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Research Article

AN ALTERNATIVE APPROACH ON BIOREMEDIATION OF HEAVY METALS IN TANNERY EFFLUENTS WASTE USING *STREPTOMYCES* SP.

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ABSTRACT

Objective: The present study is conducted to investigate the abilities of microorganisms to degrade heavy metals in industrial tannery effluent sample.

Methods: Tannery effluent sample was collected from effluent treatment plant and analyzed for physicochemical parameters. The potential microbes were isolated and identified by morphological and biochemical characterization. The sample was analyzed before and after to assess the heavy metal reducing the ability of the microorganism and the respective percentage of reduction were studied using X-ray fluorescence spectrometry.

Results: The samples were initially found to be highly contaminated with chromium, nickel, and cadmium. Out of three potential isolates, the isolate *Streptomyces* sp. was found to exhibit a better reduction against chromium (25.7%), cadmium (14.6%), and nickel (23.1%) in 50 ppm at longer incubation period. Comparatively, the reduction abilities of all the three isolates against all the three heavy metals increased with the increase in the incubation period but decreased with the increase in initial metal ion concentration except in the case of *Streptomyces* sp. against nickel where the reducing ability increased with the increase in metal concentration.

Conclusion: Apparently, the present study revealed that *Streptomyces* sp. had a better remediation potential than the indigenous *Pseudomonas* sp. and *Aspergillus* sp. Ultimately, the finding of this research has shown that the *Streptomyces* sp. can be used as a potent bioremediation agent for treating tannery and industrial effluent in an eco-friendly process.

Keywords*:* Bioremediation, Tannery effluent, Heavy metals, *Streptomyces* sp.

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INTRODUCTION

Across the world, pollution is rapidly increasing due to variations in both natural and anthropogenic activities as well as controlled and uncontrolled release of solid and liquid wastes. Use of chemical fertilizers, explosives, and tar are some of the major reasons for increased contaminants such as heavy metals, organic, and inorganic compounds in the biosphere. Furthermore, industries such as textiles, electroplating, and tanneries are recognized as a serious environmental threat all over the globe [1]. These effluents possess toxic chemicals such as sulfides, chromium salts, and heavy metals that can change effluents into poisonous waste water [2,3]. Heavy metals are roughly defined as elements having a density above 6 g/cm^3. Among these elements, Co, Cu, Mn, Ni, Se, and Zn are important only in trace amounts [4]. Whereas higher levels cause severe damage to the environment [5,6]. The effluents are densely colored and foul-smelling liquids with high concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and dissolved solids. Most heavy metals such as Co, Cr, Cu, Pb, and Cd are recorded to be present in complex form [7] and have become a menace when subjected to removal. Traditional chemical methods used for cleaning effluent pollutants usually remove symbiotic microbial biota which is a major adverse effect [8]. Hence, natural processes to break down the accumulated pollutants using biological system are currently used by researchers [9]. Recent fundamental work has revealed the existence of numerous ranges of microorganisms exhibiting prominent levels of bioremediation activities [10]. Group of microbial flora such as bacteria, fungi, actinobacteria, and algae has been reported to perform various bioremediation processes under favorable conditions [11]. From the past decades, actinobacteria are recognized as extremely promising agent of potent bioactive compounds that are being relatively utilized for various commercial applications. Comparatively, actinobacteria are illustrious for their ability to produce numerous secondary metabolites, and till date, it remains matchless [12]. Although physical and chemical methods are widely used, they are less effective with high cost and have limited application [13]. During physical and chemical treatment process, massive amount of sludge is formed which is highly toxic and poses difficulty in safe disposal [14]. Microbial degradation has been reported effective in the remediation of effluents in certain quantities with reduced cost using indigenous microorganisms. The present study is to identify the indigenous microorganisms present in the tannery effluent and also to screen their bioremediation potential in removal of chromium, cadmium, and nickel present in the tannery and industrial effluents which are discarded into the environment without any treatment. Simultaneously, a comparative study was made using actinobacteria from marine soil as a source for bioremediation and their pertaining activities.

