999 | 2.980 | 0.018 |
| *B. safensis* | 0.999 | 2.790 | 0.025 |
| *B. subtilis* | 0.997 | 3.090 | 0.013 |
| Freundlich isotherm | | | |
| Isolated strains | $R^2$ | N | $K_f$ |
| *Pseudomonas sp.* | 0.983 | 5.571 | 1.102 |
| *B. cereus* | 0.963 | 4.948 | 1.309 |
| *P. fungorum* | 0.929 | 4.244 | 1.577 |
| *B. fungorum* | 0.957 | 4.119 | 1.650 |
| *B. safensis* | 0.969 | 5.023 | 1.310 |
| *B. subtilis* | 0.941 | 3.334 | 2.340 |
| Temkin isotherm | | | |
| Isolated strains | $R^2$ | B | A ($Lg^{-1}$) |
| *Pseudomonas sp.* | 0.992 | 0.409 | 1.625 |
| *B. cereus* | 0.974 | 0.435 | 1.097 |
| *P. fungorum* | 0.941 | 0.498 | 2.393 |
| *B. fungorum* | 0.971 | 0.513 | 2.970 |
| *B. safensis* | 0.981 | 0.423 | 1.030 |
| *B. subtilis* | 0.958 | 0.593 | 10.220 |

$q_m$ (mg $g^{-1}$) were calculated from the plot. Adsorption capacity ($q_m$) observed for isolated strains were as follow 0.0299 mg $g^{-1}$ (*Pseudomonas sp.*), 0.0243 mg $g^{-1}$ (*B. cereus*), 0.0187 mg $g^{-1}$ (*P. fungorum*), 0.018 mg $g^{-1}$ (*B. fungorum*), 0.025 mg $g^{-1}$ (*B. safensis*), and 0.013 mg $g^{-1}$ (*B. subtilis*). Similarly, adsorption energy ($K_L$) for isolated strains were 2.893 L $mg^{-1}$ (*Pseudomonas sp.*), 2.851 L $mg^{-1}$ (*B. cereus*), 2.988 L $mg^{-1}$ (*P. fungorum*), 2.980 L $mg^{-1}$ (*B. fungorum*), 2.790 L $mg^{-1}$ (*B. safensis*), 3.090 L $mg^{-1}$ (*B. subtilis*). Langmuir correlation coefficient ($R^2$) is higher than other models of adsorption i.e. 0.997(*Pseudomonas* sp.), 0.999 (*B. cereus*), 0.995 (*P. fungorum*), 0.999 (*B. fungorum*), 0.999 (*B. safensis*), 0.997 (*B. subtilis*). Thus, according to $R^2$ value Langmuir isotherm is found to be the most suitable isotherm model than Freundlich and Temkin isotherm model for explaining the work in biosorption of nickel by bacterial strains (Table 4). Mulik et al. (2018) observed that *Kocuria* sp. BRI 36 showed the ability for $Cr^{3+}$ and $Ni^{2+}$ adsorption from the aqueous solution. They observed the $q_m$ for Ni in the case of *Kocuria* sp. was 10.41 mg $g^{-1}$. The work suggested the application of *B subtilis* as an adsorbent for the removal of Ni (II) from an aqueous solution. Equilibrium adsorption isotherms and kinetics were also investigated suggesting Freundlich and Langmuir models are suitable models to explain the absorption capacity. Bulut et al. (2012) also suggested that the biosorption of nickel also follows Langmuir isotherm for biosorption than other isotherms.

### Freundlich isotherm for nickel biosorption

The plot was made between log$C_e$ as X-axis *vs*. log $q_e$ as Y-axis (Fig. 5a–f). Freundlich constant ($K_f$) is an indicator of the adsorption capacity and $K_f$ observed for isolated strains were 1.102 (*Pseudomonas* sp.), 1.309 (*B. cereus*), 1.577 (*P. fungorum*), 1.650 (*B. fungorum*), 1.310 (*B. safensis*), and 2.340 (*B. subtilis*). The value of n is between 1 to 10 indicates favourable absorption intensity (Sonawdekar and Gupte 2020). According to Oves et al. (2012), low $K_f$ values indicates lower heavy metal adsorption and higher $K_f$ value indicates more adsorption. It was observed that $K_f$ constant (0.153) for biosorption of nickel by

