A wide range of microorganisms present in industrial effluent, exposed to different heavy metals, develop tolerance to such metal. Adaptation to the adverse environmental condition make them resistant to different antibiotics and various characters exhibited by them need to be explored. Twenty numbers of bacteria isolated from the effluent of a steel plant showed tolerance to heavy metals like Ni, Cr, Cd, Pb, and Hg and resistance to different antibiotics. Through morphological and biochemical characterization, the isolates were identified as species of *Staphylococcus, Psuedomonas, Burkholderia, Sphingomonas, Bacillus, Paenibacillus, Lysinibacillus* and *Enterococcus*. All the isolates were tried for plant growth promoting traits. Of the twenty isolates R-57 and R-58 showed positive for IAA production, siderophore production, PO₄ solubilization, HCN production and ammonia production.
They can produce phytohormones (e.g. indole-3-acetic acid) that promote plant growth, facilitate nutrient availability (e.g. by solubilizing phosphorus and producing ammonia) can alter metal flow dynamics via siderophore release etc. PGPB can also produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which alleviates plant stress through the reduction of ethylene levels (Glick, 2012). A bacterial isolate with metal tolerant efficiency and plant growth promoting (PGP) properties can provide multiple advantages in crop productivity (Tripathy et al., 2007).

Keeping in view of this an attempt has been taken in the present study to assess the ability of bacteria isolated from effluent of a steel plant on metal tolerance and plant growth promoting traits.

**Materials and Methods**

**Collection of Sample**

Effluent sample was collected in a sterile bottle from industrial effluent from a steel plant aseptically and transferred to the laboratory and stored under refrigerated condition for further work.

**Enrichment, isolation and screening of bacterial isolates**

Effluent enrichment method was performed by placing 10 ml effluent sample in a 250 ml Erlenmeyer flask containing 100 ml sterilized luria bertani media and incubated at 37°C. After 48 hours of incubation, the turbid broth was spreaded on sterilized Luria bertani agar plate and incubated for 24 hrs.

The isolates were screened on LB agar plate amended with 50 ppm of heavy metals such as Ni, Cd, Cr, Pb and Hg separately and incubated for 48 hours. The growth of heavy metal resistant bacteria colony having different morphology were observed and Purity of the isolates were confirmed by quadrant streak plate method and preserved using 15 % glycerol stocks.

**Biochemical characterization and sugar utilization test**

Morphological Colony characteristics of the isolates were noted and grams staining were performed. Biochemical tests such as indole, methyl red, voges proskauer, citrate utilization test, oxidase and catalase test, H₂S production, motility test, and Urease production were carried out following standard method. Sugar utilisation was tested using Hicarbo Kit-A, Hicarbo Kit-B and Hicarbo Kit-C (Himedia).

**Assessment of metal tolerance**

Evaluation of all the bacterial isolate was determined by gradual increase of Cr, Ni, Cd, Pb and Hg metal concentration in LB media until the bacterial isolates failed to grow on plates over 7 days of incubation. The MIC of respective isolates was determined.

**Determination of antibiotic sensitivity test**

Antibiotic sensitivity pattern of the isolates were tested by Kirby-Bauer disc diffusion method. After incubation the resistance and sensitivity were determined according to the inhibition zone around the colony.

**PGPB traits**

The metal tolerant bacterial isolate were analyzed for different plant growth promoting traits.

**Indole acetic acid producing ability**

Bacteria were incubated for 24 h in LB broth and then they were centrifuged. An aliquot of
1 ml of supernatant was taken and transferred to a test tube that contained 2 ml of Salkowski reagent. The mixture was kept in room temperature for 30 min (Gordon and Weber, 1951) to observe change on colouration.

**Solubilization of inorganic phosphate**

For phosphate solubilization assay, fresh cultures of bacterial strains were inoculated into National Botanical Research Institute Phosphate Medium (NBRIP) (Nautiyal, 1999). Plates were incubated for 7 days and the formed with clear zone around them were considered as positive for the phosphate solubilization.

**Ammonia production**

Bacterial strains were checked for ammonia production according to Cappuccino and Sherman (1992). Fresh cultures were inoculated in to 10 ml peptone water. The production of ammonia was detected by adding 500µl of Nessler’s reagent to each tube.

**Siderophore production**

Detection of Siderophore production was carried out by inoculating the bacterial isolates on Chrome Azurol S (CAS) plate assays (Schwyn and Neilands, 1987).

