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Investigation of heavy metal resistance in some bacterial strains isolated from industrial soils

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Abstract: Soil samples were collected from an industrial area in Kazanlı, Mersin, Turkey. Six heavy metals, Cr, Cu, Ni, Co, Cd, and Zn, were tested. ICP-AES analyses were carried out to detect the heavy metal levels of the soil samples. The analysis of test samples revealed high levels of Ni and Cr. A total of 272 *Pseudomonas* spp. and 161 *Bacillus* spp. strains were isolated from soils from 4 different sampling locations. The maximum tolerable metal concentrations (MTCs) of Cr, Cu, Ni, Co, Cd, and Zn for each isolate were determined. Resistance to Cr was found in 73.9% of the bacterial strains isolated from soil samples, whereas 26% of the isolates exhibited resistance to Ni, 18.4% to Zn, 11.5% to Cd, 9.2% to Co, and 7.3% to Cu. Observed maximum MTCs were 5 mM for Zn and Cu, 3 mM for Ni, and 2 mM for Cr. Plasmids of sizes 1.8, 2.1, and 28 kb were detected in most *Pseudomonas* spp. strains with resistance to Cu, Cr, Zn, and Ni.

Key words: Heavy metal resistance, *Pseudomonas*, *Bacillus*, industrial soils

Endüstriyel topraklardan izole edilen bazı bakterilerde ağır metal dirençliliğinin araştırılması

Özet: Bu çalışmada, Kazanlı / Mersin – Türkiye, endüstri bölgesinden toprak örnekleri toplanmıştır. Cr, Cu, Ni, Co, Cd, and Zn olmak üzere altı ağır metal test edilmiştir. Toprak örneklerindeki ağır metal seviyesini belirlemek için ICP-AES analizleri yapılmıştır. Analizler test örneklerinde yüksek düzeyde Ni ve Cr bulunduğunu göstermiştir. Dört farklı örnekleme bölgesinden toplam 272 adet *Pseudomonas* spp. ve 161 *Bacillus* spp. suşu izole edilmiştir. Her bir izolatın Cr, Cu, Ni, Co, Cd ve Zn için MTC değerleri belirlenmiştir. Toprak örneklerinden izole edilen bakterilerin % 73,9'u Cr'a karşı direnç gösterirken, % 26'sı Ni, % 18,4'ü Zn, % 11,5'i Cd, % 9,2'si Co ve % 7,3'ü Cu'a karşı dirençli bulunmuştur. En yüksek MTC'ler, Zn ve Cu için 5 mM, Ni için 3 mM, Cr için 2 mM bulunmuştur. Cu, Cr, Zn ve Ni'e direnç gösteren *Pseudomonas* spp. suşlarında 1,8, 2,1 ve 28 kb büyüklüğünde ortak plazmidler saptanmıştır.

Anahtar sözcükler: Ağır metal dirençliliği, *Pseudomonas*, *Bacillus*, endüstriyel topraklar

Introduction

Since the industrial revolution, humans have been unwittingly participating in a massive experiment in natural selection. By use of chemical compounds, people have altered the selective environment of all organisms. This has had a particularly strong effect on the organisms with the shortest generation times, such as bacteria. The widespread use and production of heavy metals, antibiotics, and organic compounds has altered the bacterial environment and selection for certain genotypes, including resistance to heavy metals and antibiotics (1).

Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, they tend to accumulate in soils and sediments (2). While some heavy metals are required in trace amounts as nutrients, they become strongly inhibitory for microorganisms at relatively low concentrations (3,4). Toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than do essential metals (5). Toxicity results from alterations in the conformational structure of nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance (5).

To survive under metal-stressed conditions, bacteria have evolved several types of adaptation mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and the reduction of the heavy metal ions to a less toxic state (2). Bacteria have adapted to heavy metals through a variety of chromosomal-, transposon-, and plasmid-mediated resistance systems (5).

The purpose of this study was to determine the heavy metal levels of contaminated industrial soil in the vicinity of Kazanlı, Mersin, Turkey, and also to determine the maximum tolerable metal concentration (MTC) for some bacteria isolated from the same region. The plasmid contents of metal-resistant bacteria were also investigated.

Materials and methods

Sampling location

Soils samples were collected from four locations in Kazanlı, Mersin, Turkey (Figure 1). The samples were collected in sterilized flasks. Each location was sampled once. Soil samples were stored at 4 °C for further study.

