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Isolation, Characterization and Identification of Microorganisms from Distillery Effluent Contaminated Soil and Ex- situ Bioremediation of Contaminated Soil

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ABSTRACT: In the present study the effluent samples were collected from the distillery industry situated at Kasauli, Solan, Himachal Pradesh, India. Then the samples were subjected to heavy metal analysis and isolation of heavy metal resistant bacteria. The study revealed the presence of six heavy metals i.e. Pb, Cd, Zn, Cu, Mg and Cr in the distillery effluent contaminated soil. All the heavy metals were above the permitted limits, i.e. Pb (189.02 mg/kg), Cd (5.59 mg/kg), Zn (590 mg/kg), Cu (1065.27 mg/kg), Mg (409 mg/kg) and Cr (196.20 mg/kg). The four isolates were selected based on heavy metal tolerance and antibiotic resistances. These heavy metal resistant strains were characterized and identified on the basis of 16S rRNA sequencing as *Proteus vulgaris, Bacillus subtilis, Micrococcus luteus* and *Bacillus cereus.* These selected isolates were examined for their heavy metal uptake capacity and found that isolates had potential for metal uptake. Ex-situ bioremediation was carried out using microbial consortium of four selected isolates and results revealed that the highest percentage of degradation was observed in T3 treatment i.e. bacterial consortium amended with nutrients.

Key words: Distillery effluent, heavy metals, 16S rRNA sequencing, ex-situ bioremediation

INTRODUCTION

In recent years, the pollution of natural resources such as air, water and soil has become one of the most important ecological problems on the planet. There is a great concern for rapidly deteriorating quality of water. The causes of water pollution are many, however urbanization, industrialization and ever-increasing populace are major factors (Alao *et al*., 2010). Water is said to be polluted, when its quality or composition is altered either naturally or as a consequence of human activities and it become unsuitable for drinking and less suitable for domestic, agricultural, industrial, recreational and other uses. Industrialisation contributing to water pollution has reached the alarming situation. The industries, which are substantial from the water pollution point of view are distilleries, sugar industries, textile mills, steel industry, tanneries, oil refineries, fertilizer units, pesticide plants, chemical industries, thermal power plants and metal work industries (Manivasakam, 1987). Distilleries, the alcohol producing industries, are generally characterized under the 17 most polluting industries by the Central Pollution Control Board. In India there are around 330 distilleries, the total installed capacity is about 3500 million litres of alcohol (Hati *et al*., 2007; AIDA, 2005). Distilleries produce an enormous amount of wastewater (spent wash) with massive quantity of organic and inorganic nutrients, thus having high Na, K, Ca, Mg, TKN, BOD and COD load. Distilleries producing alcohol from molasses are considered among the most polluting agrobased industries (Joshi *et al*., 2000). For production of each liter of alcohol, 12–15 liter of effluent is produced. Approximately 40 billion liters of effluent is generated per annum from 330 distilleries in the country. The study showed that the distillery effluent was highly loaded with organic pollutants along with some toxic heavy metals such as Fe, Cd, Cr, Mn and Pb (Ale *et al*, 2008). Heavy metal pollution present in industrial effluent, cause great risks to human health. The most common heavy metals that were found in polluted water include arsenic, copper, cadmium, lead, chromium, nickel, mercury and zinc. The release of these metals without proper treatment poses a significant threat to general wellbeing because of their persistence, biomagnification and accumulation in the food chain. Severe effects include reduced growth and development, cancer, organ damage, nervous system damage and in extreme cases, death.

Exposure to some metals, for example mercury and lead, may also cause development of autoimmunity in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, the kidneys, circulatory system and nervous system (Rajendran *et al*., 2003; Johnson and hallberg, 2005; Oelofse *et al*., 2007). Effluents from industries have noticeable amounts of metallic cations like zinc, copper, iron, manganese, lead, nickel and cadmium (Chopra *et al.,* 2009). These effluents when go into the soil increases organic carbon content, heavy metal accumulation and the chances of their entrance in the food chain and that may ultimately cause significant bioaccumulation (Maldonado, 2008). Therefore, the challenge for the safe disposal of the effluent cannot be ignored. Environmentalists and government are looking for cheap, efficient, effective and long lasting solutions for wastewater treatment and recycling. In developing nations like India, physico-chemical techniques of waste water treatment are certainly cost intensive and cannot be employed in all industries. Hence, in recent years, the biological treatment system has turn out to be popular and has helped in developing relatively efficient, low cost waste treatment systems (Vishakha *et al*., 2013). In order to design an proficient biological waste water treatment, it is important to distinguish the microbiota composition of the wastewater and to identify the strains, which metabolize organic compounds and degrades the toxic heavy metals (Ahring *et al*., 2001; Janczukowicz *et al*., 2007).

