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Bioremediation of Heavy Metals from Tannery Wastewater effluents using bacterial isolates

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Abstract: Bioremediation is a simple and environmentally friendly solution for solving environmental problems. In our study three bacterial strains (IS1, IS2 and IS3) were isolated from tannery wastewater and were identified using biology system Biolog ™ microplate as *pseudmonas* sp., *Klebsiella* sp. and *Enterobacte*r sp. respectively. These strains were examined for their ability to reduce Pb^{+2}, Cd^{+2} and Cr^{+3} in tannery wastewater effluent. Some parameters, such as temperature, pH, and incubation duration, were optimized in order to improve adsorption efficiency of heavy metals. The perfect optimum conditions were found to be temperature of 35°C, pH of 5, and a 48-hour incubation time. Pseudomonas sp. showed the best removal percent 76% and 75% for lead and cadmium respectively while klebsiella sp. showed the highest removal percentage for chromium 74%. A mixture of the three isolated bacterial strains was examined to see whether they could be combined in their heavy metal bio-degradation activity or not, the test revealed synergism between the three isolated strains, with higher removal percentages 78 %, 77% and 78% for Pb^{+2}, Cd^{+2} and Cr^{+3} respectively.

Keywords: Biological treatment, Biosorption, Heavy metal removal, Tannery wastewater and Bacterial isolates

1. INTRODUCTION

The substantial expansion in urbanization caused by population growth naturally raises water consumption, which in turn causes a rise in wastewater from the development of commercial, industrial, and human activity. Therefore, research into novel technologies and alternative wastewater treatment techniques is beneficial, as long as the techniques are highly effective and environmentally safe [1]. One of the most difficult tasks in environmental biotechnology is bioremediation of wastewater containing heavy metals such as lead, cadmium and chromium. Heavy metals are non-degradable elements and hence persist in the ecosystem, posing severe health risks when they come in contact with humans as a result of anthropogenic activity [2].

One of the most critical environmental problems that directly endangers human health is heavy metal pollution [2]. Further, their toxicity is a major source of pollution in the aquatic environment [3]. Different types of heavy metals are used by different types of industries, such as petrochemicals, pesticide production, chemical industry, oil refineries, foundries, smelters, tannery industries, etc., and their effluent containing harmful compounds is discharged into the aquatic environment either directly or indirectly [4]

Heavy metals like copper, iron, and zinc are required in trace amounts by living organisms, but due to their toxicity and accumulating behavior, excessive doses of these metals are harmful to the organism [5]. In Egypt, tanneries are considered one of the major contributing sources of pollution due to their potential of producing highly varying loads of toxic effluent; hence, the quantity of tanned leather produced is 125 million square feet, with Old Cairo representing 75%. The demand for different types of leather products has increased in Egypt, leading to the creation of large tannery projects such as Al Robaiky region. In Egypt, the tanning industry is one of the most significant sources of national income. There are over 300 tannery projects in Egypt, and the majority of them use the chromium tanning method. The chromium salt tanning method is a low-cost operating and manufacturing process that is quick, reliable, and effective. More than 65% of the used chromium salts react with leathers, while the chromium leftover remains in the effluent [6].

Chemical precipitation, chemical oxidation and reduction, complexation, and ion exchange are all common procedures for eliminating heavy metals. When the initial heavy metal levels are low (< 50 ^10 wt), such methods may be unsuccessful or highly costly [7]. Biomass removal of heavy metals has a numerous advantage. To start with, the removal operations are extremely quick. Secondly, biopolymers found outside of cells and walls, like phospholipids, chitin, and polysaccharides, and proteins have been shown in a variety of microorganisms to dramatically improve metal immobilization and complexation. Furthermore, heavy metal reduction by biomass tends to be reversible [7], Biomass could be utilized again for heavy metal treatment, as metals can be extracted from it using many kinds of treatment techniques. Consequently, microbial biomass removal has been identified as a possible replacement for existing heavy metal waste discharge treatment technology [7].