Heavy metal analysis

The levels of Cr, Cu, Ni, Co, Cd, and Zn in soil samples were analyzed with a Varian® Liberty Series II inductively coupled plasma atomic emission spectrometer (ICP-AES). Element analyses were carried out by using the nitric acid digestion method described by Sangün and Özdilek (6). The blank was prepared in the same manner as the samples. Cr, Cu, Ni, Co, Cd, and Zn standards were prepared from High Purity® multistandards for ICP-AES. The values of the elements and blank obtained from the ICP-AES were calculated, and values were expressed as mg/kg of dry mass.

Isolation and identification of *Pseudomonas* and *Bacillus* spp. from soil

From each soil sample, 1 g of soil was suspended in 10 mL of sterile distilled water and vortexed for 1 min. Soil suspensions were then centrifuged at 2000 rpm for 10 min and 0.1 mL of supernatants were diluted and plated onto GSP agar for the selection of genus *Pseudomonas*. GSP agar had the following composition: soluble starch, 20 g/L; Na-L-glutamate, 10 g/L; $MgSO_4$, 0.5 g/L; KH_2PO_4, 2 g/L; phenol red, 0.6 g/L; agar, 12 g/L; pH 7.2 (7). After the incubation at 30 °C for 24 h, colonies that showed a red precipitation halo were selected for identification and further characterization. To select the gram-positive spore-forming *Bacillus* spp., the soil sample was suspended in sterile distilled water and vortexed and centrifuged as described above. Supernatant fluids were transferred to a glass tube with a screw cup and pasteurized at 80 °C for 10 min in a water bath, and then 0.1 mL aliquots of appropriate dilution were placed on nutrient agar plates. Plates were incubated at 30 °C for 24 h. The isolates were identified on the basis of morphological, trophic, and biochemical traits, according to *Bergey's Manual of Systematic Bacteriology* (8).

Determination of MTCs

For testing heavy metal resistance, the metals Cr, Cu, Ni, Co, Cd, and Zn, used as $K_2Cr_2O_7$, $CuSO_4$, $NiCl_2$, $CoCl_2$, $CdCl_2$, and $ZnCl_2$, were added to sterilized nutrient agar medium in concentrations varying from 0.1 to 5 mM. Plates were then spot inoculated and incubated at 30 °C for 2 days (9).

The MTC of heavy metals was designated as the highest concentration of heavy metal that allowed growth after 2 days (10). The sensitive and resistant range of MTC was described on the basis of the standard strain *Escherichia coli* ATCC 11230. A concentration above the MTC value of the control strain was accepted as resistant range. All of the experiments were replicated 3 times.

Plasmid isolation and electrophoresis

Plasmid DNAs were isolated from the bacteria by a minipreparation method (11). The isolated plasmids were characterized by agarose gel electrophoresis according to the standard procedure of Sambrook and Russel (11). Agarose gel electrophoresis was performed through horizontal slab gel of 0.8% agarose submerged in Tris-HCl, boric acid, and EDTA (TBE) running buffer at 70 V for 2 h. DNA bands were then stained with ethidium bromide for 15 min and visualized on a UV transilluminator. The molecular sizes of the plasmids were determined by comparison with a supercoil DNA size marker (Sigma D5292) using a gel documentation system (UVP).

Results

ICP-AES analysis of industrial soils

ICP-AES was used for determination of the heavy metal contents of the contaminated soils. Soil samples were taken from 4 different locations in the vicinity of a heavy metal industrial area in Kazanlı, Mersin, Turkey (Figure 1). The heavy metal concentrations of the soil samples from these locations are shown in Table 1. Forest soil from a different geographical region was used as the control group. Table 1 gives a clear picture of the contamination of soils treated with industrial applications by a variety of heavy metals. The concentrations of Cr, Cu, Ni, Co, and Zn in the soils were high as compared with the values of the control soil. However, the concentration of Cd in the soils was found to be less when compared with the control soil. Cr was found to be 249.2-565.9 mg/kg, Cu 25.7-41.5, Ni 95.5-333.8, Co 16.3-22.8, and Zn 38.1-65.9 at different sampling sites (Table 1).