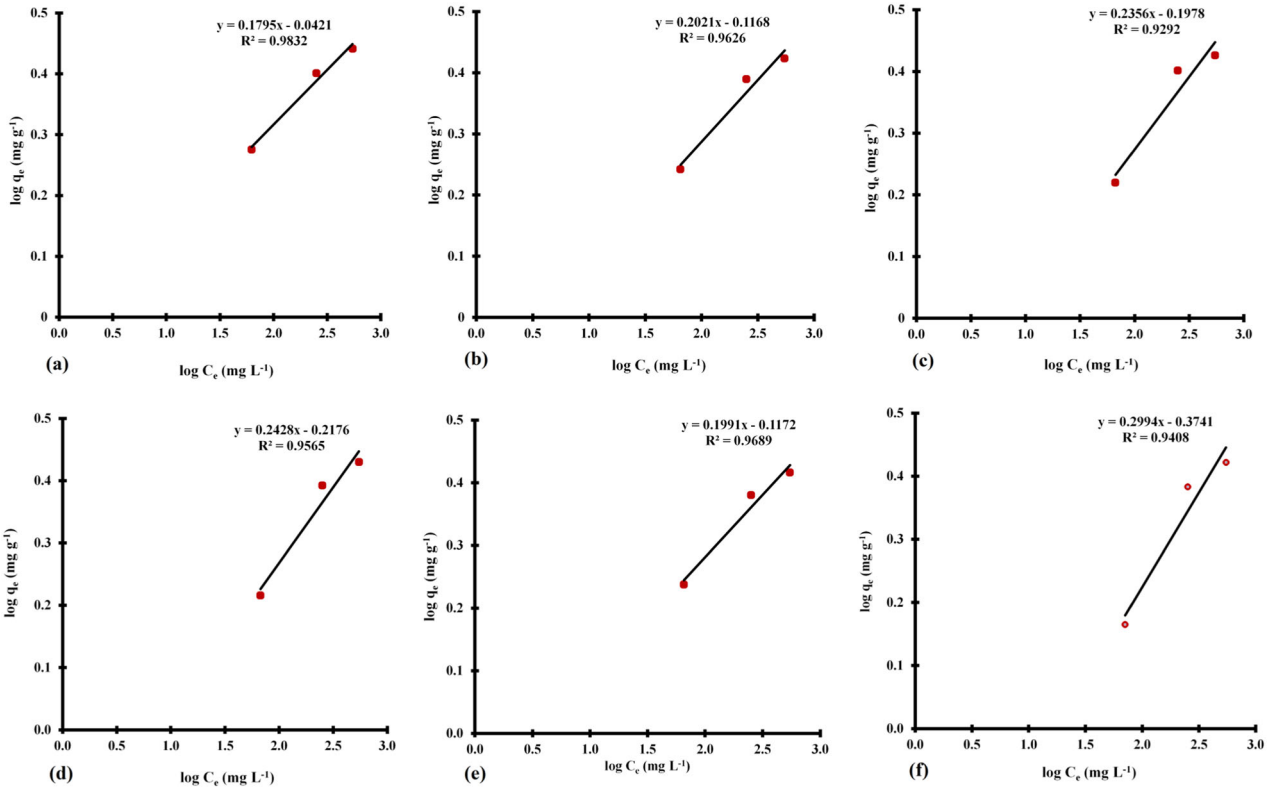


**Fig. 5** Freundlich isotherm of nickel adsorption for isolated strains (**a**) *Pseudomonas* sp. (**b**) *B. cereus* (**c**) *P. fungorum* (**d**) *B. fungorum* (**e**) *B. safensis* (**f**) *B. subtilis*

*Bacillus thuringiensis* strain OSM29 isolated from north Indian soil contaminated by industrial effluent is lower than *P. fungorum* (1.577), *B. fungorum* (1.650), & *B. subtilis* (2.340) in our study. In present study highest $K_f$ value for *B. subtilis* (2.340) is highest. Thus, suggested high nickel absorption and it also supported the absorption percentage as observed in case of treatment of synthetic nickel salt solution (Table 3) and nickel electroplating (Table 6).

### Temkin isotherm for nickel biosorption

The determination of Temkin isotherm was performed by plotting $q_e$ as Y-axis versus lnCe as X-axis to obtain constant A and B (Fig. 6a–f). The value of Temkin isotherm constant A for isolated strains were 1.625 (*Pseudomonas* sp.), 1.097 (*B. cereus*), 2.393 (*P. fungorum*), 2.970 (*B. fungorum*), 1.030 (*B. safensis*), and 10.220 (*B. subtilis*). Similarly, Temkin isotherm constant B for isolated strains were 0.409 (*Pseudomonas* sp.), 0.435 (*B. cereus*), 0.498 (*P. fungorum*), 0.513 (*B. fungorum*), 0.423 (*B. safensis*), and 0.593 (*B. subtilis*). Thus, *B. subtilis* has maximum constant value among others. The correlation coefficient is comparatively lower than Langmuir isotherm. Therefore, biosorption equilibrium cannot be observed according to Temkin isotherm (Bulut et al. 2012; Mulik et al. 2018).

The binding affinity of the metal ion is also explained based on these equilibrium adsorption isotherms and it depends on the functional group present on the capsule or wall of bacterial biomass i.e., the negatively charged functional group of bacterial wall hold on the positively charged cation. The groups which interact with metal ions in the cell wall of bacteria are hydroxyl carbonyl, carboxyl, thioester, sulfonate, amines, etc. Like in the case of *Bacillus subtilis* $COO^-$, C=O, active carboxyl, phosphate, amide act as a functional group which plays a crucial role in deciding binding affinity for metal ions (Pan et al. 2007; Abdel-Monem et al. 2010) and is explained based on the Langmuir isotherm and Freundlich isotherm.

