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ISOLATION AND CHARACTERIZATION OF HEAVY METAL-REDUCING MICROBES FROM TANNERY EFFLUENT AND ITS ACTIVITY ON IMMOBILIZED BEADS

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Keywords:

Heavy metal tolerance, Tannery effluent, Reduction, Bioremediation, inductively coupled plasma-optical emission spectrometry, Immobilization

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ABSTRACT: Heavy metal pollutants in tannery effluent can be removed using the bioremediation technique. This study intends to extract bacteria that may reduce heavy metals and molecularly characterize them using 16S rRNA PCR in samples taken from tannery effluent. It also studies their cell-free immobilized enzymatic activity. Thirteen bacterial isolates were identified based on morphological, cultural, and biochemical characteristics. The top four isolates, A, B, sp5, and sp3, were identified as *Brevundimonas sp*, *Proteus mirabilis*, and *Alcaligenes faecalis* by morphological, cultural, biochemical, MALDI-TOF Analysis, and molecular characteristics using 16S rRNA. These organisms showed good tolerance to zinc, nickel, and chromium. When heavy metals like zinc and chromium have reduction properties, by ICP-OES to assess the concentration of heavy metals. Further investigation, beads were immobilized, and its activity was analyzed.

INTRODUCTION: Bioremediation [1] refers to biological systems that remove ecological pollutants from the air, gas, and industrial effluent states. The ability of microorganisms to captivate, assemble, and reduce common pollutants has enchanted contamination [1]. Bioremediation decreases the impact of products created from environmental change activities, such as urbanization and agricultural processes. Organic pollutants are generally more susceptible than heavy metals, including free radicals.

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Oxidation increases the solubility level in water in vulnerability degraded by the addition of water. Biodegradation converts the elements of hydrogen and carbon to carbon dioxide and water. Bioremediation provides the best solutions for heavy metals [7]. Heavy metal can be removed by various bioremediation techniques [16].

The process of treating animal skins and hides to generate leather is known as tanning. A tannery [7] is a facility that preserves skins. Sulphites, ammonium salts, and calcium salts are found in the effluents from tannery procedures and de-liming bating. These hairs are plucked and immersed in water for two days. Sulphuric acid, chrome, chlorides, sodium bicarbonate, and sulfates are found in the pickling and chrome tanning effluents. High hazardous pollutants like sulphides in an animal's skin tanning are used to identify tannery

effluents. Numerous research investigations on the treatment of tannery effluent have recorded a variety of procedures, including flotation, electrochemical treatment, sedimentation, coagulation, filtration, ultra-filtration and reverse osmosis process. Because so many chemicals are employed in turning animal hides into leather, the tanning industry is the dirtiest. Chromium salts, phenolics, tannins, and organic debris are among the substances that are routinely emitted into the environment by tanneries.

Tannery wastewater [7, 9] is defined by the following parameters: suspended solids (SS), chromium, sulphides, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) [12]. Total dissolved salts (TDS) @125°C, Carbonate as Caco3, Bicarbonate as Caco3, Residual Sodium Carbonate, Sodium Adsorption Ratio, Sulphate, Chloride, Nitrate, iron, potassium, Calcium, Magnesium, Sodium, Total dissolved salts (TDS) @125°C, Carbonate as Caco3, Bicarbonate as Caco3, Residual Sodium Carbonate, Sodium Adsorption Ratio Gelatin and glue derived from tannery leftovers. Limited fleshings, trimmings, and shavings are used to make foam-generating compounds. The fleshings can be used to make manure, animal feed, amino acids, grease, and other products.

Heavy metals contain harmful or unpleasant substances. Zinc (Zn), chromium (Cr), and nickel are examples of heavy metals (Ni). The tannery effluent [20] contains 5.50 and 11.0 mg/L of chromium [3], respectively. Zinc concentrations in tannery effluent range between 6.00 and 7.00 mg/L, below WHO. Allowed limits. Numerous factors, including the exposure route, chemical species, dose, and age, gender, genetics, and nutritional status of the exposed individuals, all affect how dangerous they are. Due to their high toxicity, arsenic, cadmium, chromium, lead, and mercury are among the priority metals that concern public health. These metals were considered toxicants known to cause multiple organ injuries even at low exposure levels [9]. The US Environmental [23] Protection Agency and the International Agency for Research on Cancer classify them as human carcinogens (known or probable). This study looks at their manufacture, use, and prevalence in the environment, the possibility of human exposure, and the cellular mechanisms underlying their toxicity, genotoxicity, and carcinogenicity. Due to their low concentrations (ppb to 10ppm) in many environmental matrices, heavy metals were also categorized as trace elements. Bioavailability was impacted by physical elements such temperature, phase association, adsorption and sequestration. Chemical factors affect lipid solubility, complexation kinetics, octanol/water partition coefficients, and thermodynamic equilibrium. Additionally, biological aspects including species characteristics, trophic relationships, and physiological and biochemical flexibility have a big impact.

With oxidation [15] states varying from chromium (II) to chromium (VI), chromium (Cr) is an element that is naturally present in the crust of the planet [8]. The trivalent [Cr (III)] form of chromium elements, which is stable, is found in ores like ferrochrome. The hexavalent form of [Cr (VI)] is the second stable state. Nature does not include the chromium element [Cr (0)]. There are several natural and phylogenetic sources of chromium that end up in the environment (air, water, and soil), with industry having a substantial impact [15]. Among the sectors with greater chromium subscriptions are those that process metal, have tannery facilities, create chromate, weld stainless steel, produce ferrochrome, and produce chrome pigment. Metallurgical, refractory and other industrial operations, in particular, are the main sources of chromium-releasing air and wastewater.