Chromium toxicity

One of the most abundant heavy metals in the whole study area was Cr (Table 1). For all strains, the resistance was found to be in the range of 0.5-2.0 mM of $K_2Cr_2O_7$. *Pseudomonas* spp. strains showed a high frequency of resistance, 95.1%, for Cr in location B2 (Table 2). This frequency was calculated to be 97.3% in location B3 for *Bacillus* spp. strains (Table 3).

Copper toxicity

The resistance to Cu was found to be in the range of 3.0-5.0 mM of $CuSO_4$. The Cu-resistant strains were only found in location B2 (Table 2). In this location, the frequency of Cu resistance was calculated to be 26% in *Pseudomonas* spp. strains. Interestingly, no Cu-resistant *Bacillus* spp. strains were found in any locations.

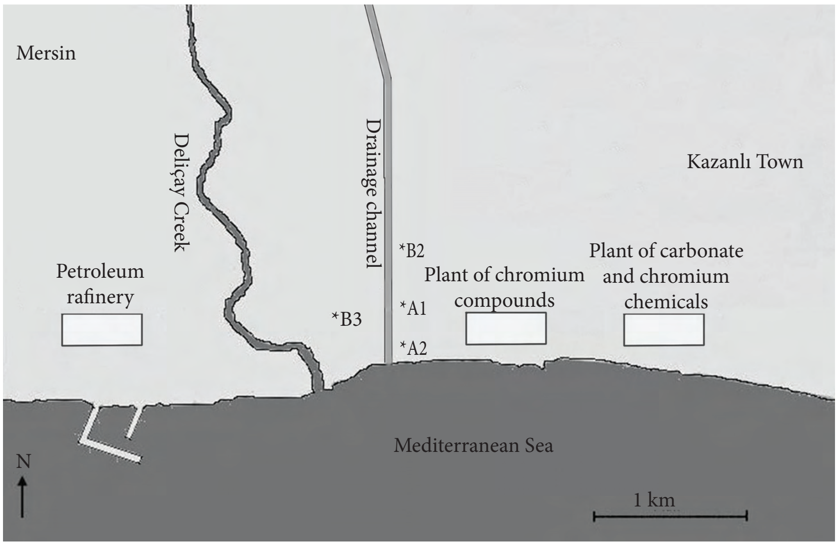

Figure 1. Sampling locations. Soils samples were collected from 4 locations in Kazanlı, Mersin, Turkey. These locations were numbered A1, B1, B2, and B3.

Table 1. Heavy metal concentrations in soil samples from different locations.

Location	Soil pH	Cd	Co	Cr	Cu	Ni	Zn
A1	8.3	0.2	16.3	565.9	25.7	95.5	38.1
B1	7.8	0.8	19.9	431.0	31.4	255.4	65.9
B2	7.5	0.8	20.3	276.8	41.5	272.5	63.1
B3	8.6	0.1	22.8	249.2	36.7	333.8	47.5
Control*	7.3	1.0	8.1	107.4	13.2	56.2	26.0

* Forest soil sample was used as the control group.
The values of the elements were calculated as mg/kg dry mass.

Table 2. Incidence of metal resistance in bacterial isolates from location B2.

Metal	MTC Control Str.	Sens. Rang.	Tot. No. Sens. Str.	Res. Rang.	Tot. No. Res. Str.	Freq. Res.
K_2Cr_2O7	0.4 mM	<0.1-0.4 mM	6 (○)	0.5-2.0 mM	117 (○)	95.1%
			3 (●)		38 (●)	92.6%
$CdCl_2$	0.4 mM	<0.1-0.4 mM	123 (○)	0.5-0.8 mM	0 (○)	0%
			41 (●)		0 (●)	0%
$ZnCl_2$	1.0 mM	<0.1-1.0 mM	70 (○)	2.0-5.0 mM	53 (○)	43%
			38 (●)		3 (●)	7.3%
$NiCl_2$	1.0 mM	<0.1-1.0 mM	106 (○)	2.0 mM	17 (○)	13.8%
			41 (●)		0 (●)	0%
$CoCl_2$	0.4 mM	<0.1-0.4 mM	114 (○)	0.5-0.8 mM	9 (○)	7.3%
			36 (●)		5 (●)	12.1%
$CuCl_2$	2.0 mM	<0.1-2.0 mM	91 (○)	3.0-5.0 mM	32 (○)	26%
			41 (●)		0 (●)	0%

MTC Control Str.: Maximum tolerable concentration of control (*E. coli* ATCC 11230) strain
Tot. No. Sens. Str.: Total number of sensitive strains
Res. Rang.: Resistant range
Sens. Rang.: Sensitive range
Tot. No. Res. Str.: Total number of resistant strains
Freq. Res.: Frequency of resistance
(○): *Pseudomonas* spp. (total 123 strains)
(●): *Bacillus* spp. (total 41 strains)

Table 3. Incidence of metal resistance in bacterial isolates from location B3.

