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ASSESSMENT OF HEAVY METALS TOLERANCE OF BACTERIAL ISOLATES FROM EFFLUENT OF METAL PROCESSING INDUSTRIES

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ABSTRACT In this study heavy metals tolerance of bacterial isolates from effluent of metal processing industries was carried out. The microbiological analysis total of 6 industrial effluents samples were collected from various industries, and total of 42 isolates were obtained among these isolates were screed for heavy metals tolerance/resistance, among them one isolate SR6a (*Achromobactor*) was resistant to Cu at higher concentration (2000ppm) and considered as potential heavy metal resistance isolate. The some isolates such SR3f (*Bacillus*), SR4g (*Achromobactor*), SR5c (*Bacillus*), and SR3a (*Pseudomonas*) were multi heavy metal resistance ones. All these isolates from metal industrial effluents showed the heavy metal resistance against Copper, Nickel, Cadmium, Zinc and Mercury. the isolate SR6a identified as *Achromobactor* sp. was able to tolerate the heavy metal up to 3000 ppm concentration and can be very useful for the application in the environmental bioremediation.

KEYWORDS : Heavy metals tolerance, bacterial isolates, effluent and metal processing industries.

INTRODUCTION

Industrial wastes containing toxic heavy metals were characterized by their differences rather than their similarities. These heavy metals can arise from a wide variety of industrial processes. The quality and the quantity of the wastes containing toxic heavy metals are dependent upon their industrial sources. In recent years, concern has increased over heavy metal pollution, as all heavy metals are potentially harmful to most organisms at some level of exposure. The release of increasing quantities of heavy metals and their salts in terrestrial and aquatic environment and their accumulation in living and non-living systems endanger life. Since the second part of 20th century, there has been growing concern over the diverse effects of heavy metals on humans and aquatic ecosystems. Environmental impact of heavy metals was earlier mostly attributed to industrial sources (Modi, 1996; Pazirandeh *et al,* 1998; Roane and Pepper, 2000; Vieira and Volesky, 2000).

A significant part of the anthropogenic emissions of heavy metals ends up in wastewater. Major industrial sources include surface treatment processes with elements such as Cd, Pb, Mn, Cu, Zn, Cr, Hg, As, Fe and Ni, as well as industrial products that, at the end of their life, are discharged in wastes. Major urban inputs to sewage water include household effluents, drainage water, business effluents (e.g., car washes, dental uses, other enterprises, etc.), atmospheric deposition, and traffic related emissions (vehicle exhaust, brake linings, tires, asphalt wear, gasoline/oil leakage, etc.) transported with storm water into the sewerage system. For most applications of heavy metals, the applications are estimated to be the same in nearly all countries, but the consumption pattern may be different. For some applications which during the last decade has been phased out in some countries, there may, however, today be significant differences in uses (Hui *et al.,* 2005; Karvelas *et al.,* 2003; Ahluwalia *et al.,* 2007).

In recent years, metal production emissions have decreased in many countries due to strict legislation, improved cleaning/ purification technology and altered industrial activities. Today and in the future, dissipate losses from consumption of various metal containing goods are of most concern. Therefore, regulations for heavy metal containing waste disposal have been tightened (McGrath *et al.,* 1995; Bhutada and. Dahikar, 2017).

A wide variety of microorganisms including bacteria, fungi, yeast and algae interact with metals. The structural and functional complexity of microbes help them to interact with heavy metals in several ways. The interactions of microorganisms with heavy metals can be broadly classified as metabolism dependent (active) and metabolism independent (passive). They can also be classified on the basis of the site of metal interaction viz. extracellular, exocellular and intracellular (Veglio and Beolchini, 1997). Intrinsic bacteria, which are capable of metal accumulation, existing in soil on or near the site of contamination have adapting mechanisms to the contaminant. Naturally occurring bacteria that are capable of metal accumulation, have been extensively studied since it is difficult to imagine that a single bacterium could be capable to remove all heavy metals from its polluted site (Clausen, 2000). Therefore, there is an urgent need for the treatment of metal processing industry waste. Waste management strategies adopted in India have failed to keep pace with the industrial growth and urbanization.