METHODS

Study area

For the isolation of microorganism in the present study, Sample-I (effluent) was collected from effluent treatment plant (PTIET Co., Ltd.) at Pallavaram, Chennai, Tamil Nadu. Sample-II (soil) was collected from Muttukadu backwater (near South Chennai, Tamil Nadu) which extends for a distance of 20 km from the mouth of the estuary.

Collection of samples

1 L of tannery effluent sample was procured from a combined effluent treatment plant at Pallavaram, Chennai (India), in sterile specimen

bottles and transported to the laboratory in an ice pack. One of the bottles was acidified with concentrated nitric acid to bring down pH up to 2.0, while the other bottle was stored at 40°C to arrest any biological activity.

Analysis of physicochemical parameters

Several physicochemical parameters on tannery effluent sample were performed for determination of temperature, color, smell, pH, COD, and BOD. All the tests were performed according to standard methods [15].

Heavy metal analysis in effluent

A suitable volume of effluent was taken and filtered through Whatman No.1 filter paper, and pH was adjusted using weak base (Na_2CO_3) to 7.0. The sample was analyzed for heavy metal concentration by X-ray fluorescence (XRF) spectrometry (using minipal 4 bench top model) as described by Zarazua *et al.* [16].

Isolation of microorganisms

The collected effluent samples were directly used for isolation of bacteria and fungi. The sample was serially diluted and spread over the surface of nutrient agar (NA) and potato dextrose agar (PDA) plates without addition of metal and incubated. The marine soil samples were serially diluted and streaked on starch casein agar (SCA) amended with 50 mg/ml of fluconazole at pH 7.5 to inhibit fungal contaminates. Then, all the plates were kept at 28°C for 7-10 days.

Screening of potential isolates

Primary screening

The morphologically different isolates were inoculated on NA, PDA, and SCA (for actinobacteria) medium amended with Cr^{+6}, Cd^{+2}, and Ni^{+2} 50 mg/L concentration (i.e., $K_2Cr_20_7$, $CdCO_3$, and $NiCl_2$) and incubated at appropriate condition. Finally, all the isolates were characterized using morphological and biochemical methods.

Secondary screening

To determine heavy metal depletion ability of various isolates, a stock metal solution of Cr^{+6}, Cd^{+2}, and Ni^{+2} were prepared by dissolving AR grade salt sources such as $K_2Cr_20_7$, $CdCO_3$, and $NiCl_2$ in sterile distilled water. Working metal solution was then prepared using the stock solution for concentration of 50 ppm (2,500 cps), 100 ppm (5,000 cps), and 150 ppm (7500 cps) for each of the above metal. Three different concentrations (50, 100, and 150 ppm) of heavy metals in the working solution were prepared in NA, SDA, and SCA for bacteria, fungi, and actinobacteria, respectively. A loop full of log phase culture was inoculated into the appropriate amended medium in 100 ml Erlenmeyer's flask. The inoculated flasks were incubated in rotary shaker at 120 rpm with 32°C for 72-144 hrs for bacteria and 120 rpm with 27°C for 6-10 days for fungi and actinobacteria. 10 ml of each was taken after the end of incubation period and adjusted to pH 7.0. The heavy metal reduction analysis was carried out using XRF spectrometry as described by standard method [16,17]. The concentration of metal left in solution was recorded at 3rd and 6th day, 6th and 9th day, and 10th and 13th day of bacteria, fungi, and actinobacteria inoculated broth, respectively.

RESULTS AND DISCUSSION

Physical character of the effluent sample such as color, odor, pH, and temperature was analyzed. The effluent appeared black in color with foul smell, and the pH was recorded as 8.75 with temperature of 29°C. Color is the primary contaminant to be recognized in textile industry waste due to the presence of unused dye in water, which affects esthetic as well as biological activity of the ecosystem [18]. The data revealed high BOD (1200 mg/L) and COD (3800 mg/L) of the effluent were found to have exceeded the Tamil Nadu Pollution Control Board (TNPCB) standard, which may be due to high content of organic pollutant in the effluent. Similar finding was conducted and reported that BOD and COD values were very higher than permissible limit of TNPCB [19]. The most potential isolates were identified as *Pseudomonas* sp., *Aspergillus* sp., and *Streptomyces* sp. by characterization studies.

