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Analysis of electroplating industry effluent and bioprospecting of heavy metal resistant microbial diversity

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ABSTRACT

Electroplating industry effluent is discharging heavy metals like Ni, Zn, Mg, Mn, Pb, Cu and Cr into soil and water bodies. The challenge is to properly tackle the waste disposal so that the industrial solid wastes do not contribute any type of pollution. Keeping all these perspectives in view, analysis of Industrial effluent was done by ICP-MS results from which showed that the concentration of lead (4.09mg/L) and zinc (376.28mg/L) were beyond the standard limits. Further heavy metal resistant microbes isolated from contaminated soil were identified as *Providencia sp*. and *Enterobacter sp*., which could be potential remedial measure for bioremediation of heavy metal polluted sites.

KEY WORDS: POLLUTION, ELECTROPLATING, BIOREMEDIATION AND ICP-MS

INTRODUCTION

Population has been increasing exponentially and industrialization activities are polluting our environment by depositing heavy metals (Marzan *et al.*, 2017; Shifaw, 2018). The need to control toxic materials particularly, heavy metals discharged from industrial effluent is currently increasing in our environment. These days most of the rivers receive millions of gallons of domestic waste and industrial effluent containing pollutants of varying characteristics from simple nutrient to highly toxic substances (Nivruti *et al.*, 2013). Although industries in India abide by the guidelines of Central Pollution Control Board (CPCB) but environment situation is still

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far from satisfactory (Lokhande *et al.,* 2011). Most of these defaulting industries are petrochemical industries, distilleries, sugar mills, leather processing industries, agrochemicals, paper mill, pesticides and pharmaceutical industries. Heavy metals are accumulated in soils and plants, interfere with physiological activities of plants such as nutrient absorption, photosynthesis and cause reductions in plant growth. Dietary intake of heavy metals through consumption of plants has long term detrimental effects on human health (Saikia *et al.,* 2015).

A quandary before the scientists is how to tackle the toxic contaminants that endanger the environmental. Many novel approaches to clean-up the environment are being developed and many are already in practice such as conventional physico-chemical methods like electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption (Dixit *et al.,* 2015). Bioremediation is an environmentally friendly and potentially very effective alternative to physical remediation methods. Bioaccumulation a process dependent upon metabolic potential of microorganisms whereas, biosorption or bioaccumulation is a metabolically passive process, meaning it does not require energy, amount of contaminant a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface, (Hansda *et al.,* 2016).

MATERIAL AND METHODS

Collection of sample: Industrial waste water samples and nearby landfill soil was collected from two different battery recycling industries. A cold chain was maintained while transporting the samples to laboratory at Kurukshetra University, Kurukshetra. Samples were stored at 4˚C for analysis of electroplating industry effluent and screening for heavy metal resistant bacterial strains.

Determination of physicochemical properties of effluent samples: Analysis of physiochemical parameters in the industrial effluent samples was carried out. Different parameters such as pH, COD, BOD, chloride estimation, Total residual chlorine, conductivity and salinity, TDS and oil and Grease were estimated as per the Indian Standard Methods and American Public Health Association (APHA., 1981). Heavy metal contents were determined with the help of Inductive Coupled Plasma-Mass Spectroscopy using APHA 3125B (APHA., 1992).

Isolation and Identification of Bacterial Strains: Isolation of heavy metal resistant bacteria was done by using spread plate method on sterile nutrient agar. Plates were incubated at 37˚C for 48 hrs. After 48 hrs, plates were observed for growing bacterial colonies. On the basis of morphological differences (e.g. shape, size and color) different bacterial colonies were picked up and repeatedly streaked on nutrient agar plates to get pure culture (Raja *et al.,* 2009). Identification of isolated bacterial cultures was done on the basis of morphological, physiological and biochemical characteristics. Different tests were used to identify the isolates according to Bergey's Manual of Systematic Bacteriology (Bergey *et al.,* 2005). Isolated colonies of purified strains grown on solidified agar plates were observed and data was recorded regarding the form (circular, filamentous and irregular); elevation (flat, convex and umbonate); margin (entire, undulate, erose and filamentous) and optical feature (opaque, translucent and transparent) of the colonies (Pelczar *et al.,* 1958). Cells were observed after Gram staining under the microscope (oil immersion, 100 X) (Duguid *et al.,* 1989). For biochemical characterization Hi25™ Enterobacteriaceae Identification Kit was used. The kit contains a combination of 25 biochemical tests.

