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ORIGINAL ARTICLE

Biosorption of Heavy Metal (Mn^{2+}) by Thermophilic Bacterial Strains Isolated from Surya Kund Hot Spring, Yamunotri, Uttarakhand

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Abstract
This investigation aimed to identify the bioremediation potential of Mn^{2+}-resistant bacterial strains cultured from the Surya Kund hot spring, Yamunotri, Uttarakhand. In this study, eight heavy metal-resistant isolates belonging to two phyla, i.e., *Firmicutes* and *Proteobacteria, were investigated for their* Mn^{2+} *biosorption potential.* The metal tolerance potential of all the thermophilic bacterial strains was determined by MIC. Bioremediation assay of these metal-resistant strains was performed for Mn^{2+} through the live and dead biomass of the bacterial cell. The evaluation of the bioremediation rate of metal ions through bacteria was done by AAS. All the selected bacterial strains were evaluated with effective biosorption rates for Mn^{2+}. *Acinetobacter sp.* LSN-10 (YII-1) has been showing the highest potential for the removal of Mn^{2+} in both live (41.202%) and dead biomass (64.721%) conditions. The bioremediation rate of dead biomass was observed quite higher in comparison to bioremediation through live bacterial cells in the maximum number of isolates. This study may provide a new eco-friendly and cost-effective approach to dealing with metal toxicity. However, further investigation is needed to identify the most effective applications and potential limitations of this method.

Keywords Thermophiles · Bioremediation · Hot spring · Mn^{2+} · Heavy metals

Introduction

In the present time, anthropogenic activities such as extensive industrialization, technological advancement, and urbanization have been raising the number of metal pollutants in the environment and become the key sources of metal pollution all over the world [1]. As, Cd, Ni, Cr, Cu, Pb, and Zn are the most commonly found toxic metal ions of industrial wastewater [2]. The contamination of toxic heavy metal ions in air, water, soil, and soil

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sediment has been creating an alarming situation for our biological ecosystem [3]. Various natural and anthropogenic resources are the main source of metal pollution. Industries such as electro-osmosis, electrolysis, metallurgical mining, distilleries, tanneries, manufacturing of electrical appliances, paints, electroplating, textiles, plastics, rubber, leather, paper, cosmetics, food, pharmaceuticals, production of iron, steel, energy, fertilizers, pesticides, and aerospace including atomic energy installations are some main sources of metal pollution in the environment [4, 5].

Although some metal elements such as micro and macro elements are necessary for the growth and development of the human body only in the prescribed concentration, if the concentration of heavy metals may increase above their prescribed limits, they could become mutagenic, teratogenic, carcinogenic, and dangerous for human health [3, 6]. These heavy metals cause serious threats to the ecosystem and living organisms by altering the enzymatic activities; disrupting the cell membrane; denaturing the DNA; damaging the liver and kidney; and causing birth defects, mental and physical retardation, and skin lesions [3, 5, 7]. Manganese (Mn) is an important micronutrient for many living organisms because it is required for the growth and survival of bacteria, fungi, plants, and humans. Mn^{2+} can become a health and environmental problem because, at high concentrations, this metal could be toxic to human health. Exposure to a high concentration of Mn^{2+} ion is harmful to human health as it causes insomnia, loss of appetite, and problems with memory. The metal element is usually found in wastewater and drainages of different industrial sectors. The removal of manganese metal ion is notoriously difficult because of the high stability of the Mn^{2+} in aqueous solutions. Due to the toxic effect of this metal, it is necessary to remove excess amount of Mn^{2+} from the environment [8]. Some other highly toxic metals such as arsenic, cadmium, and lead are examples of some lethal elements, as they can be "carcinogenic for both, humans and animals," while lead exposure is responsible for 3% of cerebrovascular diseases worldwide [9].

The increased number of populations, lack of awareness, and avoidance of environmental sustainability have been playing a key role in the disposal of metal pollutants into the environment regularly, resulting in the bioaccumulation of these metals into different trophic levels of the food chain [3]. Therefore, it is necessary to develop a sustainable and cost-effective bacterial bioremediation approach to deal with this problem of metal toxicity effectively. However, there are many conventional physicochemical methods such as precipitation, ion exchange, electrochemical, and reverse osmosis available for the removal of heavy metals but these methods are very costly and found least effective [2]. In comparison to these conventional methods, the processes of bioremediation are preferable as these are environmentally safe and techno-economically feasible and do not generate any toxic by-products [3]. Bioremediation is involved in degrading, removing, altering, immobilizing, or detoxifying various chemicals and physical wastes from the environment through the action of bacteria, fungi, and plants [10]. According to a report from the United States Environmental Protection Agency (USEPA), bioremediation accounts for 24% of the remediation technologies selected for soil and groundwater remediation [11].