MATERIALS AND METHODS

A. Sample collection

Effluent samples were collected from the distillery industry located at Kasauli, 25.5 Kms away from Solan town, under the Solan district, Himachal Pradesh. Samples were stored at 4°C for further analysis.

B. Analysis of soil samples

Soil samples were irrigated with different concentrations of distillery effluent for 30 days and then analysis of the samples was done.

C. Heavy metals analysis (Aqua Regia Method)

Well mixed samples of soil 2 g each were taken in 250 ml glass beakers and digested with 8 ml of aqua regia (mixture of concentrated hydrochloric (HCl) and nitric acids (HNO_3) in the HCl: HNO_3 ratio of 3:1) on a sand bath for 2 hrs and refluxed. The digested mixture was centrifuged at 1500 rpm and the supernatant analysed for heavy metals on Atomic Absorption for concentration by using a specific cathode lamp. AAS was calibrated for each element using a standard solution of known concentration before sample injection (APHA, 1992).

D. Isolation of heavy metal resistant bacteria

Bacterial strains were isolated from distillery effluent contaminated soil by serial dilution method (Adebayo *et al*., 2010). For the selective isolation of heavy metals resistant bacteria, heavy metals incorporated basal media with initial concentration of 10mg/l of lead, copper, chromium, manganese, zinc and iron were used. After initial screening, the isolated bacterial strains were tested for resistance against lead, copper, chromium, manganese, zinc and iron with nutrient agar in concentrations ranging from (20-1000mg/l). The plates were allowed to solidify at 37°C for 24hrs. Growth of the bacterial culture was determined visually as positive or negative. Relative growths of the bacterial isolates were expressed as the percentage of those obtained in untreated control which was taken as 100%.

E. Maximum tolerance of heavy metals

The metals used in the study and detailed procedure to determine the tolerance property, in terms of Maximum Tolerable Concentration (MTC) was carried out (Schmidt and Schlegel, 1994).

F. Determination of antibiotic resistance

The antibiotic resistance was done by standard agar well diffusion method. 100 µl of fresh bacterial cultures were spread on the plates. The antibiotics such as Ciprofloxacin (30µg/ml), Gentamycin (10 g/ml), Ampicillin (10µg/ml), Kenamycin (30µg/ml) and Streotomycin (10 µg/ml) were poured in the wells. The plates were incubated at 37ºC for 24hrs. Inhibition zones in diameters were measured in mm using a caliper. Strains were classified as Resistant (R), Intermediate (I) and Susceptible (S) according to the criteria recommended by the National committee for clinical Laboratory Standards, 2001.

G. Molecular and biochemical characterization

Selected heavy metal resistant isolates were grown on nutrient agar (Himedia, India). The shape and colors of the colonies were examined under the microscope after Gram staining. Biochemical tests were used to identify the isolates according to Bergey's Manual of Determinative bacteriology (Holt *et al*., 1994). Extraction of DNA from bacterial isolates was done as per the protocol described by Atashpaz *et al* (2010).

H. 16S rRNA sequencing of isolates

Polymerase Chain Reaction amplification and sequencing of the extracted DNA samples was done by Eurofins, India. In this case, the first step of the 16S rRNA sequencing was to separate the DNA from the isolates. Then the evaluation of the quality was done in 1-2% Agarose Gel. A fragment of 16S rRNA gene was amplified by Polymerase Chain Reaction from the isolated DNA.

A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel that was continued with purification of PCR amplicon in order to remove the undue containments. The forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 27F and 1492R primers using BDT v3.1 cycle sequencing kit on an ABI 3730xl Genetic Analyser. Consensus sequence of rRNA gene was generated from forward and reverse sequence data using aligner software. The 16S rRNA gene sequence was used to carry out Basic Local Alignment Search Tool with the database of the National Center for Biotechnology Information Genbank database. Based on the maximum identity score, first ten sequences were selected and aligned using the multiple alignment software program. Clustal W. On the basis of percentage similarity a phylogenetic tree was constructed (Saitou and Nei, 1987; Kumar *et al*., 2016).

