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ORIGINAL ARTICLE

Isolation, Screening and Biochemical characterizations with multiple traits of Heavy Metal Tolerant Rhizobacteria from Mining Area and Landfill site

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ABSTRACT
The aim of the study was to isolate and characterizations of heavy metal tolerate rhizobacteria from rhizosphere soil near plant growing near mining area and landfill site. A total of 91 rhizobacterial colonies isolated, 51 rhizobacteria were isolated from Zawar mines area, Udaipur, Rajasthan, India and 40 rhizobacteria were isolated from Pirana landfill site. Ahmedabad, Gujarat, India. Total Six rhizobacteria (SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38) showed higher tolerances against heavy Metals, which was subjected to biochemical characterization and extracellular enzyme production. Optimized growth conditions for all six isolates were at temperature 37°C and pH 7. Maximum Inhibition Concentration was performed using Nutrient enriched Media (Nutrient Agar and Luria Bertani Media) and Nutrient Deficient Media (Minimal salt media) in both solid agar plate and liquid broth condition. The rhizobacterial isolate SMHMZ4 could resist the higher concentration of Cd^{2+} up to 1500 µg/ml in all three liquid broths and 1100 µg/ml, 1500 µg/ml, and 900µg/ml on NA, LB, and MSM agar plate respectively. The SMHMZ46 isolates could resist higher concentrationsofPb^{2+} and Ni^{2+} up to 2000 µg/ml and 5000 µg/ml, respectively in all three liquid broths and on solid agar plate Pb^{2+} 1700 µg/ml, 2100 µg/ml, and 800 µg/ml on NA, LB, and MSM agar plate respectively and Ni^{2+} up to 4500 µg/ml, 4500 µg/ml, and 2100 µg/ml on NA, LB, and MSM agar plate respectively. Also, Antibiotic sensitivity of rhizobacterial isolates was performed. Isolated heavy metal tolerates rhizobacteria may be advantageous for potential agents for bioremediation and in groups with phytoremediation of heavy metals in the contaminated environment.
Keywords: *Rhizobacteria, Heavy Metals, Mining area, Landfill site, Minimum Inhibition Concentration (MIC), Bioremediation, Phytoremediation*

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INTRODUCTION

Increasing population**,** the speedy growing mine tailings and industrial waste, the disposal of metal and land application of chemical fertilizers, waste sludge, and wastewater irrigation may result in heavy metal pollution of agricultural lands and water resources [2,8,9,12,13]. Soil contamination with heavy metals like cadmium, arsenic, nickel, copper, lead, zinc, aluminum, and mercury is one in every of the world's major environmental issues, leading to major negative concerns on ecosystems, crop yield, and human health [6,17,21].

Mining action produces a large amount of discarded rocks and tailing generated from mining and smelting activity that becomes a source of metal contamination throughout the course of your time [6]. The Heavy metals square measure cannot be degraded and destroyed; they are stable and persistent environmental contaminants. Numerous different heavy metals present in mines area and landfill site is also drained off during the rainy season, which result in metal percolating and drawn into watersheds down streams. In the food chain, various heavy metals may be transferred and accumulated in the bodies of humans and animals, which is able to most likely causes DNA damage and carcinogenic effects on their mutagenic ability [8,19].

A Times of India article concerning municipal solid dumpsite illumined that the Pirana Landfill site collects roughly 4000 tons of waste a day and most of it is discarded in the landfill unprocessed. What's more, the noxious chemical waste and by-products generated by several industries/factories within the proximity of the landfill site were conjointly dropped haphazardly. One of the explanations behind heavy metal-polluted soil is an unmanaged waste product. Pirana-mountain of garbage having a height of 200m not solely affects the aesthetics, however, conjointly have unsafe effects on neighboring soil as well as in groundwater [15].

In the current study, we are inspecting tolerance of three heavy metals cadmium, lead and nickel has no important biological function, but it is well recognized for its exceedingly toxicity, bioaccumulation and bio magnification through the food chain [31]. According the agreements on environmental protection and the World Health Organization (WHO), Cadmium (Cd) is among the top ten in the black list. Due to bio magnification and bioaccumulation a property, Cadmium is deliberate a extremely toxic pollutant [16]. Among these toxic metals, Pb has the best injurious impact on human health. Most Pb concentrations originate in the environment are connected to such human activities as metal mining and smelting, leaded petroleum consumption, lead battery production, the agricultural practice of sewage sludge, and uncontrolled dumping of industrial wastes. Chronic exposure to Pb increases the risks of high blood pressure, hyperactivity, anemia, and diseases of the nervous system and kidneys, and infertility [37].