MATERIALS AND METHODS

Collection of Effluent Samples: The effluents sample were collected from different metal processing industry of Marathwada region. Samples were collected in a plastic bucket and then thoroughly mixed on a piece of clean cloth and the lumps were broken using wooden pestle and mortar and were air dried (Tandon, 1993). After collection, a portion of each sample was immediately transferred to laboratory and stored at 4^{0}C for microbial analysis.

Isolation and identification of Bacteria

One mL of water sample was added to 9 mL of sterile distilled water and a tenfold serial dilution was done, and the lower, middle, and high dilutions were plated in duplicate into nutrient agar (Himedia, Mumbai), MacConkey agar, and potato dextrose agar plates already prepared. These were incubated at 37°C for 18–24 hrs for total bacteria and coliforms. Colonies on plates were observed and counted and the population density was estimated; bacterial colonies were picked according to their cultural morphology on the plates and these were streaked on new nutrient agar plates for pure colonies (Nwachukwu and Apata, 2013).

Morphological characterisation of bacterial isolates

The morphological characteristics of isolates were observed and recorded and this was the basis for the isolation of colonies. The cell shape and arrangements of isolates were determined following the standard procedures of basic stain, gram stain (Nwachukwu and Apata, 2013).

Screening of the heavy metal tolerance bacteria

Screening of the isolates from the different domestic wastes and industrial wastes were done using the conventional plate techniques. The different concentrations of the heavy metals ranging from (200 ppm-2000 ppm) were added in the nutrient agar media and the respective results were recorded.

Preparation of 2000 ppm stock solutions of different heavy metals:

Nickel

Dissolve 2.0 g of nickel in 40ml of conc. nitric acid. Dilute to 1 litre in a volumetric flask with deionized water. It will give the stock solution of 2000 ppm.

Zinc

Dissolve 2.0 g of zinc metal in 60ml of 5M hydrochloric acid. Dilute to 1 litre in a volumetric flask with deionized water. It will give the stock solution of 2000 ppm.

Mercury

Dissolve 2.0 g of mercury metal in 40ml of 5M nitric acid. Dilute to 1 litre in a volumetric flask with deionised water. It will give the stock solution of 2000 ppm.

Copper

Dissolve 2.0 g of copper metal in 100ml of 5M nitric acid. Dilute to 1 litre in a volumetric flask with deionised water. It will give the stock solution of 2000 ppm.

Cadmium

Dissolve 2.0 g of cadmium metal in 40ml of 5M hydrochloric acid and two drops of nitric acid. Dilute to 1 litre in a volumetric flask with deionised water. It will give the stock solution of 2000 ppm.

RESULTS AND DISCUSSION

In this preliminary study, the bacterial isolates from the effluent from metal processing industries of Marathwada region investigated revealed metals resistance strains probably as a result of selective pressure from metal pollution in waste wastewater and this is of public health concern. A total of 6 industrial effluents samples were collected from various industries, shown in fig. 1. From these 6 industrial effluent samples, a total of 42 isolates were obtained and identified on basis of standard morphological, biochemical and sugar fermentation characteristics by using determinative bacteriology of Bergey's manual. Among these 6 industrial effluent samples, a total of 42 isolates were obtained, among these isolates *Achromobacter* sp. (8), predominantly obtained followed by *Bacillus* sp. (5), *Shigella* sp. (7), *Salmonella* sp. (7), *Pseudomonas* sp. (5), *Corynebacterium* sp. (3), *Staphylococcus* sp. (3), *Proteus* sp. (2), *Exigoubacterium* sp. (1) and *Microbacterium* (1).