As a result, microbial biomass removal has been identified as a viable alternative to conventional technologies for heavy metal waste discharge treatment processes. Industrial wastewater treatment plants are considered as the major harmful metal contaminants source such as lead, nickel, and cadmium that found in water bodies [8]. Lead is a non-essential heavy metal and a general toxin in the heavy metal family. It's a multi-polluting substance that pollutes land, water, and the atmosphere [9]. Lead is found in lead-based gasoline paints, bullets, batteries, and alloys, and so penetrates the environment and human food chain. Adults absorb 5-15 percent of interest lead, with just about 5% of it being retained. Lead in the blood at concentrations of <.5-0.8 µg/ml causes a variety of problems. According to the Worldwide Agency for Research on Cancer (IARC), lead is a type 2B carcinogen. Lead measurement for exposure monitoring is critical because of its extremely lethal effects [10]. Chromium stands out as an important inorganic contaminant, particularly in tannery effluents with concentrations ranging from 10 to 1000 mg/L, significantly exceeding discharge restrictions limits. Furthermore, there are serious concerns to human health and the environment when chromium oxidizes to its hazardous form, Cr (VI). In muscles, bones, and blood, the accumulated Cr levels vary from 0.14 to 3.2 ppm, 1.8 ppm, and 0.0052 ppm, respectively. As a heavy metal, chromium can accumulate through time and result in chronic exposure that can be toxic and cause several kinds of pathophysiological defects, such as anemia, burns, sores, especially in the stomach and small intestine, damage to sperm and the male reproductive system, and effects on other biological systems [11]. Exposure to cadmium can cause lung damage and flu-like symptoms. However, prolonged exposure can cause lung, bone, and kidney diseases. Also, excessive levels can irritate the stomach and cause diarrhea and vomiting [12].

Bio sorption is the process of withdrawing metal or metalloid species, compounds, and particles from a solution using inexpensive biological materials [13]. Bacteria, algae, fungi, plants, waste from industrial or agricultural sources and other polysaccharide materials are all common sources of inexpensive biosorbents for heavy metal removal [14]. Overall, most types of biomaterials employed for biosorption were shown to have good biosorption capabilities for all types of metal ions. Many researchers have investigated the use of laboratory-cultivated microorganisms, or microbial biomass produced by various processing companies or wastewater treatment units, in biosorption for heavy metal removal [15,16].

The term "mechanism of biosorption" refers to the process by which microbial cells absorb heavy metals through biosorption mechanisms as shown in Fig. 1. It can also be separated into two categories: metabolism-dependent bioaccumulation, which involves sequestration, redox processes, and species-transformation techniques, and metabolism-independent biosorption, which mostly happens on the cell surface [17, 18]. Biosorption can be done passively by dead biomass or living cells via surface complexation onto the cell wall and surface layers [19]. Bioaccumulation is influenced by several physical, chemical, and biological techniques, including intracellular and extracellular processes, in which biosorption has a restricted and unclear involvement in these processes [19].

In this study, we investigated the potential of bacterial isolates (*pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp.) as a heavy metal removal agent for microbial biomass (Pb^{+2}, Cd^{+2} and Cr^{+3}) from tannery wastewater. Under varying pH, equilibration duration, and temperature conditions, we conducted tests utilizing biomass from *pseudomonas* sp., *Klebsiella* sp., and *Enterobacter* sp. Reusing and renewing the bacterial biomass will significantly lessen the operation costs for the removal of heavy metals from aqueous solutions.

