IJPSR (2020), Volume 11, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 September 2019; received in revised form, 27 December 2019; accepted, 10 March 2020; published 01 September 2020

ISOLATION OF CADMIUM DEGRADING MICROORGANISMS FROM ELECTROPLATING, STEEL AND BATTERY INDUSTRY

Sneh Lata [1], Sukhminderjit Kaur [1] and Tulika Mishra [*2]

University Institute of Biotechnology [1], Chandigarh University, Gharuan - 140413, Punjab, India.
Department of Immunology and Microbiology [2], Aureus University, Aruba.

Keywords:

Biochemical oxygen demand, Cadmium, Cadmium tolerance, Heavy metals, pH, Microorganisms

Correspondence to Author:
Sneh Lata

University Institute of Biotechnology, Chandigarh University, Gharuan - 140413, Punjab, India.

E-mail: sneh.ahluwalia@gmail.com

ABSTRACT: Increase in industrialization has raised the levels of heavy metal pollution in the country. In order to combat with this problem, many strategies were developed till date, and among them, the use of microorganisms is the most successful technique. The present study was undertaken for isolation, identification, and characterization of heavy metal (Cd) tolerant bacteria from industrial effluents of electroplating, steel and battery industry SAS Nagar, Punjab and Chandigarh (India). The effluents were initially tested for physicochemical parameters (BOD, COD, TDS, TSS, *etc*.).The cadmium content was quantified through spectrophotometric assay. Initially, a total of 20 bacterial isolates were isolated and screened, then the effect of selected strain on all the parameters of test samples was checked. On the basis of performance to cadmium tolerance, only two isolates were selected. These bacteria could tolerate cadmium up to 1000 ppm and 1500ppm. These isolated bacteria may be helpful for the bioremediation of heavy metal contaminated industrial effluents.

INTRODUCTION: Heavy metals are toxic in nature. Cadmium was discovered by Stromeyer and Hermeny in 1817. A very large amount of cadmium is released into the environment [1]. Cadmium is released into rivers through weathering of rocks, forest fires, *etc*. Human's activities are also responsible for the deposition of cadmium by the manufacturing process. So it is entering into the soil and directly into the food chain by absorption through roots. Health hazards to plants, animals, aquatic life, and humans by heavy metals are reported [2]. Tannery industries are the main sources and causes of such toxic pollutants, such as cadmium [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(9).4403-10

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4403-10

Unprocessed tannery effluents are released into water sources In Bangladesh, which mainly serves as the reason for heavy metal toxicity [5, 6]. Many microorganisms have developed a different resistant mechanism for heavy metals [7].

Keeping above in view, the present study was designed for the analysis of the physicochemical parameters of the electroplating industry, steel, and battery industry of area SAS Nagar (Punjab, India) and Chandigarh. This study also involves the measurement of cadmium content in these samples by spectrophotometric assay. Also, the strains were isolated from the soil sample of the same area against cadmium, and they were checked for tolerance for different concentrations of cadmium.

MATERIALS AND METHODS:

Sample Collection: Fifteen Soil samples were collected in and near the Industrial Area, Mohali, as well as Chandigarh. Soil and wastewater samples were collected. These samples were placed in

sterile plastic bags and bottles, respectively. Bags containing soil samples were first labeled and then placed in the laboratory. Samples were maintained at a temperature of 4 ºC in order to avoid contamination.

Measurement of Physical Parameters:

Color of Soil: Color of soil sample was brown with blackish /grey tinge. It was observed by seeing the color of the soil sample.

pH: To 10 g of air-dried soil 50 ml. of distilled water was added to prepare a suspension (1:5 w/v). The suspension was kept over a shaker for 30 minutes. By using the pH meter, the pH of the soil sample was measured. The pH meter was calibrated using [10] buffers of pH 4.0, 7.0, and 10.0 prior to its use [8].

Estimation of Physicochemical Parameters: Various samples (sludge sample, effluent sample) of electroplating Industry, Steel Industry, and battery manufacturing Industries, water sample of various reservoirs has been collected where effluent from the industries are discharged and also the samples from the focal point of various areas. The samples have been tested for BOD, COD, TS, TDS, and TSS [9].

Biochemical Oxygen Demand: BOD is defined as the amount of dissolved oxygen needed by the aerobic organisms in order to breakdown the organic material that is present in the water sample at a certain temperature and for a specific period of time. BOD usually determines the degree of organic pollution in water. It is usually measured in milligrams of oxygen consumed per liter of the sample during 5 days of incubation at 20 ºC.

Chemical Oxygen Demand: Chemical oxygen demand (COD) is the measure of oxygen equivalent to the organic content of the sample that is susceptible to oxidation by a strong chemical oxidant. The intrinsic limitation of the test lies in its ability to differentiate between the biologically oxidizable and inert material.

TSS (Total Suspended Solids): Determines the dry weight of particles in a filter. It is a parameter to test the quality of water. It is measured in mg/L. Filter paper A was weighed. Then 10 ml of water sample was taken, filtered the water sample through weighed filter paper. Filter paper was allowed to dry (in the oven) Again weighed the filter paper B.

TDS (Total Dissolved Solids): It is the measure of a combination of all the organic and inorganic substances present in a liquid (sample) in ionized or granular form. It is a parameter to test the water quality. China Dish was weighed properly. 10ml filtrate (left after filtration). Then that filtrate was transferred to china's dish and boiled the filtrate in china dish until the water was evaporated. Again the china dish was weighed.