Table 1. Microbial analysis of Effluent Samples Collected from different Metal processing Industries

Sr. No	Name of isolates	No.of Isolates
1.	*Achromobacter* sp.	8
2.	*Bacillus* sp.	5
3.	*Shigella* sp.	7
4.	*Salmonella* sp.	7
5.	*Pseudomonas* sp.	5
6.	*Corynebacterium* sp.	3
7.	*Staphylococcus* sp.	3
8.	*Proteus* sp	2
9.	*Exigoubacterium* sp.	1
10.	*Microbacterium*	1
Total isolates		42

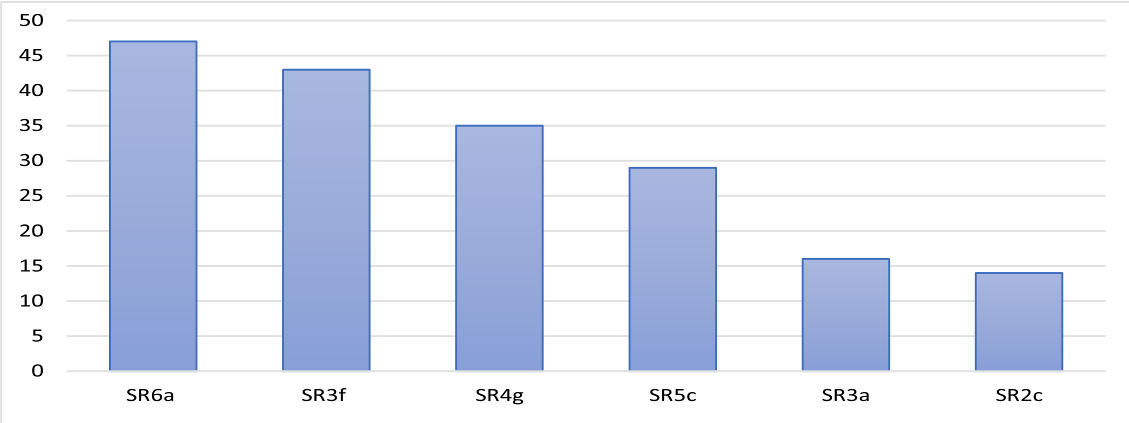
Table 2: Growth of heavy metal tolerant bacteria on different concentration of heavy metals

Sr.No	Isolates code	Metals	200ug	400µg	600µg	800µg	1000µg	1200µg	1400µg	1600µg	1800µg	2000µg	Name of isolates
1	**SR2c**	**Cu**	+	+	+	+	-	-	-	-	-	-	***Exigoubacterium***
		Ni	+	+	-	-	-	-	-	-	-	-	
		Cd	+	+	-	-	-	-	-	-	-	-	
		Hg	+	+	-	-	-	-	-	-	-	-	
		Zn	+	+	+	+	-	-	-	-	-	-	
2	SR3a	**Cu**	+	+	+	-	-	-	-	-	-	-	*Pseudomonas*
		Ni	+	+	+	+	+	+	-	-	-	-	
		Cd	+	+	-	-	-	-	-	-	-	-	
		Hg	+	+	-	-	-	-	-	-	-	-	
		Zn	+	+	+	-	-	-	-	-	-	-	
3	**SR3f**	**Cu**	+	+	+	+	+	+	+	+	+	+	***Bacillus***
		Ni	+	+	+	+	+	+	+	+	+	-	
		Cd	+	+	+	+	+	+	+	-	-	-	
		Hg	+	+	+	+	+	+	+	+	-	-	
		Zn	+	+	+	+	+	+	+	+	+	-	
4	SR4g	**Cu**	+	+	+	+	+						*Achromobactor*
		Ni	+	+	+	+	+	+	-	-	-	-	
		Cd	+	+	+	+	-	-	-	-	-	-	
		Hg	+	+	+	+	+	+	+	+	+	+	
		Zn	+	+	+	+	+	+	+	+	+	+	
5	**SR5c**	**Cu**	+	+	+	+	+	+	+	+	+	+	*Bacillus*
		Ni	+	+	+	+	+	+	-	-	-	-	
		Cd	+	+	+	+	+	-	-	-	-	-	
		Hg	+	+	-	-	-	-	-	-	-	-	
		Zn	+	+	+	+	+	+	-	-	-	-	
6	**SR6a**	**Cu**	+	+	+	+	+	+	+	+	+	+	***Achromobactor***
		Ni	+	+	+	+	+	+	+	+	+	+	
		Cd	+	+	+	+	+	+	+	+	-	-	
		Hg	+	+	+	+	+	+	+	+	+	-	
		Zn	+	+	+	+	+	+	+	+	+	+	