The most prevalent chromium [14] assembly in the environment is the hexavalent [Cr (VI)]. Numerous executive and non-executive authorities have identified the hazardous adulterant hexavalent chromium [Cr (VI)] (US Department of Health and Human Services) as a human carcinogen. Depending on its level of oxidation, chromium [17] poses a variety of health concerns that can range from mild metal lethality to severe toxicity. Because only Cr (III) was present in the air, water, soil, and biological materials, it was once thought that anything containing Cr (VI) was artificial. The World Health Organization's drinking water standard for Cr (VI) is 50 g per litre, yet it has been discovered that Cr (VI) levels in surface and ground waters are greater. Metal is a crucial process in surface treatment and metal deposition

for increased product life and ornamentation, despite nickel contamination in the environment. Depending on the particular needs of the objects, metals including nickel, copper, zinc, and chromium can be used for electroplating. Significant volumes of metal ions are discharged into the effluent during the washing of the electroplating tanks [5].

Businesses that manufacture storage batteries, zinc base casting and silver refineries, for instance, all have Ni (II) in their effluents. Higher nickel concentrations cause lung, nasal, and bone cancer. Nickel compounds enter the food chain in both humans and animals. One of the negative effects of nickel exposure is dermatitis (the Ni itch) and another is acute Ni toxicity [5].

Due to its resistance to corrosion from the environment, zinc is used to galvanise iron in order to prevent it from rusting. Anode materials for galvanic cells, the cyanide method for the preparation of gold and silver, the parks process for the desilverization of lead, and the manufacturing of zinc white and a variety of precious alloys like brass, German silver, and delta metal all use zinc [10].

Zinc salts are used to make ceramics, textiles, fertilizers, pigments, photographic paper, batteries, wood preservatives, catalysts, and accelerators for rubber vulcanization. It is uncommon, but excessive zinc ingestion might cause stomach distress and diarrhoea.

Using a simple and quick method, developmental conditions for getting maximal intensity in ultrasonic baths are given [13]. Bath sonication is an indirect sonication technique that uses a water bath. Ultrasonic energy is delivered to a water bath and subsequently into a vessel or several tubes in bath sonication. This approach works best with extremely little samples [24].

It is often not guaranteed that all the polymers will pass through this region when using probe sonicators for sonication and mixing because the power is concentrated into a very small volume with weak forces acting. Sound energy is applied to a liquid containing particles using an ultrasonic bath or probe. It is claimed that high-speed mixing combined with probe sonication is effective [16]. The confinement of a cell or enzyme in a matrix is known as Immobilization. Natural, synthetic, and inorganic materials all benefit from immobilization procedures. Making enzymes more stable, active, and reusable can be accomplished by enzyme immobilization [4, 6], which is frequently utilized. Enzymes or cells can be immobilized using various techniques, including copolymerization, trapping, adsorption, and covalent bonding. *Aspergillus oryzae's* aminoacylase [4] was the first enzyme to be immobilized using immobilization cells.

An enzyme becomes insoluble and inactive when calcium chloride is combined with a sodium alginate solution and enzyme solution mixture. Conditions like pH and temperature can now be changed more easily as a result.

Increasing industries require advanced solutions to rectify the effluent generated by the beverage and leather industries. Leather industries are one of the major wastewater generating hubs, which generate a lot of heavy metals like chromium, nickel, zinc, etc. These heavy metals are difficult to remove from the water before it lets out into the freshwater lake after treatment. Effluents generally contain heavy metals but also more bacteria that can thrive in such an environment. Bioremediation is one such solution to remove heavy metals.

For example, *Bacillus spp* [10] can convert hexavalent chromium (VI) into chromium (III). Our study aims to isolate heavy metals reduction and molecular characterization of the bacteria using 16S [19] rRNA PCR in samples collected from the tannery industry and to study its cell-free immobilized enzymatic activity. These microbes may produce enzymes that can reduce some of the heavy metals. These can be extracted and immobilized into sodium alginate beads, which can do an enzymatic activity, so the beads can be used at industrial and household levels to eliminate heavy metal content.

MATERIALS AND METHODS:

Sample Processing: Effluent samples were collected from Sugan Leather Enterprises, Nagalkeni-Chrompet. The sample involved effluent and was collected in falcon tubes and preserved at approximately 4°C to prevent contamination and allow the sample to stay longer.

Measurement of Physicochemical Parameters: Physicochemical parameters like chromium, nickel, zinc, pH@25℃, Sulphate, Chloride, Nitrate, iron, potassium, Calcium, Magnesium, Sodium, TDS@125℃, Carbonate as Caco3, Bicarbonate as Caco3, Residual Sodium Carbonate, Sodium Adsorption Ratio of the effluent was determined [12].

Screening and Isolation of Bacteria: The nutrient agar was sterilized at 121°C for 15 min and allowed to cool at 40-45℃. The effluent was serially diluted in 9 ml of saline water, and then 1 ml of sample was added to the first test tube to have 10^{-1} to 10^{-9} and then 0.1ml was screened by standard spread and pour plate method and observed at 37℃ for 24 h. After 24 h of incubation, plates were observed for different morphological appearances [12].