Estimation of Cadmium Content in Various Samples: The samples have been tested and quantified for Cadmium through spectrophotometric assay [10]. Alizarin red system was used for the preparation of the standard curve of cadmium. Different unknown samples were analyzed for the cadmium concentration by using a standard curve. The absorption spectra of the cadmium-alizarin Red S system in 0 05M H_2SO_4 medium was recorded using the spectrophotometer. The absorption spectrum of the cadmium-alizarin Red S is a symmetric curve with the maximum absorbance coefficient is shown in **Fig. 1.** In all instances, measurements were made at 422 nm against a reagent blank.

Screening and Isolation of Microorganism for Bioremediation: Primary screening of cadmium resistant bacteria was done to isolate the desired bacteria [11]. Sludge samples of various industries, soil samples from banks of water reservoirs, soil samples near the focal points, and effluent samples have been taken and after dilution tested on PDA (Potato dextrose agar medium) and NA (Nutrient agar medium). Various strains have been checked for resistance for cadmium, and pure cultures have been isolated by the standard method of the streak plate method. Out of 20 strains only two were selected for their tolerance of high concentration of cadmium

Effect of Selected Strain on all the Parameters of Test Samples: The strains showing maximum efficiency incubated with various test samples for 24-120 h at various pH. After incubation, the test samples also checked for pH, BOD, COD, TS, TDS, Fluoride, and Chloride.

Heavy Metal Tolerance of Isolated Strains: To assess the ability of isolates to resist Cadmium, isolated microbial strains inoculated on a Nutrient agar/ potato dextrose agar or ionic media (BHB) plates supplemented with different concentration (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mM) of cadmium [12].

RESULTS AND DISCUSSION:

Analysis of Physiological Parameters (KR Aneja 2001): The physiological parameters like BOD, COD TDS, TSS fluoride, and chloride content was analyzed in fifteen samples from different industries, and it is clear from **Table 1, 2, 3** that values of all parameters are high. The BOD values ranged from 22 mg/L-47mg/L. The COD values ranged from 230mg/L-540mg/L. Similarly TDS and TSS values ranged from 312mg/L-985 mg/L and 9mg/L-22mg/L respectively. These results are in a very close pattern in various industrial effluent discharges [13]. The fluoride and chloride content in samples were high. The greater values of all the parameters showed that it might be due to the use of chemicals, *i.e.*, may be organic or inorganic in nature. High values of TDS are due to higher dissolved solids. But the range of TSS values is within the permissible limits that cannot cause damage to agricultural land and aquatic life.

TABLE 1: PHYSICAL PARAMETER FOR SAMPLE OF ELECTROPLATING INDUSTRY

S. no.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
pH	5.5	6	8.9	7	8.5	6.2	8.3
Soil Color	Brown	Brown grey tinge	Brown Black tinge	Black	Brown grey tinge	Brown	Brown grey tinge
BOD	35	26	24	29	37	27	39
COD	430	470	540	510	520	370	490
TDS	870	980	912	611	719	514	312
TSS	12	11	16	13	09	15	10
Fluoride	1.2	1.02	1.3	2.7	2.8	1.56	2.9
Chloride	440	617	890	450	780	278	345

TABLE 2: PHYSICAL PARAMETER FOR SAMPLE OF STEEL INDUSTRY

S. no.	Sample 8	Sample 9	Sample 10	Sample 11
pH	7.12	6.02	5.67	7
Soil Color	Brown	Brown grey tinge	Black	Brown grey tinge
BOD	32	35	29	40
COD	370	540	450	230
TDS	775	695	423	393
TSS	20	22	15	17
Fluoride	3.7	2.9	3.5	1.8
Chloride	509	611	786	621

TABLE 3: PHYSICAL PARAMETER FOR SAMPLE OF BATTERY INDUSTRY

S. no.	Sample 12	Sample 13	Sample 14	Sample 15
pH	7	8.5	6.7	8.9
Soil Color	Brown	Black	Brown grey tinge	Brown black tinge
BOD	22	36	21	42
COD	260	390	470	560
TDS	817	970	985	812
TSS	14	16	20	15
Fluoride	1.09	3.6	2.7	1.6
Chloride	519	617	712	690

Spectrophotometric Assay of Cadmium: Spectrophotometric assay was done, and a straight line curve was obtained. By using this curve, the concentration of cadmium was determined and found out that the concentration was far above the maximum permissible level, as recommended by FEPA and WHO. The range of concentration of cadmium was 1.02-3.95. It was maximum in sample no. 1 of the battery industry, which shows that the samples were heavily contaminated with toxic cadmium metal. A synthetic reagent CMHBH (cinnamaldehyde-4-hydroxybenzoylhydrazone) was used to find out the toxic metal concentration from wastewater [14].