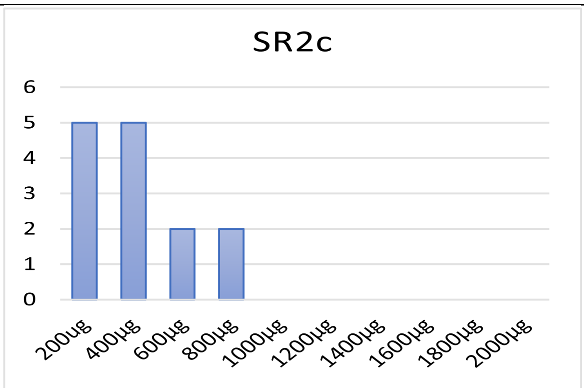
Recently, microbial bioremediation has appeared as an alternative technique to such traditional chemical treatments (Brierly, 1990). Microorganisms like bacteria, fungi, algae and actinomycetes have effectively sequestered heavy metals (Wong and So, 1993). These have been used to remove metals from polluted industrial and domestic effluents on a large scale. Microbiological detoxification of polluted water is economical, safe and sustainable (Eccles, 1995). It is well recognized that microorganisms have a high affinity for metals and can accumulate both heavy and toxic metals by a variety of mechanisms (Rehman *et al.,* 2008). The screening for the isolates from domestic sewage wastes have shown that probably all the isolates were resistant to one of the selected five heavy metals. There are a total 42 isolates of domestic sewage samples, among them one isolate SR6a (*Achromobactor*) was resistant to Cu at higher concentration (2000ppm) and considered as potential heavy metal degrading isolate. The some isolates such SR3f (*Bacillus*), SR4g (*Achromobactor*), SR5c (*Bacillus*), SR3a (*Pseudomonas*) and SR2c (*Exig,ubacterium*) were multi heavy metal degrading ones. All these isolates from metal industrial effluents showed the heavy metal resistance against Copper, Nickel, Cadmium, Zinc and Mercury. The results of study of Jaysankar *et al.,* (2003), showed that bacteria highly resistant to mercury that were isolated from seawater and sediment samples were capable of growth at 50 ppm of mercury. Also, Ready *et al.,* (2007) investigated the effect of amalgams exposure on mercury resistant bacteria, the mercury chloride was used at concentrations ranging from 0.125 to 512 µM and bacteria with a $HgCl2$ MIC of >325 µM were regarded as Hg resistant. One of the reasons for such high resistance among the isolated bacteria in this study could be related to the way of isolating mercury-resistant bacteria and it is of great import.

From the Table no.1, it was observed that, there are total six isolates, five from industrial effluents and one from domestic sewages sample were shown growth on 2000 ppm concentration of heavy metals are considered as potential heavy metal degrading isolates. These isolates included as SR6a (*Achromobactor*) SR3f (*Bacillus*), SR4g (*Achromobactor*), SR5c (*Bacillus*), SR3a (*Pseudomonas*) and SR2c (*Exigoubacterium*).

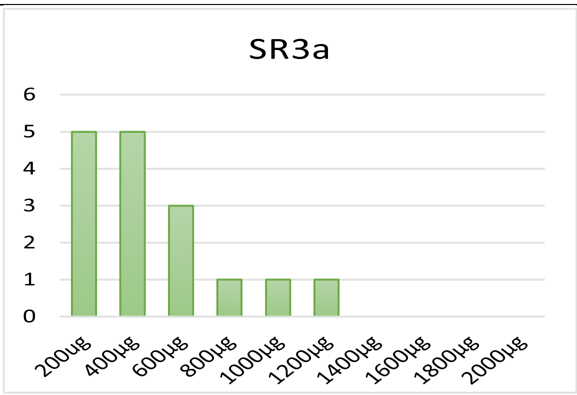
Graph 1: Potent heavy metal tolerant bacteria