Multiple Metal Tolerance Capacity: Thirteen isolates (A, B, sp5, sp3, 10^{-3}, 10^{-5}, pp3, pp6, pp4C, pp4D, sp3A, sp3B, sp5A) were picked from the spread and pour plates and streaked on heavy metal containing media supplemented with 50 mg/L, 100 mg/L, 200 mg/L of Zinc, Nickel and Chromium and kept for incubation for 24 hrs for heavy metal tolerance [11].

Characterization of Bacterial Isolates: The bacterial isolates were characterized based on their cultural, morphological and biochemical characteristics as described in Bergey's manual for the identification of bacteria. For the activities of gram staining, catalase, oxidase, lecithinase and lipid hydrolysis were analyzed. The four isolates (A, B, sp5, sp3) were characterized using MALDI-TOF and 16s Rrna [2, 11] sequencings and were analyzed with the database of NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

Determination of Heavy Metals: 3.125g of Luria Broth (LB) with 40 ppm of Zn, Cr was mixed with each 125 ml of distilled water and autoclaved at 121°C for 15 minutes before being put into a conical flask and incubated for 24 h at 37℃. A heavy metal test was performed by Inductively coupled plasma-optical emission spectrometry and compared with standard and control [28].

Sonication and Protein Estimation: For sonication [21], heavy metal and non-heavy metal-induced samples were sonicated at 20-40 kHz for 30 minutes. The samples were centrifuged at 5000 rpm for 15 min, and the supernatant was collected and stored.

The samples were prepared in different concentrations from S1 to S6, and different volumes of BSA were added with distilled water along with 200 µl of bradford. Triplicates were kept for the samples and analyzed at 595 nm for brad ford assay for protein estimation [27].

Immobilization: 1% sodium alginate and calcium chloride was prepared in each 100 ml of distilled water in two different beakers. Sodium alginate was mixed well with heavy metal-inducing and non-heavy metal-induced samples in the beaker separately. Those samples were induced in calcium chloride through the filler, and immobilization beads were formed and analyzed [22].

RESULTS:

Physicochemical Characteristics of Tannery Effluent: Standard techniques were used to measure the physicochemical characteristics. The effluent was green in color, chromium, nickel, zinc, pH@25℃, Sulphate, Chloride, Nitrate, iron, potassium, Calcium, Magnesium, Sodium, TDS@125℃, Carbonate as Caco3, Bicarbonate as Caco3, Residual Sodium Carbonate, Sodium Adsorption Ratio of the effluent was also analyzed. Zinc 0.21 mg/L, Nickel 0.24 mg/L, 2,156 mg/L, 63 mg/L, and 528 mg/L are among the heavy metals found in effluent. Carbonate as Caco3 is nil, while bicarbonate as Caco3 is 147.25 mg/L.

Other researchers have produced similar findings. This sample has an electrical conductivity of 5400 S/cm, and a total dissolved salts test of 3521 mg/L.

Other characteristics evaluated in the effluent sample included sodium, chloride, nitrate, iron, potassium, calcium, magnesium and sodium was determined to be 897.3 mg/L, 1300.51 mg/L, 3.2 mg/L, 0.24 mg/L, 2,156 mg/L, 63 mg/L, and 528 mg/L. Carbonate as Caco3 is nil, but the Bicarbonate Caco3 test results are 147.25mg/ L. Other researchers have observed similar findings in **Table 1**.

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF EFFLUENT

Parameters	Unit	Effluent
Chromium	mg/L	5.83
Nickel	mg/L	<DL (1.0)
Zinc	mg/L	0.21
pH@25℃	mg/L	7.92
Electrical conductivity@25C	µS/cm	5400
Sulphate	mg/L	897.3
Chloride	mg/L	1033.51
Nitrate	mg/L	3.2
Iron	mg/L	0.24
Potassium	mg/L	9
Calcium	mg/L	156
Magnesium	mg/L	63
Sodium	mg/L	528
TDS at 105 C	mg/L	3521
Carbonate as Caco3	mg/L	Nil
Bicarbonate as Caco3	mg/L	147.25
Residual Sodium Carbonate	m.eq/L	Nil
Sodium Adsorption Ratio	mg/L	8.99

Screening and Isolation of Bacteria from Tannery Effluent: More colonies were grown in the spread and pour plates at the same sample location. Totally thirteen single bacterial colonies were isolated and were able to tolerate 50 mg/L, 100 mg/L, 200 mg/L of Zn, Ni, Cr in nutrient agar after 24 hrs of incubation. This collected sample consists of heavy metal tolerance bacteria **Fig. 1** and **2**.