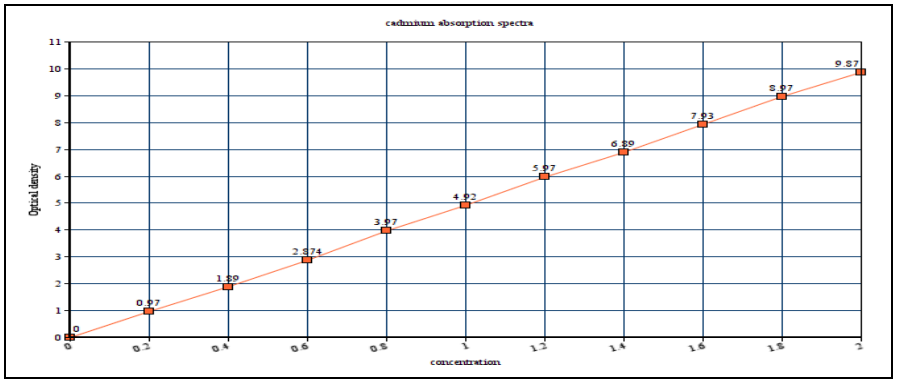

FIG. 1: PLOT SHOWING THE SPECTROPHOTOMETRIC ASSAY OF CADMIUM

TABLE 4: CADMIUM CONC. FROM ELECTROPLATING SAMPLES

Samples	1	2	3	4	5	6	7
Cadmium conc.	1.12	2.24	1.87	2.92	3.45	2.76	1.02

TABLE 5: CADMIUM CONC. FROM STEEL SAMPLES

Samples	8	9	10	11
Cadmium conc.	2.89	1.95	2.99	3.02

TABLE 6: CADMIUM CONC. FROM BATTERY SAMPLES

Samples	12	13	14	15
Cadmium conc.	3.97	1.02	2.87	1.34

Isolation of Microorganisms: A total of 20 different strains were isolated from different samples and purified by using nutrient agar media. Out of those, only four were selected on the basis of their resistance to cadmium.

Effect of Selected Strain on all the Parameters of test samples: The selected strains were then tested for all the parameters in order to justify their ability to minimize the cadmium effects, and it is clear from **Fig. 2, 3, 4** and **5** that the strains were potent enough to reduce all the parameters *i.e.*, physiological parameters and to make them fall in permissible limits. The readings of all parameters were taken after every 24 h and continued till 120 h. Each and every culture showed a variation in their efficacy and ability.

