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Heavy metal removal by isolates from domestic and industrial waste water

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Abstract

Rapid industrialization has released industrial contaminants, particularly heavy metals into water bodies. With regard to removal of heavy metals, bioremediation is an advantageous technique which is comparatively safer and cheaper. This study focused on industrial zones of Marathwada region which have various types of industries. It was attempted to evaluate the potential of heavy metal removing microbial flora from industrial effluent and domestic sewage for bioremediation. Six species were considered as potentially heavy metal removing isolates. These isolates included *Pseudomonas* spp. (DMb5 (Cu), *Achromobacter* spp. (MPb2 (Ni), *Pseudomonas* spp. (MPb3 (Cu), *Exigoubacterium* spp. (MPb2 (Hg), *Pseudomonas* spp. (PW4 (Cu), Uncultured *Microbacterium* (MPb3 (Ni). All these isolates were able to remove toxic heavy metals present in waste water within incubation period of 24hr, 48 hr and 72 hrs respectively. ICP-AES analysis revealed that the incubation period of 72 hr was best suited for heavy metal removal by isolates. Hence, the study concluded that bacterial strains have shown high degree of heavy metal resistance and could be explored as candidates for waste water bioremediation processes particularly heavy metal contaminated water bodies.

Keywords: Heavy metals, bioremediation, isolates, incubation, degradation

1. Introduction

Human activities such as mining operations, fuel combustion, application of agricultural chemicals and discharge of industrial waste, have resulted in accumulation of metals such as copper, chromium, cadmium, lead, mercury, zinc etc in the environment [1]. Heavy metals play indispensable role in cell growth and in metabolic function. Despite their enormous physiological significance they are needed only in trace concentration and become toxic for the cell when the physiological levels are exceeded. Similarly metal ions for which no physiological function has been shown can be detrimental when they enter the cell in concentrations exceeding tolerance limit. Heavy metals cause adverse reaction in different organs such as lungs, kidneys, liver and hamper biological function of central nervous function, reproductive system and result in birth defects in humans [2]. Metals like Pb, Cd and Hg induce oxidative stress indirectly by displacing native metals from cellular binding sites. In addition, at high levels both essential and non-essential metals bind cell membranes, alter enzyme specificity, disrupt cellular functions and damage DNA structure [3]. Different organisms have evolved a variety of mechanisms to detoxify metals. In eukaryotes, route of detoxification involves synthesis of chelating molecules that bind to the metal ions and mediate their transport out of the cells or their sequestration into sub—cellular compartments.

Heavy metals get accumulated through the food chain; contaminate drinking water reservoirs and fresh water habitats leading to serious ecological and health effects [4]. On account of their adverse effects the presence of metals in soil and ground poses serious challenge to environmental managers as the options for remediation are limited. These contaminants are not degradable; remediation alternatives are limited to excavation of contaminated soil and pump and treat processes for contaminated ground water [5]. Physico-chemical techniques such as incineration, ion-exchange, filtration, chemical precipitation, adsorption and reverse osmosis are available for heavy metal removal. But these techniques are associated with high energy requirements, incomplete removal, waste product generation and are financially expensive [6].
It has been reported that micro-organisms become adapted to these environments by acquisition of specific resistance systems [7]. The interest in interactions of heavy metals with micro-organisms has increased and has focused in particular on the selection of metal-resistant micro-organisms from polluted waters and the possibility of using the microbes for detoxifying polluted environments [8]. With regard to removal of heavy metals, bioremediation is an advantageous technique which is comparatively safer and cheaper [9]. A study found that industrial effluent site of river Nagavali in Andhra Pradesh contained various bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. These bacteria were able to grow in heavy metal solutions of Cu, Hg, Co and Zn. The potency of a white rot fungus *Phanerochaete chrysosporium* was evaluated to remediate chromium from fortified solution. It was found that 99.7% Cr (VI) was removed after 72 hours [10]. Copper tolerant bacteria were isolated from soil samples located near metal industries. Strain N1c and strain N5a showed maximal bioremoval efficiency of 82% and 75% respectively. The 16S rRNA gene sequence of N1c was 96% similar to *Achromobacter* sp and that of N5a was similar to *Pseudomonas stutzeri* [11]. It was reported that tannery effluent contaminated soil can be remediated by micro-organisms. Isolated *Bacillus* sp was found to reduce 85.9% of chromium from medium after 96h [12].
In this background this study is focused on Shendra MIDC (19°53’20”N 75°29’3” E) and Chikalthana MIDC (19°53’15”N 75°21’59”E) which are industrially populated areas in Marathwada region. This region has different types of industries including metal processing, pharmaceutical, paint, distilleries and the domestic waste disposal sites. This region has previously never been studied before for presence of potential microbial bioremediation agents. The research attempts to evaluate the potential of heavy metal degrading microbial flora from heavy metal contaminated industrial effluent and domestic sewage for bioremediation [13].