Microbial bioremediation is an important and prominent example of bioremediation. Microbial action mainly catalyzes the two processes of bioremediation mechanism. First is metal immobilization and second is metal mobilization, which could help detoxify toxic metal into its simpler form [2]. The process of metal immobilization and mobilization makes the process of bioremediation more efficient and effective for metal detoxification. Microorganisms are involved in bioremediation through their protein action and enzymatic pathways that act as biocatalysts and facilitate the progress of biochemical reactions to degrade the desired pollutant. Although structural uniqueness, extra polymeric substances, cell wall composition, and genetic composition of microbial species also become

an important source to enhance the rate of bioremediation, the efficiency of bioremediation depends on many factors, including chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population, and environmental factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) [10, 12].

Among all the types of microbial bioremediation, bacterial bioremediation is a more promising alternative. The unique properties of bacterial cells and specific metal detoxification mechanisms can make the process of bacterial bioremediation more efficient and feasible to reduce the toxicity of heavy metals [13]. In the condition of massive accumulation of toxic metals, the majority of bacterial species have developed genetic resistance mechanisms and specific metabolic pathways against metals to handle their toxicity [9].

The living and dead biomass of the bacteria can be effectively used as biosorbents for the removal of heavy metal ions [14]. The negative charge of bacterial cells is present due to various anionic structures on their cell surfaces that promote the binding of metal cations [14] Therefore, bacteria are good adsorbents because of having surfaces for the absorption of chemicals like teichoic acid, small size and lipo-polysaccharides, and their high surface-to-volume ratio [15].

To simplify complexity, most investigations on metal elimination employ dead biosorbents as the favored alternative. Autoclaving a bacterial biosorbent improves its capacity to biosorb heavy metal, possibly because the cell wall is degraded, exposing potential possible binding sites that can accommodate more metal ions. Biosorption of heavy metal ions is a main mechanism of dead biomass bioremediation. Dead biosorbents have multiple advantages in comparison with their live counterparts, such as high efficiency, no requirement for growth media or nutrients, reduced waste sludge generation, and low cost. Using dead bacterial biomass also reduces the concern of introducing alien microbes, a possible risk of microbial infection, and the cost of maintenance.

Also, live biosorbents possess a unique collection of benefits. They can transport adsorbed heavy metals inside cells and change the nature of heavy metal ions to minimize toxic effects [16]. However, only a few studies have evaluated the capability of dead and life biosorbents to adsorb hazardous heavy metals [17].

Hot water springs represent harsh environmental conditions across the entire Himalayan region [18]. Hot water ponds have water temperature higher than atmospheric temperature in surroundings. The temperature of geothermal springs is influenced by the temperature of the magma (for volcanic springs), the depth of penetration, and the nature of conduits of water to the surface. The geochemical dissolution of the rock increases with temperature and that may increase the presence of metal ions in hot spring [19]. These springs deposit high concentrations of various metal ions on the surface of the Earth. These metal deposits can become significant point sources for heavy metal pollution of streams (e.g., arsenic, mercury, cadmium, zinc, lead, manganese, and cadmium).

Hot springs are research hot spots not only for the geologists and ecologists but also for microbiologists as they are considered rich repertoire of thermophilic bacteria [20]. Bacteria thriving in hot springs may get metal resistance due to availability of heavy metals that are released through some geological processes and weathering of rocks that make them able to develop a metal-resistant system to adapt with toxic metal concentrations of atmosphere [19, 21]. Since many metals, such as arsenic, copper, manganese, and iron, are naturally present in thermal springs, therefore, these springs are the common habitat of various heavy metal-resistant bacterial species. Screening of bacterial isolates from hot

springs reveals potential bacterial candidates with diverse biophysical properties that can be useful in bioremediation of hazardous polluted water [22].

Thermophiles have stable thermozymes that make them more resistant to toxic heavy metals [23, 24]. Mathew et al. [25] reported that a thermophile bacterium also produced a thermostable S-amino-transaminase that is highly active at temperatures up to 80 °C [26, 27] and can be used to degrade metal pollutants. Oxidoreductases and oxygenases are some other bacterial enzymes that also play an important role in bioremediation. Quinone reductase, chromate reductase, flavin reductase, flavin oxido-reductase, nitroreductase, and superoxide dismutase are some enzymes that help to remediate chromium. Arsenate reductase helps to remediate arsenic, selenate reductase, thioredoxin reductase (TrxA and TrxB), fumarate reductase (FccA), nitrite reductase (NirBD), and oxyanion reductase remediate selenium; superoxide dismutase also removes lead, iron reductase removes iron, and mercury reductase remediates mercury from the environment [28]. Manganese superoxide dismutase is an important enzyme that removes toxic Mn^{2+} from the environment [29].

The enzymatic catalysis of thermophilic bacteria helps in the solubilization of toxic metals from their higher oxidation state to their lower oxidation state [2, 30]. The thermophilic species of bacteria can actively participate in the solubilization and precipitation of toxic heavy metals that may contribute to the transformation of a polluted environment. In this study, we have studied the Mn^{2+} metal ion-resistant properties and bioremediation potential of some thermophilic bacterial strains that were isolated from the Surya Kund hot spring, Yamunotri, Uttarakhand, India. The metal bioremediation of potential bacterial strains was analyzed through atomic absorption spectroscopy (AAS). This novel research is linked with isolation of thermophilic bacteria from Surya Kund hot spring, Yamunotri, and their role in bioremediation. Current research may shade the light on the importance of hot springs located in famous pilgrim sites of Uttarakhand Garhwal Himalaya to remove toxic metal ions from the environment.