I. Metal uptake capacity of isolates using microwave plasma atomic emission spectroscopy

The efficient heavy metal tolerant bacterial isolates were further tested for their removal by inoculating 1 ml of cell suspension containing 10^8- 10^9 cells ml^{-1} into 100 ml nutrient broth in 250 ml conical flasks, containing 25 mg/l concentration of each heavy metals (Pb, Cd, Cr, Zn, Mn and Cu). After 72 hrs of incubation at 30^0C, optical density was recorded at 640 nm on the spectrophotometer and bacterial biomass was harvested by centrifugation at 8000 rpm for 10 min. The harvested bacterial biomass was dried in hot air oven at 80^oC and weighed. The dried bacterial biomass was digested in nitric and perchloric acid (3:1). After digestion, digested bacterial biomass was filtered using whatman filter paper no. 42 and volume of the filtrate was made up to 50 ml. Heavy metal content in digested bacterial biomass was estimated by Microwave Plasma Atomic Emission Spectroscopy (MPAES).

J. Ex- situ bioremediation of heavy metal contaminated soil

Biodegradation experiments

Standard inoculums having a concentration of 1.5×10^8 colony forming units (CFU)/ml was used. The ability of bacterial isolate to remediate heavy metal contaminated soil sample was performed by carrying out the biodegradation experiment in soil for 60 days under room temperature (30 ± 3°C). The experiments were carried out in rectangular plastic trays of 30 cm × 23 cm × 6 cm (Length × Width × Height) containing 2.5 kg soil. Prior to starting the experiment, the water-holding capacity (WHC) and pH of the soil were determined. Experiments were conducted with three treatment combinations: Treatment A: Soil + sterile distilled water (abiotic control), Treatment B: Soil + bacterial isolates and Treatment C: Soil + bacterial isolates + nutrients (poultry litter) in the ratio (80:20).

For each experimental unit, soil was inoculated with 1L bacterial consortium 1.5×10^8 cell concentration. The bacterial density in different treatment units was determined by serial dilution method and optical density was checked after 24 to 48 hrs of incubation at 37°C. The extent of biodegradation of heavy metal was determined by atomic absorption spectroscopy.

RESULTS AND DISCUSSION

A. Heavy metal analysis of soil

Analysis of the soil for the presence of heavy metals was carried out using atomic absorption spectroscopy (AAS). In the present study, six heavy metals i.e. Lead (189.02 mg/kg), Cadmium (5.59 mg/kg), Zinc (590 mg/kg), Copper (1065.27 mg/kg), Manganese (409 mg/kg) and Chromium (196. 20 mg/kg) were detected in the distillery effluent contaminated soil (Table 1). The concentration of all the heavy metals was higher in effluent irrigated soil as compared to the control, which was irrigated with tap water. The concentration of almost all the heavy metals exceeded the standard limit of heavy metals, set for agricultural soil. (Ale *et al*.,2008; Kumar and Chopra, 2011) also found the presence of heavy metals Fe, Cd, Mn, Pb, Zn, Cu in the soil irrigated with distillery effluent by ASS and observed that most of the heavy metals were above toxic level. The present results are also consistent with the findings of (Anyakora *et al*., 2013; Vajihabanu *et al*., 2015).

B. Isolation and metal resistant evaluation

A total of 30 microbial isolates were obtained through the serial dilution method, using 10 mg/L concentration of heavy metals (Table 2). These isolates were then subjected to secondary screening.

Table 1: Heavy metals detected in effluent contaminated soil by AAS.

Heavy metals detected (mg/kg)	Control soil	Soil irrigated with effluent	Standard limits of heavy metals		
			EU (mg/kg)	UK(mg/kg)	USA(mg/kg)
Lead (Pb)	14.68	189.02	300	70	300
Cadmium(Cd)	0.70	5.59	3.0	1.4	3.0
Zinc (Zn)	70.89	590	300	200	200-300
Copper(Cu)	12.98	1065.27	140	63	80-200
Manganese(Mn)	128.13	409	-	-	-
Chromium (Cr)	19.27	196.20	180	70	300

The bacterial isolates capable of tolerating high metal salt concentrations ranging from 10-1000 mg/L were selected. Out of which, 4 isolates showed resistance against high concentrations of metal salts used.