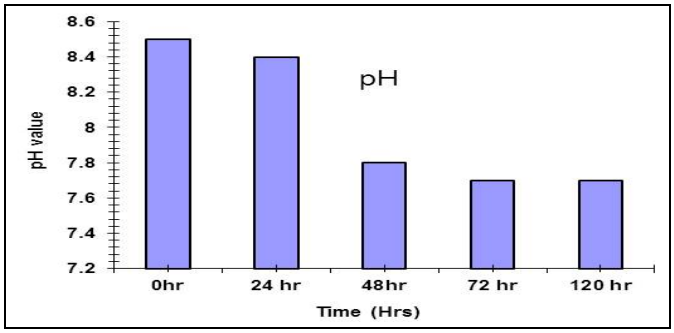

FIG. 2A: EFFECT OF ECD006 ON pH

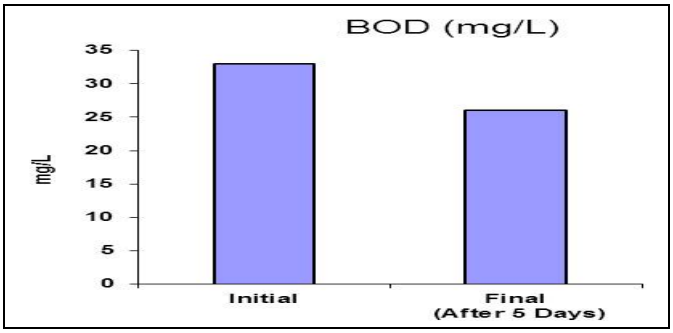

FIG. 2B: EFFECT OF ECD006 ON BOD

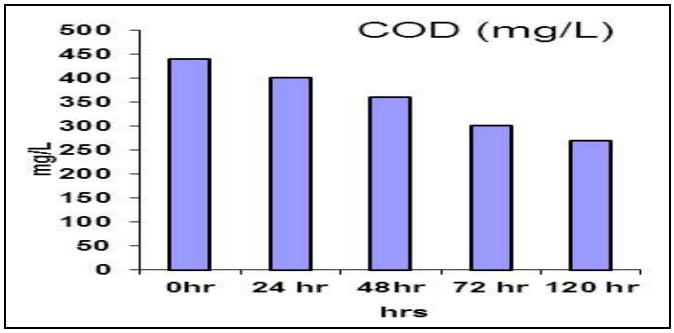

FIG. 2C: EFFECT OF ECD006 ON COD

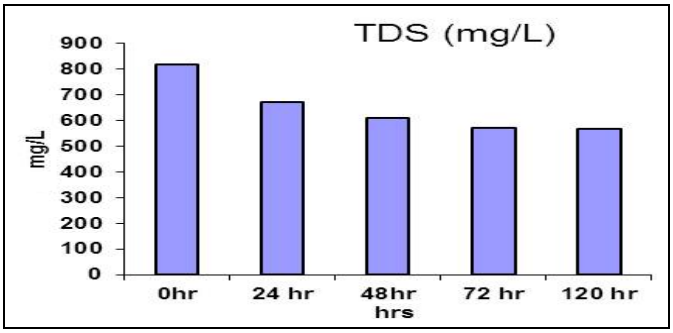

FIG. 2D: EFFECT OF ECD006 ON TDS

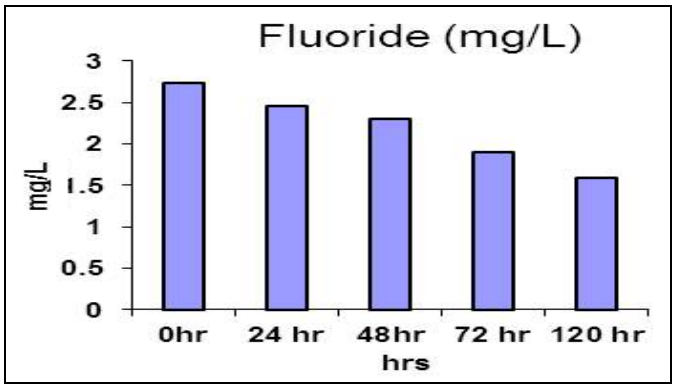

FIG. 2E: EFFECT OF ECD006 ON FLUORIDE

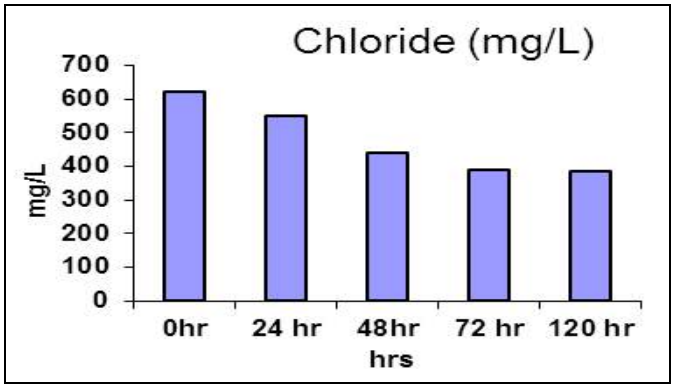

FIG. 2F: EFFECT OF ECD006 ON CHLORIDE CONTENT

The novel resistant *Bacillus salmalaya* strain 139SI was studied [15] and was potent enough to significantly reduce the chemical oxygen demand.

Microbial degradation of paper and pulp industry waste was studied [16]. They utilized fungus for the same purpose.