Metal	MTC Control Str.	Sens. Rang.	Tot. No. Sens. Str.	Res. Rang.	Tot. No. Res. Str.	Freq. Res.
K_2Cr_2O7	0.4 mM	<0.1-0.4 mM	25 (○)	0.5-2.0 mM	25 (○)	50%
			1 (●)		37 (●)	97.3%
$CdCl_2$	0.4 mM	<0.1-0.4 mM	44 (○)	0.5-0.7 mM	6 (○)	12%
			38 (●)		0 (●)	0%
$NiCl_2$	1.0 mM	<0.1-1.0 mM	42 (○)	2.0 mM	8 (○)	16%
			37 (●)		1 (●)	2.6%
$NiCl_2$	1.0 mM	<0.1-1.0 mM	50 (○)	-	0 (○)	0%
			38 (●)		0 (●)	0%
$CoCl_2$	0.4 mM	<0.1-0.4 mM	36 (○)	0.5-0.8 mM	14 (○)	28%
			37 (●)		1 (●)	2.6%
$CuCl_2$	2.0 mM	<0.1-2.0 mM	50 (○)	-	0 (○)	0%
			38 (●)		0 (●)	0%

MTC Control Str.: Maximum tolerable concentration of control (*E. coli* ATCC 11230) strain
Tot. No. Sens. Str.: Total number of sensitive strains
Res. Rang.: Resistant range
Sens. Rang.: Sensitive range
Tot. No. Res. Str.: Total number of resistant strains
Freq. Res.: Frequency of resistance
(○): *Pseudomonas* spp. (total 50 strains)
(●): *Bacillus* spp. (total 38 strains)

Nickel toxicity

The most abundant heavy metal in the soils of all locations was nickel, as compared with the values of the control soil (Table 1). The resistance was found to be in the range of 2.0-4.0 mM of $NiCl_2$ and 76% of *Pseudomonas* spp. strains were resistant to Ni in location B1 (Table 4). This resistance was calculated to be 7.5% in location A1 for *Bacillus* spp. strains. No Ni-resistant bacteria were found in B3.

Cobalt toxicity

Cobalt was found about 2.5 times more in all locations as compared with the control soil (Table 1). The resistance to Co was found to be in the range of 0.5-0.8 mM of $CoCl_2$ and 28% of *Pseudomonas* spp. strains isolated from location B3 were resistant to Co (Table 3). For *Bacillus* spp. strains, the frequency of resistance was found to be only 12.1% in location B2. No Co-resistant *Bacillus* spp. strains were found in location A1 (Table 5).

Zinc toxicity

The concentrations of Zn were approximately 1.5-2 times higher in all locations than the control soil (Table 1). For all strains, the resistance was found to be in the range of 2.0-5.0 mM of $ZnCl_2$; 43% of *Pseudomonas* spp. strains were resistant to Zn in location B2 (Table 2). This frequency was calculated to be 9.5% in location B1 for *Bacillus* spp. strains (Table 4). No Zn-resistant bacterial strains were found in location A1.

Cadmium toxicity

The resistance to Cd was found to be in the range of 0.5-1.0 mM of $CdCl_2$. *Pseudomonas* spp. strains showed a maximum frequency of resistance of 12% for this metal in location B3 (Table 3). *Bacillus* spp. strains were found to be sensitive to Cd in all locations.

Plasmid profiles of some bacterial strains from contaminated soils

To determine the plasmid contents in bacteria from the soil samples, plasmid DNAs were isolated from the bacterial strains that showed the highest MTC values for each heavy metal. The plasmid incidence of these strains is shown in Table 6. According to the electrophoretic separation, 1.8, 2.1, and 28 kb common plasmids were detected in some *Pseudomonas* spp. strains with resistance to Cu, Cr, Zn, and Ni, but no plasmids were detected in *Bacillus* spp. strains.

Table 4. Incidence of metal resistance in bacterial isolates from location B1.