Determination of Minimum Inhibitory Concentration (MIC) of metals for growth of bacterial colonies: The MIC of the heavy metals (Pb, Cd, Cu, Ni, Zn and Mn) for different isolated strains (VX1, VX2, VX3, VX4 and VX5) was determined according to Summers and Silver with some modifications (Summers and Silver., 1972).Maximum resistance of the selected isolates against increasing concentrations of heavy metals was evaluated by agar plating techniques until the strains unable to grow colonies on the agar plates (Xin *et al.,* 2006). The lowest concentration of heavy metals that completely prevent the growth is known as MIC (Yilmaz., 2003).

Antibiotic Resistance Test: The isolated bacterial strains were checked for antibiotic resistivity and sensitivity using single disc diffusion method (Bauer *et al.,* 1966).

RESULTS AND DISCUSSION

Data given in the table 1 shows analytical data of various physiochemical parameters set according to General standards for Discharge of Environment Pollutants, Part A: Effluent as per Schedule VI of the Environment (Protection) Rules 1986.

A total of 5 morphologically different bacterial colonies were picked and streaked on nutrient agar plates. These bacterial strains were isolated from lead battery manufacture and recycling small scale industries and named as VX1, VX2, VX3, VX4 and VX5. After that all these bacterial strains were tested for tolerance level against six heavy metal salts (Pb, Cd, Cu, Zn, Mn and Ni) as shown in table 2.

Gram staining was performed with all the isolated bacteria to characterize their morphological features. VX1 and VX2 was found gram negative rod. VX3, VX4 and VX5 were found gram positive rod. Results of physiological characterization are given in the table 3.

Table 1. Analysis of industrial effluent of electroplating industry.			
No	**Characteristics**	**Lead-battery industrial effluent**	**EPA standards**
1.	Color and odor	Turbid	-
2.	TDS	1750mg/L	2100
3.	pH value	5.5	5.5 to 9
4.	Temperature	27˚C	45˚C
5.	Oxidation and Reduction Potential (ORP)	1570mV	
6.	Conductivity	4200µS	3000 µS
7.	Salinity	3.5ppm	-
8.	Oil and grease mg/L	2.403	10
9.	Dissolved oxygen mg/L	10.3	-
10.	Cadmium (as Cd) mg/L	0.024	2
11.	Copper (as Cu) mg/L	2.9	3
12.	Iron (as Fe) mg/L	0.144	3
13.	Lead (as Pb) mg/L	4.09	0.1
14.	Manganese (as Mn) mg/L	1.98	2
15.	Nickel (as Ni) mg/L	0.328	3
16.	Zinc (as Zn) mg/L	376.28	5

VX3 and VX5 were grown at temperatures ranging from 20 to 45˚C. Optimal temperature for both the strains was 30 to 35. Bacterial strain VX3 and VX5 have shown the ability to grow at salt concentration as high as 6%. Both the isolates have the capability to grow at pH ranging from 6-10. Their optimum pH range was found to be 6 to 8. Two best bacterial strains VX3 and VX5 were analyzed for biochemical characteristics. For this a total of 25 biochemical tests were performed on the Hi25™ enterobacteriaceae Identification Kit. This kit contains two test strips. Strip 1 contains 12 biochemical tests and strip 2 contains 12 carbohydrate metabolism tests. The results of biochemical tests are shown in table 4.

Minimum inhibitory concentration (MIC) of various strains showed minimum concentration at which bacterial strain is able to survive efficiently. From this study it was conclude that at initial VX3 and VX5 showed promising results against maximum heavy metals. VX4 showed moderate growth against Mn, Zn and Pb. VX2 and VX1 were only resistant to Mn.

Table 5 shows that bacterial strains VX3 and VX5 showed resistance against kanamycin but sensitive towards other antibiotics.