2. Experimental

2.1 Sample collection

A total of 50 samples were collected, among them 25 effluent samples were collected from various industries like pharmaceutical, distilleries, metal processing plants, paint industry etc and 25 samples were collected from domestic sewage water samples. Effluent samples were collected in dry, sterile polypropylene containers and transported immediately to Sakolkar Life Sciences and Research Centre, Shendra MIDC, Aurangabad. These containers were maintained at 4°C to ensure the minimal biological activity. Processing of samples for isolation of bacteria was carried out within 3h of sample collection.

2.2 Physico-Chemical Analysis

Parameters such as pH, color, turbidity, temperatures, BOD (Biological oxygen demand) and COD (Chemical oxygen demand) of all collected samples were analyzed and also heavy metal degradation analysis was carried out in the laboratory [14].

2.3 Isolation of Bacteria

The bacterial species were isolated from the collected water samples with the help of conventional serial dilution technique [15]. For the pure culture of bacteria single colonies were picked and streaked on the nutrient agar plates containing different concentrations (200 to 2000 ppm) of different heavy metals (Cu, Ni, Hg) under sterile conditions. These concentrations were selected on the basis of previous studies reported in the literature.

Pure cultures of strains which showed growth on plates containing 2000 ppm heavy metal concentration were grown on slants by stab and streak method for storage and subsequently for identification and biochemical characterization of bacterial isolates. The isolates which have shown growth on heavy metal concentration (2000 ppm) were considered as potential degraders. Among the isolated bacteria, 6 isolates were named as MP(b2)Ni, MP(b3)Ni, MP(b2) Hg, DM-B5-Cu, PW4-Cu, MPb3-Cu found potent heavy metal degraders. Hence, for cumulative identification these isolates were sent to NCCS Pune for the 16s rRNA. Further to this confirmative identification these isolated species were inoculated in nutrient broth containing heavy metal concentration (2000 ppm) incubated for three different time periods at 20-30^{o}C temperature. After completion of incubation the samples were centrifuged to separate the supernatant and the separated supernatant samples were then sent to IIT, Powai Mumbai for analysis of the heavy metal degradation by using ICP-AES (ARCOS, M/S Spectro, Germany).

3. **Results and discussion**

The physico-chemical analysis (Table 1) of samples collected from industrial effluents and domestic sewage water show that samples were black, greyish, brown in color having pH in range of 5-9 and having pungent and irritating odor. Color of the effluent might be due to the presence of biodegradable and non-biodegradable high molecular weight organic compounds. pH in range of 5-9 may be due to the presence of salts of sodium, potassium and chromium etc. The temperature of samples was in range of 20-30^{o}C. All the samples were found turbid. BOD of industrial effluent was 12-25 mg/g where as domestic sewage was 20-25 mg/g COD. COD of industrial effluents was 180-230 mg/g and of domestic sewage was 190-210 mg/g.