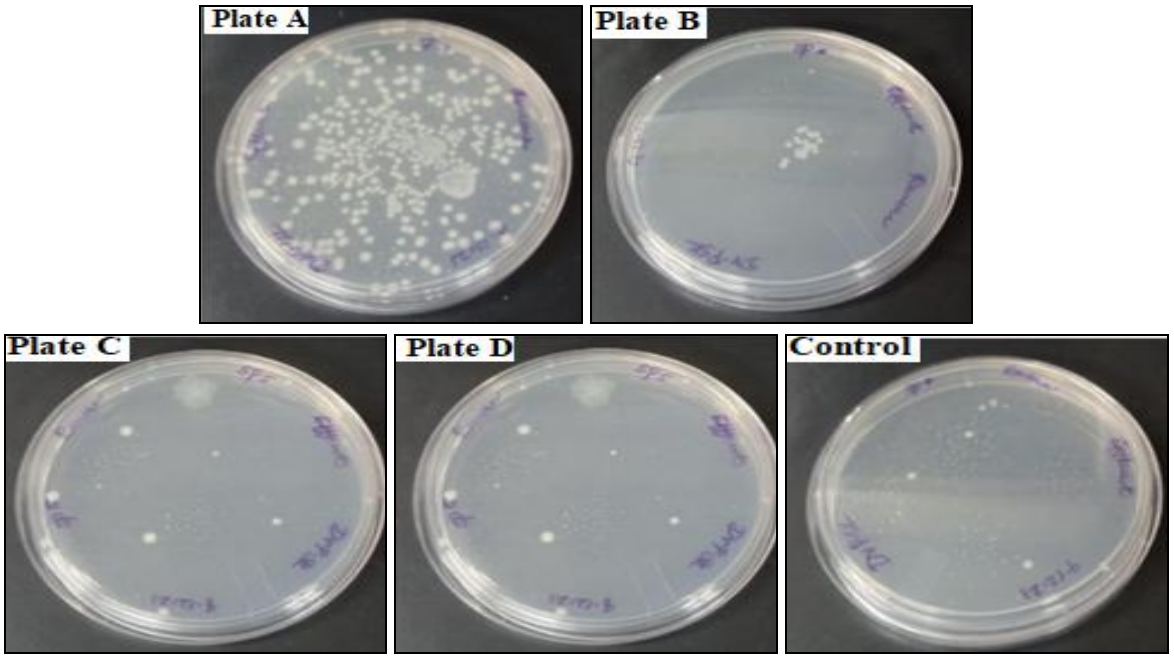
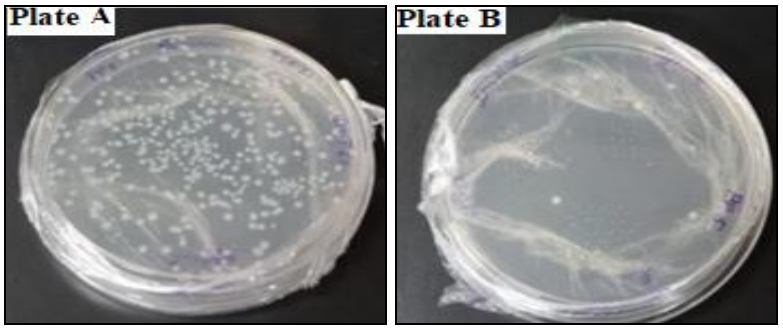


FIG. 1: SPREAD PLATE



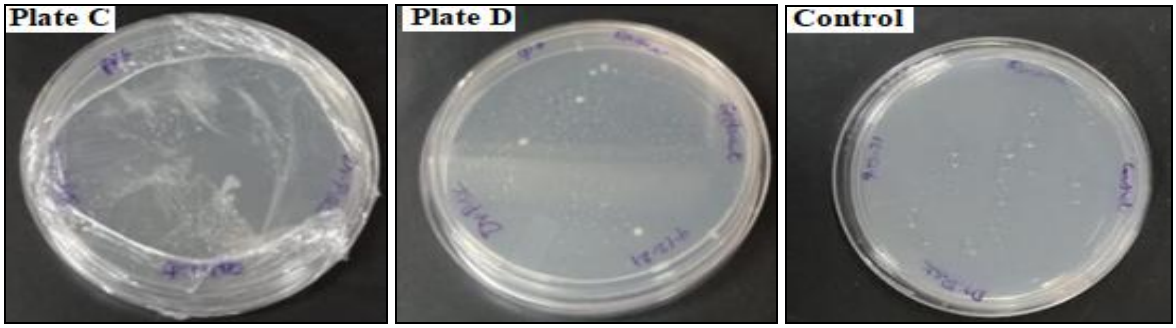


FIG. 2: POUR PLATE

Comparative Analysis of Multiple Heavy Metal Tolerance Capacity: Total of thirteen bacterial isolates, among them four isolates (A, B, sp5, sp3) were selected for further study.

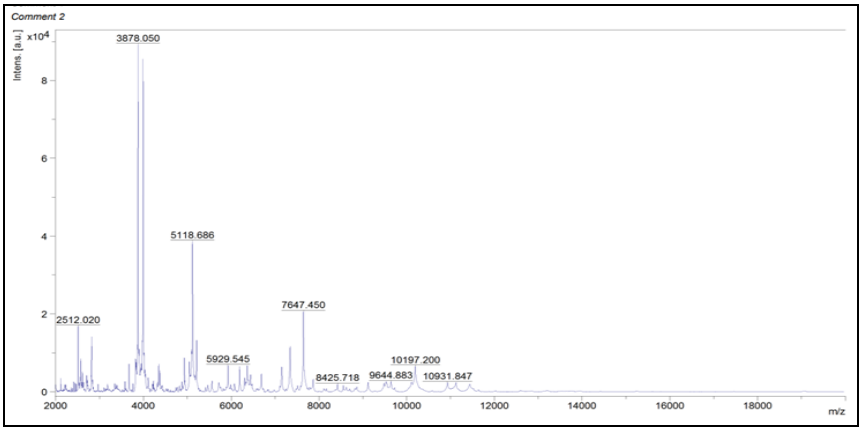
In this experiment, four isolates showed good tolerance in 50 mg/L, 100 mg/L, 200 mg/L of Zn, Ni, Cr.