C. Determination of maximum tolerance concentrations (MTC) of resistant bacteria

The result showed that, isolate 3 has highest MTC value for Mn (1000 mg/L), Pb (600 mg/L) and Cu (300 mg/L), whereas isolates 14 has highest MTC value for Mn (1000 mg/l). Isolate JK4 has MTC value for Pb (600 mg/l) and Cr (300 mg/l). Maximum MTC value (1000 mg/l) for Mn was recorded in isolates 3 and14. The maximum MTC value (600 mg/L) for Pb was recorded in isolates 3 and JK4. The maximum MTC value (300 mg/L) for Cr was recorded in the isolates 14, JK4 and JK5. The maximum MTC value (300 mg/L) for Cu was recorded in isolate 3, whereas the maximum MTC value (175 mg/L) for Zn was recorded in isolates 14, JK1 and JK5. In this study all the strains showed resistance in the order of metal concentrations of Mn> Pb>Cr>Cu>Zn>Cd (Table 3). This varying response of selected bacteria might be due to the difference in their cell wall composition or due to variations in resistance mechanisms (Lucious *et al*., 2013).

D. Determination of antibiotic resistance pattern

Antibiotic resistance was determined by agar well diffusion method (Table 4). Inhibition zone was noted after 24hrs of incubation. Strains were considered susceptible, when the inhibition zone was 17 mm or more in diameter. In this method, all the isolates 3, 14, JK4 and JK5 were found resistant to two or more different groups of antibiotics. Such isolates were regarded as multidrug resistant. This number further increased when the intermediate resistance was also accounted for resistant strains. These isolates were regarded as multidrug resistant. Heavy metals as well as the antibiotic resistance among bacterial population may be an indication of risk to the safety. The association between resistance to antibiotics and heavy metals has been reported (Dhakepalker and Chopade, 1994; Verma, 2001). A correlation between metal tolerance and antibiotic resistance in bacteria were found to exist. Due to the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are expected to be transferred together in the environment (Vajiheh and Naser, 2003; Tuckfield and Arthur, 2007).

E. Characterisation of bacterial isolates

The 4 selected resistant strains were subjected to morphological and biochemical tests. The strains 14, JK4 and JK5 were gram positive except isolate 3, as it retained pink colour during gram staining. This was followed by biochemical analysis, the details of which are summarized in Table 5.

F. Identification of bacterial isolates

Isolation of genomic DNA. Extraction of DNA from the selected bacterial isolates was done as per the procedure described by Atashpaz *et al.* (2010).

Table 2: Growth of heavy metal resistant bacteria on different concentrations of heavy metals.

Bacterial isolates	Lead (mg/l)	Cadmium (mg/l)	Chromium (mg/l)	Manganese (mg/l)	Copper (mg/l)	Zinc (mg/l)	Range
3	600	10	20	1000	300	50	10-1000
14	500	10	300	1000	150	350	10-1000
JK4	550	10	300	300	150	50	10-550
JK5	600	50	300	500	200	350	50-600

Table 3: Level of heavy metal resistance at highest concentration of selected isolates.

Bacterial isolates	MTC for various metals (mg/l)
3	Mn (1000), Pb (600), Cu (300)
14	Mn (1000), Zn (350), Cr (300)
JK4	Pb (600), Cr (300)
JK5	Zn (350), Cr (300)

Table 4: Multiple drug resistant (MDR) pattern of isolates for different antibiotics.

S. No	Name of antibiotics	Isolates			
		3	**14**	**JK4**	**JK5**
1	Ampicillin	0 (R)	24 (S)	32 (S)	0 (R)
2	Ciprofloxacin	27 (S)	11 (R)	0 (R)	11 (R)
3	Streptomycin	0 (R)	27 (S)	11 (R)	14 (I)
4	Kanamycin	0 (R)	16 (I)	0 (R)	0 (R)
5	Gentamycin	12 (R)	16 (I)	25 (S)	22 (S)

Table 5: Characterisation of bacterial isolates.

S. No	Biochemical test	Isolates			
		3	14	JK4	JK5
1	Catalase Test	-	+	+	+
2	Oxidase Test	-	-	-	+
3	Methyl red test	+	+	-	+
4	Voges proskauer test	-	+	-	-
5	Citrate utilization test	-	+	-	+
6	Carbohydrate fermentation test	+	+	-	+
7	Indole test	+	-	-	+
8	Mannitol salt test	-	+	+	+
9	Motility test	M	NM	N M	M

Positive (+), Negative (-), Motile (M), Non Motile (NM)

The quality and intactness of the extracted DNA was examined by running on 1% agarose gel which contain 1 µg/ml ethidium bromide. The extracted DNA molecules were used as templates for the amplification of 16 S rRNA genes.

Amplification of 16S rRNA. For bacterial classification generally sequencing of 16 S rRNA gene was used as an important identification tool (Clerck *et al*., 2004). The reasons include its presence in almost all bacteria; its function has not changed over time and the 16 S rRNA gene (1,500 bp) is large enough to provide a genus and species identification of isolates (Fig. 1) (Janda and Sharon, 2007). The DNA samples of all the bacterial isolates were run on the agarose gel and the bands were visualized when observed under the Gel doc.