Materials and Methods

Microorganisms and Culture Conditions

Eight bacterial strains were isolated from the Surya Kund hot spring, located at Yamunotri Temple, District Uttarkashi, Uttarakhand, India (Fig. 1), in our previous work [27]. These strains include *Acinetobacter* sp. strain LSN1-10 (YII-1), *Pseudomonas aeruginosa* strain MLTBM2 (YII-16), *Acinetobacter* sp. strain L161 (YII-19), *Parageobacillus toebii* NBRC 107807 strain DSM 14590 (Y8-I), *Pseudomonas punonensis* strain LMT03 (Y9-II), *Paenibacillus thiaminolyticus* strain NBRC 15656 (Y10), *Pseudomonas alloputida* strain BG-3 (Y12-II), and *Enterobacter hormaechei* strain A26 (Y19). These strains were pre-selected based on their highest heavy metal tolerance. Their sequences have been deposited in GenBank with accession numbers MZ330689 (YII-1), MZ330857 (YII-16), MZ092845 (YII-19), MZ164963 (Y8-I), MZ164970 (Y9-II), MW917243 (Y10), MZ164972 (Y12-II), and MZ330682 (Y19). All the isolates were successfully grown at 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, 75 °C, and 80 °C. Optical density (O.D.) was taken at 600 nm after 24 h of incubation broth at 60 °C, 65 °C, 70 °C, and 75 °C to determine the optimum growth. The optimum growth was observed at 65 °C for all the isolates. In the same way, all isolates were successfully grown at various pH ranges such as 6, 7, and 8. The optimum growth was observed at pH 7. Therefore, the bacterial cultures were routinely grown at a temperature of 65 °C in Tryptone Soya Agar (TSA) medium (enzymatic digests of casein and soybean meal at 7.3 ± 0.2) for 24 h.

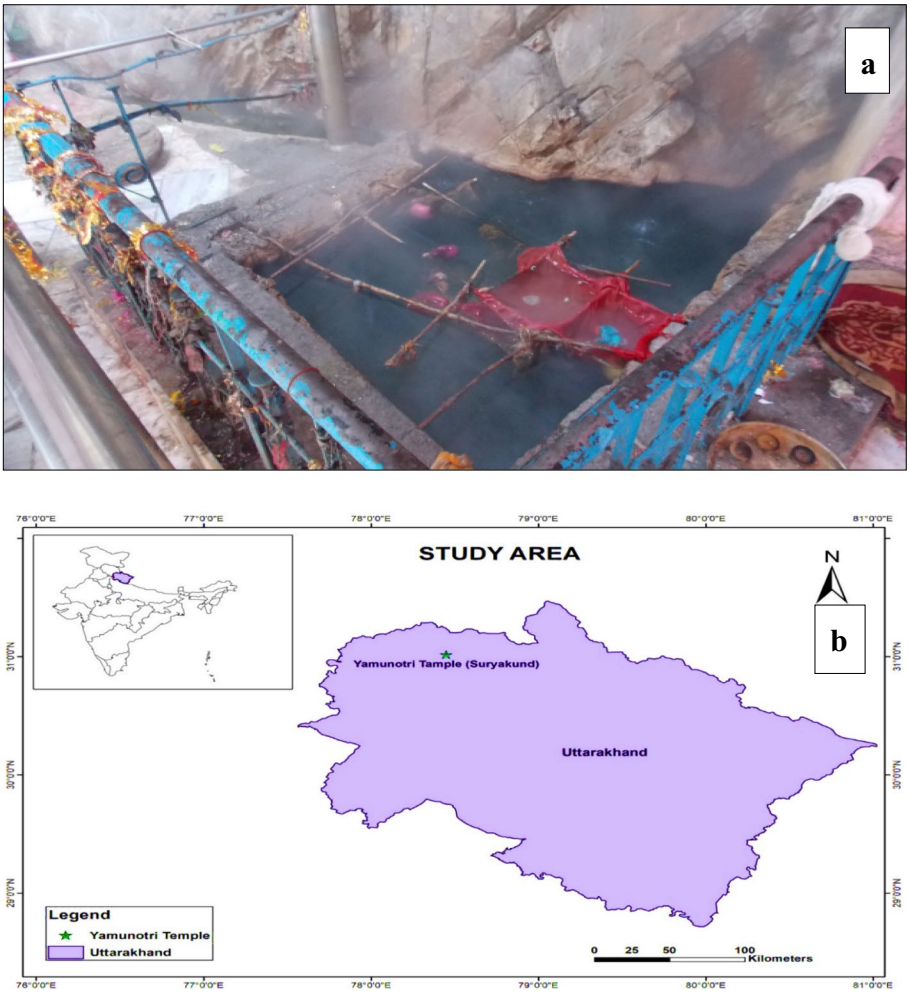

Fig. 1 Study site: **a** Surya Kund hot spring, Yamunotri, India; **b** geographical location of the study site in map