Metal	MTC Control Str.	Sens. Rang.	Tot. No. Sens. Str.	Res. Rang.	Tot. No. Res. Str.	Freq. Res.
$K_2Cr_2O_7$	0.4 mM	<0.1-0.4 mM	6 (○)	0.5-2.0 mM	44 (○)	88%
			4 (●)		38 (●)	90.4%
$CdCl_2$	0.4 mM	<0.1-0.4 mM	48 (○)	0.5-0.8 mM	2 (○)	4.1%
			42 (●)		0 (●)	0%
$ZnCl_2$	1.0 mM	<0.1-1.0 mM	39 (○)	2.0-3.0 mM	11 (○)	22%
			38 (●)		4 (●)	9.5%
$NiCl_2$	1.0 mM	<0.1-1.0 mM	12 (○)	2.0-3.0 mM	38 (○)	76%
			42 (●)		0 (●)	0%
$CoCl_2$	0.4 mM	<0.1-0.4 mM	42 (○)	0.5-0.6 mM	8 (○)	16%
			40 (●)		2 (●)	4.7%
$CuCl_2$	2.0 mM	<0.1-2.0 mM	50 (○)	-	0 (○)	0%
			42 (●)		0 (●)	0%

MTC Control Str.: Maximum tolerable concentration of control (*E. coli* ATCC 11230) strain
Tot. No. Sens. Str.: Total number of sensitive strains   Res. Rang.: Resistant range   Sens. Rang.: Sensitive range
Tot. No. Res. Str.: Total number of resistant strains   Freq. Res.: Frequency of resistance
(○): *Pseudomonas* spp. (total 50 strains)   (●): *Bacillus* spp. (total 42 strains)

Table 5. Incidence of metal resistance in bacterial isolates from location A1.

Metal	MTC Control Str.	Sens. Rang.	Tot. No. Sens. Str.	Res. Rang.	Tot. No. Res. Str.	Freq. Res.
$K_2Cr_2O_7$	0.4 mM	<0.1-0.4 mM	48 (○)	0.5-0.6 mM	1(○)	2%
			20 (●)		20 (●)	50%
$CdCl_2$	0.4 mM	<0.1-0.4 mM	47 (○)	0.5-1 mM	2 (○)	4%
			40 (●)		0 (●)	0%
$ZnCl_2$	1.0 mM	<0.1-1.0 mM	49 (○)	-	0 (○)	0%
			40 (●)		0 (●)	0%
$NiCl_2$	1.0 mM	<0.1-1.0 mM	36 (○)	2.0-4.0 mM	13 (○)	26.5%
			37 (●)		3 (●)	7.5%
$CoCl_2$	0.4 mM	<0.1-0.4 mM	48 (○)	0.5-0.7 mM	1 (○)	2%
			40 (●)		0 (●)	0%
$CuCl_2$	2.0 mM	<0.1-2.0 mM	49 (○)	-	0 (○)	0%
			40 (●)		0 (●)	0%

MTC Control Str.: Maximum tolerable concentration of control (*E. coli* ATCC 11230) strain
Tot. No. Sens. Str.: Total number of sensitive strains Res. Rang.: Resistant range Sens. Rang.: Sensitive range
Tot. No. Res. Str.: Total number of resistant strains Freq. Res.: Frequency of resistance
(○): *Pseudomonas* spp. (total 49 strains) (●): *Bacillus* spp. (total 40 strains)

Table 6. Plasmid contents of the metal-resistant *Pseudomonas* spp. strains.

Bacterial strain	MTC	Number of plasmids	Plasmid sizes (kb)
A1P41	3 mM $NiCl_2$	2	1.8, 2.1
B2P55	5 mM $ZnCl_2$	2	1.8, 2.1
B2P77	5 mM $ZnCl_2$	2	1.8, 2.1
B2P99	5 mM $CuCl_2$	2	1.8, 2.1
B2P115	5 mM $CuCl_2$	2	1.8, 2.1
B2P117	5 mM $CuCl_2$	2	1.8, 2.1
B2P123	5 mM $CuCl_2$	2	1.8, 2.1
B2P110	5 mM $CuCl_2$	3	1.8, 2.1, 28
B2P60	2 mM $K_2Cr_2O_7$	2	1.8, 2.1