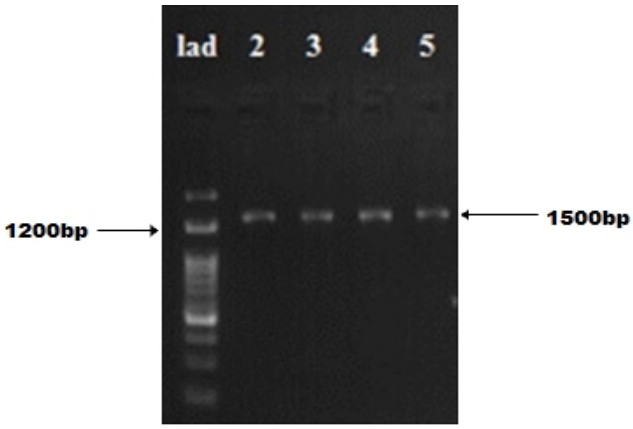


Fig. 1: Gel showing amplification of 16S rRNA amplicon. Lane 1 showing DNA ladder. Lane 2-5 showing band of amplicon of Isolate 3, 14, JK4, JK5.

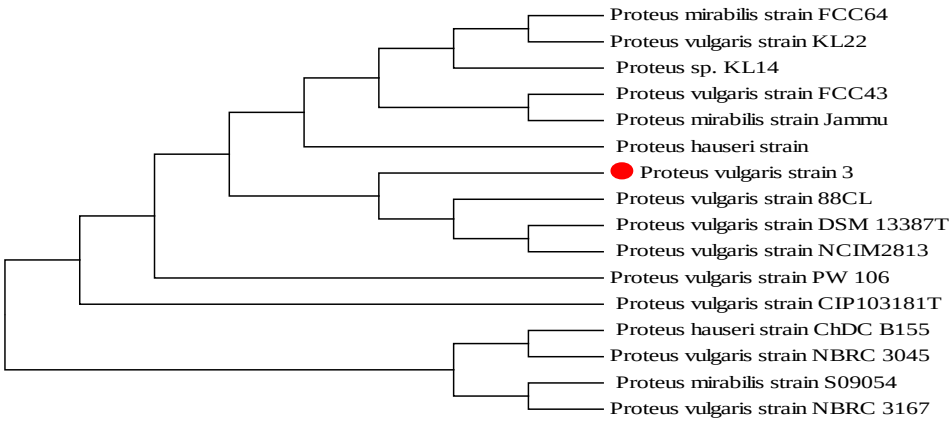


Fig. 2. A Phylogenetic tree constructed based on 16s rRNA of isolate 3.

The sequencing of the 16 S rRNA gene was done. Based on the 16 S rRNA sequences, phylogenetic dendrograms were constructed to know the genetic relationship between the bacterial isolates. The 16S rRNA sequences of strain 3, 14, JK4 and JK5 were deposited in the NCBI database under the accession numbers **MF490435**, **MF276915**, **MF490436** and **MF490437**, respectively. The identification of the isolates and their phylogenetic dendrograms were shown in the (Figs. 2-5).

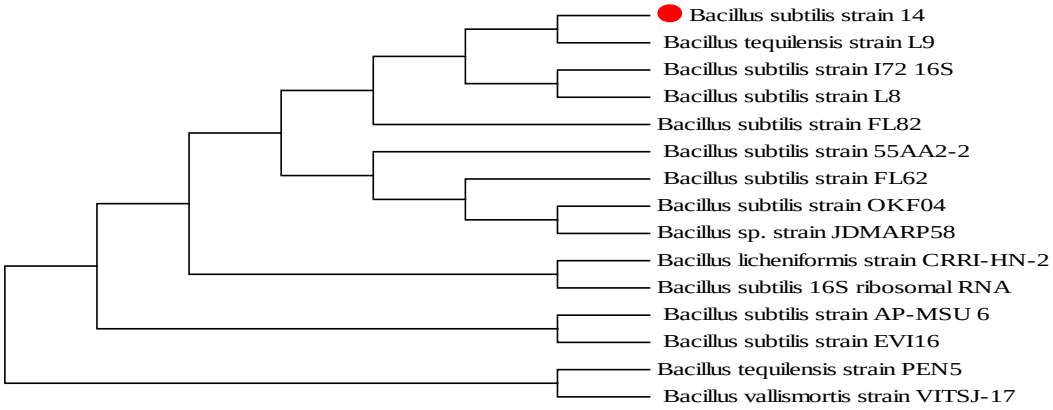


Fig. 3. A Phylogenetic tree constructed based on 16s rRNA of isolate 14.