Proteobacteria is the second largest phylum, which are composed of mostly gram-negative, mesophilic and neutrophilic bacteria. The members of this phylum are found in diverse classes (alpha-, beta-, gamma-, and epsilon Proteobacteria) [31]. The phylum Proteobacteria includes a total of six bacteria, i.e., *Acinetobacter* sp. strain LSN1-10, *Pseudomonas aeruginosa* strain MLTBM2, *Acinetobacter* sp. strain L161, *Pseudomonas punonensis* strain LMT03, *Pseudomonas alloputida* strain BG-3, and *Enterobacter hormaechei* strain A26.

The Firmicutes are a phylum of bacteria, most of which have gram-positive cell wall structure. They have round cells, called cocci (singular coccus), or rod-like forms (bacillus). Many Firmicutes produce endospore, which are resistant to desiccation and can survive extreme conditions [32]. Two bacteria *Parageobacillus toebii* NBRC 107807 strain DSM 14590 and *Paenibacillus thiaminolyticus* strain NBRC 15656 belong to the phylum Firmicutes.

Preparation of Heavy Metal Stock Solution

The heavy metal solution (1000 mg/l) of Mn^{2+} was prepared by dissolving its 100% pure chloride salt ($MnCl_2 \cdot 4H_2O$) with sterilized distilled water [33]. After the solid is completely dissolved, dilute the solution to a final volume with deionized (distilled) water. Further filtered solution of metal was digested with a few drops of conc. HNO_3 to avoid any precipitation. Working concentration of heavy metal mixture was obtained by serial dilutions. The stock solutions of 1000 ppm were stored in the dark at 4 °C.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of heavy Mn^{2+} at which no colony growth occurs was determined by the agar dilution method [34]. Sterilized metal-containing agar plates were prepared with an initial metal concentration of 100 mg/l. Plates were inoculated with bacterial strains and incubated at 65 °C temperature for 24 h. Bacterial growth, depending on the individual bacterial isolates, was observed and transferred to other agar plates by streak plate method containing higher metal concentration until the isolates failed to grow. The dilution of heavy metal which completely inhibited the bacterial cell growth in the medium was considered MIC [34].

Assessment of Bioremediation Potential of Bacterial Isolates

The assessment of absorbed metal concentration was done by atomic absorption spectrophotometer (AAS). AAS is a technique used for determining the concentration of a particular metal element within a sample. The atomic absorption spectrophotometer (Varian Model—AA 240) was used for the detection of metal ion. All the samples were run sequentially with their standards. The procedure of AAS is given below:

Sample Preparation

Transfer a measured volume (50 ml) of well-mixed, acid-preserved sample to a flask or beaker. Add 5 ml con. HNO_3 and a few boiling chips or glass beads. Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 to 20 ml). Continue heating and add conc. HNO_3 as necessary until digestion is complete as shown by a light-colored, clear solution. Do not let the sample dry during digestion. Wash down the flask or beaker walls with water and then filter if necessary. Transfer the filtrate to a 100-ml volumetric flask with two 5 ml portions of water, adding these rising to the volumetric flask. Cool, dilute to mark, and mix thoroughly. Take portions of this solution for required metal determinations.

Standardization

Select at least three concentrations of each standard metal solution to bracket the expected metal concentration of a sample. Aspirate blank and adjust zero of the instruments. Then, aspirate each standard in turn into flame record absorbance. Prepare a calibration curve by plotting on linear graph paper the absorbance of standard versus their concentrations. For instruments equipped with direct concentration readout, this step is unnecessary.

Data Analysis and Calculations

Calculate the concentration of each ion, in mg/l for trace elements and in mg/l for more common metals, by referring to the appropriate calibration curve. Alternatively, read the concentration directly from the instrument readout if the instrument is so equipped. If the sample has high values, multiply it by the appropriate dilution factor.

The evaluation of the bioremediation potential of bacterial strains was done by two methods. First is through live bacterial cells [34] and second through dead biomass of bacteria [35]. The result was compared with the control to calculate heavy metal degradation capacity (%) as follows.

$$\%\text{of heavy metal utilized} = \frac{\text{initial metal concentration} - \text{final metal concentration}}{\text{initial metal concentration}} \times 100$$

Bioremediation of Mn^{2+} by Live Bacterial Cell One hundred microliters of the overnight-cultured bacteria was inoculated into 20 ml of the broth medium and incubated at 150 rpm for 48 h at 65 °C. One hundred milligrams per liter of sterilized solution of Mn^{2+} was added in the medium and incubated the same for another 24 h. The bacterial cells were separated by centrifugation at 10,000 rpm for 15 min. The supernatant was collected and digested with 2% conc. HNO_3 and used for the estimation of the metal biosorption rate of bacteria through atomic absorption spectroscopy (AAS) [35].