**HCN production**

The HCN production by the bacterial isolates was determined by adapting the method of Ahmad *et al.*, (2008). Fresh cultures were inoculated in LB medium supplemented with glycin (4.4g/lit) for 24 h at 30°C. On the top of each plate, a sterilized filter paper (Whatman no.1) soaked in 2% sodium carbonate prepared in 0.5% picric acid solution was placed. Plates were incubated at 30°C to study HCN production.

**Results and Discussion**

**Enrichment, isolation and screening of Bacterial isolates**

A total of sixty bacterial isolates (R-1 to R-60) were isolated from effluent sample. Amongst them twenty bacterial isolates were found to be tolerant to Ni, Cr, Cd, Pb, and Hg. Out of these twenty isolates, six bacterial isolates were gram negative and fourteen were gram positive. All the 20 isolates exhibited tolerance against all the five heavy metals up to a minimum concentration of 50 ppm (Table 1).

**Biochemical characterization and sugar utilization test**

On biochemical variability and sugar utilization, it was observed that among 20 isolates only R-14 was positive for indole production and rest isolates were negative. Ten isolates were positive for methyl red and ten showed negative result.

Eight isolates showed positive for Voges Proskauer reaction and twelve were negative for the test. Twelve of the twenty bacterial isolates utilized citrate. Fourteen bacteria were positive for oxidase, seventeen were positive for catalase and nine isolates could produce hydrogen sulphide.

Among twenty, fifteen isolates were found to motile and thirteen isolates had the ability to produce urease enzyme. Total 35 numbers of sugars were taken for test. The results are described in table 2. 3 and 4. According to above biochemical reaction the isolates were identified by ABIS online are in table 5.

**Tolerance for heavy metal concentrations (in ppm)**

All the 20 bacterial isolates isolated from industrial effluent were found to be tolerant to
Ni, Cr, Cd, Pb and Hg. Isolates R-3, R-4, R-10, R-22, R-26, R-35, R-43, R-44, showed the highest tolerance of 1350 ppm to the metal Ni (Fig. 1). Isolate R-37 was able to tolerate Cr metal up to the concentration of 1650 ppm (Fig. 2).

Most of the bacteria were tolerant to 120 ppm of Cd concentration (Fig. 3). For Pb all the organisms were found to be tolerant at the concentrations of 75 ppm. Most of the isolates were tolerant to 50 ppm concentration and both R-37 and R-56 were tolerant to 100 ppm of Hg (Fig. 4 and 5).

**Antibiotic susceptibility test**

Table 6 depicts that all the isolates resisted to all most all the commonly used antibiotics taken for this study. All the isolates are susceptible for penicillin B. All the bacteria were resistant to nalidixic acid, azithromycin, streptomycin, ciprofloxacin, kanamycin, and amoxycilin. A few organisms were susceptible for rifampicin, gentamycin, amikacin, cefonicid and tetracycline. Sarma et al., 2010 opined that the resistance capacity may develop due to the metal contaminated environment in isolates.

**Table 1** Grams staining and morphological characteristics of the isolates

| Isolates | Gram’s reaction | Morphological character                                                   |
|----------|-----------------|-------------------------------------------------------------------------|
| R-3      | +ve cocci       | Creamy, cerrated, dry round ring at the centre, sorounded by layers     |
| R-4      | +ve cocci       | Cerrated margin, ring at centre                                          |
| R-10     | +ve rod         | Small, round, Creamy, shiny, centre elevated and dry                     |
| R-14     | -ve rod         | Small, round, greenish brown, elevated shiny                             |
| R-22     | +ve cocci       | Creamy, flat, dry, surrounded by wet margin.                            |
| R-26     | +ve cocci       | White, flat surrounded by dry margin                                    |
| R-35     | +ve cocci       | Small, creamy, dry, round, flat                                         |
| R-37     | -ve rod         | Light green, spreaded colony                                            |
| R-43     | -ve rod         | Flat, cerrated margin                                                   |
| R-44     | +ve cocci       | Mucoid colony, folded, wrinkled                                         |
| R-45     | +ve cocci       | White, cerrated margin, ring at centre                                  |
| R-51     | +ve rod         | Small, white, round                                                     |
| R-52     | +ve cocci       | Small, Yellow, round                                                    |
| R-53     | +ve rod         | Mucoid, cerrated margin                                                 |
| R-54     | +ve cocci       | Mucoid, elevated colony,                                                |
| R-55     | +ve rod         | Small, round, white, elevated colony                                    |
| R-56     | -ve rod         | Small, round, watery, cerrated margin                                   |
| R-57     | -ve rod         | Lemon green, oily, spreaded                                             |
| R-58     | -ve rod         | Green, and mucoid colony                                                |
| R-60     | +ve cocci       | Round, offwhite                                                         |
**Table 2** Biochemical characterization of the isolates

