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REMOVAL OF HEAVY METAL BY BACTERIAL SPECIES ISOLATED FROM EFFLUENT DISCHARGE OF METAL PROCESSING INDUSTRIES

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ABSTRACT

Heavy metals containing industrial effluent cause health hazards to plants, animals, aquatic life and humans increasing pressures on the flora and fauna. In this study heavy metal degrading bacteria from effluent discharge of metal processing industries were studied. 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. The ICP-AES analysis of the isolates incubated for the 24hr shows that the isolate SR3a and SR2c have the removal efficiency of 44 and 45 percent respectively. Isolate SR5c, can remove the heavy metal with removal efficiency of 52 percent. All the three remaining isolates such as SR6a, SR3f and SR4g have the same removal efficiency of the heavy metal mercury, copper, and nickel respectively, which was calculated as 55 percent. 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.

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INTRODUCTION

Heavy metal pollution is a major environmental problem which reduces crop production and food quality due to excessive application of agricultural inputs like fertilizers, pesticides, and mulch have resulted in the heavy metal contamination of soils (Su, 2014). Most of the pesticides are organic compounds, and a few are organic—inorganic compounds or pure minerals, and some pesticides contain Hg, As, Cu, Zn, and other heavy metals (Arao *et al.,* 2010). The introduction of heavy metals in variousforms in the environment can results inconsiderable modifications of the microbial communities and their activities (Sheik *et al.,*2012).Although heavy metals are toxic to humans as well as microbes, metals and microorganisms have co-existed since early history (Silver and Phung, 1996) and their survival in polluted environment depends on intrinsic biochemical and structural properties, physiological, or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Ehrlich, 1997; Wuertz and Mergeay, 1997). Generally, the strategy adopted by microorganisms aims to avoid thebuild-up of excess metal levels, and thus to prevent the onset of toxicity symptoms. These heavy metal resistant microbes develop the various mechanisms which help in detoxification and removal of the heavy metalfrom polluted environment (Ahmed *et al.,* 2005). 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(Pazirandeh *et al,* 1998; Roane and Pepper, 2001; Vieira and Volesky, 2000). In recent years, metal production emissions have decreased in many countries due to strict legislation, improved cleaning/ purification

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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). The microbes are biochemically discovered and their potential to resist heavy metals such as zinc and copper will be determined. In the past two decades, there have been recent advances in bioremediation techniques, with the goal being to effectively restore polluted environments in an eco-friendly approach, and at a very low cost. 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(Veglio and Beolchini, 1997). Several heavy metals have been tested using bacterial species like *Flavobacterium, Pseudomonas, Enterobacter, Bacillus,* and *Micrococcus* sp. Their great biosorption ability is due to their high surface-to-volume ratios and potential active chemisorption sites (teichoic acid) on the cell wall (Mosa *et al.,* 2016). Bacteria are more stable and survive better when they are in mixed cultures (Sannasi *et al.,* 2006). Therefore, consortia of cultures are metabolically superior for the biosorption of metals and are more appropriate for field applications (Kader *et al.,* 2007). 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 effluents from metal processing industry and waste management strategies. The present study was carried out to explore the heavy metal resistance and degradation capabilities of microbial diversity present into metal processing industry effluent.

MATERIALS AND METHODS

Study area and sample collection: 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). Collected samples were preserved at 4^0C for physicochemical and microbial analysis. Various parameters like pH, temperature, and color of effluent were documented at the sampling site using methods recommended by APHA (2005).

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.

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 Table 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).

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. 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