Bioremediation of Mn^{2+} by Bacterial Dead Biomass One milliliter of the overnight grown culture of the thermophilic isolate was inoculated in 500-ml broth medium and incubated at 65 °C and 150 rpm for 24 h. Bacterial cells were separated by centrifugation at 10,000 rpm for 30 min. The obtained cell biomass of each bacterium was dried at 80 °C for 24 h and grind to make cell powder [35] that was further autoclaved to kill the bacterial cells. The complete death of cells was confirmed by inoculating some amount of powder in the broth medium. To check the metal biosorption rate of bacterial dead biomass, 200 mg of dried powder was suspended into 10 ml of 100 mg/l of Mn^{2+} solution for 24 h at 200 rpm and 65 °C. This solution was centrifuged at 10,000 rpm for 20 min and filtered to eliminate residual cell debris. The collected supernatant was digested with 2% conc. HNO_3 and used to check the metal biosorption rate through atomic absorption spectroscopy (AAS) [35].

Results and Discussion

Microorganisms and Culture Conditions

The water samples were collected from the western region of the Garhwal Himalayas at an altitude of 3291 m and geographic coordinates of 3101′ 0.12″ N 78027′ 0″ E. The water body is thoroughly independent of temperature-affecting environmental factors. The temperature of the spring was found 86.5±0.2 °C. The water was colorless and pH was recorded as slightly alkaline 7.3±0.2. The temperature of hot springs remains the same throughout the year and is not affected by the seasonal variation of the surroundings; the same observation of the alkaline nature of this hot spring is also reported by Ranawat and Rawat [36]. Another study of two hot springs from Himachal, India, by Kumar et al. [37],

also reported alkaline hot springs with high temperatures (85–95 °C). However, these studies reported pH of the same site was slightly higher. The nine hot springs situated in Tato Field, Tatta Pani, and Murtazaabad, in Pakistan, were reported highly diverse in their environmental conditions and ranging in temperature from 60 to 95 °C and in pH from 6.2 to 9.4 [38].

In the present study, the growth temperature requirement for most of the isolates was 40–80 °C. All the isolates were able to grow at a temperature of 80 °C. Therefore, they could be classified as thermophilic bacteria according to Brock [39]. The optical density (O.D.) was measured at 600 nm to analyze bacterial growth at optimum temperature and pH range. The optimum temperature for most of the isolates was 65 °C (Table 1 and Fig. 2) and optimum pH range was 7 (Table 2 and Fig. 3). The temperature of the site and temperature requirements show relevance with the hot springs of Manikaran, Himachal Pradesh

Table 1 Growth density of bacterial strains at different temperature range

Bacterial isolate	Temperature			
	OD at 60 °C	OD at 65 °C	OD at 70 °C	OD at 75 °C
YII-1	1.38	1.59	0.47	0.12
Y8-I	0.87	1.25	0.29	0.00
YII-16	0.07	0.09	0.00	0.00
YII-19	0.04	0.82	0.00	0.00
Y10	0.02	0.74	0.00	0.00
Y12-II	1.27	1.34	0.37	0.01
Y-19	1.20	1.39	0.27	0.00
Y9-II	1.09	1.14	0.11	0.00

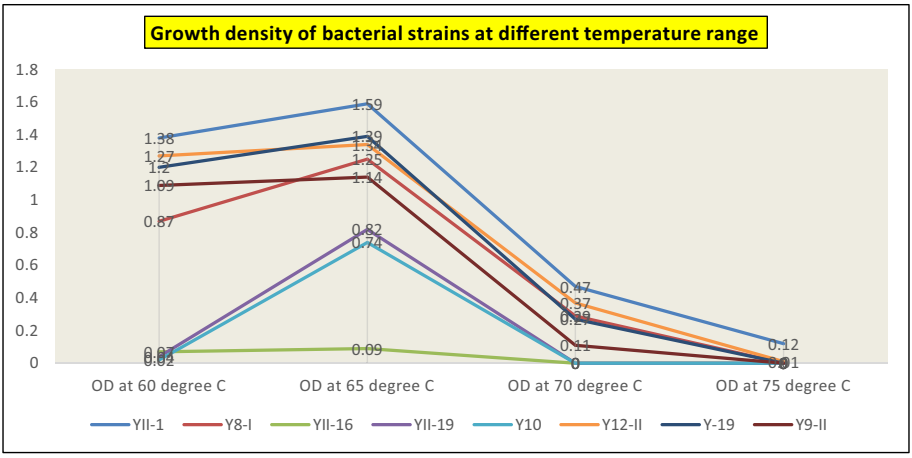

Fig. 2 Observed optimum temperature, i.e., 65 °C, for the growth of bacterial strains

Table 2 Growth density of bacterial strains at different pH range

Bacterial isolate	pH		
	OD at pH 6.0	OD at pH 7.0	OD at pH 8.0
YII-1	0.02	0.35	0.00
Y8-I	0.00	0.02	0.00
YII-16	0.01	0.38	0.00
YII-19	0.02	0.36	0.00
Y10	0.02	0.43	0.00
Y12-II	0.03	0.24	0.00
Y-19	0.00	0.03	0.00
Y9-II	0.05	0.64	0.00

