Pakistan Journal of Science (Vol. 69 No. 3 September, 2017)

PHYSIOCHEMICAL ANALYSIS AND HEAVY METAL TOLERANCE POTENTIAL OF BACTERIA FROM TEXTILE EFFLUENTS

A.M. Afzal, M.H. Rasool*, M. Waseem and B. Aslam

Department of Microbiology, Government College University Faisalabad, Pakistan
Corresponding author's email: drmhrasool@gcuf.edu.pk

ABSTRACT: Heavy metal tolerant bacterial species were isolated from the textile effluents collected from Faisalabad, Pakistan. Thirty samples were subjected to heavy metal analysis. Nickel and Cobalt were selected for further study on the basis of their predominant concentration in all samples. Thirteen samples were screened out as positive for Ni tolerant bacteria and 03 of them had typical characteristics which tolerated Ni up to 08 mM. These species were identified as AMIC1 (*Klebsiella spp.*), AMIC2 (*Bacillus spp.*) and AMIC3 (*Bacillus spp.*). These indigenous strains exhibited pronounced tolerance to Ni and Co and showed multi metal resistance (MMR) potential. Bacteria isolated from sample $SarDP_2$ were able to tolerate Co up to 06mM and exhibited MMR to Ni, Co and Cr (1:1:1) up to 5.5mM. Isolates from $RgrDP_3$ were able to tolerate Co up to 07mM and showed MMR to Ni, Co and Cr (1:1:1) up to 4.5mM. Similarly, isolates from $SarDP_5$ were able to tolerate Co up to 6.5mM and exhibited MMR to Ni, Co and Cr (1:1:1) up to 4.5mM. It was concluded that these species had significant heavy metal tolerance potential and may be used for the development of effective bioremediation agents to detoxify textile effluents at industrial surroundings within natural environments.

Keywords: Heavy metals, Bacteria, Textile effluents, Faisalabad.

(Received 10-05-2017 Accepted 18-08-2017).

INTRODUCTION

Heavy metal contamination of industrial effluents is one of the main universal environmental distresses. Depending upon soil pH, heavy metals became mobile in soil and a small part of the total mass became available to living organisms (Hookoom and Puchooa, 2013). Release of heavy metals in effluent waste poses a menace to public health because of its persistence, biomagnifications and accumulation in food chain (Issazadeh *et al.,* 2014). Cadmium, chromium, mercury, lead, nickel, cobalt and copper are frequently present in the industrial wastewater as reported by Smrithi and Usha (2012).

People living in the vicinity of the dumping sites are facing various health problems by the metal contamination of drinking water and food (Chisti, 2004). Heavy metals accumulate in different organs and cause numerous diseases (Ozer and Pirincci, 2006). The bioremediation of heavy metals using microorganisms got a great deal of attention (Singh *et al.,* 2010). Microorganisms that are able to survive well in high concentration of heavy metals are of great interest as bioremediation agents. Specifically, it conducts bioaccumulation based on the incorporation of metals inside the living biomass or biosorption, in which metal ions are adsorbed at the cellular surface by different mechanisms reported by Vijayaraghavan and Yun (2008). The advantages of using microbes for bio-remediation include natural occurrence, cheap production, easy availability to treat large volumes of wastewater due to rapid kinetics and high selectivity in terms of removal and recovery of specific metals.

Faisalabad is the 3rd biggest city in Pakistan after Karachi and Lahore. It is the 2nd biggest city in the province of Punjab after Lahore, and a major industrial center. The city is also known as the "Manchester of Pakistan". Due to the heavy industrialization different types of wastes are being produced by the different industries. Several studies conducted on heavy metal contaminated sites have confirmed a high diversity of microorganisms. Indigenous organisms have the ability to adopt according to the prevailing environments (Haq and Shakoori, 2000). Heavy metals are non biodegradable and many of them have harmful effects on living organisms up to certain concentration (Mansouri *et al.,* 2012). Microorganisms have adopted ways to endure the metals either by presence of heavy metals through efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Haferburg and Kothe, 2010).