Table 1: Physico- Chemical Analysis of waste water

SNo	Physico-chemical parameter	Industrial effluent	Domestic effluent
1.	Color	Black, greyish, brown	Black, greyish
2.	pH	5.7-9	5-9
3.	Odor	Pungent	Pungent
4.	Temperature	20-30^{o}C	20-30^{o}C
5.	Turbidity	Turbid	Turbid
6.	BOD (mg/g)	12-25	20-25
7.	COD (mg/g)	180-230	190-210

From figure 1 it is clear that from 25 industrial effluent samples a total of 58 isolates were obtained, among these isolates *Shigella* sp (11) were predominantly obtained followed by *Bacillus* sp. (9), *Salmonella* sp (9), *Achromobacter* sp. (8), *Pseudomonas* sp. (7), *Corynebacterium* sp. (5), *Staphylococcus* sp. (4), *Proteus* sp. (3), *Exigoubacterium* sp. (1) and uncultured *Microbacterium* (1).

A total of 25 domestic sewage samples were collected and a total of 45 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 isolates *Salmonella* sp. (14), was predominantly obtained followed by *Achromobacter* sp. (8), *Shigella* sp. (7), *Proteus* sp. (6), *Bacillus* sp. (3), *Pseudomonas* sp. (3), *Corynebacterium* sp. (2) and *Staphylococus* sp. (2) as shown in Figure 2.

Screening for isolates was done from domestic sewage samples and it was found that all 45 isolates were resistant to one of the selected five heavy metals. However, one isolate DMB5Cu was resistant to Cu at high concentration of 2000 ppm and therefore, was considered as a potential heavy metal degrading isolate. In a similar manner five isolates were identified from industrial effluent and showed growth on 2000 ppm concentration of heavy metals. These species were considered as potentially heavy metal degrading isolates. These isolates included *Pseudomonas* spp. (DMb5 (Cu), *Achromobacter* spp. (MPb2 (Ni), *Pseudomonas* spp. (MPb3 (Cu), *Exigoubacterium* spp. (MPb2 (Hg), *Pseudomonas* spp. (PW4 (Cu), Uncultured *Microbacterium* (MPb3 (Ni). For the confirmative identification purpose these isolates were proceeded for 16s rRNA.