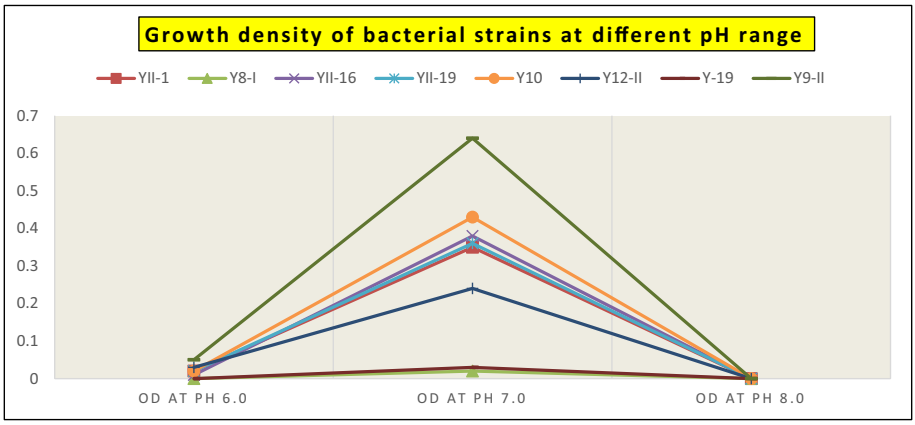

Fig. 3 Observed optimum pH 7.0 for the growth of bacterial strains

[40]. Thermophilic bacteria are an important tool for research as they have value in both basic and applied biology. In the present study, an attempt was made to explore the bioremediation potential of bacterial strains of Surya Kund hot spring. From the bioremediation point of view with the thermophilic bacteria from this site, no literature was found. So, this study area fulfills the research objective of the study.

Determination of Minimum Inhibitory Concentration (MIC)

All eight bacterial isolates YII-1, Y8-I, YII-16, YII-19, Y9-II, Y10, Y12-II, and Y19 were found resistant against selected Mn^{2+} through MIC. The MICs of Mn^{2+} heavy metal ions toward the 8 bacterial isolates are shown in Table 3**.** For Mn^{2+}, MIC was highest for Y10 (375 mg/l) strains and lowest for YII-1 and Y12-II (300 mg/l) strains (Fig. 4).

Table 3 MIC of Mn^{2+} tolerated by the isolated bacterial strains

Isolate	Metal concentration (mg/l)
YII-1	300
Y8-I	325
YII-16	325
YII-19	325
Y10	375
Y12-II	300
Y-19	350
Y9-II	375

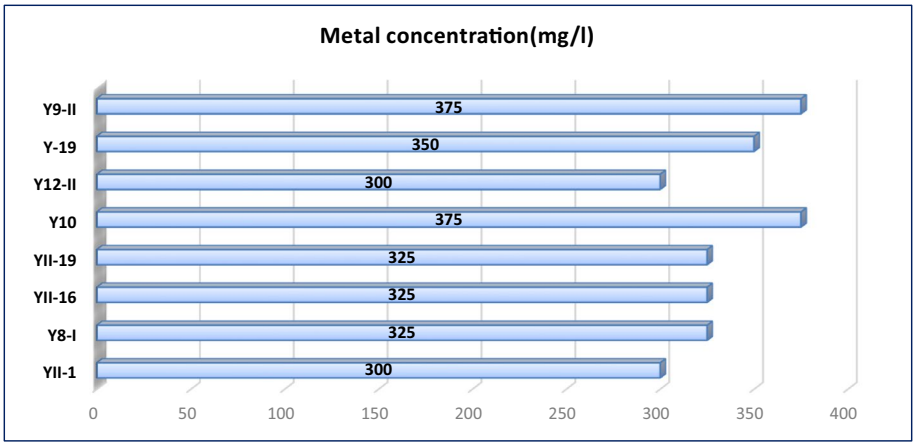

Fig. 4 Heavy metal tolerance potential and MIC of the isolated strains

Assessment of Bioremediation Potential of Bacterial Isolates

1. Bioremediation of Mn^{2+} by Live Bacterial Cell

Atomic absorption spectroscopic analysis of the control and collected supernatant estimated that the concentration of Mn^{2+} ions has been decreased after 24 h of incubation. The density and growth of culture were increased after inoculation of heavy metal but with slow rate in comparison to the growth of culture without any metal ion. The details of tested isolates and metal are given in Table 4. The concentration of manganese in the control reported by AAS analysis was 98.382 mg/l. The highest and lowest percentage of metal biosorption has been detected in YII-1 (41.202%) and YII-16 (25.068%) (Fig. 5); both strains belong to the phylum Proteobacteria.