Characterization and Identification: Thirteen heavy metal tolerance isolates (A, B, sp5, sp3, 10^{-3}, 10^{-5}, pp3, pp6, pp4C , pp4D, sp3A, sp3B, sp5A) were characterized based on their morphological and biochemical characteristics as described in Bergey's manual for the identification of bacteria **Table 2**.

TABLE 2: BIOCHEMICAL TEST

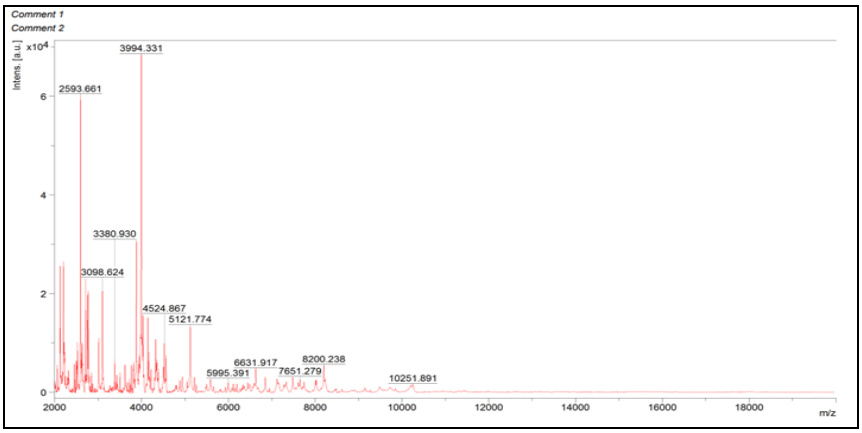
Bacterial isolates	Gram nature	Catalase test	Oxidase Test	Lipid hydrolysis test	Lecithin's test	Colony morphology
sp5	Gram negative	+	+	-	+	Yellow color
sp3	Gram negative	+	+	-	+	White color
10^{-5}	Gram negative	-	-	-	-	White color
10^{-3}	Gram negative	+	+	-	-	White color
A	Gram negative	+	-	+	+	Milky White color
B	Gram negative	+	+	-	+	White color
pp4D	Gram negative	+	+	-	+	Pale yellow color
sp3B	Gram negative	-	+	+	+	White color
sp5A	Gram negative	+	+	+	+	Pale yellow color
Pp3	Gram negative	-	-	+	-	White color
Pp6	Gram negative	-	-	-	+	White color
sp3A	Gram negative	+	+	-	-	Pale yellow
Pp4C	Gram negative	-	+	-	-	Light yellow

Matrix-assisted Laser Desorption/ Ionization-time of Flight (MALDI-TOF) Mass Spectrometry (MS): Bacterial isolates A depicts *Alcaligenes faecalis* which has a 2.05 log score. Isolate B shows *Thauera terpenica*, which has a log score of 1.275; isolate sp5 shows *Alcaligenes faecalis* which has a log score of 1.804; isolate sp3 shows *Proteus mirabilis,* which has a 1.706 log score, as shown in **Fig. 3, 4, 5, 6**.



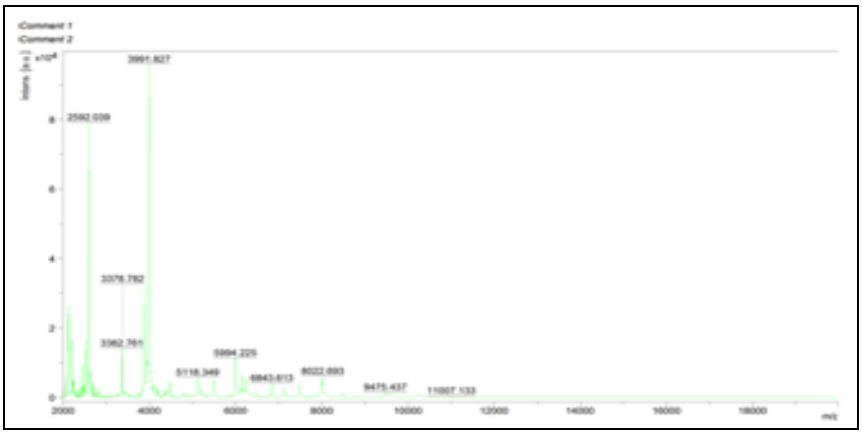
Mo	Detected Species	Log(Score)
	Alcaligenes faecalis ssp faecalis DSM 30030T HAM	2.050
	Alcaligenes faecalis ssp faecalis DSM 30033 DSM	2.003
	Alcaligenes faecalis ssp faecalis 052 NF24 NFI	1.982
	Alcaligenes faecalis ssp faecalis DSM 30030T DSM	1.975
	Alcaligenes faecalis ssp phenolicus DSM 16503T DSM	1.867
	Alcaligenes faecalis 2015028830 1 MVD	1.805
	Alcaligenes faecalis ssp parafaecalis DSM 13975T HAM	1.760
	Alcaligenes faecalis ssp faecalis DSM 6174 DSM	1.736
	Alcaligenes faecalis DSM 13644 DSM	1.727
	Alcaligenes faecalis ssp faecalis DSM 2576 DSM	1.411