## Plant growth-promoting (PGP) activities of isolated bacteria strains

The strains were grown in a nickel-containing medium and analyzed for the IAA and EPS production ability. The maximum amount IAA was produced by *Bacillus cereus* strain ($33.9 \pm 0.25$ mg $L^{-1}$) followed by *Bacillus subtilis* strain ($26.3 \pm 0.18$ mg $L^{-1}$), *Bacillus safensis* ($19.8 \pm 0.049$ mg $L^{-1}$), and *Paraburkholderia fungorum* ($13.92 \pm 0.01$ mg $L^{-1}$) (Fig. 7). This indicated that the bacterial isolates were able to utilize L-tryptophan as a precursor to

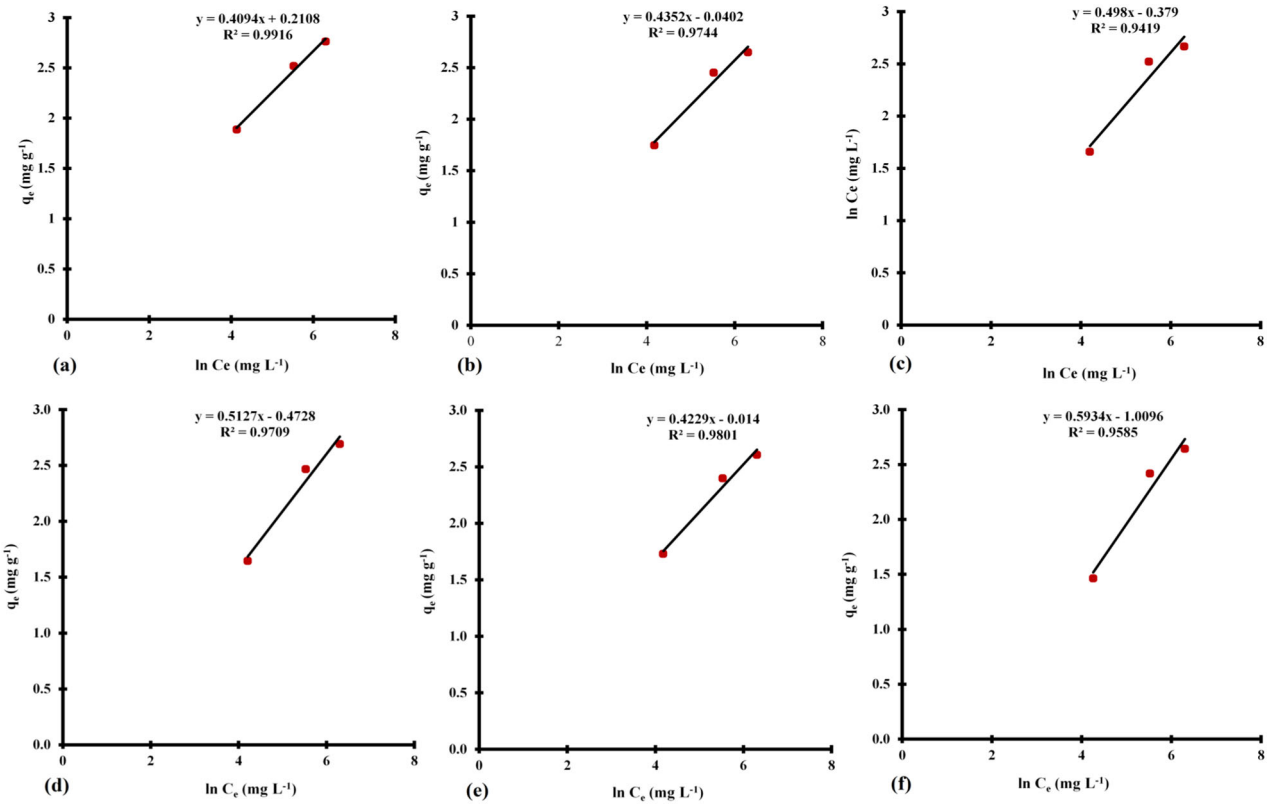


**Fig. 6** Temkin isotherm of nickel adsorption for isolated strains (**a**) *Pseudomonas* sp. (**b**) *B. cereus* (**c**) *P. fungorum* (**d**) *B. fungorum* (**e**) *B. safensis* (**f**) *B. subtilis*

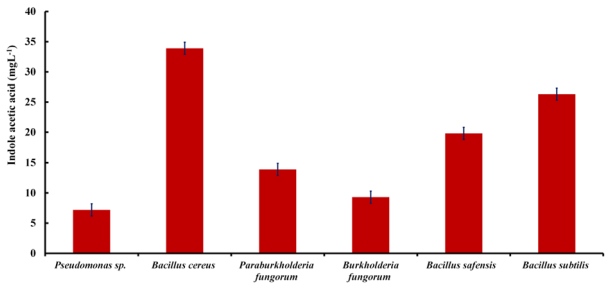


**Fig. 7** Indole acetic acid production potential of isolated strains

growth and IAA production and the minimum IAA (7.2 ± 0.037 mg $L^{-1}$) was obtained by *Pseudomonas sp*.

Similarly, Rajkumar and Freitas (2008), demonstrated that heavy metal plant growth-promoting bacterial strains could increase the growth of plants growing in zinc, nickel, and copper contaminated soil. Rana et al. (2020) also demonstrated that the inoculation of endophytic bacteria *Acinetobacter guillouiae* EUB2RT.R1 had led to an overall increase in wheat plant biomass. This endophytic presence of bacterial strain also led to increasing Zn and Fe content in the plant which are essential for plant growth. Zhu and She (2018) found that the isolates which belong to the genera of *Klebsiella, Kocuria*, and *Pantoea* showed the maximum potential to produce IAA, ranging from 17.09 to 58.66 g·$mL^{-1}$. They also observed that *Sporosarcina, Bacillus, Erwinia*, and *Pseudomonas* species isolates produced a very low amount of IAA, ranging from 1.21 to 9.88 g·$mL^{-1}$. Similarly, Chen et al. (2020) also observed that *Stenotrophomonas sp. S20* and *Pseudomonas sp. P21* as nickel resistant bacteria had a high IAA production potential of 18.0 and 28.5 mg $L^{-1}$ respectively.