Table 4 Metal absorption percentage of live isolated bacterial strains

Metal	Isolate	Initial metal conc. (mg/l)	Final metal conc. (mg/l)	Bioremediation (%) with live bacterial cells
Mn^{2+}	Y9-II	98.382	65.210	33.172
	Y8-I		71.952	26.430
	YII-19		61.00	37.382
	Y19		69.329	29.053
	YII-16		73.314	25.068
	Y10		64.440	33.942
	Y12-II		69.357	29.025
	YII-1		57.180	41.202

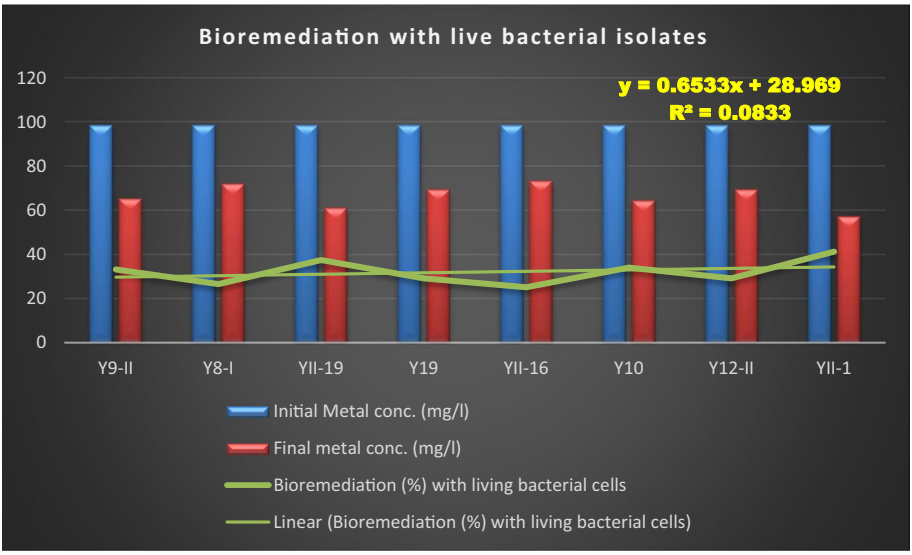

Fig. 5 Rate of Mn^{2+} absorption through live cells of resistant strains

2. Bioremediation of $Mn^{2+}$by Bacterial Dead Biomass

The density and growth of culture were increased after inoculation of heavy metal but with slow rate in comparison to the growth of culture without any metal ion. Atomic absorption spectroscopic analysis revealed that the maximum biosorption of manganese was found in YII-1 (64.721%) and the minimum in YII-16 (24.792%) (Fig. 6). AAS results of biosorbed metal conc. in samples are given in Table 5 along with bioremediation % of each tested isolate. The concentration of manganese in the control reported by AAS analysis was 98.106 mg/l. The highest and lowest percentage of metal biosorption has been detected in YII-1 (64.721%) and YII-16 (24.792%). These two strains belong to the phylum Proteobacteria.

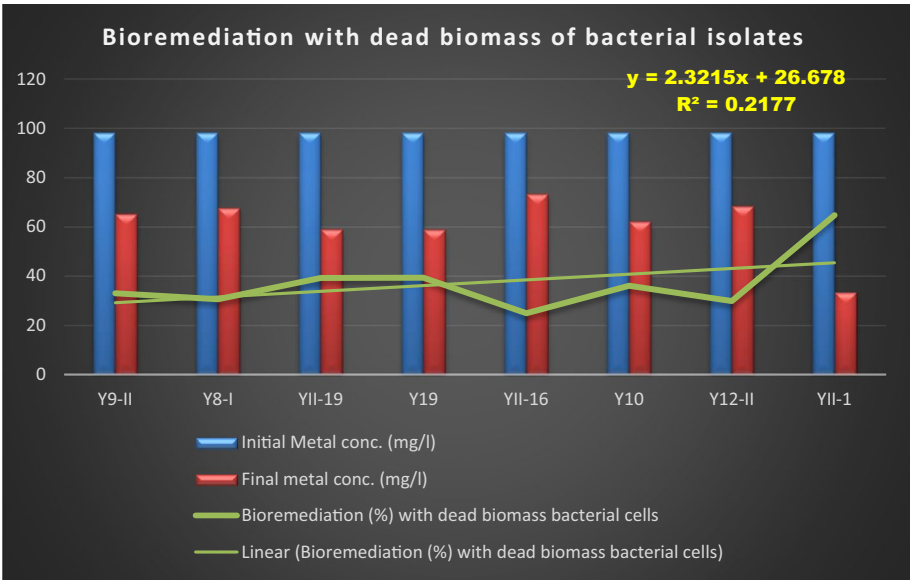

Fig. 6 Rate of Mn^{2+} absorption through dead biomass of strains

Table 5 Metal absorption percentage of dead biomass of isolated strains

Metal	Isolate	Initial metal conc. (mg/l)	Final metal conc. (mg/l)	Bioremediation (%) with dead biomass bacterial cells
Mn^{2+}	Y9-II	98.106	65.210	32.896
	Y8-I		67.608	30.498
	YII-19		58.937	39.169
	Y19		58.898	39.208
	YII-16		73.314	24.792
	Y10		62.139	35.967
	Y12-II		68.357	29.749
	YII-1		33.385	64.721