Table 2. Multiple heavy metal resistance and MIC against five bacterial isolates.					
Metal	**MIC (µg/ml) concentration of metals in mM**				
	VX1	**VX2**	**VX3**	**VX4**	**VX5**
Nickel (Ni2+)	1	1	4	1	12
Lead (Pb)	1	3	15	4	15
Cadmium (Cd)	1	1	4	1	1
Copper (Cu)	1	2	6	1	4
Zinc (Zn)	3	4	6	4	6
Manganese (Mn)	4	6	25	10	30

Table 3. Physiological characteristics of bacterial strains (VX1, VX2, VX3, VX4 and VX5)			
Characteristic		**Name of bacterial strain**	
		VX3	**VX5**
Temperature	20	+	-
	25	++	+
	30	+++	+++
	35	+++	+++
	40	++	+
	45	-	-
Salt Tolerance	2	+++	+++
	4	++	++
	6	+	+
	8	+	-
	10	-	-
pH	4	-	-
	6	+	+
	7	+++	+++
	8	+++	+++
	10	+	+
	12	-	-
(+, scant growth) (++, moderate growth) (+++, abundant growth) (-, no growth)			

Table 4. Biochemical tests of bacterial strains (VX3 and VX5)			
S. No.	Names of biochemical test	Name of bacterial strain	
		VX3	VX5
1.	ONPG	-	+
2.	Lysine utilization	-	-
3.	Orinthin utilization	-	+
4.	Urease detection	-	-
5.	Phenylalanine Deamination	+	V
6.	Nitrate reduction	+	+
7.	H2S production	-	-
8.	Citrate utilization	V	+
9.	Voges Proskauer's	-	+
10.	Methyl Red	V	-
11.	Indole test	+	V
12.	Malonate utilization	-	V
13.	Esculin hydrolysis	-	+
14.	Arabinose	-	+
15.	Xylose	-	+
16.	Adonitol	+	-
17.	Rhamnose	-	+
18.	Cellobiose	-	+
19.	Melibiose	-	+
20.	Saccharose	V	+
21.	Raffinose	-	+
22.	Trehalose	-	+
23.	Glucose	+	+
24.	Lactose	-	+
25.	Oxidase test	-	+
+ Positive (more than 90%), - Negative (more than 90%) and V= 11-89% Positive.			

Our study deals with two main aspects, firstly, analysis of untreated electroplating industrial effluent according to IOS methods set under EPA norms and secondly, biologically remediating this problem caused due to this waste to the environment.Electroplating industry effluent is discharging heavy metals like Ni, Zn, Mg, Mn, Pb, Cu and Cr into soil and water bodies. Lead is one of the most abundant heavy metals in nature. Maximum permissible limit for lead in drinking water is 0.01 mg/L and for industries is 0.1 mg/L. But the industrial sample analysed in our studies contained extremely might amount of lead after that is (4.09 mg/L) after analysis was extremely high. In our study extremely high concentration (376.28 mg/L) of zinc was present in the industrial effluent samples which are very high as compared to the EPA standards (5 mg/L). The concentration value of 2.9 mg/L obtained for Cu was on the line of accepted standard. However, rest of the metal concentrations were within the acceptance limits (Ni 0.328mg/L, Fe 0.144mg/L, Cd 0.024mg/L, Mn 1.98mg/L). Similar studies have also been done by (Khan *et al.*, 2008). Khan studied the effect of heavy metals in contaminated crops irrigated with waste water and its associated health risks. Results indicate that there is a substantial buildup of heavy metals in waste water-irrigated soils, collected from Beijing (China).

Table 5. Antigram study of isolated bacterial strains against different antibiotics.			
Antibiotic	Disc content (mcg)	Diameter of zone against bacterial isolates (mm)	
		VX3	VX5
Neomycin	30	25	29
Ciprofloxacin	5	30	27
Amoxicillin	10	15	12
Kanamycin	5	8	9
Ceftazidime	30	16	21
(mcg- microgram)			

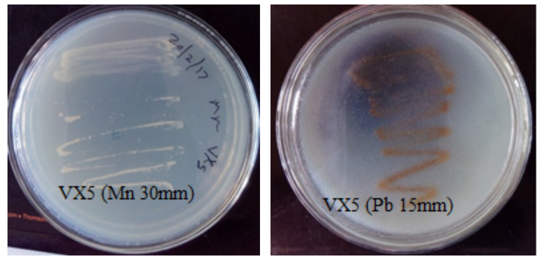

FIGURE 1. Bacterial strain VX5 growing at various metal concentrations Mn 30mm and Pb 15mm