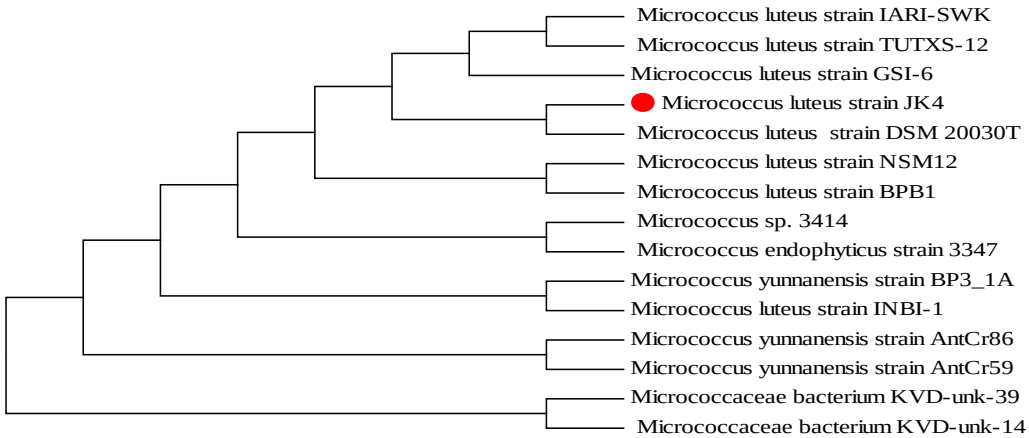


Fig. 4. A Phylogenetic tree constructed based on 16s rRNA of isolate JK4.

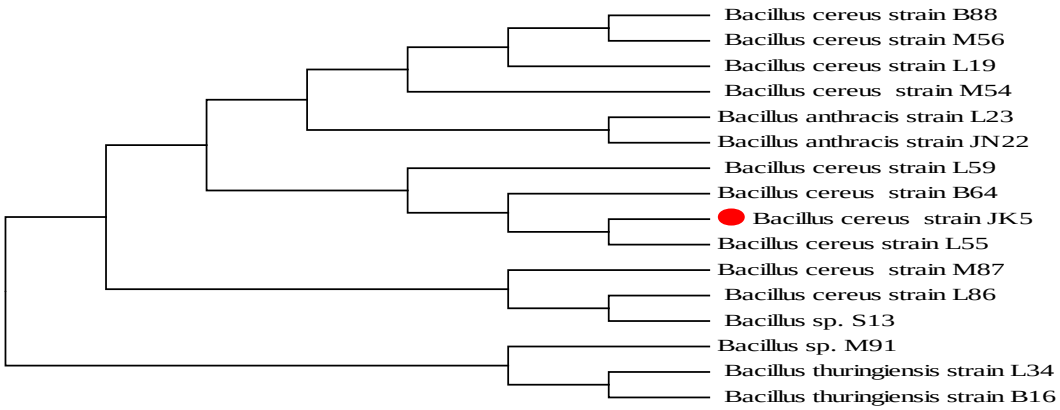


Fig. 5. A Phylogenetic tree constructed based on 16s rRNA of isolate JK5.

G. Metal uptake capacity of heavy metal resistant bacteria

The metal uptake capacity of heavy metal resistant bacteria was analysed by Microwave Plasma Atomic Emission Spectroscopy (MPAES). All the isolates selected after primary screening that showed growth on high concentration of metals were further screened. Selected 4 isolates that were multi drug and multi heavy metal resistant were selected for metal uptake capacity (Fig. 6). The isolates 3, 14, JK4 and JK5 showed the maximum uptake capacity for cadmium and minimum for copper. The uptake capacities of 4 isolates are summarized in (Table 6). The metal uptake capacity of the bacteria increases with an increase in the concentration of metals (Vimala and Das, 2009). Increase in electrostatic interactions involving sites, lowers the affinity for metal ions (Al-Garmi, 2005). A number of metal complex formation sites in biological system have been reported, that helps in metal uptake. These include accumulation in the cell wall, protein polyphosphate complexes and complex of the carboxyl groups of the peptidoglycan in the cell wall (Vieira and Volesky, 2000).

Table 6: Metal uptake capacity of heavy metal resistant bacterial isolates by MPAES.