Nickel is being wide employed in varied industries such as electroplating, leather tanning, pulp process, wood preservation and steel manufacturing and is discharged into wastewater and neighboring environment by these industries. This is of key fear because of the non-degradable nature of nickel [35]. Nickel is typically originated in Ni (0) or Ni (II) state due to the constancy of these species in water. Nickel is a vital compound for bacterial metabolism [35] and is used as a co- factor by several well categorized microbial enzymes like urease, Ni-superoxide dismutase, hydrogenase, carbon monoxide dehydrogenase, and methyl coenzyme M reductase and acetyl CoA synthase/decarboxylase, as well as some forms of glyoxalase [22], but at higher concentrations nickel becomes toxic [27].

The microorganisms exist in in such polluted tailings and contaminated lands have advanced mechanisms to tolerate the occurrence of heavy metals by complexation, efflux, or reduction of metal ions or to habit them as terminal acceptors in aerobic respiration [36]. Despite the toxic stress, micro-organisms that tolerate high metal concentrations and more rapidly decompose pollutants are more likely to survive. Therefore, microbial–based remediation technique is a better alternative to reduce metal pollution of such contaminated sites [10,33].

The solicitation of heavy metal tolerates rhizobacteria is an auspicious approach for increase metal bioavailability in heavy metal polluted soil. The objective of present study was to isolate and characterization of Cadmium, lead and Nickel tolerate rhizobacteria from heavy metal polluted soil. Isolated heavy metal tolerate rhizobacteria may be advantageous for potential agents for bioremediation and in grouping with phytoremediation of Cd^{2+}, Pb^{2+} and Ni^{2+} contaminates soil.

MATERIAL AND METHODS

Sample location

The rhizosphere soil sample were collected from two location, one from plants growing near the Zawar mines area, Udaipur, Rajasthan, India [31] and another from Pirana landfill site, Ahmedabad, Gujarat, India. The soil samples were composed using the sterilized spatula and kept in sterile zipper plastic bags in cold conditions till shifted to a laboratory for more studies and store at 4°C [31].

Media and chemical used in present study viz. Nutrient agar, Peptone, Beef extract, Yeast extract, Trypton, and Agar powder were procured from HiMedia Laboratories, Mumbai, India. Chemicals viz. Glucose, NaCl, $(NH_4)_2SO_4$, KH_2PO_4, K_2HPO_4, $CaCl_2$, $MgSO_4$, $FeSO_4$ were procured from Fine chemicals (P) Ltd. New Delhi, India. While heavy metal viz. $CdCl_2$, $Pb(NO_3)_2$, $NiCl_2.7H_2O$ were purchased from SRL Pvt. Ltd. Mumbai, India.

Heavy metal stock solution preparation

The heavy metal stock solutions of Cd^{2+}(5000 ppm), Pb^{2+} (5000 ppm) &Ni^{2+}(5000 ppm) were prepared by using $CdCl_2$, $Pb(NO_3)_2$, $NiCl_2.7H_2O$ respectively in double-distilled water and autoclaving at 121^0C and 15psi for 15min.

Isolation of heavy metal tolerates rhizobacteria

From the rhizosphere soil samples, isolation of heavy metal tolerates rhizobacteria were carried out using the enrichment broth method. One gram of rhizosphere soil sample inoculated in Nutrient broth having 100 µg/ml heavy metals (Cd^{2+}, Pb^{2+} and Ni^{2+}) and incubated on a shaker at 150 RPM for 24h. The serial dilution method used for isolation, for that enriched broth was serially diluted up to $10^{-5.}$ Then 0.1ml of

the highest dilution was spread on nutrient agar plates having 100 µg/ml heavy metals (Cd^{2+}, Pb^{2+} and Ni^{2+}), incubated at 37°C for 24 to 72h [5,23,34]. With the idea of morphological distinct colonies were selected (e.g. size, shape, and color) and repeatedly streaked on nutrient agar plates to acquire pure cultures and stored at 4^0C for further studies.

Biochemical characterization and Identification of the heavy metal tolerate rhizobacteria

On the basis of their morphological, physiological, cultural, and biochemical characteristics heavy metal tolerate rhizobacteria were characterized and identified. The isolated heavy metal tolerates colonies were characterized on the basis of gram staining, Catalase test, oxidase test, IMVIC test, and Carbohydrate utilization [11].