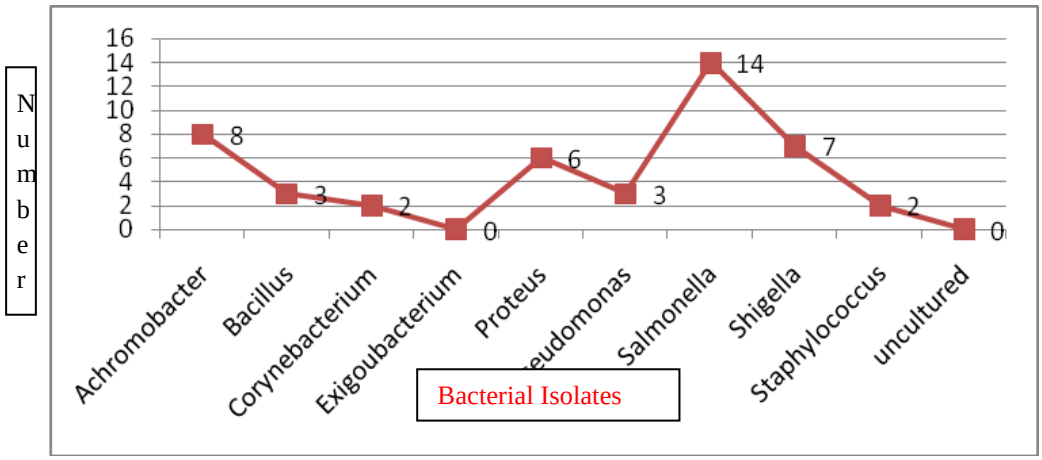

Figure 1: Total Isolates from Industrial Effluents

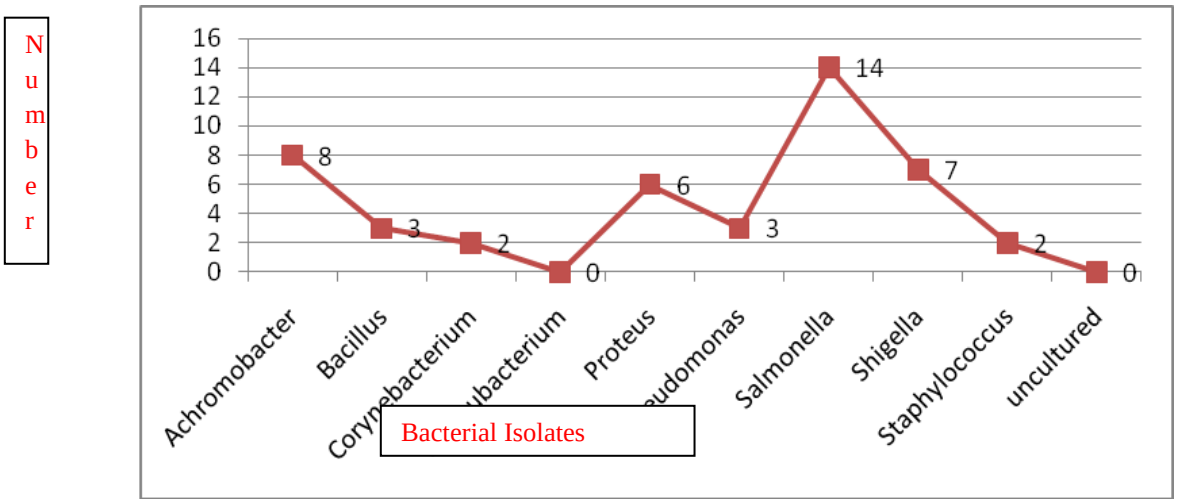

Figure 2: Total Isolates from Domestic Sewage samples

Table 2: ICP-AES analysis of heavy metal degradation by isolates after 24 hr

Sr. No.	Code of Isolate	Time Interval				Name of Isolate
		24 hr			Removal Efficiency (%)	
		Control	Test	Metal removed		
1.	DMb5(Cu)	254	142	112	44	*Pseudomonas sp.*
2.	MPb2(Ni)	280	154	126	45	*Achromobacter sp.*
3.	MPb3(Cu)	367	173	194	52	*Pseudomonas sp.*
4.	MPb2(Hg)	441	195	246	55	*Exigoubacterium sp.*
5.	PW4 (Cu)	376	167	209	55	*Pseudomonas sp.*
6.	MPb3(Ni)	467	210	257	55	*Uncultured Microbacterium sp.*

ICP-AES analysis of the isolates incubated for 24 hr (Table 2) showed that the isolate DMb5 (Cu) and MPb 2(Ni) had removal efficiency of 44 and 45 per cent respectively. While the isolates MPb 2 (Hg), PW4 (Cu) and MPb3 (Ni) were able to remove heavy metal mercury, copper and nickel with removal efficiency of 55%.

Table 3: ICP-AES analysis of heavy metal degradation by isolates after 48 hr

Sr. No.	Code of Isolate	Time Interval			Removal Efficiency (%)	Name of Isolate
		48 hr				
		Control	Test	Metal removed		
1.	DMb5(Cu)	254	125	129	50	*Pseudomonas Sp.*
2.	MPb2(Ni)	280	140	140	50	*Achromobacter Sp.*
3.	MPb3(Cu)	367	160	207	56	*Pseudomonas Sp.*
4.	MPb2(Hg)	441	180	261	59	*Exigoubacterium Sp.*
5.	PW4 (Cu)	376	154	236	59	*Pseudomonas Sp.*
6.	MPb3(Ni)	467	198	269	57	*Uncultured Microbacterium Sp.*

The ICP-AES analysis of the isolates incubated for 48h (Table 3) revealed that the isolates D M b5 (Cu) and MPb2 (Ni) had the same removal efficiency of 50 percent. Isolate MPb3 (Cu) was able to remove heavy metal with removal efficiency of 56 per cent. While MPb2 (Hg) and PW4 (Cu) had the same heavy metal removal efficiency of 59 percent (Table 3).