High oxidation and reduction potential (ORP) is convenient for quickly disinfecting the water but leads to decrease in pH and increase in dissolved solids. Increase in the number of free ions present in water body leads to progressive rise in conductivity and salinity. Sample analysis revealed values of ORP (1570mV), conductivity (4200mS), TDS (1750) and salinity (3.5ppm) which are intolerant to most of the microbial species. Dissolved oxygen is necessary for aquatic ecosystem. Dissolved oxygen level when drops below 5mg/L cause stress to aquatic life. Oil and grease reduces the availability of dissolved oxygen affecting the aquatic system. Sample analysed had oil and grease at a permissible limit of 2.403mg/L. DO value of our sample was found to be 10.3mg/L. Similar study was carried out by Sugumaran (2014) for the analysis of electroplating industry effluent which showed high concentration of suspended solids (200 or 300 mg/L), heavy metals (300 to 600 mg/L) and cyanide.

Metals exert toxic effect on microorganisms through various mechanisms resulting into development of metal resistant microbial diversity in these habitats. Their potential skills could be used for bioremediation in heavy metal industries. In the present study, we have isolated 12 bacterial isolates from two different samples. Six heavy metal salts ($MnSO_4$, $C_4H_6O_4Pb$, $CuSO_4$, $ZnSO_4$, $CdCl_2$ and $NiSO_4$) which were screened for heavy metal tolerance against bacterial strains VX1, VX2, VX3, VX4 and VX5 were selected after primary screening at conc. of 1mM for further study.The presence of Pb, Mn, Cu, Cd, Zn and Ni in growth medium had direct effect on colonial morphology and growth of isolated bacterial strains. The isolates showed different tolerance level for these metals.

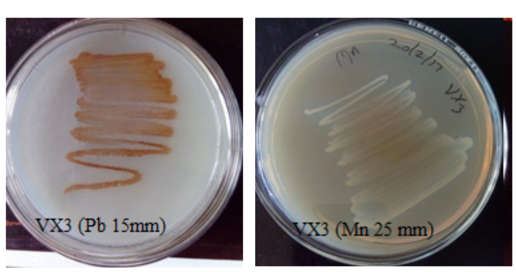

FIGURE 2. Bacterial strain VX3 growing at various metal concentrations Mn 25mm and Pb 15mm.

Bacterial strain VX3 and VX5 have shown high resistance towards lead. Both the bacterial strains were identified as *Providencia sp.* and *Enterobacter sp.* These species could tolerate as high as 15mM concentration of lead. This study is quite significant because very limited species of bacteria show so much resistance for lead till now.Roane (1999) carried out study in which the overall mechanisms of a lead-resistant *Pseudomonas marginalis* and *Bacillus megaterium*. These bacterial strains tolerated lead concentration up to 2.5mM which is significantly lower than the concentration tolerated by VX3 and VX5. In addition, VX3 has also shown good resistance against Pb (15mM), Cd (4mM), Cu (6mM), Zn (6mM), Ni (4mM) and Mn (25mM). Similarly VX5 showed high resistance against Pb (15mM), Cd (1mM), Cu (4mM), Zn (6mM), Ni (12mM) and Mn (30mM).

CONCLUSION

Heavy metals are important to human beings in many aspects, especially in the manufacturing of important products, such as accumulators (Pb), utensils (Al), mercury-arch lamps and a wide range of other products. But the toxic effects, when unduly exposed, could be potentially life threatening hence, cannot be neglected. Heavy metals possess high threat to mankind and other living organisms. The effluent collected from the contaminated site contained high amount of heavy metal contamination, particularly Zinc which was found to be 70 times more than the permissible limit. This critical situation leads to an urgent call for remediation without further affecting our environment. Further study is needed to resolve the issue of heavy metal contaminants from the soil. Considering above situation we have isolated heavy metal resistant bacteria which could survive even at higher concentrations of heavy metals. Their ability to attain life in such hostile conditions makes them ideal for bioremediation purposes. Bacterial strains VX3 and VX5 identified as *Providencia sp.* and *Enterobacter sp.* were found to be specifically resistant to lead. The isolated microbes could be potential remedial measure used for bioremediation of heavy metals polluted sites.

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