Heavy metal analysis

The heavy metal characterization and quantification from the tannery effluent sample were determined using XRF spectrometry (Fig. 1). The tannery effluent sample was analyzed for the presence of three heavy metals such as Cr, Cd, and Ni and it contains Cr 24.11 cps, Ni 396.30 cps, and Cd 4,990.81. The measurement was conducted in triplicate to minimize error, and mean values were recorded. All the concentrations were beyond the permissible limit of TNPCB. Similar study has been carried out and reported that the effluent was found to contain heavy metals such as Mn, Cr, Pb, and Zn were beyond the permissible limit [20,21].

Isolation of indigenous microorganisms

A total of 99 bacterial isolates were isolated from tannery effluent, and among this, five morphologically different isolates were identified. From the five morphologically different isolates, the most potential bacterial isolate was obtained through a repeated sub culturing on respective media amended with 50 ppm (2500 cps) concentration of Cr^{+6} at 37°C for 48 hrs. The most potential isolate showed Gram-negative, rod shaped with positive reaction against indole, methyl red, Voges-Proskauer, citrate, catalase, and oxidase and Ak/Ak reaction in triple sugar iron test. Based on the morphological and biochemical reaction, the most potential isolate was identified as *Pseudomonas* sp. Similarly, the previous study also reported that the genus *Pseudomonas* has high potential to remove Cr^{+6} (VI) [22]. Eight fungal species were observed in PDA medium. Among eight morphologically different isolates, only four of them that had metal-resistant abilities. Among the 4 potential isolates, the most resistant isolate was identified as *Aspergillus* sp. with lactophenol cotton blue stain followed by microscopic examination as described in "Hand book of soil Fungi" by Nagamani *et al.* [23]. A total of 14 actinobacterial isolates were isolated from 5 different sampling stations. Four isolates showed potential-resistant activity against heavy metals. The most potential isolates produced powdery gray-colored aerial spore mass, brown-colored reverse side on SCA medium, respectively. The isolate showed spirally twisted sporophore with 2-3 bends, globose shaped with smooth spores surface. It produced a positive reaction against catalase, amylase, and oxidase. Based on the morphological and biochemical reactions, the most potential isolate was identified as *Streptomyces* sp. All the most potent isolates from three groups were selected for secondary screening (bioremediation activity).

Uptake of heavy metals by bacteria

The most potential bacterial isolate *Pseudomonas* sp. degrade the metals Cr^{+6} at 3rd day of incubation was 23.3% (in 50 ppm), 19.4% (in 100 ppm), and 6.7% (in 150 ppm); however, in the 6th day, it uptakes 28.4% (in 50 ppm), 20.2% (in 100 ppm), and 7.8% (in 150 ppm). The percentage of Ni^{+2} uptake on 3rd day was 4.4 (in 50 ppm), 4.1 (in 100 ppm), and 0.5 (in 150 ppm), but at the 6th day, the percentage of uptake was 11.9% (in 50 ppm), 12.1% (in 100 ppm), and 20.4% (in 150 ppm). As for Cd^{+2} on 3rd day, percentage uptake was 9.0 (in 100 ppm), 7.7 (in 150 ppm), and 7.5 (in 200 ppm); however, at the 6th day, it was 14.0% (in 100 ppm), 18.5% (in 150 ppm), and 10.8% (in 200 ppm). For all metals, the percentage of reduction increases with increase of incubation period and it decreases when concentration of metal increased (Table 1). Comparatively, in this study, *Pseudomonas* sp. was found to reduce better Cr > Ni > Cd all in 6 days of incubation. Similar finding was conducted by Karmakar and Ray [24]. The study reported that a Gram-negative bacteria isolated from tannery soil reduced Cr (vi) ranging from 100 to 500 mg/at 35% within 72 hrs at pH 6 and temperature 37°C.