Screening of Heavy metal tolerates rhizobacteria

Minimum Inhibition concentration was performed with solid agar plate method and Liquid broth tube method was performed by using nutrient-rich media (Nutrient Agar Media and Luria Bertani Media) and nutrient-deficient media (Minimal Salt Media (MSM). On the solid agar plate MIC of the heavy metal tolerate bacteria was determined by gradually increasing the concentration of the heavy metal, 100 µg/ml each time on N- Agar plate (1% Peptone, 1% Beef Extract, 0.5% Sodium Chloride, 2% Agar) [9,19], LB agar plate (1% Trypton, 0.5% Yeast extract and 1% Sodium Chloride, 2% Agar) [5] and MSM plate (1 % glucose, 1 % $(NH_4)_2SO_4$, 0.1 % KH_2PO_4, 0.15 % K_2HPO_4, 0.086 % $CaCl_2$, 0.1 % $MgSO_4$, 0.02 % $FeSO_4$ and 1 % glucose and 2% Agar) [18] containing respective HMs (Cd^{2+}, Pb^{2+},and Ni^{2+}) to the strains failed to give colonies on the plate. The starting concentration used was 100 µg/ml [25].

For the MIC of the liquid broth, heavy metal tolerates bacteria which show highest resistant against heavy metals was grown in Nutrient broth, Luria bertani broth and minimal salt (MS) broth separately supplemented with Cd^{2+} (100 to 1500 µg/ml), Pb^{2+} (100 to 2000 µg/ml and Ni^{2+} (200 to 5000 µg/ml) incubate at 37 °C for 24 h. After incubation, growth was observed by taking the absorbance at 600 nm spectrophotomatrically (Shimadzu, Model No.1722) against respective broth (blank) containing the same amount of heavy metals [13]. The minimum Cd^{2+} Pb^{2+} and Ni^{2+} concentration at which bacterial isolate did not appearance growth was considered as its MIC.

Study the effect of temperature and pH on the growth of isolated bacteria

Optimal temperature and pH were determined so that this optimum parameter could be used in subsequent experiments. The isolate was grown in Nutrient broth and incubated at temperatures ranging from 28°C, 37°C, 40°C, 45°C, and Room temperature and for pH, the isolate was grown in Nutrient broth with varying pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 incubated at 37°C for 24 h at 150 rpm. The microbial growth of each sample was determined in terms of optical density at 600 nm using UV- Vis spectrophotometer (Shimadzu, Model No.1722).

Antibiotic resistant and susceptibility test

Antibiotic sensitivity of isolated heavy metal tolerates isolates were carried out using the Kirby- Bauer disc diffusion technique [4]. Antibiotic disc was purchased from 'HiMedia' and Pathoteq Biological Laboratory (India) which incorporates chloramphenicol (30 µg), ampicillin (10 µg), tetracycline (30 µg), gentamicin (10 µg), kanamycin (30 µg), Co- Trimoxazole (25 µg), amikacin (30 µg), Streptomycin (25 µg), Cefotaxime (30 µg), Piperacillin (100 µg), Ciprofloxacin (5 µg), Ceftizoxime (30 µg), Ofloxacin (5 µg), Amikacin (30 µg), Gatifloxacin (10 µg) [24]. The antibiotic disc was placed on agar plates and incubated for 24 h at 37 °C. After incubation, the organisms were categorized as sensitive or resistant to an antibiotic permitting the diameter of the zone of inhibition given in the standard antibiotic disc chart [24,25,28].

Other extracellular enzymatic activity of Heavy metal tolerates rhizobacteria

Catalase Activity

Catalase test was executed by taking 48 h old bacterial colonies grown on nutrient agar medium on a glass slide and add 3-4 drops of 3% hydrogen peroxide (H_2O_2). The gas bubbles release, which indicated a positive test for catalase activity [30].

Protease Activity

The sterile skimmed milk agar plate was used for the qualitative test for protease production. Bacterial isolates were spot inoculated on a skimmed milk agar plate and incubate at 37°C for 48 hrs. and the clear zone around the colony indicating the production of protease [14].

Amylase Activity

The starch agar plate was used for amylase production. The bacterial isolates were spot inoculated on these plates and incubated at 28°C for 48 h. After incubation, the iodine solution was flooded on plates. Yellow zones around the colony against a blue background indicated the production of amylase [7,32].

Cellulase activity

Carboxy methylcellulose (CMC) agar plates were used for cellulase production. The isolated bacterial strain was spot inoculated on Carboxy methylcellulose (CMC) agar plates and incubated at 28°C for 4-5 days. To visualize the hydrolysis zone, 1% Congo red solution was flooded on the incubated plates for 15 min. After that, the Congo red solution was emptied, and the plates were further flooded with 1 M NaCl for 10-15 min. A Yellow-orange zone was observed around the colony which indicates the production of cellulase [29].

Statistical analysis

All experiments were performed in triplicate. The relate the growth of isolates rhizobacteria in broth supplement with varied concentration of heavy metal, the obtained data were analyzed by calculating mean ± SE, and single factor analysis of variance (ANOVA). P value was calculated to see the significant results. When the results showing P value less than 0.05 were measured as significant ($P < 0.05$) and whereas a P value less than 0.01 as highly significant ($P < 0.01$). All numeric variances in the data were deliberated significantly different at the probability level of $P \leq 0.05$.