| Isolates | Indole Test | Methyl red | Voges Proskauer | Citrate utilization | Oxidase | Catalase | H$_2$S production | Motility | Urea hydrolysis |
|----------|-------------|------------|-----------------|---------------------|---------|----------|-------------------|----------|----------------|
| R-3      | -           | +          | +               | -                   | +       | -        | -                 | -        | +              |
| R-4      | -           | +          | +               | +                   | -       | +        | +                 | +        | -              |
| R-10     | -           | +          | +               | -                   | +       | +        | +                 | +        | -              |
| R-14     | +           | +          | -               | +                   | +       | +        | -                 | -        | +              |
| R-22     | -           | +          | +               | +                   | -       | +        | -                 | +        | -              |
| R-26     | -           | +          | -               | -                   | -       | +        | +                 | +        | +              |
| R-35     | -           | +          | -               | +                   | +       | +        | +                 | +        | +              |
| R-37     | -           | -          | -               | +                   | +       | +        | -                 | +        | -              |
| R-43     | -           | +          | +               | -                   | -       | -        | +                 | -        | +              |
| R-44     | -           | +          | +               | -                   | +       | -        | +                 | +        | +              |
| R-45     | -           | +          | +               | +                   | +       | +        | -                 | +        | +              |
| R-51     | -           | -          | -               | -                   | +       | +        | +                 | +        | +              |
| R-52     | -           | +          | -               | -                   | +       | +        | -                 | +        | +              |
| R-53     | -           | -          | +               | -                   | +       | +        | -                 | -        | +              |
| R-54     | -           | -          | +               | -                   | +       | +        | +                 | +        | +              |
| R-55     | -           | +          | -               | +                   | +       | +        | -                 | +        | +              |
| R-56     | -           | -          | -               | -                   | -       | +        | -                 | +        | +              |
| R-57     | -           | -          | -               | +                   | +       | +        | -                 | +        | -              |
| R-58     | -           | -          | +               | +                   | +       | -        | +                 | -        | -              |
| R-60     | -           | -          | +               | +                   | +       | +        | -                 | +        | -              |

*Legend: + (positive) reaction, - (negative) reaction.*
### Table 3: Sugar fermentation of the isolates

| Isolates | lac | Xyl | Mal | Fruc | Dext | Gal | Raf | Tre | Mel | Suc | L-ara | Man | Inu | N-agl | Glyc | Sal | Dulc | Ins |
|----------|-----|-----|-----|------|------|-----|-----|-----|-----|-----|-------|-----|-----|-------|------|-----|------|-----|
| R-3      | -   | -   | -   | +    | +    | -   | -   | +   | -   | -   | +     | -   | -   | -     | -    | -   | -    | -   |
| R-4      | -   | -   | -   | +    | +    | +   | +   | +   | +   | +   | +     | -   | -   | -     | +    | -   | -    | -   |
| R-10     | +   | -   | -   | +    | +    | -   | -   | +   | -   | -   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-14     | +   | +   | +   | +    | +    | +   | +   | -   | +   | -   | +     | -   | +   | +     | +    | +   | +    | +   |
| R-22     | -   | +   | +   | +    | -    | +   | +   | -   | +   | -   | +     | -   | +   | +     | +    | +   | +    | +   |
| R-26     | -   | +   | +   | +    | +    | -   | -   | +   | +   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-35     | -   | -   | +   | +    | +    | -   | -   | +   | -   | -   | +     | -   | -   | +     | +    | +   | +    | -   |
| R-37     | -   | -   | -   | -    | -    | -   | -   | -   | +   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-43     | -   | -   | +   | +    | +    | -   | +   | -   | +   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-44     | +   | +   | +   | -    | +    | -   | +   | -   | -   | -   | -     | -   | -   | +     | +    | +   | +    | +   |
| R-45     | +   | +   | +   | +    | -    | +   | -   | -   | +   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-51     | -   | -   | -   | -    | -    | +   | -   | +   | +   | -   | -     | -   | -   | +     | -    | +   | +    | +   |
| R-52     | -   | +   | -   | +    | +    | -   | -   | -   | +   | -   | -     | -   | +   | +     | +    | +   | -    | +   |
| R-53     | +   | +   | +   | +    | -    | +   | -   | +   | -   | +   | -     | -   | +   | +     | +    | +   | -    | +   |
| R-54     | +   | +   | -   | -    | -    | +   | +   | +   | +   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-55     | -   | -   | -   | -    | -    | +   | -   | +   | -   | +   | +     | -   | +   | +     | -    | +   | +    | -   |
| R-56     | +   | +   | -   | -    | -    | +   | -   | +   | +   | +   | -     | -   | +   | +     | -    | +   | +    | -   |
| R-57     | -   | +   | -   | +    | +    | -   | +   | -   | -   | +   | -     | -   | +   | +     | -    | +   | -    | +   |
| R-58     | -   | +   | -   | +    | -    | -   | +   | -   | -   | +   | -     | -   | +   | +     | -    | -   | +    | -   |
| R-60     | +   | +   | +   | +    | +    | -   | +   | -   | -   | +   | +     | -   | +   | +     | -    | -   | -    | +   |