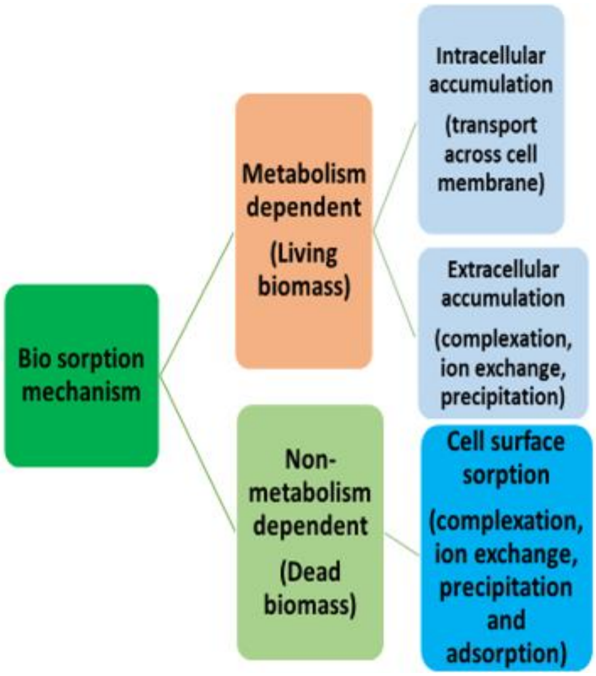


Fig. 1: Schematic diagram for biosorption mechanism.

Materials and methods

The present work carried out in the departments of Microbiology and Chemistry of water & wastewater analysis, Sanitary and Environmental Engineering Institute, Housing and Building National Research Centre.

1-Collection of samples

Samples were randomly collected from the Robkki leather industrial city in Cairo from different places such as drainage canal. Samples were collected in polyethylene bottles that had been previously cleaned with distilled water and HNO_3 (1M). The bottle was filled all the way to the top, and the cap was securely fastened to prevent any air from escaping the bottles. As soon as feasible, the obtained samples were moved to the lab so that different physicochemical properties could be examined. As advised by APHA 2017, a few factors, including color, temperature, and pH, was observed at the sampling location. [20]. The collected samples were preserved for further analysis in the laboratory.

2-Isolation and screening of heavy metal resistant bacteria:

A 1 ml sample from 10^{-3} to 10^{-7} dilutions was pour plated in nutrient agar (NA) plates after the bacteria found in the textile dye effluents were isolated using serial dilution up to 10^{-8} dilutions in buffer. The inverted plates were incubated for twenty-four hours at 37°C. As indicated in Fig. 2, growing bacterial colonies were picked, and the streaking method was used to carry out additional purification.

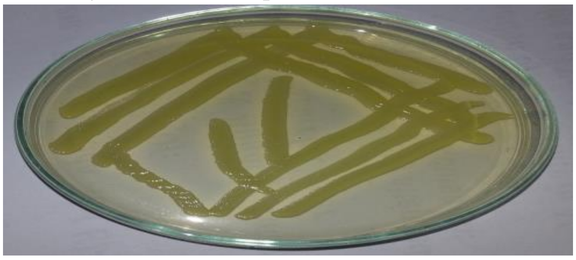


Fig.2 Streaking method for the purification of the isolated strains

In order to screen twenty different bacterial strains, they were streaked on nutritional agar enriched with Pb^{+2}, Cd^{+2}, or Cr^{+3} (as a salt of heavy metals at a dose of 20 mg/100 ml). For 48 hours, the plates were incubated at 37 °C. From the twenty isolated bacterial strains three well grown colonies were selected in nutrient agar slants in pure form for identification by BiologTMmicroplate.

3-Measuring bioremediation activity of selected isolated bacterial strains

The potential bioremediation of lead, chromium and cadmium tolerating bacterial isolated was done by batch experiment process. The chromium (as potassium dichromate), cadmium (as cadmium nitrate), and lead (as lead nitrate) supplements were added to the nutrient broth where the bacteria were cultured, the metal salt ratio was kept constant at 20 mg per 100 ml within the medium. Cells were inoculated in nutrient broth (250 ml flask) and kept at 35 °C for 48 hr in a rotary shaker at 180 rpm. Sample taken at predetermined intervals were centrifuged at 3000 rpm/min for 30 min and the supernatant were analyzed. The analysis of heavy metal ions was carried out by Flame Atomic Absorption Spectroscopy (AAS) (Thermo Scientific ICE 3000 series, China). The removal percentage % of each heavy metal was calculated for each run as follow:

The removal percentage is calculated as

$$(Ci - Cf)/Ci * 100$$

Where Ci represents the heavy metal concentration at the beginning and Cf represents the concentration at the end.