RESULT AND DISCUSSION

Isolation of heavy metal tolerates rhizobacteria

The Research was focused on the isolation and characterization of the heavy metal tolerates selected rhizobacterial strains to identify potential contenders for heavy metals bioremediation. Total 91 rhizobacteria were isolated. Out of 91 isolates, 51 rhizobacteria isolated from the Zawar mines area, Udaipur, Rajasthan, India [31] and 40 rhizobacteria isolated from Pirana landfill site, Ahmedabad, Gujarat, India. All the 91 isolates subjected to heavy metal tolerance against Cd^{2+}, Pb^{2+}, and Ni^{2+} heavy metals. The rhizobacterial isolate which revealed the highest MIC for heavy metals was designated for further study

Biochemical characterization and identification of the heavy metal tolerates rhizobacteria

Total Six rhizobacterial isolates, three from the Zawar mines area and three from the Pirana landfill site were revealed the highest MIC for heavy metals. Therefore, they were subjected to biochemical characterization and their result showed in Table 1.

Table 1: Biochemical characterization of the heavy metal tolerates rhizobacteria

Biochemical test	Culture Code					
	SMHMZ2	SMHMZ4	SMHMZ46	SMHMP4	SMHMP23	SMHMP38
Gram Staining	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Indole production Test	-	-	-	-	-	-
Methyl red Test	-	-	-	-	-	-
VP (Voges Proskauer) test	-	-	+	-	-	-
Citrate utilization Test	+	+	+	+	-	+
Hydrogen sulphide production Test	+	+	-	+	+	-
Gelatin hydrolysis Test	+	+	-	+	+	-
Dehydrogenase Test	-	-	+	-	-	+
Lead acetate paper strip Test	-	-	-	-	-	+
Litmus milk Test	-	-	-	+	+	-
Nitrate reduction Test	-	-	+	-	-	-
Tripal sugar ion Test	+	+	A/A	+	-	+
Phenylalanine deamination Test	+	+	-	+	-	-
Gelatine Hydrolysis	+	+	-	+	+	-
Carbohydrate utilization Dextrose	U	U	U,F	U	U	U,F
Galactose	U	U	U,F	U	U	U
Fructose	U	U	U,F	U	U	U,F
Mannitol	U	U,F	U,F	U	U	U,F
Lactose	U	U	U,F	U	U	U,F
Maltose	U	U	U,F	U	U	U,F
Sucrose	U	U	U,F	U	U	U,F
Salicin	U,F	U,F	U,F	U	U,F	U,F
Rhamnose	U	U	U,F	U	U	U,F
Xylose	U	U	U,F	U,F	U	U
Trehalose	U	U	U,F	U,F	U	U,F
Dulcitol	U	U	U	U,F	U	U,F
Cellobiose	U	U	U,F	U,F	U	U,F
Raffinose	U	U	U,F	U,F	U	U,F

Inulin	U	U	U	U	U,F	U,F
Adonitol	U	U	U,F	U	U	U,F
Melibiose	U	U	U,F	U	U	U,F
Arabinose	U	U	U,F	U	U	U
Galactose	U	U	U,F	U	U	U,F
Mannose	U	U	U,F	U,F	U	U,F
Sorbitol	U	U	U,F	U	U	U,F
Inositol	U	U	U	U	U	U,F

Keys: (+) Positive, (-) Negative, (F) Fermentation (Acid + Gas), (U) Utilization (Growth).