Among the isolated strains *Bacillus cereus* strain had the highest production of EPS (368 ± 2.4 mg $L^{-1}$) in the absence of nickel. With the increase in nickel concentration, the production of EPS increased, but till 100 mg $L^{-1}$, after that, it started decreasing. *Pseudomonas sp*. and *Paraburkholderia fungorum* strains have shown high EPS production in the presence of 1000 mg$L^{-1}$ nickel as compared to the absence of nickel in the medium (Table 5). Sonawdekar and Gupte (2020) observed that *Bacillus cereus* sys1 produces exopolysaccharides efficiently and acts as a potential biosorbent for cadmium (II) and copper (II) metal ion from the solution. Microbial EPS binds to different metals with different degrees of affinity and specificity, which can facilitate the adaptation of bacteria to environmental stress and the ability of bacteria to remove metal from contaminated sites. The previous work was done by Bomfeti et al. (2011, also showed microbial production of EPS significantly enhanced plant growth by facilitating biological nitrogen fixation and fulfilling the carbohydrate

**Table 5** Exopolysaccharide production by isolated strains at different nickel salt concentration

| Strains | Nickel doses | Exopolysaccharide (L $mg^{-1}$) at different Ni doses | | | |
|---|---|---|---|---|---|
| | | Control | 100 L $mg^{-1}$ | 500 L $mg^{-1}$ | 1000 L $mg^{-1}$ |
| *Pseudomonas sp.* | | 356 ± 3.2 | 380 ± 2.2 | 364 ± 3.4 | 360 ± 1.2 |
| *Bacillus cereus* | | 368 ± 2.4 | 404 ± 3.6 | 396 ± 2.8 | 340 ± 2.9 |
| *Paraburkholderia fungorum* | | 308 ± 1.6 | 352 ± 1.9 | 344 ± 2.3 | 316 ± 0.9 |
| *Burkholderia fungorum* | | 336 ± 1.9 | 364 ± 1.5 | 312 ± 3.4 | 268 ± 0.4 |
| *Bacillus safensis* | | 312 ± 2.6 | 328 ± 3.8 | 324 ± 0.7 | 292 ± 1.3 |
| *Bacillus subtilis* | | 352 ± 0.6 | 372 ± 2.9 | 324 ± 1.4 | 308 ± 3.1 |

**Table 6** Biosorption of nickel from nickel electroplating effluent using the isolated strains

| Strains | Biosorption per Biomass | | Biosorption |
|---|---|---|---|
| | (mg $L^{-1}$) | (mg $g^{-1}$) | % |
| *Pseudomonas sp.* | 913.40 ± 1.10 | 45.7 ± 0.12 | 52.0 ± 0.49 |
| *B. cereus* | 1003.50 ± 0.90 | 50.2 ± 0.27 | 57.2 ± 0.62 |
| *P. fungorum* | 938.40 ± 1.60 | 46.9 ± 0.34 | 53.5 ± 0.23 |
| *B. fungorum* | 879.10 ± 1.20 | 44.0 ± 0.06 | 50.1 ± 0.26 |
| *B. safensis* | 754.10 ± 0.70 | 37.7 ± 0.14 | 43.0 ± 0.41 |
| *B. subtilis* | 772.20 ± 0.60 | 38.6 ± 0.32 | 44.0 ± 0.53 |

Nickel concentration in untreated effluent 1791 ± 0.0

requirement of the plant. The ability of the isolates capable to produce high IAA and EPS and subsequently helps plants growth even under high nickel concentrations in soil. Also, the ability of isolates to produce EPS helps in the biosorption of the heavy metal from waste effluents thus all isolates were tested for the adsorption of nickel from nickel electroplating effluents.

## Biosorption of nickel from nickel electroplating industrial effluent

All the strains were able to remove nickel from the effluent in range of 43 ± 0.41 - 57.2 ± 0.62% with *B. cereus* showing maximum biosorption by biomass 50.2 ± 0.27 mg/g i.e., 57.2 ± 0.62% of total nickel present in the effluent (Table 6). The order of nickel removal from electroplating effluent for different isolates are as follows *B. cereus* (57.2 ± 0.62%) > *P. fungorum* (53.5 ± 0.23%) > *Pseudomonas sp.* (52 ± 0.49%) > *B. fungorum* (50.1 ± 0.26%) > *B. subtilis* (44 ± 0.53%) > *B. safensis* (43 ± 0.41%). Nickel is the main constituent in the electroplating industrial effluent and effluent has a high concentration of nickel which decreases biosorption percentage which was also observed in several earlier studies observed by Padmavathy et al. (2003), Gheethi et al. (2017). In the present study, all isolated endophytic strains remove nickel significantly.

*B. cereus* isolated from electroplating effluent by Naik et al. (2012) has shown the ability to remove chromium (Cr) and nickel (Ni) from electroplating effluent. They also observed that one of *Leucobacter* sp strain was able to remove 99% of nickel. Abdel-Monem et al. (2010) demonstrated that *P. cepacia* 120 S and *B. subtilis* 117 S could also be used for biosorption of nickel from the contaminated effluents without losing nickel removal capacity even at higher metal concentrations. At low concentrations of nickel biosorption by *P. cepacia* 120 S was higher (insert value) than *B. subtilis* 117 S. However, at high nickel concentrations in effluents, the metal removal efficiency of *B. subtilis* 117 S was much higher than *P. cepacia* 120 S.