Table 4: ICP-AES analysis of heavy metal degradation by isolates after 72 hr

Sr. No.	Code of Isolate	Time Interval			Removal Efficiency (%)	Name of Isolate
		72 hr				
		Control	Test	Metal removed		
1	DMb5(Cu)	254	110	144	56	*Pseudomonas Sp.*
2	MPb2(Ni)	280	115	165	58	*Achromobacter Sp.*
3	MPb3(Cu)	367	150	217	59	*Pseudomonas Sp.*
4	MPb2(Hg)	441	183	264	59	*Exigoubacterium Sp.*
5	PW4 (Cu)	376	149	227	60	*Pseudomonas Sp.*
6	MPb3(Ni)	467	183	284	60	*Uncultured Microbacterium Sp.*

The heavy metal removal efficiency of the isolates after 72 hr (Table 4)revealed that the isolate DMb 5(Cu) had removal efficiency of 56% while the efficiencies of PW4 (Cu) and MPb 3(Ni) were higher at 60%. These isolated species were inoculated in nutrient broth containing heavy metal concentration (2000 ppm) and were incubated for three different time periods (24 hr, 48 hr and 72 hr respectively) at 20-30^{o}C temperature.

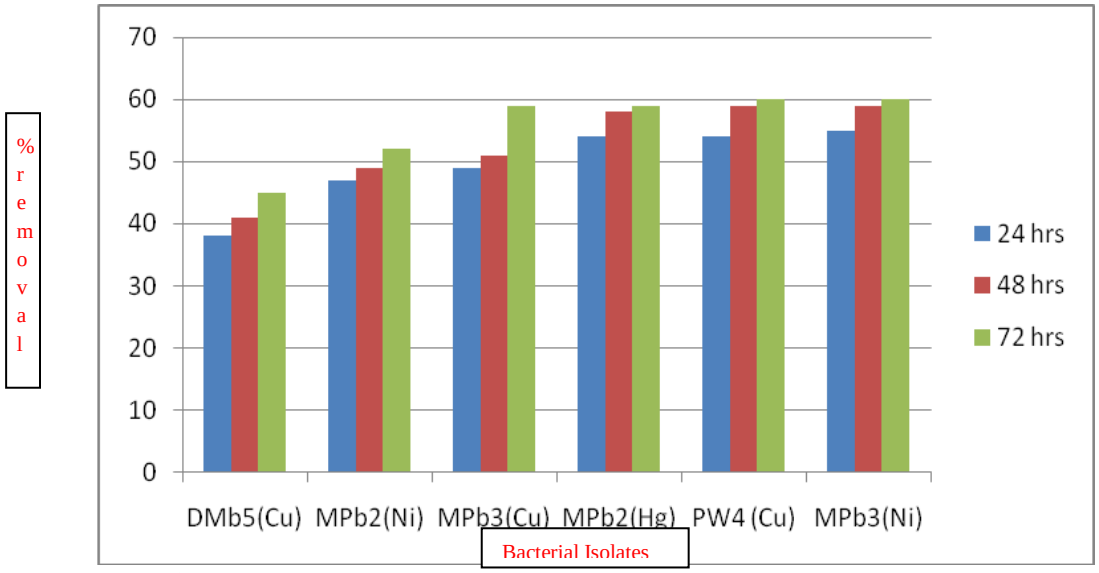

Figure 3: Heavy metal removal at 2000 ppm by isolates at 24 hr, 48 hr and 72 hr

From figure 3 it is clear that the degradation efficiency increased with the increase in incubation period. Isolates MPb2(Hg) *Exigoubacterium Sp.* , PW4 (Cu) *Pseudomonas* and MPb 3(Ni) *Microbacterium* were found to be the most efficient heavy metal removing isolates. In a study carried out in Iran to isolate high resistant degrading species for copper from Khur Mousa sediments (located in North of the Persian Gulf, Iran) 10 bacterial species were isolated from marine sediments. One strain represented high potential to grow in medium supplemented with copper. Isolated bacterium was identified as *Pseudomonas* sp, by biochemical tests. Over 70% of copper was sorbed on *Pseudomonas* sp within 150 min. Based on its ability of biosorption of copper this species was introduced as an appropriate micro-organism for bioremediation of contaminated environments [16]. Similar study was undertaken on *Psedomonas putida* and *P. fluorescens* species in Egypt. Cu(II) removal percentage ranged between 50-93% [6]. In a study on remediation of tannery contaminated soil by micro-organisms it was found that the leather tannery effluent contaminated soil had a higher pH in addition to large amount of suspended and total dissolved solids, minerals and metals like chromium, zinc and copper. It was also found to contain *Bacillus spp*. which reduced 85.9% chromium from the medium after 96h. The results also revealed that the isolated *Bacillus spp.* possessed the ability to remove other heavy metals (Ni, Cr, Cu, Zn and Cd) from the tannery effluent. The metal removing capacity increased with increase in the concentration of metals [12]. Also few biosorption studies have shown that as the concentration of biomass increases the removal efficiency of the heavy metal degradation increases as well [17]. Bacterial species *Bacillus sp*., *Pseudomonas* sp. and *Micrococcus* sp isolated from waste water were investigated for heavy metal removal. It was concluded that the species removed Cu, Cd and Pb by 69.34%, 90.41% and 84.27% respectively [18]. In a study on biosorption *Bacillus licheniformis* isolated from agricultural soil treated with sewage sludge was able to remove Cr(VI) by almost 95% [19].