Legend: + (positive) reaction, - (negative) reaction.

Lac- Lactose, Xyl-Xylose, Mal-Maltose, Fruc-Fructose, Dext-Dextrose, Gal-Galactose, Raf-Raffinose, Tre-Trehalose, Mel-Mellibiose, Suc-Sucrose, L-ara-L-arabinose, Man-Mannose, Inu-Inulin, N-agl-Sodium- gluconate, Gly-Glycerol, Sal-Salicin, Dul-Dulcitol, Ins-Inositol.
| Isolates | Sorb | Mant | Ado | Arab | Erythr | α-methyl | Rha | Celli | melz | α-mano | Xylt | ONPG | Esc | D-ara | Citr | Maln | Sorb |
|----------|------|------|-----|------|--------|--------|------|-------|------|--------|------|------|-----|-------|-----|------|------|
| R-3      | -    | +    | -   | -    | -      | +      | -    | +     | -    | -      | -    | +    | -   | -     | -   | -    | +    |
| R-4      | +    | +    | -   | -    | +      | -      | -    | +     | -    | -      | -    | +    | -   | +     | -   | -    | -    |
| R-10     | -    | +    | -   | +    | +      | -      | -    | +     | +    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-14     | +    | +    | -   | -    | -      | -      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-22     | +    | +    | -   | -    | -      | +      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-26     | +    | +    | -   | -    | -      | -      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-35     | +    | -    | -   | +    | -      | +      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-37     | +    | +    | -   | +    | -      | -      | -    | -     | -    | +      | -    | +    | +   | -     | -   | -    | -    |
| R-43     | -    | -    | -   | -    | +      | -      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-44     | -    | +    | -   | -    | +      | -      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-45     | -    | +    | -   | +    | -      | -      | -    | -     | -    | +      | -    | +    | +   | -     | -   | -    | -    |
| R-51     | +    | +    | +   | -    | -      | -      | -    | +     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-52     | +    | -    | +   | -    | -      | +      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-53     | +    | +    | +   | -    | +      | -      | -    | -     | -    | +      | -    | +    | +   | -     | -   | -    | -    |
| R-54     | +    | -    | +   | +    | -      | +      | -    | -     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-55     | -    | +    | -   | +    | -      | +      | -    | -     | -    | +      | -    | +    | +   | -     | -   | -    | -    |
| R-56     | +    | -    | +   | +    | -      | +      | -    | -     | -    | +      | -    | +    | +   | -     | -   | -    | -    |
| R-57     | +    | -    | +   | +    | -      | -      | -    | +     | -    | -      | -    | +    | +   | -     | -   | -    | -    |
| R-58     | -    | -    | -   | -    | +      | -      | -    | -     | +    | +      | +    | -    | +   | -     | -   | -    | -    |
| R-60     | -    | +    | +   | -    | -      | -      | -    | +     | +    | -      | +    | -    | +   | -     | -   | -    | -    |