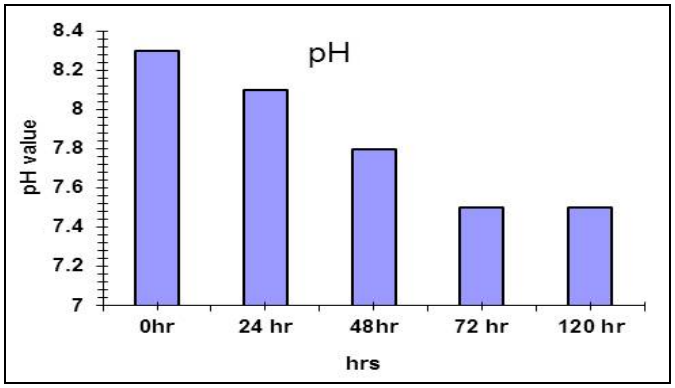

FIG. 3A: EFFECT OF STEEL Cd002 ON pH

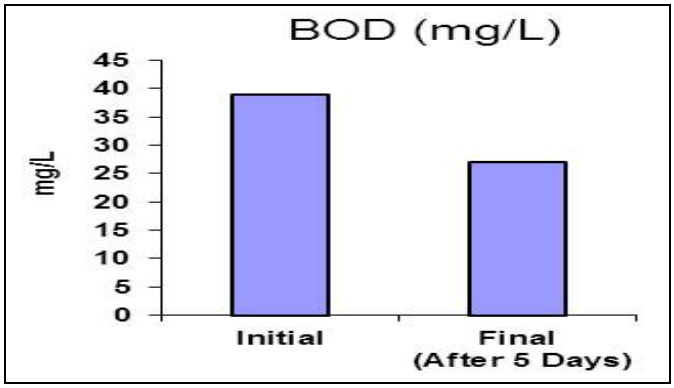

FIG. 3B: EFFECT OF STEEL Cd002 ON BOD

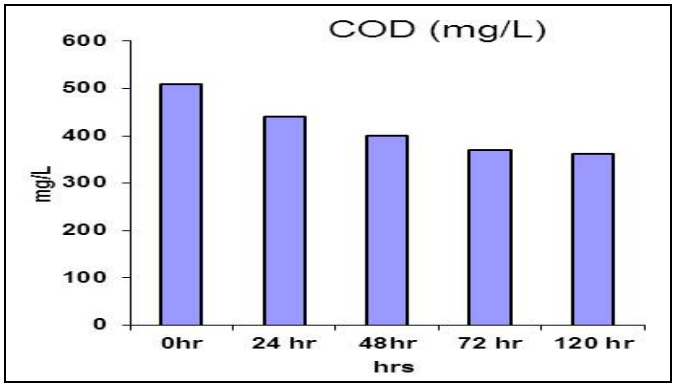

FIG. 3C: EFFECT OF STEEL Cd002 ON COD

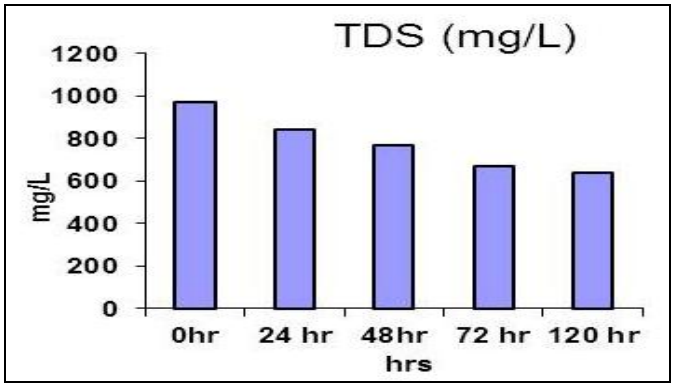

FIG. 3D: EFFECT OF STEEL Cd002 ON TDS

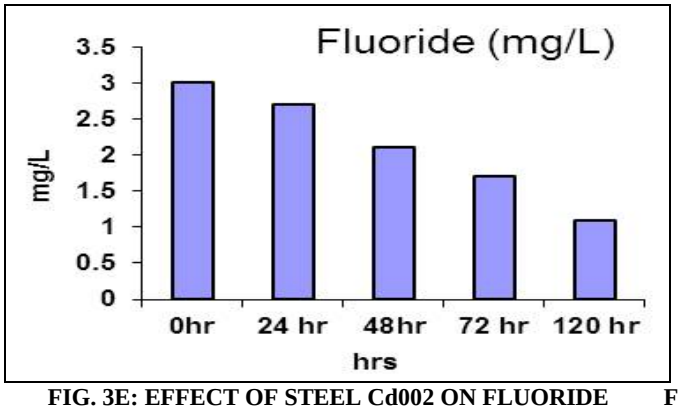

FIG. 3E: EFFECT OF STEEL Cd002 ON FLUORIDE

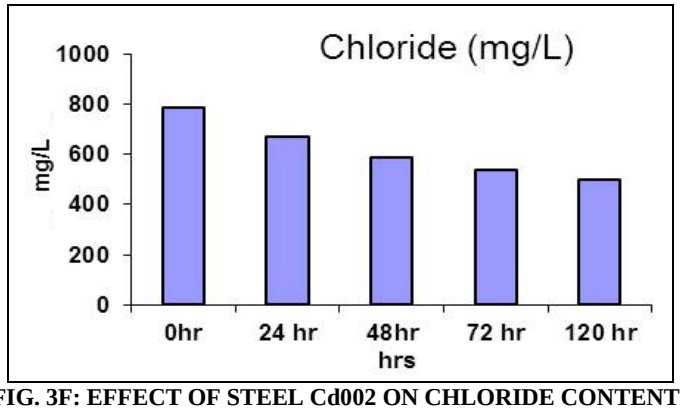

FIG. 3F: EFFECT OF STEEL Cd002 ON CHLORIDE CONTENT

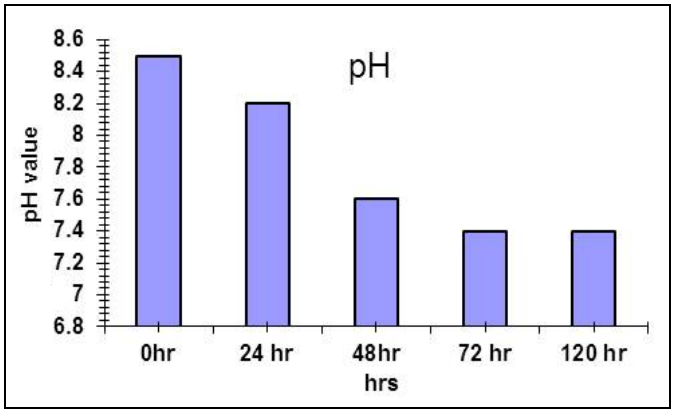

FIG. 4A: EFFECT OF ECd004 on pH

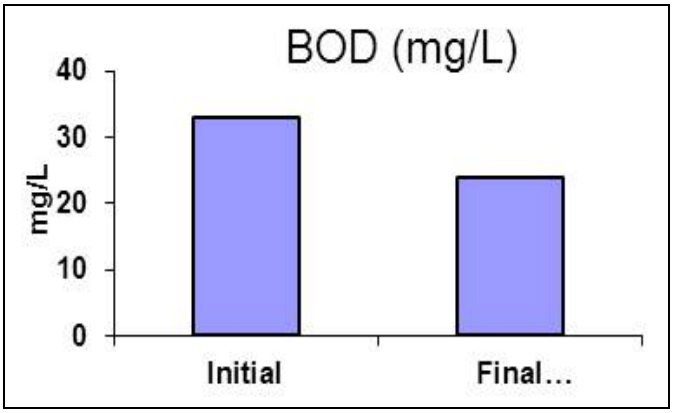

FIG. 4B: EFFECT OF ECd004 ON BOD

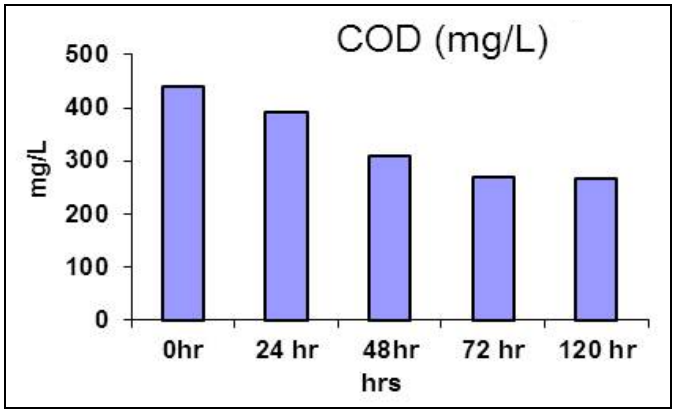