Uptake of heavy metals by fungal isolate

The potential fungal isolate *Aspergillus* sp. percentage of uptake of Cr^{+6} at 6th day was 11.2% (in 50 ppm), 3.2% (in 100 ppm), and 2.9% (in 150 ppm). However, at the 9th day of incubation, it was 11.5% (in 50 ppm), 12.8% (100 ppm), and 3.1% (in 150 ppm). For Ni^{+2} at the 6th day, percentage uptake was 4.8 (in 50 ppm), 2.6 (in 100 ppm), and 1.2 (in 150 ppm), but at the 9th day, it was 12.7% (in 50 ppm), 17.3% (in

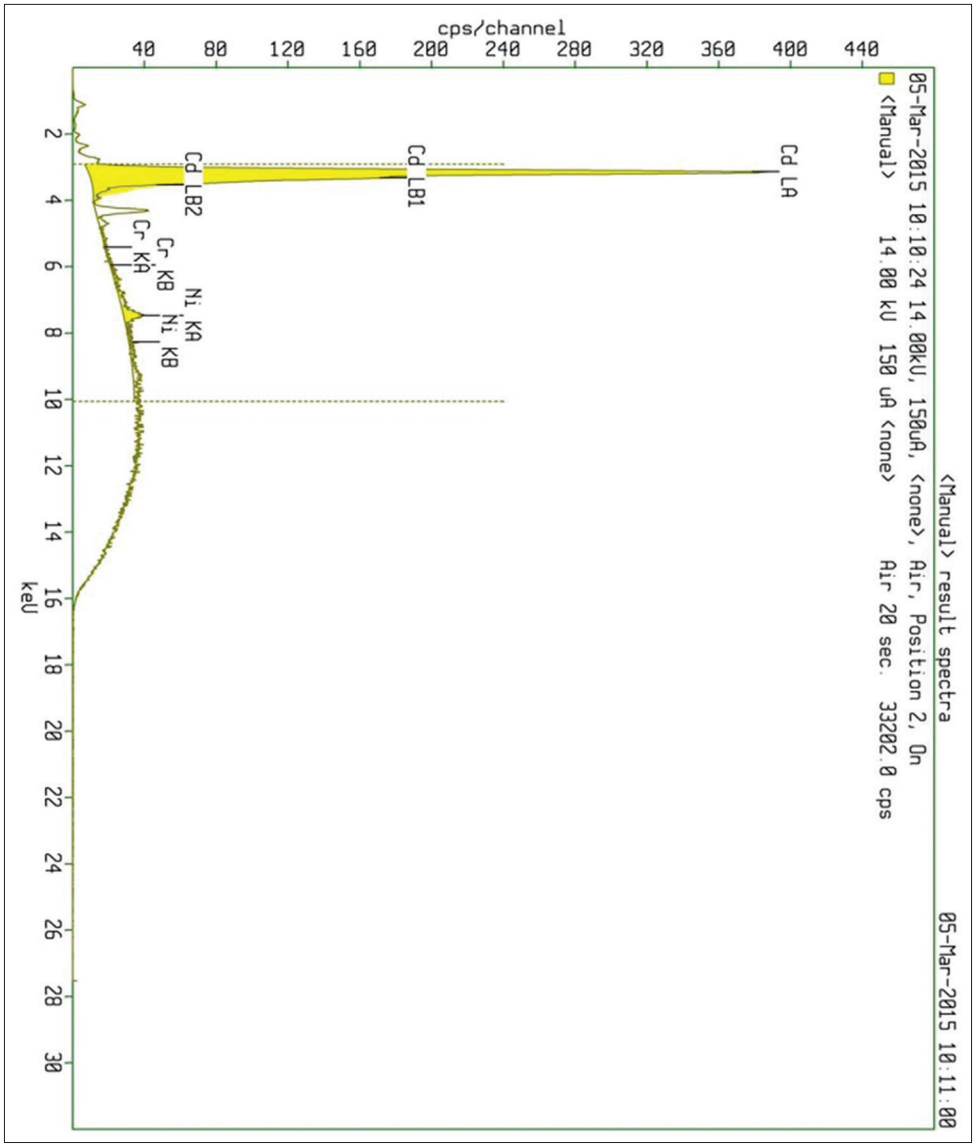

Fig. 1: X-ray fluorescence spectrometry analysis of tannery effluent sample

Table 1: Percentage of uptake of chromium, nickel, and cadmium in broth by bacterial isolate

Heavy metal	Concentration in cps/ppm	Percentage of uptake in 3rd day	Percentage of uptake in 6th day
Cr^{+6}	2500/50	23.3	28.4
	5000/100	19.4	20.2
	7500/150	6.7	7.8
Ni^{+2}	2500/50	4.4	11.9
	5000/100	4.1	12.1
	7500/150	0.5	26.4
Cd^{+2}	5000/100	9.0	14.0
	7500/150	7.7	18.5
	10,000/200	7.5	10.8