Sr. No	Parameters	Unit	Bacterial isolates			
			3	14	JK4	JK5
1	Lead (Pb)	%	6.61	3.60	1.63	6.72
2	Cadmium (Cd)	%	10.1	4.85	16.6	9.18
3	Zinc (Zn)	%	6.52	2.63	6.54	5.71
4	Copper (Cu)	%	0.05	0.05	0.03	0.03
5	Manganese (Mn)	%	2.91	0.91	2.35	1.35
6	Chromium (Cr)	%	1.24	0.74	0.15	1.12

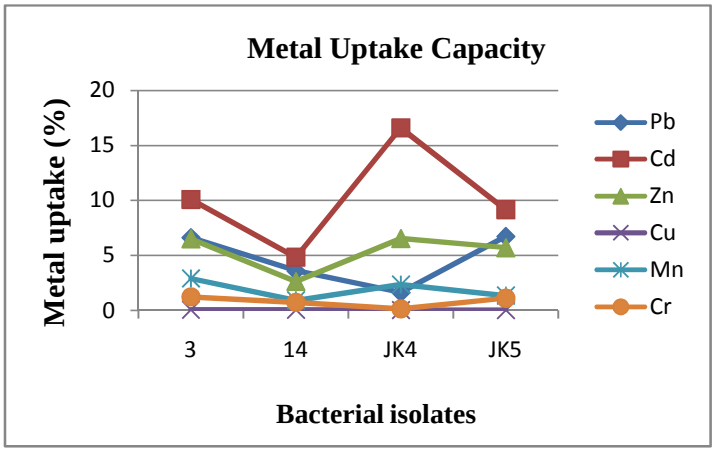


Fig. 6. Metal uptake capacities of selected heavy metal resistant isolates.

H. Ex-situ bioremediation of heavy metal contaminated soil

Bioremediation of heavy metal contaminated soil was carried out with 4 bacterial isolates (3, 14, JK4 and JK5), which showed high resistance to different concentrations of heavy metals and antibiotics. The bacterial density in the untreated control soils varied from 0.660 to 0.823 at 600nm (Table 7). The addition of bacterial consortium in the contaminated soil led to an increase in values to approximately 0.721 to 0.911. The addition of the bacterial consortium with nutrient to contaminated soil led to a steady increase in the bacterial density from 0.837 to 1.073, after 60 days. After 60 days, the population was maximum in nutrient amended soil, indicating the role of nutrients in the enhancement of bacterial population (Fig. 7).

Prior to bioremediation process water holding capacity and pH of soil was analysed. The water holding capacity of the soil was 37.4% and pH of the soil was 6.1 After 60 days of incubation, the highest percentage of degradation was observed in the treatment (T3). The addition of only bacterial consortium had shown the least effect on the degradation of heavy metals after 30 and 60 days of inoculation. As compared to T1 and T2 treatments, T3 showed the maximum degradation of heavy metals (Table 8). The highest percentage of degradation was found upon the amendment of nutrients to the bacterial consortium [Fig. 8 (a) & (b)].

Table 7: Microbial density during bioremediation process

Treatments	15 days	30 days	45 days	60 days
T1	0.660 ±0.30	0.702 ±0.28	0.785 ±0.35	0.823 ±0.27
T2	0.721 ±0.43	0.785 ±0.31	0.840 ±0.33	0.911 ±0.47
T3	0.837 ±0.27	0.914 ±0.34	0.966 ±0.40	1.073 ±0.38

Each value is mean ±SD of three individual observations
T1- control,T2- Soil+ bacterial isolates, T3- Soil+nutrients+bacterial isolates

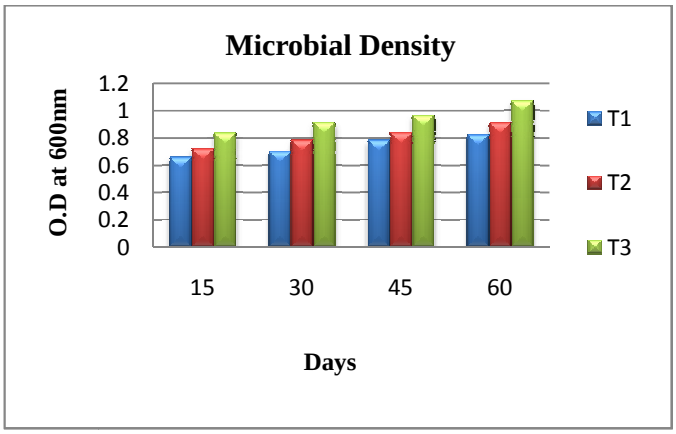
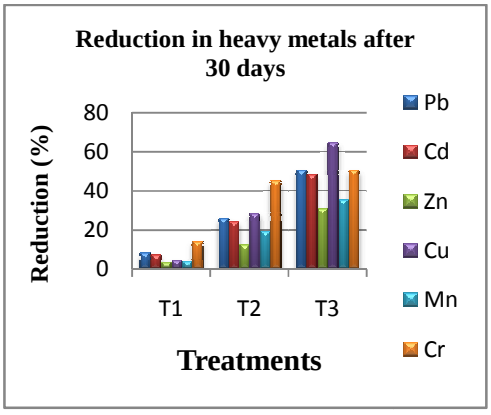