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

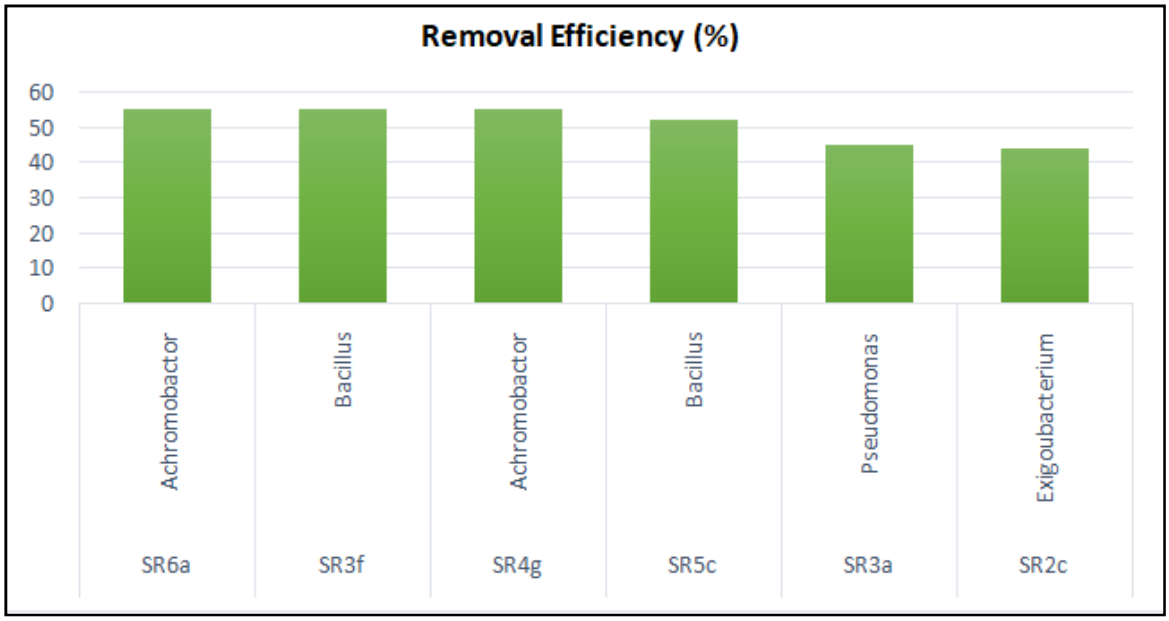

Fig. 1. Heavy Metal degradation after 24h

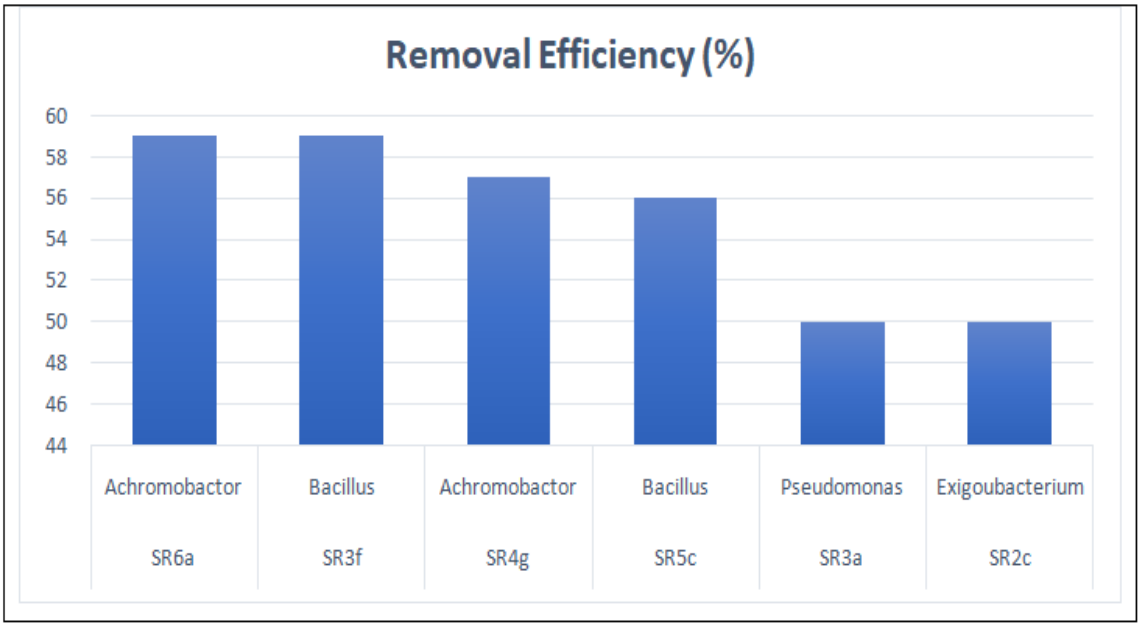

Fig. 2. Heavy Metal degradation after 48h

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

Sr.No	Isolates code	Metals	200µg	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	+	+	+	+	+	+	+	+	+	+	

(*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 (Table 2).

The ICP-AES analysis of the isolates incubated for the 24hr shows that the isolate SR3a and SR2c have the removal efficiency of 44 and 45 percent respectively. Isolate SR5c can remove the heavy metal with removal efficiency of 52 percent.