4-Applying the optimized conditions on the raw tannery wastewater

After we have determined the best optimum conditions for the heavy metal removal, Biodegradation of heavy metals experiments were conducted in 250 ml of different flasks holding 100 ml of tannery effluent collected samples and the best optimum conditions were adjusted for the best selected isolates and removal percentage was calculated as mentioned above and also an synergism/antagonism test was performed between the bacterial isolates to know if they might be combined together in their biodegradation activity of heavy metals in tannery wastewater or not.

Results and Discussion

1-properties of the collected tannery wastewater

The physiochemical analysis showed that raw tannery wastewater has high loads of pollutants as shown in Table 1 this is average of five collected samples from different sites:

Table 1 physiochemical characteristics of raw tannery wastewater

S.N	Parameter	Average of collected samples
1	color	Brownish to dark brownish color
2	pH	5.5 - 6
3	odor	Foul smell
4	Pb^{+2}	198 mg/l
5	Cd^{+2}	227 mg/l
6	Cr^{+3}	543 mg/l

As shown in Table 1 tannery wastewater contains a large variety of toxic metals as chromium, cadmium and lead. These heavy metals were present in the tannery wastewater due to the use of chemicals in the tanning process. The chemicals used in the tanning process include chromium, which is used to tan the leather, lead, and cadmium are biocides [21]. The presence of these heavy metals in the tannery wastewater can cause serious environmental and health problems by accumulating in the soil and water, and can be dangerous to both plants and animals [21].

2-Screening and identification of heavy metal resistant bacteria

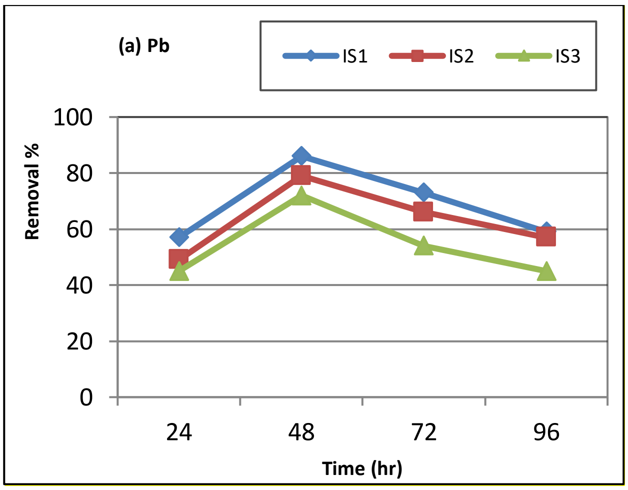
The screening was done by cultivating twenty isolates on nutrient agar enriched with heavy metal salts (Pb^{+2}, Cd^{+2} and Cr^{+3}) out of the twenty isolates seven were selected as the heaviest metal resistant bacteria and then identified by BiologTM microplate [22], Three bacterial strains were identified from the identified isolates, which included some repetitions (IS1, IS2 and IS3) as pseudmonas sp., Klebsiella sp. and Enterobacter sp. respectively.

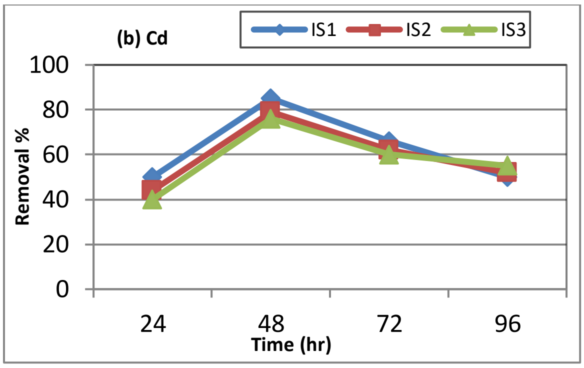
3- The impact of cultivation time on heavy metal removal using isolated bacterial strains.