The textile zone is playing a vital role in the export of the country but at the same time a lot of environmental pollution is being produced by this zone. Therefore, present study was aimed to analyze the industrial effluents of Faisalabad, for the isolation and identification of indigenous bacteria to explore their potential of tolerance against heavy metals founds in such effluents.

MATERIALS AND METHODS

Sample collection: Six main drains present in and around Faisalabad receiving the textile effluents and surrounding different textile industries (n= 20) were selected. From each drain, 05 samples were collected keeping the distance of about 1000 meter between two points. In this way 30 samples were collected and tagged with specific sample codes. Samples were collected in sterile plastic bottles using aseptic techniques, transported on ice to Postgraduate Research Lab of Department of Microbiology, Government College University Faisalabad for further processing following the protocol as described by Baby *et al.* (2014).

Physico-chemical parameters: The physico-chemical parameters of the effluent samples were determined for all samples. The pH was determined by digital pH meter, Electric conductivity (EC) was quantified using EC meter, Dissolved Oxygen (DO) using DO meter, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were analyzed by titration method described by Nanda *et al.* (2011). Total Dissolved Solids (TDS), Total Suspended Solids (TSS) and Total Solids (TS) were determined following the standard procedures (Anonymous, 2005).

Heavy metals analysis: Samples were processed for determination of heavy metals i.e. Cobalt (Co), Chromium (Cr), Nickel (Ni), Lead (Pb) and Zinc (Zn). Samples were digested following the protocol as described by Sinha and Paul (2014) and metal analysis was done using Atomic Absorption Spectrophotometer (AAS) following the conditions described in AOAC (1990).

Enumeration of bacteria: All the collected effluent samples were serially diluted tenfold in sterile distilled water up to 10^{-5} dilutions and 0.1mL from each dilution inoculated in triplicate on pre-sterilized nutrient agar plates. All the plates were incubated at 37^0C in incubator for 24 hours. Plates containing 250 to 300 colonies of bacteria were selected, colonies were counted using automated digital colony counter and numbers of bacteria were calculated as per following formula:

CFU/ml of original sample = No. of colonies on plate X reciprocal of dilution factor x 10

Heavy Metal Tolerant bacteria: Isolation of heavy metal tolerant (HMT) bacteria of Ni and Co was done through spread plate method as described by Samanta *et al.* (2012). 500ml nutrient agar was prepared in distilled water adding 1.5g beef extract, 2.5g peptone, 2.5g sodium chloride and 7.5g agar. 05ml of 100mM Ni $(NO_3)_2$ was added. Similarly, 05ml of 100mM $CoCl_2$ was added in 500ml nutrient agar media. 0.1ml from each sample dilution was inoculated onto nutrient agar plates having 01mM of Ni and Co and was incubated at 37°C for 2 days then observed for bacterial growth (Samanta *et al.,* 2012). The number of Ni and Co tolerant bacteria were calculated and compared with bacterial counts without adding heavy metals and percentages of Ni and Co tolerant bacteria were calculated as per following formula.

Percentage of Metal tolerant bacteria = No. of tolerant bacteria (Ni or Co) x 100
No. of bacteria without metal

MTC of heavy metal was selected as the highest concentration of heavy metal that allowed visible bacterial growth after 48 to 96 hours of incubation. The increasing concentration of both heavy metals (Ni and Co) i.e. (0.5, 1, 1.5, 02, 2.5, 03, 3.5, 04, 4.5, 05, 5.5, 06, 6.5, 07, 7.5, 08, 8.5, 09, 9.5 and 10mM) were added in pre-sterilized nutrient agar plates for testing the MTCs of isolates (Vashishth and Khanna, 2015). MMR of bacteria was determined by inoculating them on nutrient agar medium incorporated with Ni, Co and Cr in equal concentration i.e. 1:1:1 (Saini and Pant, 2016).