FIG. 3: BACTERIAL ISOLATE A - *ALCALIGENES FAECALIS*- 2.05



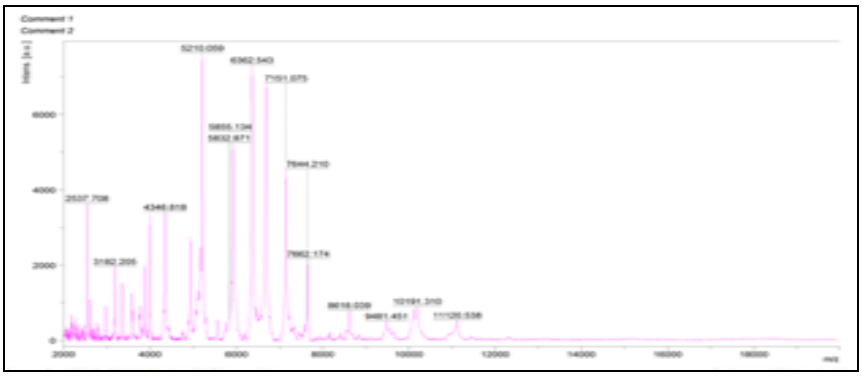
Mo	Detected Species	Log(Score)
	Thauera terpenica 58Eu MPB	1.275
	Rhizobium radiobacter B178 UFL	1.261
	Clostridium cadaveris 1074 ATCC 25783T BOG	1.250
	Lactobacillus paracasei ssp paracasei DSM 20207 DSM	1.242
	Trichosporon debeurmannianum VML	1.229
	Paraclostridium bifermentans 2274 CCUG 35556 A BOG	1.221
	Pseudomonas brenneri CIP 106646T HAM	1.210
	Paeniglutamicibacter gangotriensis DSM 15796T DSM	1.202
	Candida parapsilosis ATCC 22019 THL	1.184
	Lactobacillus ingluviei DSM 14792 DSM	1.173

FIG. 4: BACTERIAL ISOLATE B- THAUERA TERPENICA- 1.275



Mo	Detected Species	Log(Score)
	Proteus mirabilis 13210 1 CHB	1.706
	Proteus mirabilis 9482 2 CHB	1.430
	Proteus mirabilis DSM 50903 DSM	1.421
	Proteus mirabilis RV412 A1 2010 06b LBK	1.365
	Proteus mirabilis DSM 30115 DSM	1.347
	Proteus mirabilis (PX) 22086112 MLD	1.273
	Proteus mirabilis DSM 788 DSM	1.245
	Proteus mirabilis DSM 18254 DSM	1.206
	Acinetobacter calcoaceticus DSM 30006T HAM	1.174
	Streptococcus infantarius BRB	1.158

FIG. 5: BACTERIAL ISOLATE SP3-*PROTEUS MIRABILIS*-1.706



Mo	Detected Species	Log(Score)
	Alcaligenes faecalis ssp faecalis DSM 30030T HAM	1.804
	Alcaligenes faecalis ssp faecalis DSM 6174 DSM	1.754
	Alcaligenes faecalis ssp faecalis DSM 30030T DSM	1.735
	Alcaligenes faecalis ssp parafaecalis DSM 13975T HAM	1.695
	Alcaligenes faecalis ssp phenolicus DSM 16503T DSM	1.691
	Alcaligenes faecalis ssp faecalis 052 NF24 NFI	1.632
	Alcaligenes faecalis ssp faecalis DSM 30033 DSM	1.601
	Alcaligenes faecalis DSM 13644 DSM	1.598
	Alcaligenes faecalis ssp faecalis DSM 2576 DSM	1.543
	Alcaligenes faecalis ssp parafaecalis DSM 13975T DSM	1.472

FIG. 6: BACTERIAL ISOLATE SP5-*ALCALIGENES FAECALIS*- 1.804

Molecular Characterisation: Species were identified using the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/), 100bp ladder, 16S rRNA forward and reverse primer were used to analyze the plasmid and sequencing were done. *Brevundimonas sp, Proteus mirabilis, Alcaligenes faecalis were identified* **Fig. 7.**

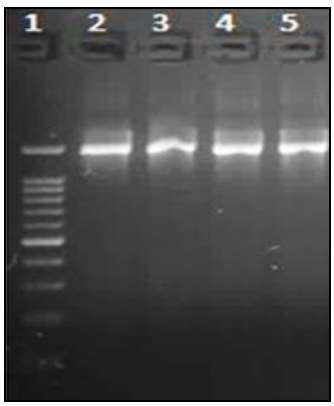


FIG. 7: LANE 1-100BP LADDER, LANE 2 SHOWS *BREVUNDIMONAS SP*, LANE 3- SHOWS *PROTEUS MIRABILIS*, LANE 4 AND 5 SHOWS *ALCALIGENES FAECALIS*

Determination of Heavy Metals: To measure heavy metals, (Cr and Zn) to determine reduction capacity, the samples were analyzed by inductively coupled plasma-optical emission spectrometry and compared with the control. All four isolates A, B, sp5, sp3 were reduced in concentration mg/L shown in **Fig. 8 & 9.**