For IS1, IS2 and IS3 adsorption of lead increases at optimum cultivation time 48 hr and then starts to decrease again where maximum adsorption of lead was 86%, 79% and 72% respectively as shown in Fig 3.

For Cd^{+2} the maximum adsorption was 85%, 79 and 76% for IS1, IS2 and IS3 respectively at optimum incubation time of 48 hr., also for Cr^{+3} the maximum adsorption time was at 48 hr. where it reaches 85%, 90% and 80% for IS1, IS2 and IS3 respectively. These results may be attributable to the effect of microbial growth associated with the optimal biomass and the optimal incubation process, at which a high level of heavy metal adsorption occurred these results were in correspondence with Merina et el. [23].

As indicated in Figure 2, Metal ion bio sorption is divided into two steps: an early step in which the rate of uptake is fast and high (typically around 48 hours with approximately 90 percent uptake) and a second, later step in which equilibrium is achieved. Metal absorption is rapid and efficient in the first stage due to the availability of unoccupied active sites for metal absorption. As time passes, the rate of bio sorption slows due to the saturation of active sites [24].





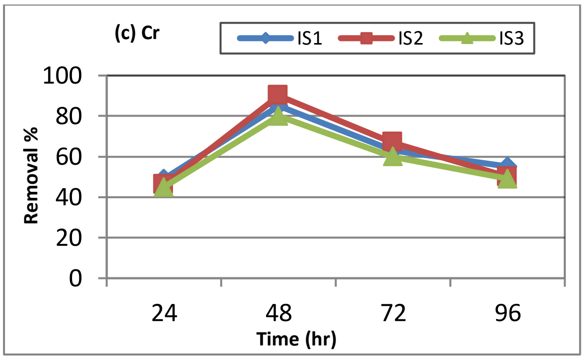


Fig. 3 Effect of the adsorption time on removal of (a)Pb^{+2}, (b) Cd^{+2} and (c) Cr^{+3}

4-Effect of temperature on heavy metal removal by using isolated bacterial strains

The temperature of the medium is a very important parameter for heavy metal adsorption, as it induces bacterial growth and also the solution medium of the metal ion. The maximum removal for Pb^{+2}, Cd^{+2}, and Cr^{+3} was observed at optimum temperature 35 °C for all isolated strains after 48 hr, as shown in Fig. 4. For IS1 the maximum removal for Pb^{+2}, Cd^{+2} and $Cr^{+3}$was 76%, 75% and 70% respectively. For IS2 the maximum removal for Pb^{+2}, Cd^{+2} and Cr^{+3} was 69%, 70% and 78% respectively, while for IS3 the maximum removal for Pb^{+2}, Cd^{+2} and $Cr^{+3}$was 62%, 67% and 65% respectively these results were in correspondence with Olukanni et el. and Merina et el. [23, 25].According to Salaudeen [24] improvement in metal uptake with increase in temperature from 21°C to 35°C this could be assigned to an increase in the number of active adsorption sites or a decrease in the thickness of the adsorbent's boundary layer. Although high temperatures increase bio sorption, they can also degrade the physical structure of the biosorbent.