Identification of Bacteria: Heavy metal tolerant bacteria were identified on the basis of colony characters, morphology and biochemical profile. All identification tests were performed following the protocols mentioned in Bergey’s Manual of Determinative Bacteriology (1994).

RESULTS AND DISCUSSION

Physico-chemical analysis in the present study revealed that all the 30 effluent samples collected from industrial drains of Faisalabad Pakistan had pH from 7.73±0.22 to 8.28±0.33, Electric Conductivity from 114.58±4.82 to 136.96±8.74, Dissolved Oxygen from 1.90±0.28 to 2.55±0.55, Chemical Oxygen Demand from 174.38±33.65 to 191.50±23.84, Biological Oxygen Demand from 70.00±13.80 to 76.66±9.34, Total Suspended Solids from 147.73±10.16 to 193.60±23.63, Total Dissolved Solids from 3241.1±175.6 to 3905.8±204.3 and Total Solids from 3388.9±185.5 to 4094.0±215.5 (Table-1).

Heavy metal analysis through Atomic Absorption Spectrophotometer revealed the presence of Ni (0.168±0.035 to 0.230±0.019 Mm), Co (0.128±0.053 to 0.216±0.008 mM), Cr (0.031±0.020 to 0.098±0.018 mM), Pb (0.026±0.023 to 0.240±0.160 mM) and Zn (0.218±0.068 to 0.336±0.016 mM) in all the 30 samples (Table-2). As the concentration of Ni and Co was significantly higher as compared to other heavy metals in all effluent samples therefore these two metals were selected for further study.

Regarding the results of heavy metal tolerant bacteria, out of 30 samples 13 were found to have Ni tolerant bacteria. Two samples collected from drain surrounding the textile units located at small industrial estate and main Sargodha road, Faisalabad ($SarDP_1$ and $SarDP_5$) and one from Raja Ghulam Rasool Nagar ($RgrDP_3$) revealed the presence of bacteria which tolerated Ni up to 08mM. AMIC1 were G-ve rods of Klebsiella spp. whereas other two strains (AMIC2 and AMIC3) were G+ve rods belonging to Bacillus spp.

Heavy metal tolerant (HMT) bacteria were assumed to occur mainly in metal-contaminated sites. Studies showed that presence of metals and other physicochemical parameters played an important role in developing metal tolerance in indigenous bacteria of specific site (Shi *et al*., 2013). The pH of specific metal contaminated site control the solubility of metals (Klimek, 2012). The pH values of all effluent samples in the present study revealed non-significant differences at all localities and ranged from 7.73±0.22 to 8.28±0.33. BOD and COD tests were performed to quantify the relative oxygen reduction effect of waste contaminants (Samudro and Mangkoedihardjo, 2010). Results revealed that effluent samples belonged to highly contaminated wastewaters. So it could be assumed that existing bacteria in a metal stressed environment can develop metal tolerance (Margesin and Schinner, 2001).

It was evident from the results that bacteria from sample $SarDP_2$ were able to tolerate Co up to 06mM and exhibited MMR to Ni, Co and Cr (1:1:1) up to 5.5mM. Isolate from $RgrDP_3$ was able to tolerate Co up to 07mM and showed MMR to Ni, Co and Cr (1:1:1) up to 4.5mM. Similarly, isolate from $SarDP_5$ was able to tolerate Co up to 6.5mM and exhibited MMR to Ni, Co and Cr (1:1:1) up to 4.5mM.

These three bacterial strains which were able to tolerate the maximum concentration of heavy metals isolated from effluent samples $RgrDP_3$, $SarDP_2$, and $SarDP_5$ were named as AMIC1, AMIC2 and AMIC3, respectively. After Gram's staining, it was found that bacterial strain AMIC1 was G-ve rods whereas AMIC2 and AMIC3 were G+ve rods. After examination of colony characteristics on selective and differential media, biochemical and sugar fermentation test results, it was confirmed that bacterial strain AMIC1 isolated from effluent sample $RgrDP_3$ was *Klebsiella* spp. whereas AMIC2 and AMIC3 isolated from $SarDP_2$ and $SarDP_5$ were *Bacillus* spp.