Discussion

The ICP-AES analysis of the heavy metals in 4 different soils is summarized in Table 1. Our findings indicate that high levels of Ni, Cr, Cu, Co, and Zn were present in the industrial soils as compared with the levels of those metals in forest soil. However, except for Ni and Cr, these values were below the limits of the environmental law of Turkey. Chromium should not exceed 100 mg/kg (pH > 6) and nickel should not exceed 75 mg/kg (pH 6) as acceptable and maximum values in soil, according to the Turkish soil pollution control regulation. The concentrations of Cr and Ni were found to be quite high in all locations. The concentration of Cr was 565.9 mg/kg in location A1. This location is very close to the chromium storage zone of the chromium compounds factory (Figure 1). The concentration of Ni was 333.8 mg/kg in location B3. This location is near a petroleum refinery in the industrial area (Figure 1). We found that the Cr and Ni values in these locations were 5 times higher than the legal limits. Heavy metals could be spread to these locations in different probable ways. For example, high concentrations of Cr and Ni might be due to the application of waste waters from factories to soil for irrigation purposes, because the drainage channels are so close to the open storage zone of the solid wastes of the chromium factory. Second, the heavy metals might be transferred from the open metallic waste storage zone to the soils by means of the wind. Several environments have a high level of chromium because it spreads in the soil and water through industrial activity, such as steel production, wood preservation, and leather tanning. Several agronomic practices, including the use of organic biomass, like sewage sludge or fertilizers based on hydrolyzed leather that contains varying degrees of chromium, also contribute to soil contamination (12). Some studies have been done to show heavy metal contamination in industrial soils. Malik et al. (13) used atomic absorption spectrophotometry to detect heavy metal contents of agricultural and industrial soils in India. It was reported that high levels of Fe, Cr, Zn, Cu, Pb, and Ni were present in the samples, compared with agricultural soil. However, our results indicate that the Cr and Ni concentrations in industrial soils in this area are dramatically higher than the values of the soils that were presented in that paper.

A total of 272 *Pseudomonas* spp. and 161 *Bacillus* spp. were isolated from industrial soils in 4 different sampling locations (Figure 1). These bacteria were tested for their resistance to several different heavy metals: Cr, Cu, Ni, Co, Cd, and Zn. The MTCs were used to determine the metal tolerance of bacteria isolated from all locations. However, it is well known that there are no currently acceptable concentrations of metal ions that can be used to distinguish metal-resistant and metal-sensitive bacteria (14). Thus, the sensitive and resistant range of MTC has been described on the basis of the standard strain. In the present study, 73.9% of the total bacteria isolated from soil samples harbored resistance to Cr, whereas 26% of the isolates exhibited resistance to Ni, 18.4% to Zn, 11.5% to Cd, 9.2% to Co, and 7.3% to Cu. A maximum MTC of 5 mM for Zn and Cu, 3 mM for Ni, and 2 mM for Cr was observed. *Pseudomonas* spp. strains showed a maximum frequency of resistance of 95.1% for Cr in location B2. The Cr concentration was found to be 276.8 mg/kg in this location. This Cr level was more tolerable for bacteria than the levels found in locations A1 and B1. The frequency of resistance to Cr in *Pseudomonas* spp. strains was observed to be very low in location A1. The Cr concentration was calculated to be 565.9 mg/kg in this location. It is thought that high Cr concentrations in this soil sample could have selected for Cr resistance in bacteria. In general, *Bacillus* spp. strains showed more tolerance to Cr than *Pseudomonas* spp. in all locations. It was reported that gram-positive bacteria were more Cr(VI)-tolerant than gram-negative bacteria (12). This was reported by other authors who widely documented the high presence of tolerant/resistant gram-positive bacteria in soil polluted with heavy metals (15,16). In the current study, the resistance was found to be in the range of 0.5-2.0 mM of $K_2Cr_2O_7$ for all bacteria. *Pseudomonas* spp. strains isolated from the soil of a municipal dump were found to be least tolerant to Cr, with MTCs between 1 and 1.7 mM, in our previous study (17). Malik et al. (13) isolated a total of 70 bacterial isolates from industrial and agricultural soils and tested for their resistance against several heavy metals. They reported that a higher percentage of the industrial isolates were resistant to different metals, compared with the agricultural isolates. They indicated that 88.8% of isolates from industrial soil were found to harbor resistance to Ni, 80% to Cr, 82.8% Zn, and 71.4% to Cd (13). In all locations, nickel-resistant bacteria were found to be very few, although there was a high nickel concentration in the soils. *Pseudomonas* spp. strains were able to reach a frequency of resistance of 76% for Ni in only location B1. No Ni-resistant bacteria could be found in B3. The Ni concentration in this location was calculated to be 333.8 mg/kg. It is possible that the bacterial strains that were tested could not tolerate such high Ni levels. Both *Pseudomonas* spp. and *Bacillus* spp. strains were shown to be less tolerant to Co and Cu, but Zn-tolerant *Pseudomonas* spp. strains were more common than *Bacillus* spp., in general. Heavy metal-resistant microorganisms are thought to naturally occur, primarily in metal-impacted soils. Roane and Kellogg found resistant *Pseudomonas* isolates (cadmium MTC of 1.2 mM) in heavy metal-contaminated soil (18). It has also been reported that nickel-resistant bacteria, *Alcaligenes xylosoxidans* 31A, isolated from a galvanization tank, tolerated relatively high concentrations of nickel (40 mM), cobalt (20 mM), zinc (10 mM), cadmium (1 mM), and copper (1 mM) (10).