FIG. 4C: EFFECT OF ECd004 ON COD

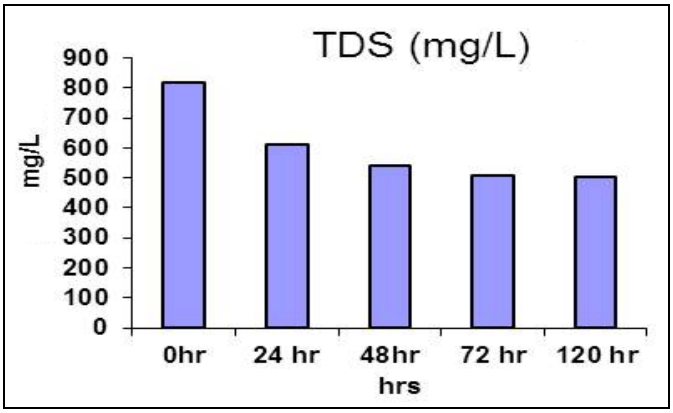

FIG. 4D: EFFECT OF ECd004 ON TDS

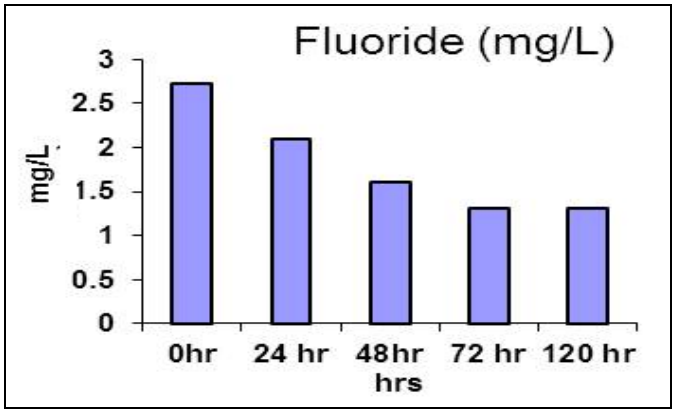

FIG. 4E: EFFECT OF ECd004 ON FLUORIDE

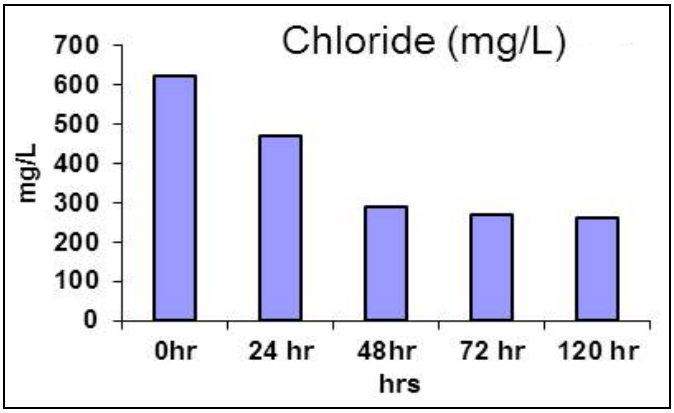

FIG. 4F: EFFECT OF ECd004 ON CHLORIDE CONTENT

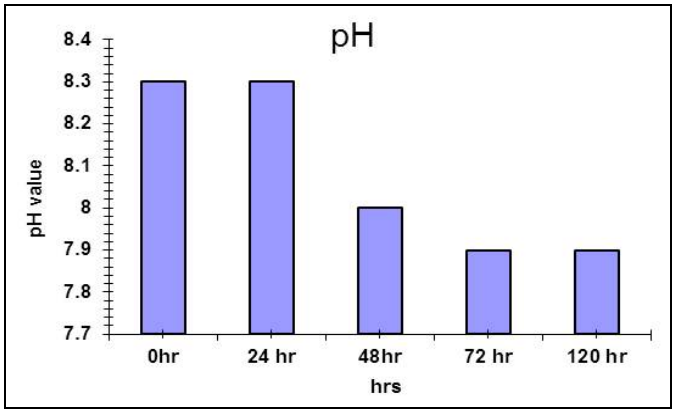

FIG. 5A: EFFECT OF STEEL Cd005 on pH

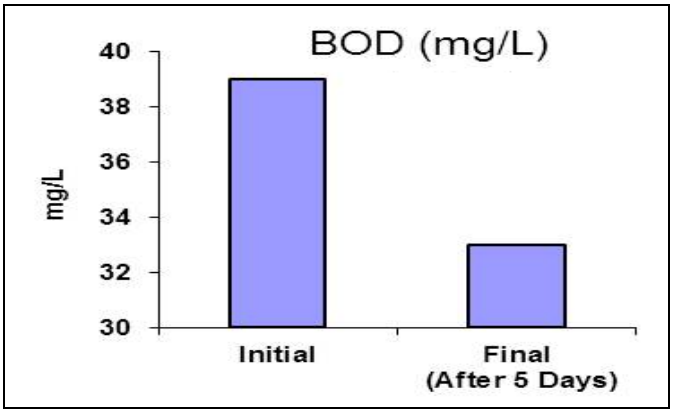

FIG. 5B: EFFECT OF STEEL Cd005 ON BOD

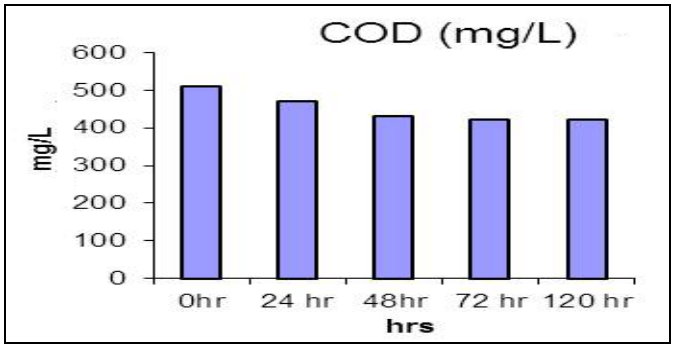

FIG. 5C: EFFECT OF STEEL Cd005 ON COD

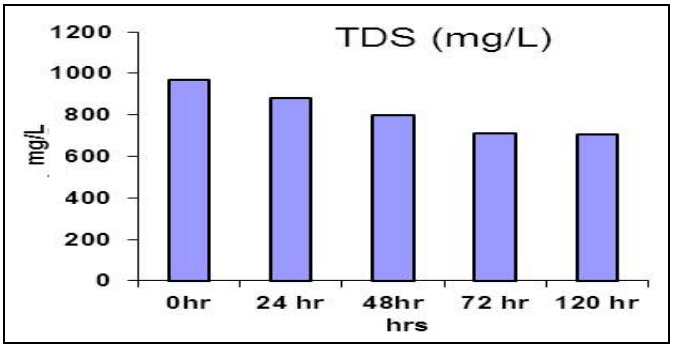

FIG. 5D: EFFECT OF STEEL Cd005 ON TDS

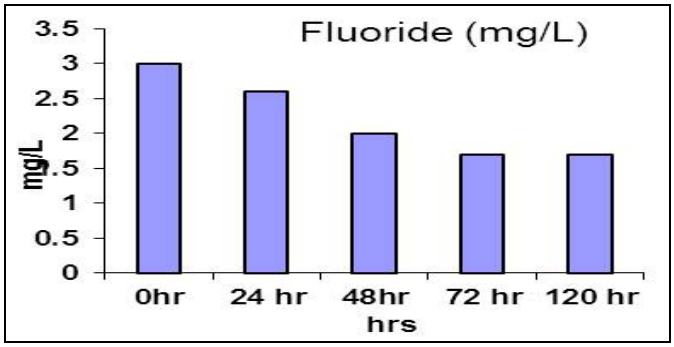

FIG. 5E: EFFECT OF STEEL Cd005 ON FLUORIDE