In a study, El Hameed *et al.* (2015) achieved the similar results by isolating the fungi from phosphatic sources and found a negative correlation between isolates and metal concentrations. Selvi *et al.* (2012) also isolated and characterized HMT bacteria from tannery effluents and found that all the isolates i.e. *Escherichia coli*, *Bacillus* spp., *Pseudomonas* spp., *Flavobacterium* spp. and *Alcaligenes* spp. exhibited tolerance to heavy metals in the respective order; Pb> Cu> Zn> Cr> Hg. Similarly, Raja *et al.*, (2006) carried out a study for the isolation and characterization of metal tolerant *Pseudomonas aeruginosa* strain and found that isolates showed biosorption potential against all four tested metals i.e. Cd, Cr, Pb and Ni and the biosorption pattern was found as: Cr (30%) < Cd (50%) < Pb (65%) < Ni (93%).

Alboghobeish *et al.*, (2014) also isolated Nickel resistant bacteria (NiRB) from wastewater polluted with different industrial sources. Similarly Ahirwar *et al.* (2016) isolated and characterized heavy metal resistant bacteria from industrial affected soil and found that bacterial strains identified as *Pseudomonas vulgaris*, *Pseudomonas fluorescence* and *Bacillus cereus* were found to be the most efficient strains in terms of metal resistance.

It was concluded that three native bacterial strains i.e. AMIC1 (*Klebsiella spp.*), AMIC2 (*Bacillus spp.*) and AMIC2 (*Bacillus spp.*) exhibited pronounced tolerance to Ni and Co and also showed MMR. The biosorptive potential of these strains could be evaluated in future through different *in-vitro* analyses and may be a potential candidate to be utilized in future for the development of bioremediation agents to detoxify textile effluents at industrial surroundings within natural environments in Pakistan.

Table 1. Physico-Chemical properties of textile effluent water samples collected from in and around Faisalabad.

Parameters		pH	EC	DO	COD	BOD	TSS	TDS	TS
*NEQS limits		6-10	**NG	**NG	150	80	150	3500	**NG
Sr. No.	Sample Code	-	(µS/cm)			(mg/L)			
1	$KhrDP_1$	7.51	117.7	1.2	115	47	210	3740	3950
2	$SarDP_1$	7.86	119.4	1.19	203	82.2	180.3	3643	3823.3
3	$JhuDP_1$	8.66	125.2	1.55	118	46.6	155.66	3450.70	3606.36
4	$SatDP_1$	8.62	156.3	2.06	264	104.8	230.45	3980	4210.45
5	$RgrDP_1$	7.02	103.7	3.88	261	105.3	240	4100	4340
6	$SamDP_1$	7.03	104.9	3.76	257	103.4	237.65	3950	4187.65
7	$KhrDP_2$	7.55	127.6	2.78	224	88.6	160.9	3553	3713.9
8	$SarDP_2$	7.83	120.7	1.67	165	67	135.75	3350	3485.75
9	$JhuDP_2$	7.9	118.9	1.26	208	83.6	155	3290	3445