Fig. 7. Microbial densities of heavy metal resistant bacterial isolates.

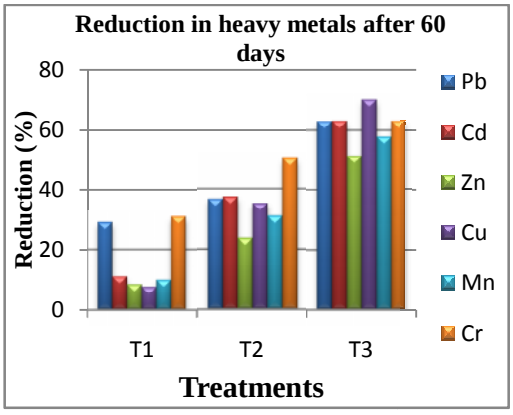
Table 8: Degradation of heavy metals by indigenous bacterial consortium.

Heavy metals (mg/kg)	Initial conc. of heavy metals	T1		T2		T3	
		30 Days	60 Days	30 Days	60 Days	30 Days	60 Days
Lead	189.02	173.36	134.03	140.19	119.66	93.42	70.12
Cadmium	5.59	5.18	4.97	4.23	3.50	2.88	2.07
Zinc	590	570.41	540.09	516.77	451.85	407.36	288.27
Copper	1065.27	1018.07	984.26	762.05	689.31	376	320.10
Manganese	409	392.94	368.10	330.47	282.05	264	173.14
Chromium	196.20	168.53	135.21	107.41	97.38	96.99	72.67

T1- control, T2- Soil+ bacterial isolates, T3- Soil+nutrients+bacterial isolates



(a)



(b)

Fig. 8. Percentage reductions of heavy metals in different treatments after 30 and 90 days.

The present findings are in accordance with the result of (Sabate *et al*., 2004; Chang, 2011), ho reported that the rate of bioremediation increases with increase in bacterial count, amended with nutrients. Addition of nutrients has been reported to enhance the degradation process (Leahy and Colwell, 1990; Barathi and Vasudevan, 2003).

CONCLUSION

Environmental laws have become stringent, discharge of the effluent within the permissible limit is mandatory in the developed and developing countries. The distillery wastewater treatment methods practiced by large-scale holders comprise physicochemical methods requiring a large surface area for the set up of effluent treatment plant and technically trained personnel with efficient management skills. It adds to the cost of the treatment process, making it cost intensive. Therefore, biological treatment methods are considered to be ideal and economical. In the present investigation six heavy metals (Pb, Cd, Zn, Cu, Mg and Cr) were detected in distillery effluent contaminated soil. Isolation and screening was carried out to isolate heavy metal resistant bacteria capable of tolerating high concentrations (1000 mg/l) of metal salts. Four selected strains were characterized and identified on the basis of biochemical tests and 16S rRNA sequencing. The four strains were confirmed as *Proteus vulgaris, Bacillus subtilis, Micrococcus luteus* and *Bacillus cereus.* These strains were examined for their ability to accumulate heavy metals and found that they exhibited good heavy metal uptake capacity. Ex-situ bioremediation was carried out with four selected strains and it was found that the highest percentage of metal degradation was observed in treatment T3 (Bacterial consortium + nutrients). The reduction in heavy metals was 150.57 & 62.9 for lead, 48.4 & 62.96 for cadmium, 30.95 & 51.14 for zinc, 64.7 & 69.95 for copper, 35.45 & 57.66 for manganese and 50.52 & 62.92 for chromium after 30 and 60 days of treatments, respectively.

Thus, the present work has clearly established the hazardous nature of distillery effluent. From the data obtained it can be concluded that long term irrigation of the soil with distillery effluent can lead to the accumulation of heavy metals and indirectly enters into the food chain thus affecting human health. Bioremediation can be used for the degradation of heavy metals as this approach is cost effective and ecofriendly.

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