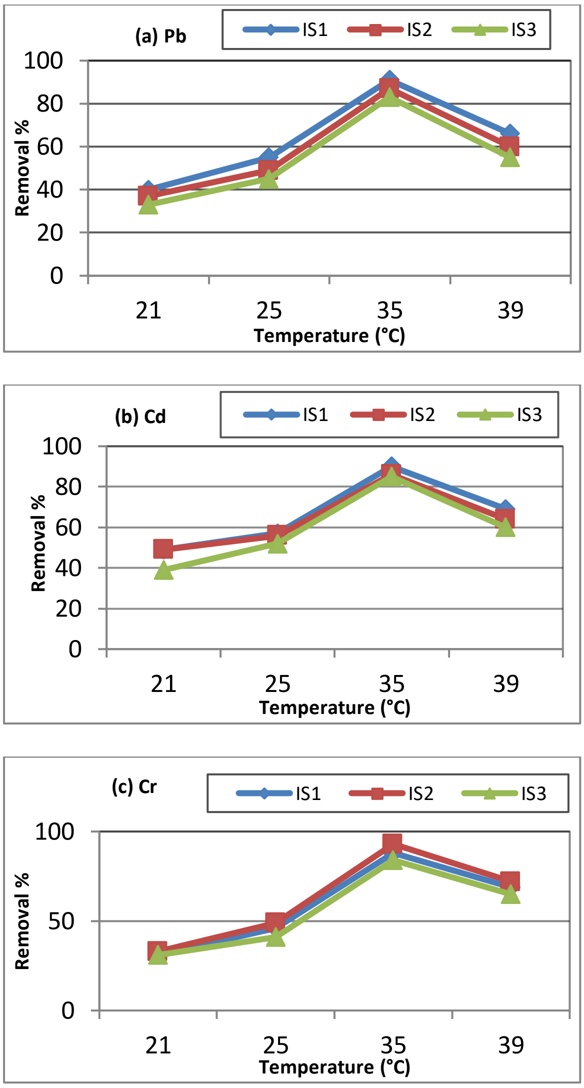


Fig. 4 Effect of the adsorption temperature on removal of (a)Pb^{+2}, (b) Cd^{+2} and (c) Cr^{+3}

5-Effect of pH on heavy metal removal by using isolated bacterial strains

pH effects on metal ion adsorption by using living cells, binding of metal ions with the cell surface and its availability in solutions is affected by pH value. As mentioned by Merina et el. [23] At low pH, cell surface sites are tightly connected to H+ ions, making them unreachable for other cations. However, as pH rises, so does the number of ligands with negative charges, resulting in greater cation binding. The increase in pH resulted in a higher negative charge on the surface of the cell, favoring electrochemical attraction and metal adsorption. In our study as shown in Fig 5 the pH range studied (3 to 9) with optimized time and temperature, all the heavy metals removal was increased at pH from 3 to 5 gradually and then decreased at pH 7 for all isolated strains where the optimum adsorption of metals was found at pH 5. for Pb^{+2} the optimum adsorption was at pH 5 where it reaches 89%, 83% and 79% for IS1, IS2 and IS3 respectively. For Cd^{+2} the optimum adsorption was also at pH 5 where it reaches 87%, 81% and 76% for IS1, IS2 and IS3 respectively and also for Cr^{+3} the maximum adsorption was at pH 5 where it reaches 88%, 91% and 82% for IS1, IS2 and IS3 respectively these results were in correspondence with Amira et el. [26].