Legend: + (positive) reaction, - (negative) reaction.
Sorb- Sorbitol, Mant- Mannitol, Ado- Adonitol, Arab- Arabinitol, Erythr- Erythritol, α-methyl- alpha-Methyl-D-glucoside, Rha-Rhamnose, Celli- Celllobiose, melz- Melezitose, α-mano- alpha-Methyl-D-Mannoside, Xylt-Xylitol, ONPG-ortho-Nitrophenyl-β-galactoside, Esc-Esculin, D-ara- D-Arabinose, Citr- Citrate, Maln- Malonate, Sorb- Sorbose.
Table 5 Biochemical identification of the isolates

| Isolates | Identification               |
|----------|------------------------------|
| R-3      | *Staphylococcus* sp.         |
| R-4      | *Staphylococcus* sp.         |
| R-10     | *Bacillus* sp.               |
| R-14     | *Burkholderia* sp.           |
| R-22     | *Staphylococcus* sp.         |
| R-26     | *Bacillus* sp.               |
| R-35     | *Staphylococcus* sp.         |
| R-37     | Unknown taxon                |
| R-43     | *Sphingomonas* sp.           |
| R-44     | *Staphylococcus* sp.         |
| R-45     | Unknown taxon                |
| R-51     | *Paenibacillus* sp.          |
| R-52     | *Staphylococcus* sp.         |
| R-53     | *Paenibacillus* sp.          |
| R-54     | Unknown taxon                |
| R-55     | *Lysinibacillus* sp.         |
| R-56     | Unknown taxon                |
| R-57     | *Pseudomonas* sp.            |
| R-58     | *Pseudomonas* sp.            |
| R-60     | *Enterococcus* sp.           |
### Table 6: Antibiotic susceptibility of the metal tolerant bacterial isolates

| Isolates | PEN | RIF | NA  | AZM | STR | GEN | CIP | KAN | AMC | CID | TET | NV | AMX |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|
| R-3      | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-4      | +   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -   | -  | -   |
| R-10     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-14     | +   | -   | -   | -   | -   | +   | -   | -   | -   | +   | +   | -  | -   |
| R-22     | +   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -   | +  | -   |
| R-26     | +   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -   | -  | -   |
| R-35     | +   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -   | -  | -   |
| R-37     | +   | +   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -  | -   |
| R-43     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-44     | +   | -   | -   | -   | -   | +   | -   | -   | -   | -   | -   | -  | -   |
| R-45     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -  | -   |
| R-51     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-52     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-53     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-54     | +   | -   | -   | -   | -   | +   | -   | -   | -   | -   | -   | -  | -   |
| R-55     | +   | -   | -   | -   | -   | +   | -   | -   | -   | -   | -   | -  | -   |
| R-56     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-57     | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  | -   |
| R-58     | +   | -   | -   | -   | -   | +   | -   | -   | -   | -   | +   | -  | -   |
| R-60     | +   | -   | +   | -   | -   | -   | +   | -   | -   | -   | -   | -  | -   |

Legend: + (Susceptible), - (Resistant).  
PEN-penicillin G, RIF-Rifampicin, NA-Nalidixic acid, AZM-Azithromycin, STR-Streptomycin, GEN-Gentamycin, CIP-Ciprofloxacin, KAN-Kanamycin, AMC-Amikacin, CID-Cefonicid, TET-Tetracyclin, NV-Novobiocin, and AMX-Amoxycilin
**Table 7** Plant growth promotion traits of the metal tolerant bacterial isolates

| Isolates | IAA   | Phosphate solubilization | Ammonia production | Siderophore production | Hydrogen Cyanide production |
|----------|-------|---------------------------|--------------------|------------------------|-----------------------------|
| R-3      | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-4      | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-10     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-14     | +Ve   | -ve                       | +Ve                | +Ve                    | -Ve                         |
| R-22     | -Ve   | -ve                       | -Ve                | -Ve                    | -Ve                         |
| R-26     | -Ve   | -ve                       | -Ve                | -Ve                    | -Ve                         |
| R-35     | -Ve   | -ve                       | +Ve                | +Ve                    | -Ve                         |
| R-37     | +Ve   | -ve                       | +Ve                | +Ve                    | -Ve                         |
| R-43     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-44     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-45     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-51     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-52     | -Ve   | -ve                       | -Ve                | -Ve                    | -Ve                         |
| R-53     | -Ve   | -ve                       | -Ve                | -Ve                    | -Ve                         |
| R-54     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |
| R-55     | -Ve   | -ve                       | -Ve                | -Ve                    | -Ve                         |
| R-56     | +Ve   | -ve                       | +Ve                | +Ve                    | -Ve                         |
| R-57     | +Ve   | +ve                       | +Ve                | +Ve                    | +Ve                         |
| R-58     | +Ve   | +ve                       | +Ve                | +Ve                    | +Ve                         |
| R-60     | -Ve   | -ve                       | +Ve                | -Ve                    | -Ve                         |