100 ppm), and 6.7% (in 150 ppm). For Cd^{+2} at the 6th day, percentage uptake was 8.7 (in 100 ppm), 6.8 (in 150 ppm), and 1.9 (200 ppm), but at the 9th day, it was 24.1% (in 100 ppm), 18.8% (in 150 ppm), and 18.8% (in 200 ppm). Therefore, the result revealed that there was increase in reduction of metals with increase in incubation period while increase in concentration caused decrease in metal reduction ability of the isolate (Table 2). Substantial number of studies had revealed similar results as reported by Han *et al.* [25], although there was little variation in pH and temperature under study.

Uptake of heavy metals by *Streptomyces* sp.

After incubation at ambient condition, the *Streptomyces* sp. percentage of uptake of Cr^{+6} at the 10th day was 21.4% (in 50 ppm), 16.3% (in 100 ppm), and 8.0% (in 150 ppm), but at 13th day, it was 25.7% (in 50 ppm), 18.9% (in 100 ppm), and 9.8% (in 150 ppm). For Ni^{+2}, the percentage of uptake at 10th day was 4.0 (in 50 ppm), 7.7 (in 100 ppm), and 20.3 (in 150 ppm), but at the 13th day, it was 14.6% (in 50 ppm), 23.1% (in 100 ppm), and 21.3% (in 150 ppm). Similarly, for Cd^{+6} at the 10th day, the percentage of uptake was 2.9 (in 100 ppm), 2.4 (in 150 ppm), and 2.0 (in 200 ppm), but at the 13th day of incubation, the percentage of uptake was 23.5 (in 100 ppm), 20.6 (in 150 ppm), and 19.8 (in 200 ppm). The result so far had shown an increase in the uptake of all the three metals along increase in the incubation period and also a decrease in the uptake ability as metal concentration increased except in Ni^{+2}. The percentage uptake of Ni^{+2} increased when concentration increased (Table 3). Similar studies were reported on the ability of *Streptomyces* sp. to degrade heavy metals as reported by El Baz *et al.* [26]. The present study revealed that marine *Streptomyces* sp. has shown a good potentiality in bioremediation of heavy metals. Significant

Table 2: Percentage of uptake of chromium, nickel, and cadmium in broth by fungal isolate

Heavy metal	Concentration cps/ppm	Percentage of uptake in 6th day	Percentage of uptake in 9th day
Cr^{+6}	2500/50	11.2	11.5
	5000/100	3.2	12.8
	7500/150	2.9	3.1
Ni^{+2}	2500/50	4.8	12.7
	5000/100	2.6	17.3
	7500/150	1.2	6.7
Cd^{+2}	5000/100	8.7	24.1
	7500/150	6.8	18.8
	10000/200	1.9	20.8

Table 3: Percentage of uptake of chromium, nickel, and cadmium in broth by actinobacteria

Heavy metal	Concentration in cps/ppm	Percentage of uptake in 10th day	Percentage of uptake in 13th day
Cr^{+6}	2500/50	21.4	25.7
	5000/100	16.3	18.9
	75000/150	8.0	9.8
Ni^{+2}	2500/50	4.0	14.6
	5000/100	7.7	23.1
	7500/150	20.3	21.3
Cd^{+2}	5000/100	2.9	23.1
	7500/150	2.4	20.6
	10000/200	2.0	19.8

reduction was recorded in bacterial isolate, especially on chromium (VI) at lower concentrations. Comparatively, in the degradation process of Ni and Cd, *Streptomyces* sp. was found to be more efficient than the indigenous *Pseudomonas* sp. and *Aspergillus* sp.

CONCLUSION

In the present study, a group of metal tolerant bacteria, fungi, and actinobacteria was isolated, identified, and utilized for biosorption of multimetal contaminated effluent. Bioremediation capabilities of all the isolates decreased with increase in the initial metal concentrations except in nickel reduction by *Streptomyces* sp. Thus, it is highly evident that metal decontamination by actinobacteria could be a suitable alternative approach to reduce metal content in a significant and cost-effective method.

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