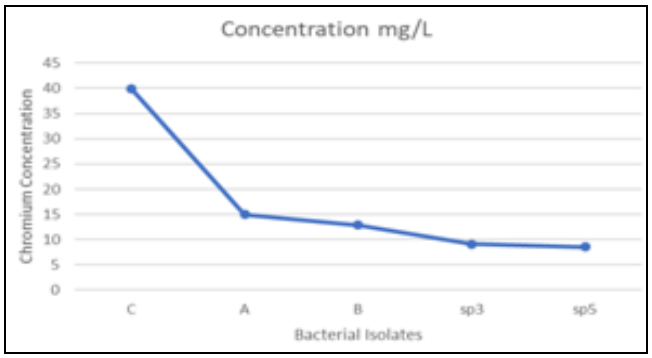


FIG. 8: CONCENTRATION OF CHROMIUM HEAVY METAL

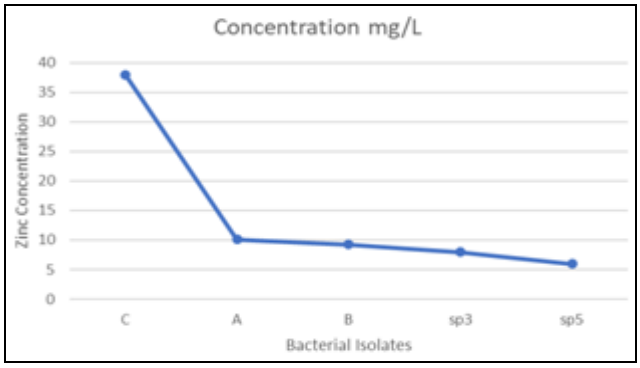


FIG. 9: CONCENTRATION OF ZINC HEAVY METAL

Sonication and Protein Concentration: Samples prepared in triplicates in various concentrations were done by BSA, distilled water and brad ford 200µl analyzed using optical density at 595 nm. Standard Graph were plotted and concentration of heavy metal-induced and non-heavy metal-induced isolates analyzed, shown in **Table 3**, **Fig. 10, 11**.

TABLE 3: BRADFORD CONCENTRATION

Concentration	Volume BSA (µl)	Distilled water (µl)		
0	0	800µl	↑	
10	10	790µl		
20	20	780µl		
40	40	760µl	Bradford 200µl	Optical density at 595 nm
60	60	740µl		
80	80	720µl		
100	100	700µl	↓	

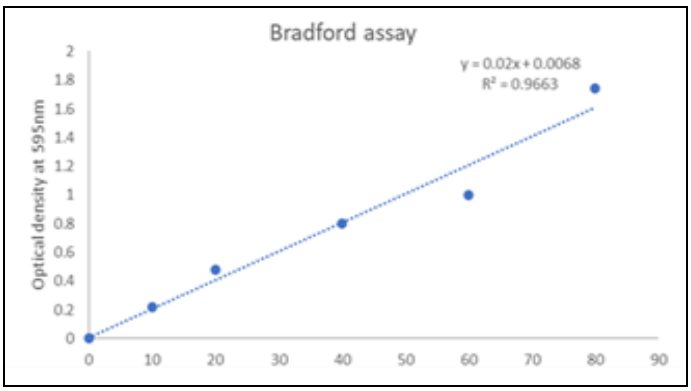


FIG. 10: STANDARD GRAPH

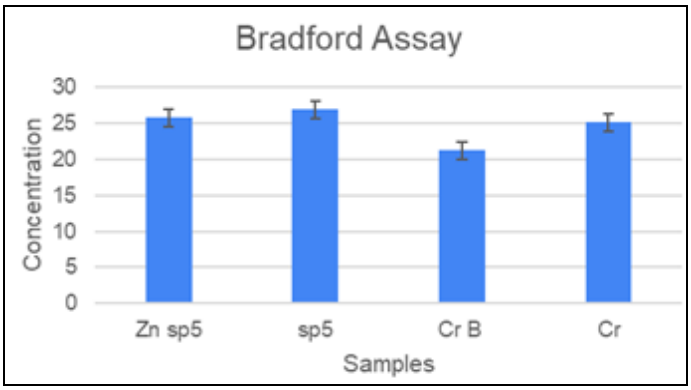
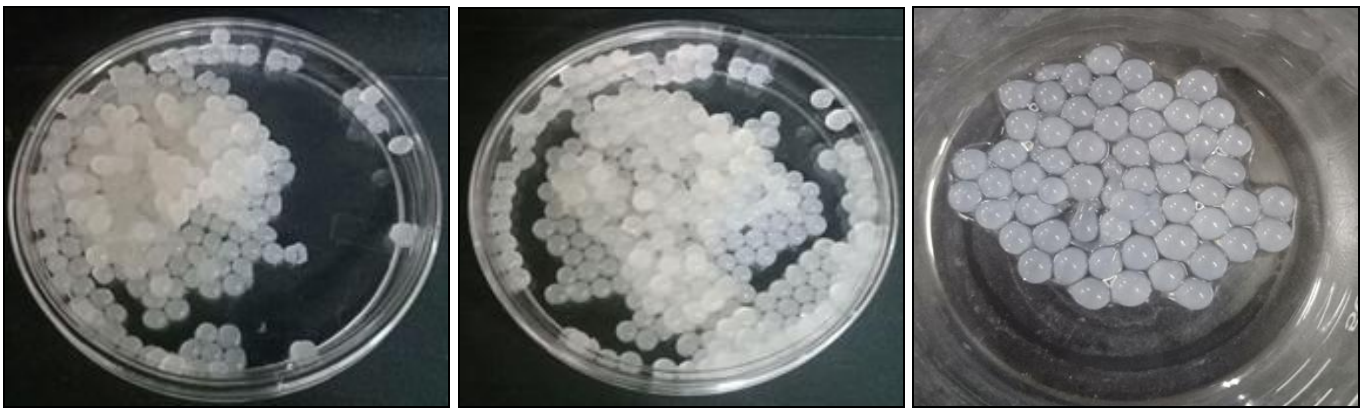