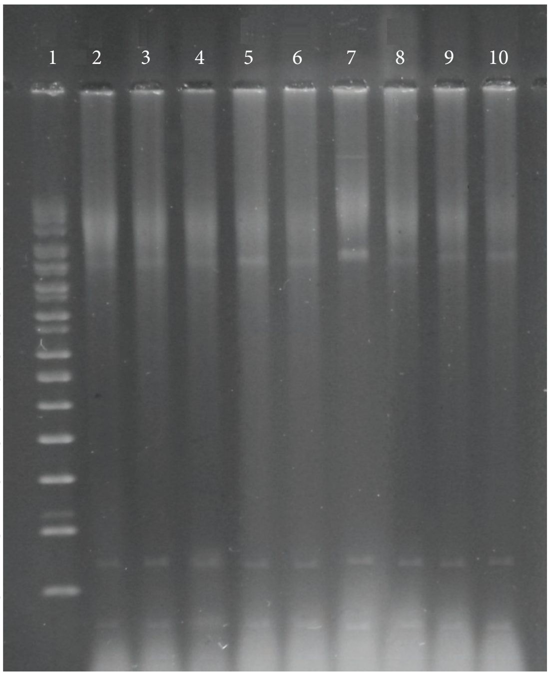

Figure 2. Electrophoretic separation profile of plasmid DNAs isolated from some of the most metal-resistant *Pseudomonas* spp. strains. Line 1: DNA ladder (11 supercoiled fragments), Line 2: plasmids from A1P41, Line 3: B2P55, Line 4: B2P60, Line 5: B2P77, Line 6: B2P99, Line 7: B2P110, Line 8: B2P115, Line 9: B2P117, Line 10: B2P123.

Plasmid DNAs were extracted and purified by miniprep isolation from the most resistant bacterial strains for each heavy metal. After agarose gel electrophoresis, 1.8, 2.1, and 28 kb common plasmids were detected in most *Pseudomonas* spp. strains with resistance to Cu, Cr, Zn, and Ni (Figure 2). Interestingly, no plasmids were detected in *Bacillus* spp. strains. Viti et al. (2003) (19) reported that most of their isolates from Cr-contaminated soil did not have plasmids. Pacheco et al. found that 100% of the resistant *Pseudomonas* strains isolated from heavy metal-polluted zones were carrying plasmids (20). Several authors suggested that the incidence of plasmid-bearing bacterial strains is greater in polluted sites than in unpolluted zones (14,21). It was found that Cu, Ni, Cd, and Cr-resistant *Pseudomonas* WP19 strains had 4 plasmids of approximately 20.8, 19.6, 8, and 4.7 kb in our previous study. Transformation and curing results suggested that nickel and copper resistance were conferred by plasmid DNA, while cadmium and chromium resistance seemed to be encoded by genes of the bacterial chromosome (17).

Conclusions

Our findings showed that heavy metal resistance among bacteria is not generally widespread in our study area, but the potential resistance to chromium was found in bacterial strains. It is thought that this resistance may be a result of long term chromium pollution in this zone.

These and future studies should provide some insight into heavy metal resistance in bacteria in industrially contaminated soil ecosystems and may demonstrate its utility in detecting environmental pollution by heavy metals.

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