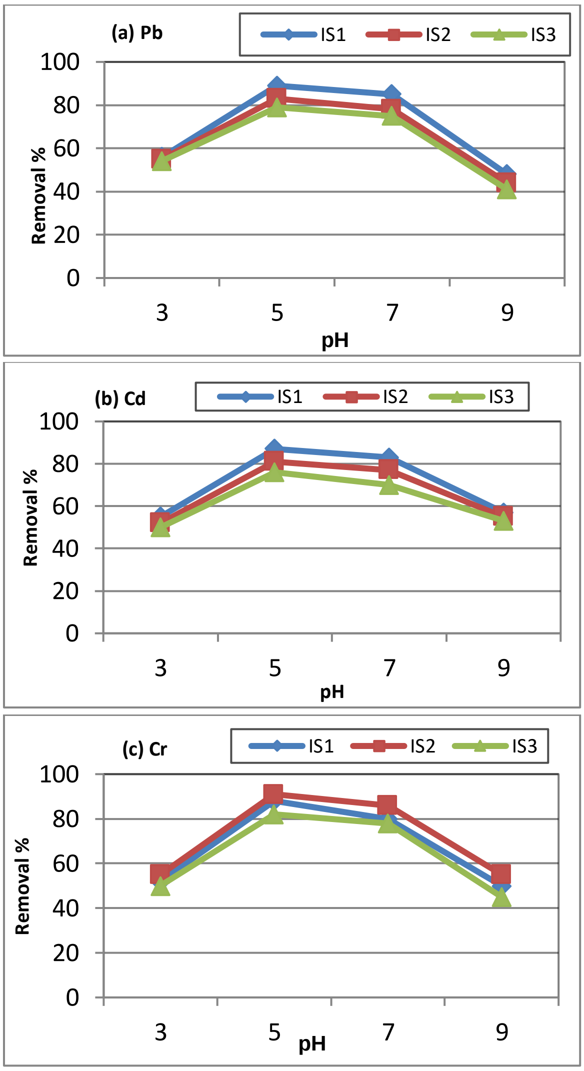


Fig. 5 Effect of the adsorption pH on removal of (a) Pb^{+2}, (b) Cd^{+2} and (c) Cr^{+3}

6- Heavy metal removal from tannery wastewater

At the most preferable optimum conditions (pH 5, temperature at 35 °c and for 48 hr. incubation time) the three best selected isolates were tested for their ability to remove heavy metal where IS1 showed the best removal percent 76% and 70% for lead and cadmium respectively while IS2 Shows the highest removal percentage for chromium 74% and for IS3 the maximum removal percentage 65%, 60% and 60% for Pb^{+2}, Cd^{+2} and Cr^{+3} respectively. The mixture of the three selected strains shows a higher removal percentage 78 %, 77% and 78% for Pb^{+2}, Cd^{+2} and Cr^{+3} respectively as shown in Fig 6 these means that there is synergistic activity between all the three strains in their ability of bioadsorption of heavy metals.

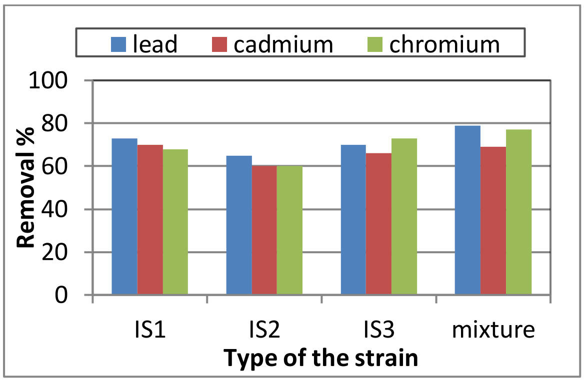


Fig. 6 heavy metal removal under optimum conditions from tannery wastewater.

CONCLUSONS

Microbial biomass is an inexpensive, highly effective biosorbent material used to eliminate heavy metals from tannery wastewater. The toxicity of heavy metals increases rapidly with industrial developments to reduce heavy metal pollution and avoid metal toxicity we use indigenous microorganisms found in wastewater which is an effective and economical bioremedial tool. Three resistant strains (*pseudmonas* sp., *Klebsiella* sp. and *Enterobacter* sp.) for Pb^{+2}, Cd^{+2} and Cr^{+3} were isolated and used for bioremediation the three strains shows significant removal percentage for lead, cadmium and chromium at optimized conditions and also shows synergism for their ability of heavy metal removal.

RECOMMENDATIONS

- The use of bacterial isolates under optimum conditions in tannery wastewater could be used in the secondary treatment stage after the pretreatment stage like sedimentation or screening to remove large particles, furthermore the biological treatment is a high-efficiency, energy-saving, and eco-friendly treatment technology for the removal of heavy metals from tannery wastewater.
- Ensure adequate nutrients (nitrogen, phosphorus) are available for microbial growth as it's a major concern for biological treatment process.

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