All the isolates were able to grow in manganese metal-containing plates. Bioremediation experiments performed with live biomass of Y9-II, YII-16, and Y12-II, related to the genus *Pseudomonas,* were able to remove Mn^{2+}. A study on bioaccumulation of heavy metals in mesophilic conditions with two *pseudomonas* strains was conducted by Hussein *et* al. [41]. In this study, 2 heavy metals were applied in combination, and Cu (II) accumulation (151.42 mg/l) and Ni (II) accumulation (54 mg/l) were reported. However, Cr (II) accumulation was also reported but the growth of Cr (VI)-resistant *Pseudomonas fluorescens* strain was directly inhibited when the Cr (VI) concentration

attained 3 mmol/L. Isolates YII-19 and YII-1 were related to *Acinetobacter sp.* and showed a good potential for the bioremediation of Mn^{2+}. YII-1 strain is 99.36% similar to *Acinetobacter sp.* LSN10 has been showing the highest potential for the removal of Mn^{2+} ions in both live (41.202%) and dead (64.721%) conditions. *Acinetobacter* sp. contributes to phosphorus removal in wastewater treatment plants [42]. Acinetobacter species such as *A. guillouiae* showed an efficient removal mechanism for the bioremediation of Cu^{2+} [43].

Detected bioremediation potential of the isolate Y19 related to the genus *Enterobacter* was found efficient about several mesophilic bacterial strains of the same genus *Enterobacter, which have* been reported as potent bioaccumulation of heavy metals [43, 44]. Y8-1 and Y10 are the Firmicutes showing efficient potential to remove manganese ions. Gupta et al. [45] suggested that Firmicutes members have the potential to reduce the concentration of sulfate and iron from the environment through bioremediation in favorable conditions.

The bioremediation with dead bacterial mass was observed quite higher in comparison to bioremediation with live bacterial cells (Fig. 7). YII-1 strain is 99.36% similar to *Acinetobacter sp.* LSN10 has been showing the highest potential for the removal of Mn^{2+} ions in both live and dead cell bioremediation, but the rate of Mn^{2+} biosorption is high in dead biomass (64.721%) in comparison to living (41.202%) cells of YII-1. The same results have also been observed in the case of the remaining isolates except Y9-II and YII-16, although the difference was negligible and considered the same. An enhancement of metal biosorption is expected in the case of dead cells as heat treatment of cells would have broken the cell wall, yielding more binding sites. Such dead biomass is more or less a particulate matter and thus can be cheaply stored and easily handled [34].

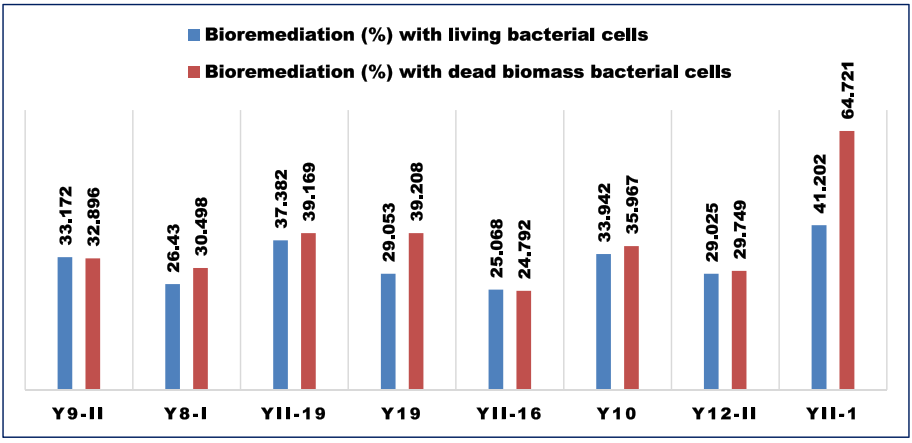

Fig. 7 Comparative analysis of Mn^{2+} absorption potential between live cell and dead biomass of thermophilic bacterial strains

Conclusion

Hot springs are an excellent model system for studying extra-terrestrial life because of their spiritual and therapeutic values. Temperature is considered one of the most important environmental variables controlling the activities and evolution of organisms. Thermophiles grow at high temperatures and have commercial importance because of their thermostability and thermoactivity. The results of this study for the bioremediation of heavy metals were promising and these bacterial isolates can be exploited further to develop efficient eco-friendly and cost-effective techniques to eliminate metal pollution from the contaminated sites. Biosorption of metal is the mechanism that is used by bacteria to remove toxic metal ions from environment. Results of bioremediation have also proved that the dead biomass is superior over the live bacterial cells; thus, the problem to maintain the live culture of bacteria in the treatment plants at contaminated sites could automatically be subsided. Therefore, this study widens the opportunities for further research to be conducted to explore more the immense significance of thermophilic bacterial isolates and scale up for field trials at the contaminated sites.

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Data Availability Not applicable

Declarations

Ethical Approval and Consent to Participate Approved with consent of participation from all the participants

Consent for Publication Not applicable

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