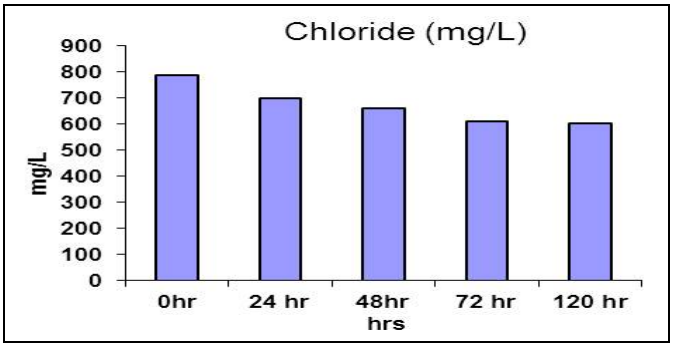

FIG. 5F: EFFECT OF STEEL Cd005 ON CHLORIDE CONTENT

Cadmium Tolerance: Above isolated strains were tested for different concentrations of cadmium. It is clear from the figures that at concentrations ranging from 100ppm -1500 ppm, the growth of bacterial strain is increasing after every 24 h. E-Cd006 strain showed maximum activity at 1000ppm. Cadmium tolerance by *pseudomonas* up to 550 µg/ml was measured [17]. Strain steel Cd006 showed good activity against cadmium conc. but the growth decreases after 48 h, especially in 1000 and 1500 ppm. The strain E Cd004 showed its maximum activity at 72 h, but after that, the activity goes decreasing.