**Fig. 1** Growth of 20 isolates at different concentrations of Ni metal
Fig. 2 Growth of 20 isolates at different concentrations of Cr metal

Fig. 3 Growth of 20 isolates at different concentrations of Cd metal

Fig. 4 Growth of 20 isolates at different concentrations of Pb metal

Fig. 5 Growth of 20 isolates at different concentrations of Hg metal
Plant growth promotion activities

Observation of pink colour in IAA tube indicates positive result. R-14, R-37 and R-56, R-57 and R-58 were found to be positive for IAA production. NBRIP Plates were incubated for 7 days and the formed with clear zone around the colony were considered as positive for the phosphate solubilization (Table 7). R-57 and R-58 showed positive result for PO$_4$ solubilisation. After adding Nessler’s reagent in the ammonia tube, the development of yellow to brown colour was considered as positive result for ammonia production. Isolates R-22, R-26, R-52, R-53 and R-55 showed positive result for ammonia production. The presence of yellow to orange halo around the bacterial growth after incubation for 72h indicated a positive result for siderophore production. R-14, R-35, R-37, R-56, R-57 and R-58 were found to be positive for siderophore. Change in colour of filter paper to orange colour was considered as positive result for HCN production. Both the isolates R-57 and R-58 were found to be positive for HCN production.

In the present investigation it is observed that all 20 bacterial isolates isolated from the industrial effluent were biochemically identified as Staphylococcus spp., Psuedomonas spp., Burkholderia sp., Sphingomonas sp., Bacillus spp., Paenibacillus sp., Lysinibacillus sp. and Enterococcus sp., and were found tolerant to 60-120ppm of Cd, 75 ppm Pb and 50-100 ppm Hg metals. These microorganisms have high degree of tolerance capacity against chromium ranging from 300-1650ppm and for nickel 680-1350ppm. Pandit et al., (2013) isolated six number of Cu, Cd, Cr and Ni tolerant bacteria from industrial effluent sample. So in this study it is evident that most of the bacterial isolates were found to be tolerant to the metal salts such as Ni, Cr, Cd, Pb and Hg with high concentrations. Pramanik et al., (2016) reported the chromium tolerant bacteria showing metal tolerant along with plant growth promotion (PGP) traits which might be helpful for plant growth promotion in chromium contaminated soil.

The results obtained in this study revealed that multiple heavy metal tolerant isolates are resistant to several antibiotics such as nalidixic acid, azithromycin, streptomycin, ciprofloxacin, kanamycin, and amoxycillin, rifampicin, gentamycin, amikacin, cefonicid and tetracycline. All the isolates are resistant to several antibiotics taken in this study except penicillin B. Kaur et al., (2015) reported that bacteria isolated from industrial effluents were found to be resistant against ampicillin, ciprofloxacin, erythromycin, kanamycin, tetracycline, vancomycin, methicillin, gentamycin, and chloramphenicol. Due to cross resistance mechanism the bacteria have tolerance more than one antimicrobial agent such as antibiotics and heavy metals (Champan, 2003). These were seemed to be an efficient plant growth promoting bacteria that could produce IAA, siderophore, ammonia, HCN and solubilize phosphate. Reports available also indicate that the potential of soil microorganism to assist plant establishment on contaminated environment through mediating nutrient mineralization and uptake by plants, production of plant growth hormones and siderophores. The beneficial effects caused by the indigenous isolates may be used as a bioinoculant that facilitates the plant growth and thus increases phytoextraction efficiency for the remediation of heavy metal contaminated sites. Psuedomonas sp. Showed potentials in metal absorption as well as PGP and biocontrol activity (Sarma et al., 2012).

The potential of the isolates can be considered as a probable candidate for the concerted
approach in the bioremediation as well as phytoremediation technology to convert metal contaminated sites to productive land and increase crop productivity.

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