Results of different studies carried out have demonstrated that the bacteria saliently affected the metal degradation rate. Hence, micro-organisms that affect the reactivity and mobility of metals can be used to detoxify heavy metals and prevent further metal contamination [20].

Study limitations
It has been reported in the literature that heat treatment, UV treatment and that with chemicals such as NaOH enhances metal biosorption by the isolates however, this was not investigated in this research. Another important limitation was that only bacterial samples were isolated and studied. It would have been better to isolate and compare the metal biosorption capacities of bacterial, algal and fungal isolates but it could not be carried out due to lack of time and resources.

Conclusions

Increased industrialization has tremendously added toxic pollutants to the environment. The most abundant pollutants in the waste water and in sewage are heavy metals such as lead, chromium, nickel, zinc, arsenic, copper etc. Heavy metals get accumulated through the food chain; contaminate drinking water reservoirs and fresh water habitats leading to serious ecological and health effects. On account of their adverse effects, the presence of metals in water and soil poses serious challenge to environmental managers as the remediation options are not only limited but also expensive. With regard to removal of heavy metals, bioremediation is an advantageous technique which is comparatively safer and cheaper. This study focused on industrial zones of Marathwada region which has different types of industries. The research attempted to evaluate the potential of heavy metal removing microbial flora from heavy metal contaminated industrial effluent and domestic sewage for bioremediation. Screening for isolates was done from both domestic sewage and industrial samples. One isolate DMB5Cu was resistant to Cu at high concentration of 2000 ppm and therefore, was considered as a potential heavy metal removing isolate. In a similar manner five isolates were identified from industrial effluent and showed growth on 2000 ppm concentration of heavy metals. These species were considered as potentially heavy metal removing isolates. These isolates included *Pseudomonas* spp. (DMb5 (Cu), *Achromobacter* spp. (MPb2 (Ni), *Pseudomonas* spp. (MPb3 (Cu), *Exigoubacterium* spp. (MPb2 (Hg), *Pseudomonas* spp. (PW4 (Cu), Uncultured *Microbacterium* (MPb3 (Ni). For the confirmative identification purpose these isolates were proceeded for 16s rRNA. All the six isolates were able to remove toxic heavy metals present in waste water under conditions of 24hr, 48 hr and 72 hrs respectively. ICPP-AES analysis revealed that the incubation period of 72 hr was best suited for heavy metal removal by isolates. Hence, the study concluded that bacterial strains have shown high degree of heavy metal resistance and could be explored as candidates for waste water bioremediation processes particularly heavy metal contaminated water bodies.

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