Nickel electroplating industrial effluent has a high nickel concentration. With a high metal concentration in the solution the biosorption percentage decreases which was observed in several earlier studies observed by Padmavathy et al. (2003) and Gheethi et al. (2017). Aslam et al. (2020) observed Ni adsorption of 48.78% by *Stenotrophomonas sp.* MB339. Further increasing the nickel concentration to 500 mg $L^{-1}$ resulted in a 10% decrease in adsorption potential of *Stenotrophomonas sp.* MB339. Thus, suggesting saturation of adsorption sites of *Stenotrophomonas sp.* MB339. However, in the present study, even at a high concentration (1791 mg $L^{-1}$) of nickel in the effluent isolated strains were able to remove a major amount of nickel from the solution. Also, kinetic saturation for nickel ion is obtained at an equilibrium time of 72 h suggesting a consortium of these isolated endophytic strains can be used as a microbial embedded filtration unit for efficient removal of nickel from nickel electroplating effluents.

## Conclusion

Nickel tolerant bacterial endophytes i.e., *Pseudomonas* sp., *Bacillus cereus*, *Paraburkholderia fungorum, Burkholderia fungorum*, *Bacillus safensis*, *Bacillus subtilis* were isolated from the V*igna radiata* plant growing in nickel-containing soil. Efficient nickel biosorption was demonstrated by all isolated endophytic strains demonstrating high nickel resistance up to 1000 mg $L^{-1}$. The biosorption potential of all the strains was confirmed by its ability to produce IAA and EPS in nickel-contaminated

medium. The favourable absorption capacity and energy obtained by Langmuir isotherm and Freundlich isotherm for all the six isolates suggested that the ability of endophytic strains for nickel biosorption. The biosorption potential was observed in all the strains of synthetic wastewater. Furthermore, the isolated strains were explored for the treatment of nickel electroplating effluents and all strains were also capable to remove nickel from electroplating effluents. Thus, isolated strains can be used as a consortium or to develop an immobilized microbe-based filtration/treatment system for large-scale treatment of industrial effluents in the future.

**Authors contributions** PV has played a vital role in the conceptualization of research ideas. SK has conducted the laboratory work and prepared the rough draft of the manuscript. RC and BK have performed the formal analysis of the results and writing of the MS. The experimental, writing, and formal analysis were supervised by PV. Also, PV and RC roles are key in the acquisition of the financial supports for the project leading to this publication. All authors have approved the final version of the manuscript.

**Funding** This work was financially supported by the Department of Biotechnology, Government of India through Project BT/304/NE/TBP/2012 and BT/PR7333/PBD/26/373/2012. The work was also supported by the Department of Science and Technology through Project IFA/2012/EAS-05 and DST-FIST (SR/FST/LSI-676/2016). B. K. acknowledges Jawaharlal Nehru Memorial Fund, New Delhi, and CSIR-SRF for providing funding for Doctoral Studies.

## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** This article does not contain any studies with animals performed by any of the authors. This article does not contain any studies with human participants or animals performed by any of the authors.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

A.O D (2012) Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherms studies of equilibrium sorption of Zn 2+ unto phosphoric acid modified rice husk. IOSR J Appl Chem 3:38–45. https://doi.org/10.9790/5736-0313845

Abd Elhady MAE, Elsayed AI, Mohamed SH et al. (2020) Isolation and identification of heavy metal tolerant bacteria from sugarcane industrial wastewater. Appl Ecol Environ Res 18:1387–1406. https://doi.org/10.15666/aeer/1801_13871406

Abdel-Monem MO, Al-Zubeiry AHS, Al-Gheethi AAS (2010) Biosorption of nickel by *Pseudomonas cepacia* 120S and *Bacillus subtilis* 117S. Water Sci Technol 61:2994–3007. https://doi.org/10.2166/wst.2010.198

Agyei NM, Strydom CA, Potgieter JH (2000) An investigation of phosphate ion adsorption from aqueous solution by fly ash and slag. Cem Concr Res 30:823–826. https://doi.org/10.1016/S0008-8846(00)00225-8

Ahalya NT, Ramachandra V, Kanamadi R (2014) Biosorption of heavy metals. Res J Chem Environ 7:71–79. http://wgbis.ces.iisc.ernet.in/energy/water/paper/biosorption/biosorption.htm

Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257. https://doi.org/10.1016/j.biortech.2005.12.006

Ahmad MSA, Ashraf M (2012) Essential roles and hazardous effects of nickel in plants. Rev Environ Contam Toxicol 125–167. https://doi.org/10.1007/978-1-4614-0668-6_6

Ahmad MS, Hussain M, Saddiq R, Alvi AK (2007) Mungbean: a nickel indicator, accumulator or excluder?. Bull Environ Contam Toxicol 78(5):319–324. https://doi.org/10.1007/s00128-007-9182-y

Akhtar MJ, Ullah S, Ahmad I et al. (2018) Nickel phytoextraction through bacterial inoculation in *Raphanus sativus*. Chemosphere 190:234–242. https://doi.org/10.1016/j.chemosphere.2017.09.136

Arifiyanto A, Apriyanti FD, Purwaningsih P, et al. (2017) Lead (Pb) bioaccumulation; Genera *Bacillus isolate* S1 and SS19 as a case study. AIP Conf Proc 1854: https://doi.org/10.1063/1.4985394