Screening of Heavy metal tolerates rhizobacteria

All the heavy metal tolerates rhizobacterial isolates used in this study exhibited very high-level tolerance against Cadmium Chloride, Nickel Chloride, and Lead nitrate in both nutrient-rich medium and nutrient-deficient medium in both conditions on the solid agar plate and liquid broth and the results of many scientists are in togetherness with the results of our study. SMHMZ2, SMHMZ4, SMHMP4, and SMHMP23 showed tolerance against Cd^{2+}, the rhizobacterial isolate SMHMZ4 could resist the higher concentration of Cd^{2+} up to 1500 µg/ml in all three liquid broths and 1100 µg/ml, 1500 µg/ml, and 900 µg/ml on NA, LB, and MSM agar plate respectively (Fig. 1, 2, and 3).SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, and SMHMP38 showed tolerance against Pb^{2+}.The SMHMZ46 isolates could resist the higher concentrations ofPb^{2+} up to 2000 µg/ml in all three liquid broths and on solid agar plate 1700 µg/ml, 2100 µg/ml, and 800 µg/ml on NA, LB, and MSM agar plate respectively(Fig. 4, 5, and 6). SMHMZ46, SMHMP38 showed tolerance against Ni^{2+}. The SMHMZ46 isolates could resist higher concentrations ofNi^{2+} up to 5000 µg/ml in both NA and LB liquid broth and 3500 µg/ml in MSM liquid broths and on solid agar plate Ni^{2+} up to 4500 µg/ml, 4500 µg/ml, and 2100 µg/ml on NA, LB, and MSM agar plate respectively (Fig. 7, 8, and 9). From the results it was evident that metal ions (Pb^{2+}, Cd^{2+}, and Ni^{2+}) significantly ($P < 0.05$) reduced the rate of growth of rhizobacteria as concentration of metal ions increased. Nath et al., isolated cadmium and lead tolerate rhizobacteria from garbage dumping site and their bacterial isolates *Pseudomonas sp* could resist up to 1400 -1800 µg/ml Cd^{2+} and 800-1000 µg/ml Pb^{2+}in the medium and *Klebsiella sp.* could resist up to 1600 µg/ml Cd^{2+} and 1100 µg/ml Pb^{2+} in the medium [24,26].Afzal *et al.*, isolated native bacterial strain from textile effluent that was identified as *Klebsiella variicola,* showed Maximum tolerable concentration against Ni (8 mM) and Co (7 mM) [3]. Ahemad *et al*., reported that their bacterial isolates (SN7, SN28, SN30) which are isolated from agriculture soil irrigated with wastewater showed a high level of tolerance against various heavy metals such asCd^{2+},Ni^{2+}, Pb^{2+} up to 100 µg/ml, 1600 µg/ml and 2400 µg/ml respectively in a nutrient medium [1].Wadood & Sabri, also reported that bacterial strains, which were isolated from wastewater, could tolerate up to range from 225 to 296 mM nickel chloride in minimal salt media [35].Kumar et al., isolated their bacterial strain from Dye industrial effluent that could resist up to 380 µg/ml Nickel chloride in a nutrient medium [20].

Study the effect of temperature and pH on growth of isolated bacteria

The isolate bacteria (Z2, Z4, Z46, P4, P23, and P38) observed to be capable of growing within a broad temperature range (28°C to 45°C) and Room temperature and broad pH range, from 4-10 and the optimum temperature and pH for its growth were 37°C and 7. The growth of the all the isolates decreased growth after 37°C and 7 pH (Fig. 10).Kumar et al., reported that their bacterial strain KL show optimal growth at 37°C and 7 pH [20].

Table 2. Antibiotic sensitivity and resistant of Cadmium, Nickel and Lead tolerates isolates

Isolates code	Resistance	Sensitive
SMHMZ2	Chloramphenicol, Ampicillin/Sulbactam, Cefotaxime, Piperacillin, Ceftizoxime, Tetracycline	Co- Trimoxazole, Ciprofloxacin, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin
SMHMZ4	Chloramphenicol, Ampicillin/Sulbactam, Cefotaxime, Piperacillin, Ceftizoxime, Tetracycline	Co- Trimoxazole, Ciprofloxacin, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin
SMHMZ46	Ampicillin/Sulbactam, Cefotaxime Piperacillin,	Chloramphenicol, Co- Trimoxazole, Ciprofloxacin, Ceftizoxime, Tetracycline, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin

SMHMP4	Chloramphenicol, Ampicillin/Sulbactam, Cefotaxime, Piperacillin, Ceftizoxime, Tetracycline	Co- Trimoxazole, Ciprofloxacin, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin
SMHMP23	Chloramphenicol, Ampicillin/Sulbactam, Cefotaxime, Piperacillin, Ceftizoxime, Tetracycline	Co- Trimoxazole, Ciprofloxacin, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin
SMHMP38	Ampicillin/Sulbactam, Cefotaxime, Piperacillin	Chloramphenicol, Co- Trimoxazole, Ciprofloxacin, Ceftizoxime, Tetracycline, Ofloxacin, Gentamycin, Amikacin, Gatifloxacin, Streptomycin, Kanamycin4

Antibiotic resistance and susceptibility test

The heavy metal tolerates rhizobacteria isolates were tested for antibiotic sensitivity. The leading isolates that are tolerant to cadmium lead and Nickel were found to be multi-antibiotic resistant. In the current study, it was detected that the isolates having high MIC values against a set of heavy metals reveals high resistance form towards a group of antibiotics (Table 2).Nath et. al.,reported that they're all the bacteria isolates (Ba/P-5, Ps/G-1, St/P-5, Pr/G-2, and Ps/P-4) were resistant to Cefixime, Methicillin, and Kanamycin. Chloramphenicol showed high sensitivity to Pr/G-2 Ps/G-1, St/P-5, Ps/P-4, St/P-5 but were resistant to K/G-1 and Ba/P-5 [24]. In case of our bacterial strain Chloramphenicol showed high resistance to SMHMZ2, SMHMZ4, SMHMP4 and SMHMP23 but sensitivity to SMHMZ46 and SMHMP38

Other extracellular enzymatic activity of Heavy metal tolerates rhizobacteria

The isolated heavy metal tolerates rhizobacteria, could resist the higher concentration of heavy metals but also produced another extracellular enzyme which will support the organism under stress and contaminated soil. (Table 3)

Table 3 Hydrolytic enzyme activity of heavy metal tolerates rhizobacterial isolates.