10	SatDP$_2$	7.95	159.7	3.67	267	107.4	140	2950	3090
11	RgrDP$_2$	8.65	125.2	1.58	120	49	130	3200	3330
12	SamDP$_2$	8.56	156.3	1.2	116	47.2	137	3370	3507
13	KhrDP$_3$	7.54	103.7	1.19	196	78.6	206	4205	4411
14	SarDP$_3$	7.39	104.9	1.55	110	44.8	195	3870	4065
15	JhuDP$_3$	8.02	127.6	2.06	280	112.6	125	2950	3075
16	SatDP$_3$	7.09	117.7	1.55	120	48	122	3149	3271
17	RgrDP$_3$	8.1	119.4	2.06	250	100.5	143	3308	3451
18	SamDP$_3$	8.23	127.6	3.88	270	108.6	147.7	3492	3639.7
19	KhrDP$_4$	7.44	120.7	3.76	254	101.8	215	4549	4764
20	SarDP$_4$	8.28	118.9	2.78	288	116	198.8	3980	4178.8
21	JhuDP$_4$	8.66	159.7	1.19	200	82.6	178	3750	3928
22	SatDP$_4$	7.51	127.3	1.55	170.8	75.8	166	3680	3846
23	RgrDP$_4$	7.86	115.8	2.06	183.6	78.2	235	4370	4605
24	SamDP$_4$	8.66	116.7	1.55	189.8	79.9	248	4567	4815
25	KhrDP$_5$	8.61	103.2	2.06	168.5	67.3	149	3482	3631
26	SarDP$_5$	7.89	114.3	2.29	105.9	40	142	3370	3512
27	JhuDP$_5$	7.58	149.5	3.5	115.3	45.2	125	2765	2890
28	SatDP$_5$	7.23	123.8	3.57	115.7	44.9	128	2950	3078
29	RgrDP$_5$	8.87	152.7	2.88	116.78	46.77	220	4140	4360
30	SamDP$_5$	8.9	134.5	2.34	110.47	42.3	184	3942	4126

*NEQS limits: National Environmental Quality Standards for wastewater discharge set by Government of Pakistan
**NG: Not given in the NEQS list.

Table 2. Heavy metal analysis in industrial effluents through atomic absorption spectrophotometer (AAS).

Sr. No.	Sample Code & No.	Concentration of heavy metals in ppm				
		Ni	Co	Cr	Pb	Zn
1	KhrDP$_1$	0.24	0.22	0.05	0.40	0.20
2	SarDP$_1$	0.27	0.28	0.11	0.16	0.32
3	JhuDP$_1$	0.24	0.14	0.05	0.00	0.10
4	SatDP$_1$	0.19	0.00	0.00	0.00	0.18
5	RgrDP$_1$	0.24	0.19	0.04	0.008	0.48
6	SamDP$_1$	0.17	0.25	0.13	0.6	0.37
7	KhrDP$_2$	0.10	0.21	0.003	0.8	0.25
8	SarDP$_2$	0.21	0.13	0.10	0.1	0.31
9	JhuDP$_2$	0.25	0.18	0.14	0.12	0.14
10	SatDP$_2$	0.20	0.20	0.06	0.00	0.30
11	RgrDP$_2$	0.19	0.21	0.08	0.00	0.27
12	SamDP$_2$	0.21	0.00	0.16	0.00	0.40
13	KhrDP$_3$	0.23	0.22	0.1	0.00	0.42
14	SarDP$_3$	0.17	0.19	0.05	0.004	0.38
15	JhuDP$_3$	0.16	0.22	0.09	0.007	0.35
16	SatDP$_3$	0.22	0.19	0.10	0.14	0.28
17	RgrDP$_3$	0.07	0.13	0.002	0.18	0.13
18	SamDP$_3$	0.14	0.20	0.00	0.16	0.10
19	KhrDP$_4$	0.17	0.24	0.00	0.002	0.20
20	SarDP$_4$	0.27	0.16	0.10	0.00	0.30
21	SatDP$_4$	0.19	0.23	0.07	0.00	0.15
22	JhuDP$_4$	0.15	0.14	0.09	0.10	0.24
23	RgrDP$_4$	0.24	0.27	0.15	0.00	0.09
24	SamDP$_4$	0.24	0.00	0.05	0.19	0.12
25	KhrDP$_5$	0.12	0.19	0.00	0.00	0.08
26	SarDP$_5$	0.23	0.17	0.00	0.10	0.37
27	SatDP$_5$	0.25	0.18	0.14	0.005	0.39
28	JhuDP$_5$	0.23	0.19	0.12	0.15	0.27
29	RgrDP$_5$	0.10	0.24	0.17	0.12	0.25
30	SamDP$_5$	0.24	0.19	0.00	0.00	0.10