Table 3. Analysis of heavy metal degradation by potential isolates after 24hr

Sr.No	Isolates code	Time interval 24hr			Removal Efficiency (%)	Name of isolates
		Control	Test	Metal removed		
1.	SR6a	467	210	257	55	*Achromobactor*
2.	SR3f	376	167	209	55	*Bacillus*
3.	SR4g	441	195	246	55	*Achromobactor*
4.	SR5c	367	173	194	52	*Bacillus*
5.	SR3a	280	154	126	45	*Pseudomonas*
6.	SR2c	254	142	112	44	*Exigoubacterium*

Table 4: Analysis of heavy metal degradation by potential isolates after 48hr

Sr.No	Isolates code	Time interval 48hr			Removal Efficiency (%)	Name of isolates
		Control	Test	Metal removed		
7.	SR6a	441	180	261	59	*Achromobactor*
8.	SR3f	376	154	236	59	*Bacillus*
9.	SR4g	467	198	269	57	*Achromobactor*
10.	SR5c	367	160	207	56	*Bacillus*
11.	SR3a	280	140	140	50	*Pseudomonas*
12.	SR2c	254	125	129	50	*Exigoubacterium*

Table 5. Analysis of heavy metal degradation by potential isolates after 72hr

Sr.No	Isolates code	Time interval 72hr			Removal Efficiency (%)	Name of isolates
		Control	Test	Metal removed (ppm)		
13.	SR6a	467	183	284	60	*Achromobactor*
14.	SR3f	376	149	227	60	*Bacillus*
15.	SR4g	441	183	264	59	*Achromobactor*
16.	SR5c	367	150	217	59	*Bacillus*
17.	SR3a	280	115	165	58	*Pseudomonas*
18.	SR2c	254	110	144	56	*Exigoubacterium*

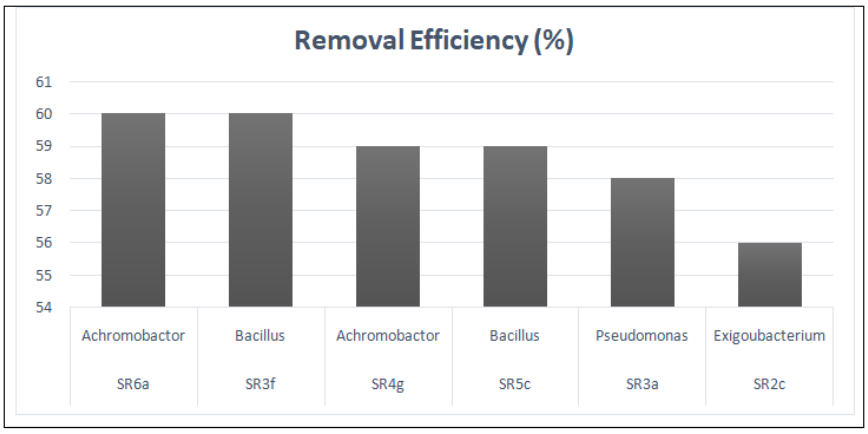

Fig. 3. Heavy Metal degradation after 72h

All the three remaining isolates such as SR6a, SR3f and SR4g have the same removal efficiency of the heavy metal mercury, copper, and nickel respectively, which was calculated as 55 percent (Table 3and Fig. 1). The ICP-AES analysis of the isolates incubated for the 48hr shows that the isolate SR3a and SR2c have the same removal efficiency of 50 percent. Isolate SR5c can remove the heavy metal with removal efficiency of 56 percent while SR4g removed 57 percent.

SR6a and SR3f have the same heavy metal removal efficiency of 59 percent (Table 4 and Fig 2). The heavy metal removal efficiency of the isolates after 72hr have shown that the isolate SR2c shows 56 percent and SR3a shows 58 percent removal efficiency. The isolate SR4g and SR5cwere 59 percent efficient in the removal of the heavy metals. The remaining two isolates SR6a and SR3fwere showing the highest heavy metal removal efficiency of 60 percent as compared to other

isolates (Table 5 and Fig. 3). De *et al.* (2008) reported a 78% reduction of chromium (Cr) using a bacterial consortium of *Acinetobacter* sp. and Arthrobacter sp. at 16 mg/L metal ion concentration. Micrococcus luteus was used to remove a large quantity of Pb from a synthetic medium. Under ideal environments, the elimination ability was 1965 mg/g (Puyen *et al.,* 2012).From the Table no.3, 4 and 5, 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*).

CONCLUSION

To conclude that the present study revealed that all the bacterial isolates were able to remove the different concentration of heavy metals. The most prominent species that were revered from all the six sites was *Achromobactor sp.*T his isolate could help in the effective bioremediation of heavy metal from the effluents of metal processing industries

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