Aslam F, Yasmin A, Sohail S (2020) Bioaccumulation of lead, chromium, and nickel by bacteria from three different genera isolated from industrial effluent. Int Microbiol 23:253–261. https://doi.org/10.1007/s10123-019-00098-w

Barzanti R, Ozino F, Bazzicalupo M et al. (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. Microb Ecol 53:306–316. https://doi.org/10.1007/s00248-006-9164-3

Bomfeti CA, Florentino LA, Guimarães AP et al. (2011) Exopolysaccharides produced by the symbiotic nitrogen-fixing bacteria of leguminosae. Rev Bras Ciência do Solo 35:657–671. https://doi.org/10.1590/S0100-06832011000300001

Bulewicz EM, Phillips LF, Sugden TM (1960) Determination of dissociation constants and heats of formation of simple molecules by flame photometry. Part 8.—Stabilities of the gaseous diatomic halides of certain metals. Trans. Faraday Soc. 1961(57):921–931. https://doi.org/10.1039/TF9615700921

Bulut Y, Gül A, Baysal Z, Alkan H (2012) Adsorption of Ni (II) from aqueous solution by *Bacillus subtilis*. Desalination Water Treat 49(1–3):74–80. https://doi.org/10.1080/19443994.2012.708201

Burrell DC (1975) Atomic spectroscopic analysis of heavy metal pollutants in water. Ann Arbor Sci Publ Inc, Ann Arbour, Mich. https://www.osti.gov/biblio/5883081-atomic-spectrometric-analysis-heavy-metal-pollutants-water

Chen J, Li N, Han S, et al (2020) Characterization and bioremediation potential of nickel-resistant endophytic bacteria isolated from the wetland plant *Tamarix chinensis*. FEMS Microbiol Lett 0–3. https://doi.org/10.1093/femsle/fnaa098

Compant S, Mitter B, Colli-Mull JG et al. (2011) Endophytes of Grapevine Flowers, Berries, and Seeds: Identification of Cultivable Bacteria, Comparison with Other Plant Parts, and Visualization of Niches of Colonization. Microb Ecol 62:188–197. https://doi.org/10.1007/s00248-011-9883-y

Corwin DL, Lesch SM (2005) Apparent soil electrical conductivity measurements in agriculture. Comput Electron Agric 46:11–43. https://doi.org/10.1016/j.compag.2004.10.005

Das S, Jean J-S, Chou M-L et al. (2016) Arsenite-oxidizing bacteria exhibiting plant growth promoting traits isolated from the rhizosphere of *Oryza sativa* L.: implications for mitigation of arsenic contamination in paddies. J Hazard Mater 302:10–18

Das S, Jean J-S, Kar S et al. (2014) Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for arsenic bioremediation. J Hazard Mater 272:112–120. https://doi.org/10.1016/j.jhazmat.2014.03.012

de Oliveira Costa LE, de Queiroz MV, Borges AC et al. (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*).

Braz J Microbiol 43:1562–75. https://doi.org/10.1590/S1517-838220120004000041

Devika M (2014) Bioremoval of Nickel Using *Pseudomonas aeruginosa*. Annu Res Rev Biol 4:538–546. https://doi.org/10.9734/arrb/2014/5410

Dipu S, Kumar AA, Thanga SG (2012) Effect of chelating agents in phytoremediation of heavy metals. Remediation 22:133–146. https://doi.org/10.1002/rem.21304

Egorova KS, Ananikov VP (2017) Toxicity of metal compounds: knowledge and myths. Organometallics 36:4071–4090. https://doi.org/10.1021/acs.organomet.7b00605

Engwa GA, Ferdinand PU, Nwalo FN, Unachukwu MN (2019) Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the Modern World - New Tricks for an Old Dog?, Ozgur Karcioglu and Banu Arslan, IntechOpen, https://doi.org/10.5772/intechopen.82511

Evangelou MW, Bauer U, Ebel M, Schaeffer A (2007) The influence of EDDS and EDTA on the uptake of heavy metals of Cd and Cu from soil with tobacco Nicotiana tabacum. Chemosphere 68 (2):345–53. https://doi.org/10.1016/j.chemosphere.2006.12.058

Febrianto J, Kosasih AN, Sunarso J et al. (2009) Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies. J Hazard Mater 162:616–645. https://doi.org/10.1016/j.jhazmat.2008.06.042

Garbisu C, Alkorta I (2001) Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236. https://doi.org/10.1016/S0960-8524(00)00108-5

Genchi G, Carocci A, Lauria G et al. (2020) Nickel: human health and environmental toxicology. Int J Environ Res Public Health 17:679

Gheethi AA, Efaq AN, Mohamed RM, et al (2017) Bio-removal of Nickel ions by *Sporosarcina pasteurii* and *Bacillus megaterium*, a Comparative Study. IOP Conf Ser Mater Sci Eng 226: https://doi.org/10.1088/1757-899X/226/1/012044

Govarthanan M, Lee GW, Park JH et al. (2014) Bioleaching characteristics, influencing factors of Cu solubilization and survival of *Herbaspirillum* sp. GW103 in Cu contaminated mine soil. Chemosphere 109:42–48. https://doi.org/10.1016/j.chemosphere.2014.02.054

Govarthanan M, Lee SM, Kamala-Kannan S, Oh BT (2015a) Characterization, real-time quantification and in silico modeling of arsenate reductase (arsC) genes in arsenic-resistant *Herbaspirillum* sp. GW103. Res Microbiol 166:196–204. https://doi.org/10.1016/j.resmic.2015.02.007