	SMHMZ2	SMHMZ4	SMHMZ46	SMHMP4	SMHMP23	SMHMP38
Catalase Activity	+	+	+	+	+	+
Protease Activity	+	+	-	+	+	-
Amylase Activity	-	-	+	-	-	-
Cellulase Activity	-	+	-	+	+	-

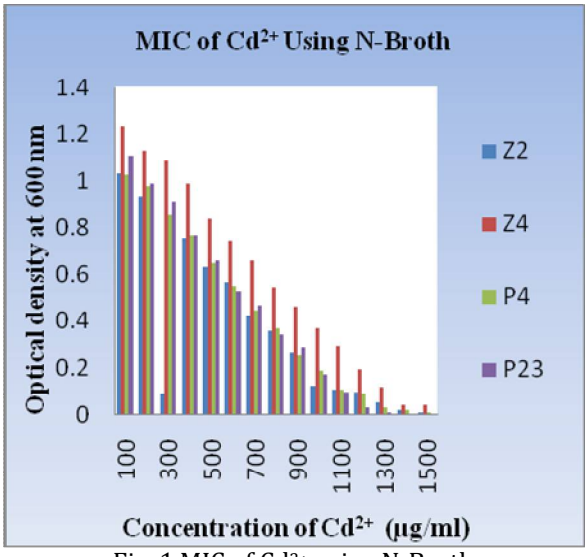

Fig. 1 MIC of Cd^{2+} using N-Broth

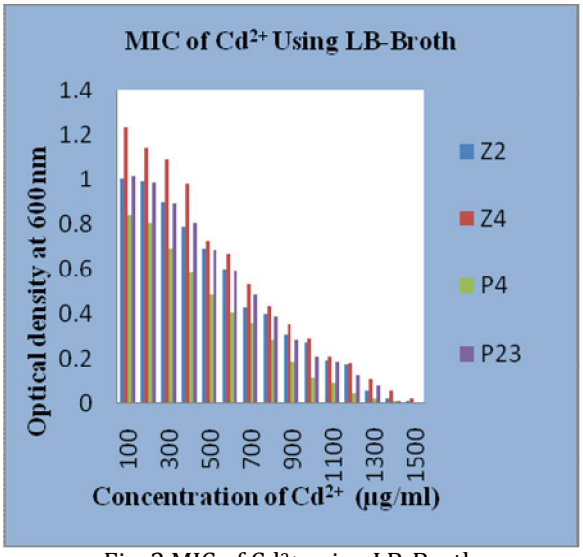

Fig. 2 MIC of Cd^{2+} using LB-Broth

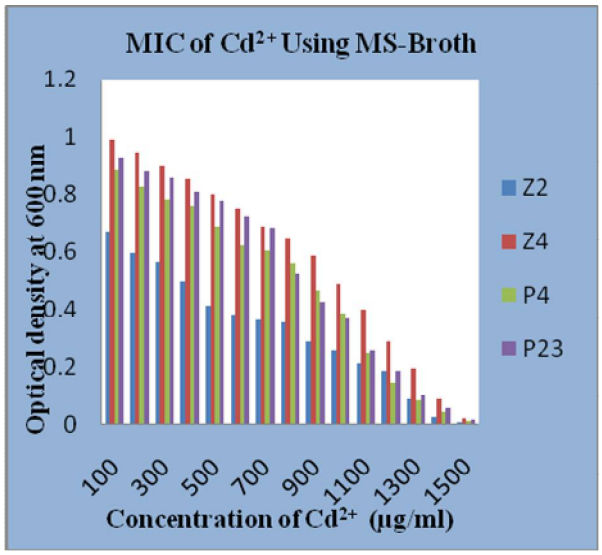

Fig. 3 MIC of Cd^{2+} using MSM-Broth

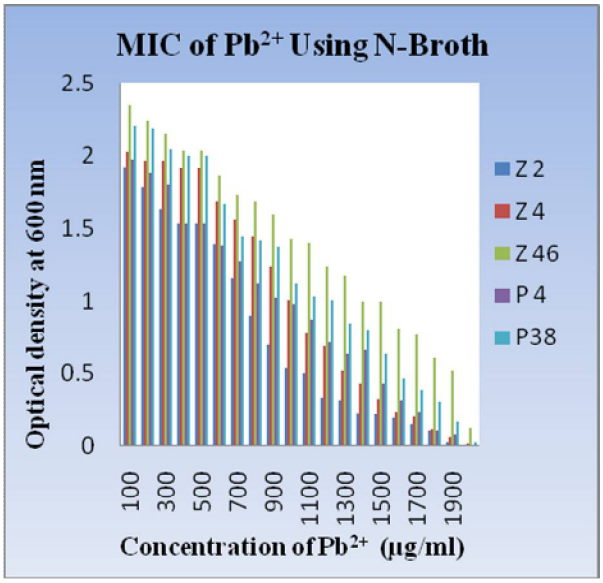

Fig. 4 MIC of Pb^{2+} using N-Broth

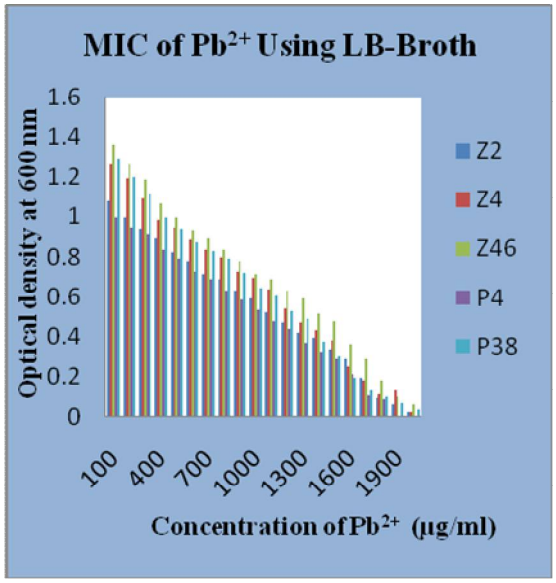

Fig. 5 MIC of Pb^{2+} using LB-Broth

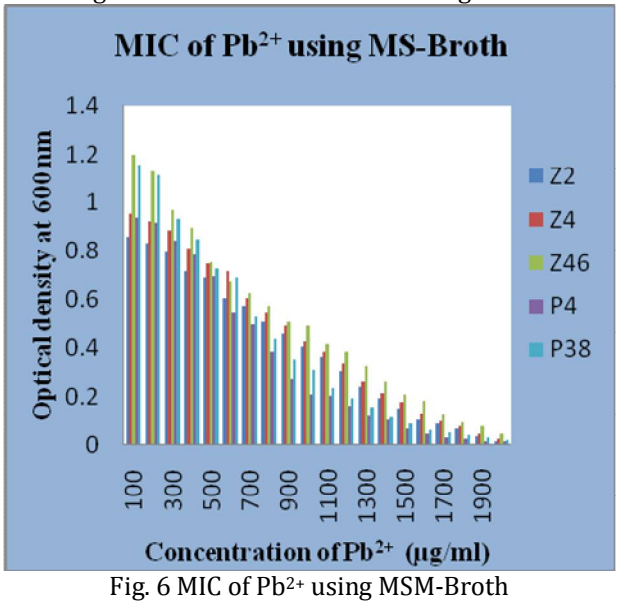

Fig. 6 MIC of Pb^{2+} using MSM-Broth

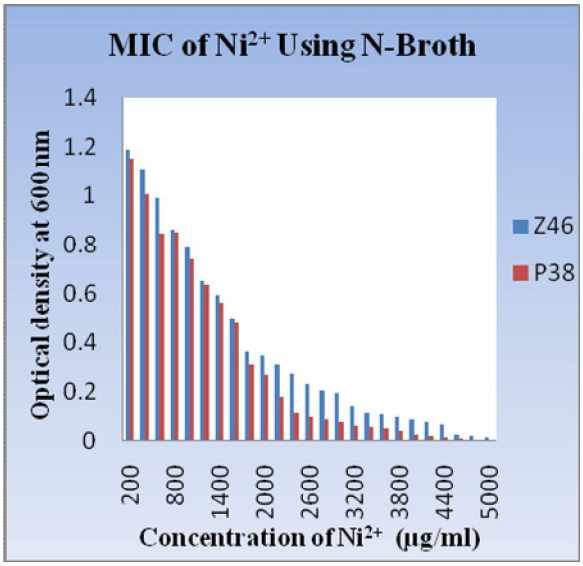