FIG. 11: CONCENTRATION OF HEAVY METAL AND NON-HEAVY METAL INDUCED ISOLATES

Immobilization: 1 % of sodium alginate and calcium chloride was prepared in each 100 ml of distilled water and then heavy metal-induced (Zinc sp5, Chromium B) and non-heavy induced (sp5, B) were prepared and analyzed by ICP-OES. Heavy metal concentration (mg/L) in Zinc and Chromium, when compared with control, concentration has been reduced in heavy metal-induced and non-heavy metal-induced beads in effluent **Fig. 12, 13.**

Non- Heavy Metalheavy Metal:



INDUCED SP5, B **INDUCED SP5, B** **CONTROL**

FIG. 12: IMMOBILIZED BEADS

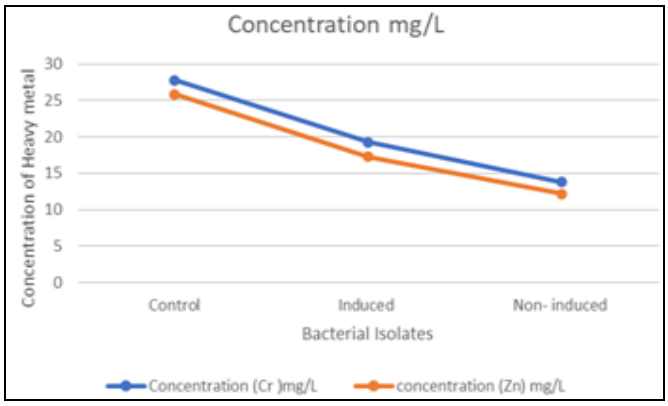


FIG. 13: CONCENTRATION OF HEAVY METAL-INDUCED AND NON-INDUCED ON IMMOBILIZED BEADS

DISCUSSION: Chennai, as an industrial city, is plagued by risks. Heavy metals are released by the leather and other sectors, endangering humans, wildlife, and agricultural settings. Even though large levels of heavy metals in our ecological system have a negative impact. Bioremediation is a safe, environmentally favourable process that can be stored in its original state.

The main goal is to isolate heavy metal-reducing microbes from tannery effluent and test their activity on immobilized beads, as well as to compare heavy metal remediation concentration and capacity for selective isolates so that they can be used to recover future hazards from tannery effluent containing toxic heavy metals.

Totally thirteen samples were identified A, B, pp4D, sp3B, sp5A, pp3, pp6,sp3A, pp4C, sp5 , sp3, 10^{-3},10^{-5} based on their morphological, cultural, biochemical characterization, and heavy metal tolerance activity in all three heavy metals zinc, nickel and chromium. The best four isolates A, B, sp5, sp3 were taken compared to all three multiple metal tolerance activities in 50 mg/L. Morphological, cultural, biochemical, and molecular analyses were used to characterize these four bacterial isolates. Bergey's manual of bacteriology was used to examine biochemical characterizations. By detecting the peptidoglycan layer, which is present in thick layers of bacteria, all isolates were determined to be gram-negative bacteria. Isolate sp5, sp3, 10^{-3}, A, B, pp4D, sp5A, sp3 shows catalase positive may be staphylococcus sp. Some isolates that were oxidase negative may be Enterobacteriaceae. In lipid hydrolysis sp5A, sp3B, pp6, pp3, and A bacterial isolates form a zone of precipitation that hydrolyses these isolates and were able to degrade. In the lecithinase test, sp5A, sp3B, pp3, A these isolates form zones of precipitation in which lecithinase in egg yolk is able to degrade. MALDI-TOF analyses were characterized and identified and carried out by DNA sequencing in which four species were analyzed: *Brevundimonas sp, Proteus mirabilis, Alcaligenes faecalis.* Two isolates were found to be

the same species, respectively. The isolates were measured for detection of heavy metals and analyzed through inductively coupled plasma-optical emission spectrometry Cr and Zn ; when compared with reference value all four isolates A, B, sp5, sp3, were reduced in concentration mg/L. The Sonication process was analyzed for cell lysis so that it may be an intracellular or extracellular protein. In Bradford assay, when compared with standard graph y=0.02x+0068, R^2=0.9663, the concentration, and heavy metal-induced and non-heavy metal-induced isolates were compared, *i.e.*, Zn sp5, B, Cr B, sp5. Then for further study, immobilization was processed by forming beads through sodium alginate and calcium chloride. Here, heavy metal and non-heavy metal-induced beads were formed separately, and control was kept. When analyzed through ICP-OES, the concentration level of heavy metal in mg/L, Zinc and Chromium, compared with control concentration, has been reduced in heavy metal-induced and non-induced beads in the effluent. Here beads were analyzed for activity and longevity so they can be revived for further purposes.

CONCLUSION: One sample was taken from the tannery sector, and thirteen isolates were tested in this investigation. Four isolates were found to have multiple heavy metal tolerance capacities. Isolates were identified as *Brevundimonas sp, Proteus mirabilis, and Alcaligenes faecalis* based on their morphological, biochemical, and molecular characterization. All of the study's findings are consistent with the idea that these three microorganisms possess valuable traits that can be used to develop bioremediation techniques that will detoxify tannery effluent at both the industrial and household levels while removing heavy metals.

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