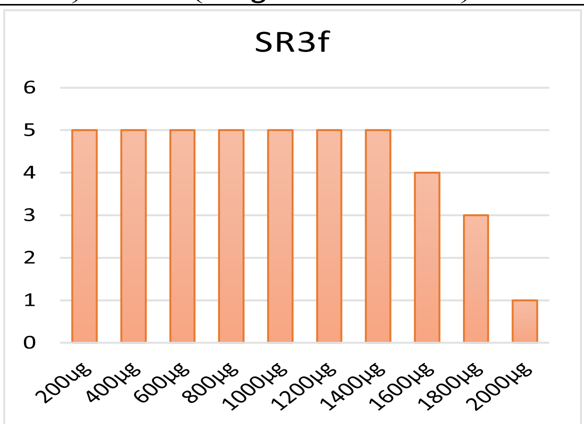
Graph 2: heavy metal tolerant bacterial isolate from effluents of metal processing industries



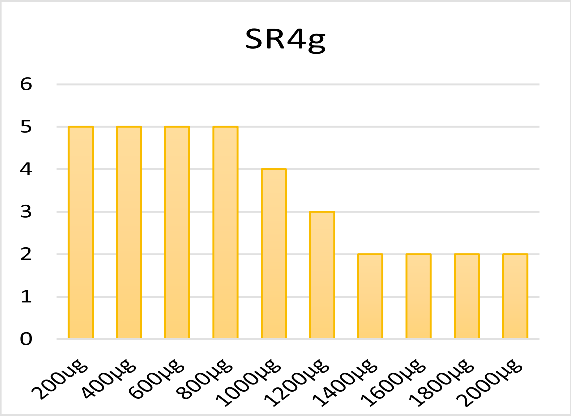
a) SR2c (*Exigoubacterium*)



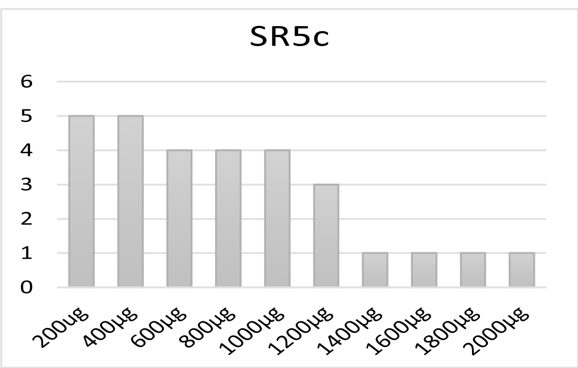
b) SR3a (*Pseudomonas*)



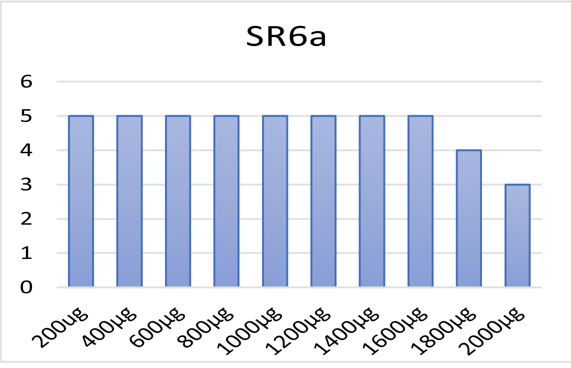
c) SR3f (*Bacillus*)



d) SR4g (*Achromobactor*)



e) SR5c (*Bacillus*)



f) SR6a (*Achromobactor*)

CONCLUSION

To conclude that the present study revealed that all the bacterial isolates were able to tolerate different concentration of heavy metals recovered from the different contaminated sites. The most prominent species that were revered from all the six sites were *Achromobactor sp.* and *Bacillus* spp., furthermore all the isolates were resistant various metals. The capability of bacteria resistance against different heavy metals may offer a beneficial tool for the simultaneous monitoring of many contaminants and pollutants in the environment.

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