Fig. 7 MIC of Ni^{2+} using N-Broth

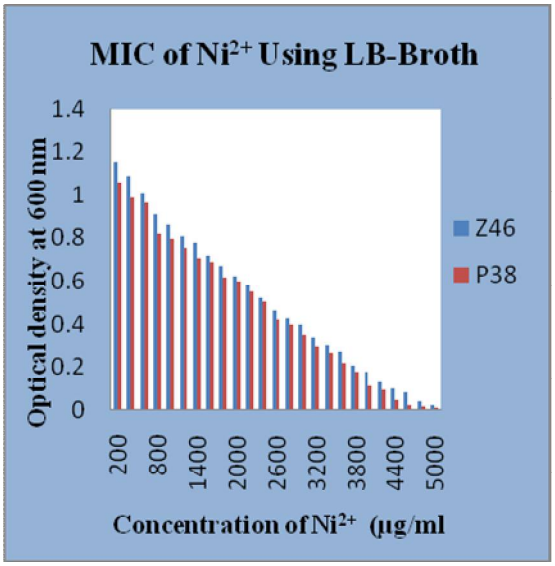

Fig. 8 MIC of Ni^{2+} using LB-Broth

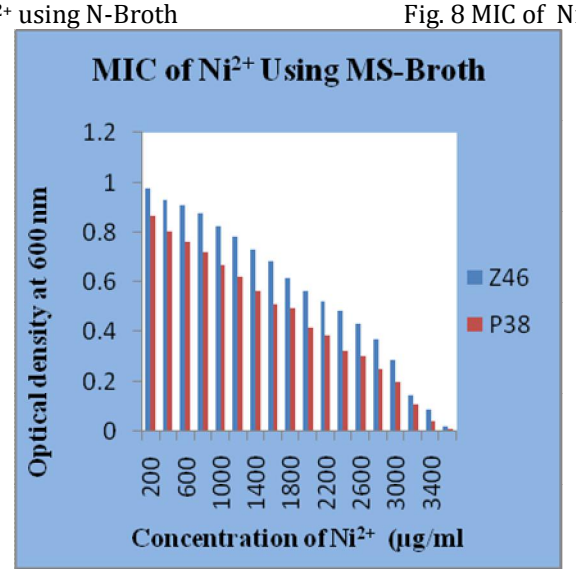

Fig. 9 MIC of Ni^{2+} using MSM-Broth

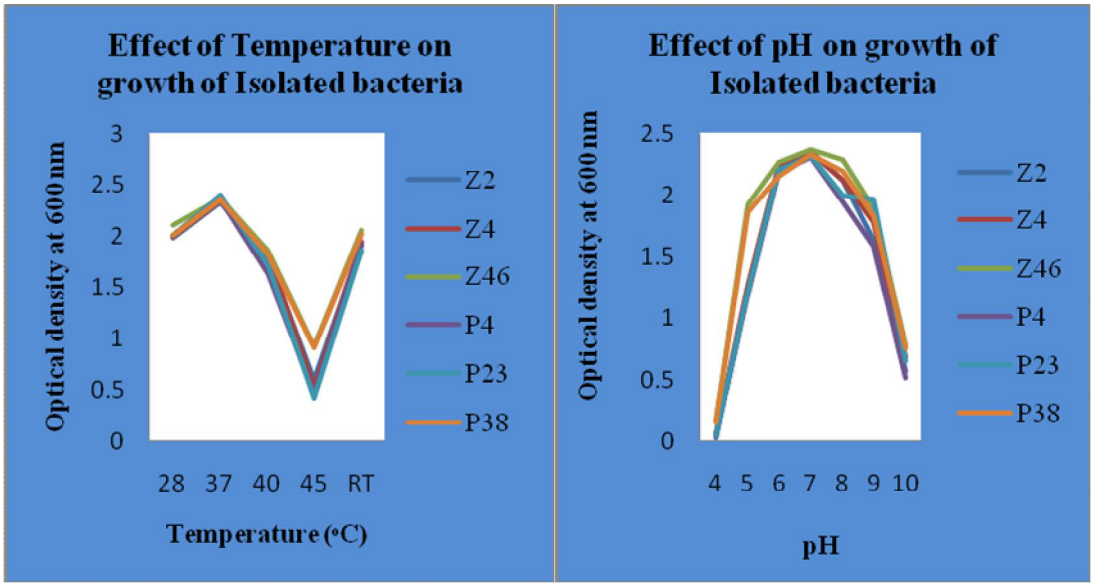

Fig.10 The effect of Temperature and pH on growth of isolated rhizobacteria

CONCLUSION

There are several techniques existing for removal of toxic heavy metals from the environment, but this study discovered some important results for metal detoxification by using microorganisms. Results of present study determine that microorganism isolated from mining area and landfill site developed the flexibility to tolerate heavy metal stress. Bacterial strains isolated in the course of this study are often efficiently used for removal of toxic heavy metals from ecosystem and reinforce the ecological balance. The use of microbes for bioremediation is highly recommended due to its low cost and environmentally friendly approach. Isolated heavy metal tolerates rhizobacteria could also be advantageous for potential agents for bioremediation and in grouping with phytoremediation of heavy metals in the contaminated environment. This nature friendly technique has turned out to be the best obtainable method which is highly proficient under heavy metal stressed environment.

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