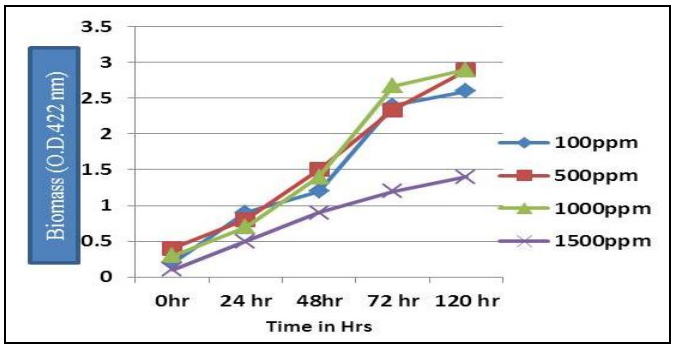

FIG. 6: CADMIUM TOLERANCE OF ELECTROPLATING STRAIN CD004

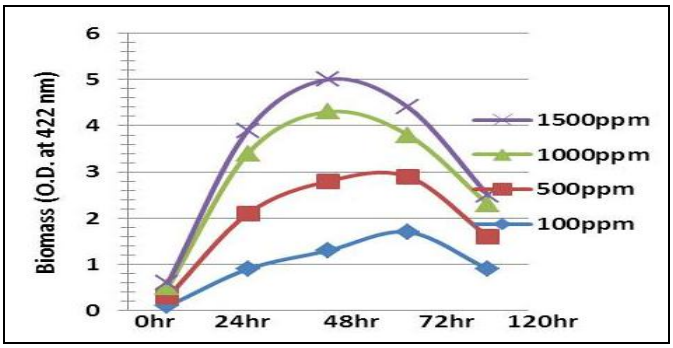

FIG. 7: CADMIUM TOLERANCE OF STEEL STRAIN CD002

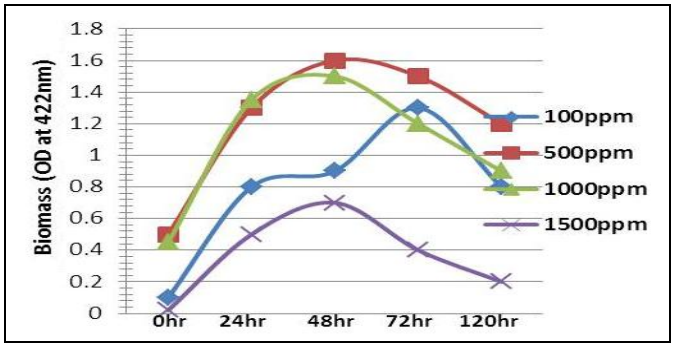

FIG. 8: CADMIUM TOLERANCE OF ELECTROPLATING STRAIN CD006

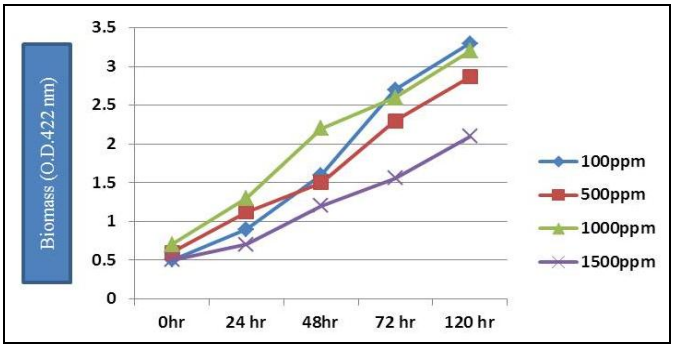

FIG. 9: CADMIUM TOLERANCE OF STEEL STRAIN CD005

CONCLUSION: Heavy metals are hazardous in nature. It is essential to detoxify them. Isolated strains showed a great effect on all physicochemical parameters of samples. Above study intended to isolate such microbial strains having actual efficiency of consuming high levels of cadmium concentrations. Two isolates E-Cd006 and S-Cd005 showed maximum growth in the presence of a high concentration of cadmium. So, these two cultures were selected for further analysis.

ACKNOWLEDGEMENT: The author(s) would like to acknowledge, Chandigarh University, Gharuan, Punjab, India, for providing a research platform and necessary facilities.

CONFLICTS OF INTEREST: All authors have none to declare.

REFERENCES:

1. Beard SJ, Hashim R, Hernandez J, Hughes M and Poole RK: Zinc (II) tolerance in Escherichia coli K-12: evidence that the zntA gene (o732) encodes a cation transport ATPase. Mole Microbiol 1997; 25: 883-91.
2. Robin RS, Muduli P, Vardhan K, Ganguly D, Abhilash R and Balasubramanian T: Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the Southwest-coast of India. J Chem 2012; 2: 191-208.
3. Mondal NC, Saxena VK and Singh VS: Impact of pollution due to tanneries on ground water regime. Curr Sci 2005; 88(25): 1988-94.
4. Zahid A, Bake KD, Hassan MQ and Flegr M: Evaluation of aquifer environment under Hazaibagh Leather processing Zone of Dhaka. Environ Geol 2006; 50: 495-504.
5. Favazzi A. Study of the impact of the main policies and environment protection measures in Africa's leather industry, Principal: Assomac Servizi Srl for UNIDO – 2002; A thesis.
6. Verheijen LA, Wiersema D, Hulshoff P, De Wit J. Livestock and the environment finding a balance: Management of waste from animal product processing. Int Agriculture Centre Wageningen, The Netherlands, International Agricultural Centre 1996.
7. Ezaka E and Anyanwa CU: Chromium (VI) tolerance of bacterial strains isolated from sewage oxidation ditch. Int J Environ Sci 2011; 1(7): 1725-34.
8. HACH. Water Analysis Handbook, 3rd edition, HACH Company, Loveland, Colorado, USA.1997.
9. Sheikh AH, Tariq M and Afsheen A: Reduction of Chemical and Biochemical Oxygen Demand after treatment of pharmaceutical effluents. Pak J Pharm 2012; 25 (1 & 2): 9-13
10. Ullah M and Haque ME: Spectrophotometric Determination of Toxic Elements (Cadmium) in Aqueous Media. Journal of Chemical Engineering 2010; 2(1): 1-12.
11. Marjan. Isolation and biochemical characterization of heavy metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. The Egyptian Jl of Aquatic Research 2017; 43(1): 65-74.
12. Hookoom M and Puchooa D: Isolation and Identification of Heavy Metals Tolerant Bacteria from Industrial and Agricultural Areas in Mauritius. Curr Res Microbiol Biotech. 2013; 1(3): 119-23.
13. Osobamiro MT and Atewolara-Odule OC: Determination of physicochemical parameters and levels of some heavy metals in industrial waste water. International Journal of Scientific& Engineering Research. 2015; 6(7): 1910-18.
14. Gopala D, Krishna N, Devanna I and Chandrasekhar KB: Direct and derivative spectrophotometric determination of cadmium in presence of micellar medium in biological material and in alloy samples using CMHBH. International Journal of Pharma and Biosciences 2010; 1(3): 1-19.
15. Arezoo D, Mohd SA and Salmah BI: Optimal reduction of chemical oxygen demand and NH3N from landfill leachate using a strongly resistant novel *Bacillus salmalaya* strain. BMC Biotechnol 2017; 17: 85-98.
16. Anuranjana JG and Vijayan N: Microbial degradation and nutrient optimization of pulp and paper industry waste water. International Research Journal of Engineering and Technology 2016; 03(8): 1919-23.
17. Abbas SZ, Rafatullah M, Ismail N and Lalung J: Isolation, identification and characterization of cadmium resistant Pseudomonas sp. M3 from industrial wastewater. Journal of Waste Management 2014; 1-6.

How to cite this article:
Lata S, Kaur S and Mishra T: Isolation of cadmium degrading microorganisms from electroplating, steel and battery industry. Int J Pharm Sci & Res 2020; 11(9): 4403-10. doi: 10.13040/IJPSR.0975-8232.11(9).4403-10.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Play store)