Table 3. Heavy metal tolerant bacterial count obtained in textile effluent water samples

Sr. No.	Sample Code & No.	CFU/mL ($\times10^5$) on Nutrient agar	CFU/mL on Nutrient agar with Ni	(%) age of Ni tolerant bacteria ($\times10^{-5}$)	CFU/mL on Nutrient agar with Co	(%) age of Co tolerant bacteria ($\times10^{-5}$)
1	$KhrDP_1$	3.4	15	4.41	20	5.88
2	$SarDP_1$	8	20	0.025	12	1.5
3	$JhuDP_1$	10	-	-	06	0.6
4	$SatDP_1$	92	-	-	-	-
5	$RgrDP_1$	101	-	-	-	-
6	$SamDP_1$	8.1	-	-	-	-
7	$KhrDP_2$	6.5	32	4.92	10	1.538
8	$SarDP_2$	3.4	25	7.35	38	11.17
9	$JhuDP_2$	22	-	-	20	0.90
10	$SatDP_2$	109	05	0.045	10	0.091
11	$RgrDP_2$	63	-	-	10	0.15
12	$SamDP_2$	5.3	-	-	10	1.88
13	$KhrDP_3$	8.9	45	5.05	40	4.49
14	$SarDP_3$	4.6	-	-	18	3.91
15	$JhuDP_3$	78	-	-	-	-
16	$SatDP_3$	7.3	-	-	-	-
17	$RgrDP_3$	86	50	0.581	40	0.465
18	$SamDP_3$	52	-	-	-	-
19	$KhrDP_4$	9.7	-	-	-	-
20	$SarDP_4$	13.5	26	1.92	20	1.48
21	$JhuDP_4$	11.7	-	-	20	0.170
22	$SatDP_4$	18	-	-	-	-
23	$RgrDP_4$	75	10	0.133	15	0.2
24	$SamDP_4$	7.1	28	3.94	32	4.5
25	$KhrDP_5$	4.9	23	4.69	30	6.122
26	$SarDP_5$	2.7	10	3.70	15	5.555
27	$JhuDP_5$	1.9	65	34.21	70	36.842
28	$SatDP_5$	7.2	30	4.16	25	3.47
29	$RgrDP_5$	9.2	40	4.34	-	-
30	$SamDP_5$	2.8	-	-	-	-

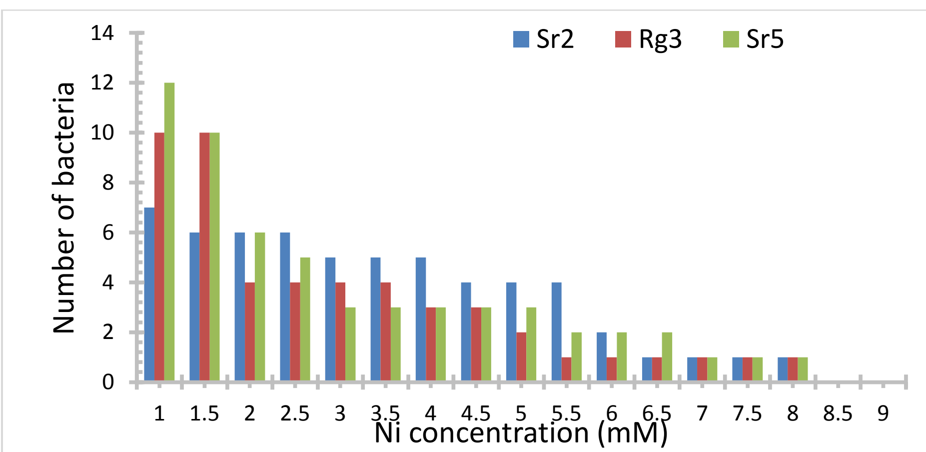

Figure 1. Graph showing effect of Ni concentration on selected tolerant bacterial strains

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