Govarthanan M, Park SH, Park YJ et al. (2015b) Lead biotransformation potential of allochthonous *Bacillus* sp. SKK11 with sesame oil cake extract in mine soil. RSC Adv 5:54564–54570. https://doi.org/10.1039/c5ra06945a

Guo H, Luo S, Chen L et al. (2010) Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium *Bacillus* sp. L14. Bioresour Technol 101:8599–8605. https://doi.org/10.1016/j.biortech.2010.06.085

Haluschak P (1981) Laboratory methods of soil analysis canada-manitoba soil survey. Methods Soil Anal. https://www.gov.mb.ca/agriculture/soil/soil-survey/pubs/laboratory_methods_of_soil_analysis.pdf

Hameed BH, Din ATM, Ahmad AL (2007) Adsorption of methylene blue onto bamboo-based activated carbon: Kinetics and equilibrium studies. J Hazard Mater 141:819–825. https://doi.org/10.1016/j.jhazmat.2006.07.049

Hasan MM, Uddin MN, Ara-Sharmeen I et al. (2019) Assisting phytoremediation of heavy metals using chemical amendments. Plants 8:1–14. https://doi.org/10.3390/plants8090295

Heidari P, Mazloomi F, Sanaeizade S (2020) Optimization Study of Nickel and Copper Bioremediation by *Microbacterium oxydans* Strain CM3 and CM7. Soil Sediment Contam 29:438–451. https://doi.org/10.1080/15320383.2020.1738335

Hseu ZY (2004) Evaluating heavy metal contents in nine composts using four digestion methods. Bioresour Technol 95:53–59. https://doi.org/10.1016/j.biortech.2004.02.008

Idris R, Trifonova R, Puschenreiter M et al. (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl Environ Microbiol 70:2667–2677. https://doi.org/10.1128/AEM.70.5.2667-2677.2004

Ishtiaq S, Mahmood S (2011) Phytotoxicity of nickel and its accumulation in tissues of three Vigna species at their early growth stages. J Appl Bot Food Qual 84:223–228

Jagetiya B, Soni A, Yadav S (2013) Effect of nickel on plant water relations and growth in green gram. Indian J Plant Physiol 18:372–376. https://doi.org/10.1007/s40502-013-0053-8

Kaur R, Sharma S, Kaur H (2019) Heavy metals toxicity and the environment. J Pharmacogn Phytochem SP1 247–249. https://doi.org/10.1007/978-3-7643-8340-4_6

Kazy SK, Sar P, Singh SP et al. (2002) Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: synthesis, chemical nature and copper binding. World J Microbiol Biotechnol 18:583–588. https://doi.org/10.1023/A:1016354713289

Khan AS, Ghaffar A, NAZb MY et al. (2017) Synthesis of nickel-copper nanocrystalline ferrite adsorbent for treatment of textile effluents. Dig J Nanomater Biostructures 12(2):235–242

Khodaverdiloo H, Samadi A (2011) Batch equilibrium study on sorption, desorption, and immobilisation of cadmium in some semi-arid zone soils as affected by soil properties. Soil Res 49:444–454. https://doi.org/10.1071/SR10156

Li H, Dong W, Liu Y, et al (2019) Enhanced biosorption of nickel ions on immobilized surface-engineered yeast using nickel-binding peptides. Front Microbiol 10: https://doi.org/10.3389/fmicb.2019.01254

Li HY, Wei DQ, Shen M, Zhou ZP (2012) Endophytes and their role in phytoremediation. Fungal Divers 54:11–18. https://doi.org/10.1007/s13225-012-0165-x

Liphadzi MS, Kirkham MB (2006) Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids. South African J Bot 72:391–397. https://doi.org/10.1016/j.sajb.2005.10.010

Loganathan P, Myung H, Muthusamy G et al. (2015) Effect of heavy metals on acdS gene expression in *Herbaspirillium* sp. GW103 isolated from rhizosphere soil. J Basic Microbiol 55:1232–1238. https://doi.org/10.1002/jobm.201500008

Ma Y, Oliveira RS, Nai F et al. (2015) The hyperaccumulator Sedum plumbizincicola harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J Environ Manage 156:62–69. https://doi.org/10.1016/j.jenvman.2015.03.024

Marzan LW, Hossain M, Mina SA et al. (2017) Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. Egypt J Aquat Res 43:65–74. https://doi.org/10.1016/j.ejar.2016.11.002

Masindi V, Muedi KL (2018) Environmental contamination by heavy metals. Heavy Met 10:115–132. https://doi.org/10.5772/intechopen.76082

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214. https://doi.org/10.1023/A:1010358708525

Mishra SR, Chandra R, Kaila AJ, Darshi BS (2017) Kinetics and isotherm studies for the adsorption of metal ions onto two soil types. Environ Technol Innov 7:87–101. https://doi.org/10.1016/j.eti.2016.12.006

Mosa KA, Saadoun I, Kumar K et al. (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids. Front Plant Sci 7:303. https://doi.org/10.3389/fpls.2016.00303

Mulik AR, Kulkarni P, Bhadekar RK (2018) Biosorption studies on nickel and chromium by *Kocuria* sp. BRI 36 Biomass 13:6886–6893. https://www.ripublication.com/ijaer18/ijaerv13n9_52.pdf

Naik UC, Srivastava S, Thakur IS (2012) Isolation and characterization of *Bacillus cereus* IST105 from electroplating effluent for detoxification of hexavalent chromium. Environ Sci Pollut Res 19:3005–3014. https://doi.org/10.1007/s11356-012-0811-6

Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333:134–139. https://doi.org/10.1038/333134a0

Oves M, Khan MS, Zaidi A (2012) Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. Saudi J Biol Sci 20 (2):121–129. https://doi.org/10.1016/j.sjbs.2012.11.006

Oyewole OA, Zobeashia SSL-T, Oladoja EO et al. (2019) Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil. SN Appl Sci 1(857):1–8. https://doi.org/10.1007/s42452-019-0879-4

Padmavathy V, Vasudevan P, Dhingra SC (2003) Biosorption of nickel(II) ions on Baker's yeast. Process Biochem 38:1389–1395. https://doi.org/10.1016/S0032-9592(02)00168-1

Pan JH, Liu RX, Tang HX (2007) Surface reaction of *Bacillus cereus* biomass and its biosorption for lead and copper ions. J Environ Sci 19:403–408. https://doi.org/10.1016/S1001-0742(07)60067-9

Rahman Z, Thomas L, Singh VP (2019) Biosorption of heavy metals by a lead (Pb) resistant bacterium, *Staphylococcus hominis* strain AMB-2. J Basic Microbiol 59:477–486. https://doi.org/10.1002/jobm.201900024

Rajappa A, Ramesh K, Nandhakumar V, Ramesh H (2015) Equilibrium and Isotherm Studies of Congo Red Adsorption Onto Commercial Activated Carbon. Int J Curr Res Chem Pharma Sci 1(3):43–48. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.677.3638&rep=rep1&type=pdf

Rajkumar M, Freitas H (2008) Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99:3491–3498. https://doi.org/10.1016/j.biortech.2007.07.046

Rana KL, Kour D, Kaur T, et al (2020) Endophytic microbes from diverse wheat genotypes and their potential biotechnological applications in plant growth promotion and nutrient uptake. Proc Natl Acad Sci India Sect B - Biol Sci. https://doi.org/10.1007/s40011-020-01168-0

Rani MJ, Hemambika B, Hemapriya J, Kannan VR (2010) Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach. African J Environmental. Sci Technol 4:77–83. https://doi.org/10.5897/AJEST09.184

Samantaray S, Rout GR, Das P (1998) Differential nickel tolerance of mung bean (*Vigna radiata* L.) genotypes in nutrient culture. Agronomie, EDP Sci 18(8-9):537–544. https://doi.org/10.1051/agro:19980804

Senthil Kumar P, Ramakrishnan K, Gayathri R (2010) Removal of nickel(II) from aqueous solutions by ceralite ir 120 cationic exchange resins. J Eng Sci Technol 5:234–245. https://doaj.org/article/c23ed87bbf514dbf8eac51a20ee576a9

Shi WY, Shao HB, Li H et al. (2009) Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J Hazard Mater 170:1–6. https://doi.org/10.1016/j.jhazmat.2009.04.097

Singh NK, Rai UN, Tewari A, Singh M (2010) Metal accumulation and growth response in *Vigna radiata* L. inoculated with chromate tolerant rhizobacteria and grown on tannery sludge amended soil. Bull Environ Contam Toxicol 84:118–124. https://doi.org/10.1007/s00128-009-9875-5

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43:246. https://doi.org/10.4103/0253-7613.81505

Sonawdekar S, Gupte A (2020) Biosorption of copper (II) and cadmium (II) by Bacillus cereus sys1 isolated from oil - contaminated site. SN Appl Sci. https://doi.org/10.1007/s42452-020-3062-z

Sun LN, Zhang YF, He LY et al. (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour Technol 101:501–509. https://doi.org/10.1016/j.biortech.2009.08.011

Tiwari S, Sarangi BK, Thul ST (2016) Identification of arsenic resistant endophytic bacteria from Pteris vittata roots and characterization for arsenic remediation application. J Environ Manage 180:359–365. https://doi.org/10.1016/j.jenvman.2016.05.029

Tognacchini A, Rosenkranz T, van der Ent A et al. (2020) Nickel phytomining from industrial wastes: Growing nickel hyperaccumulator plants on galvanic sludges. J Environ Manage 254:109798. https://doi.org/10.1016/j.jenvman.2019.109798

Torabia N, Kardel F (2019) Biosorption of nickel by *Halobacillus* sp. Kn57 isolated from the Miankaleh Wetland, Iran. Int J Aquat Biol 7:280–290. https://doi.org/10.22034/ijab.v7i5.704

Wang LK, Chen JP, Hung Y-T, Shammas NK (2017) Heavy metals in the environment. CRC press, Boca Raton

Wang Y, Luo Y, Zeng G et al. (2020) Characteristics and in situ remediation effects of heavy metal immobilizing bacteria on cadmium and nickel co-contaminated soil. Ecotoxicol Environ Saf 192:110294. https://doi.org/10.1016/j.ecoenv.2020.110294

Weyens N, Truyens S, Saenen E et al. (2011) Endophytes and their potential to deal with co-contamination of organic contaminants (toluene) and toxic metals (nickel) during phytoremediation. Int J Phytoremediation 13:244–255. https://doi.org/10.1080/15226511003753920

Zhu Y, She X (2018) Evaluation of the plant-growth-promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*. Can J Microbiol 64:253–264. https://doi.org/10.1139/cjm-2017-0529