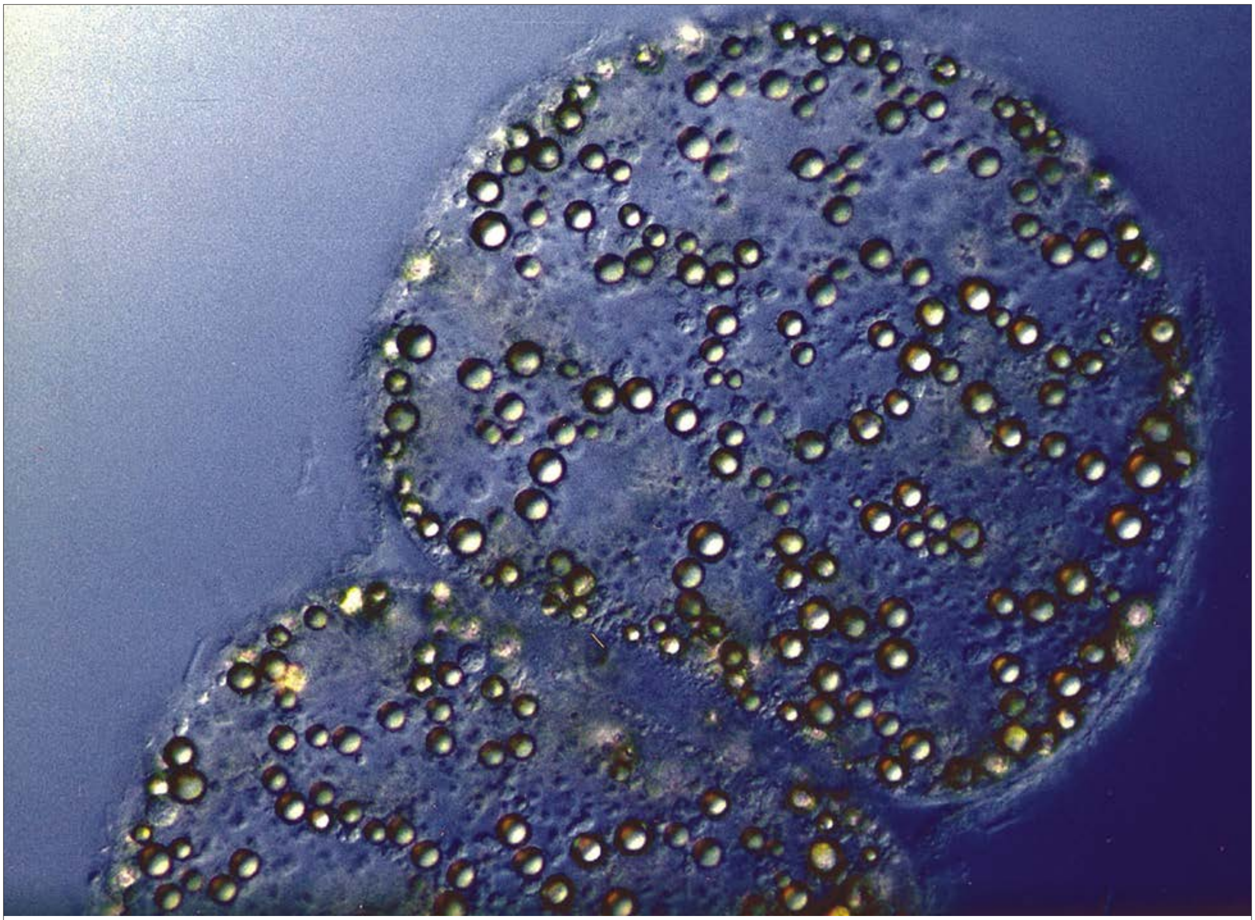

Microbiology
Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 4 (1): 12-21, 2014
ISSN 2153-0696 / DOI: 10.3923/mj.2014.12.21

Prevalence of Heavy Metal and Antibiotic Resistance in Bacterial Isolates from Metal Polluted Soils

Munees Ahemad and Abdul Malik
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, U.P., India

Corresponding Author: Munees Ahemad, Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, U.P., India Tel: +91-571-2702945

ABSTRACT

Owing to continual industrialization and exploitation of metal-containing agricultural products in current farming practices, environmental pollution is burgeoning with the release of toxic metal species and antibiotic products and concomitantly affecting both soil health and water quality. Therefore, this study was undertaken to determine the level of heavy metal and antibiotic resistance patterns in bacterial isolates from agricultural soils irrigated with metal polluted wastewater. A total of 34 bacteria isolates were recovered from soils and characterized as pseudomonads (67%), bacilli (15%) and staphylococci (18%) following standard methods. Further, resistance of bacterial isolates to heavy metals and antibiotics was determined by the plate dilution and disc diffusion methods, respectively. This study showed that 14.7% isolates were resistant to Hg^{2+}, 2.9%-Cd^{2+}, 91.2%-Cu^{2+}, 79.5%-Zn^{2+}, 91.2%-Ni^{2+}, 97%-Pb^{2+}, 94.1%-Cr^{3+} and 79.4%-Cr^{6+}. Furthermore, all bacterial isolates showed resistance to one to eight antibiotics in different combinations and more than 80% isolates exhibited resistance to neomycin, cloxacillin and amoxicillin. Bacterial isolates possessing co-resistance to the maximum number of heavy metals as well as exhibiting multiple antibiotic resistance patterns were found to harbor plasmid DNA. The results of this study inferred that the agricultural soil irrigated with metal polluted wastewater is a rich source of resistant bacteria to heavy metals and antibiotics and bacteria isolated from these sites have substantial resistance to both heavy metals and antibiotics. Moreover, these sites may be exploited to isolate the multiple metal and antibiotic resistant bacteria of environmental significance.

Key words: Antibiotic resistance, metal resistance, plasmid DNA, soil, bacteria, wastewater

INTRODUCTION

Various industrial and agricultural processes and anthropogenic activities like, smelting of metalliferous surface finishing industry, fertilizer and pesticide industry, sewage sludge, energy and fuel production, mining, agriculture, leather finishing, metallurgy, electroplating, faulty waste disposal, electrolysis, electro-osmosis, photography, electric appliance manufacturing have directly or indirectly release toxic heavy metals enormously into air, water and soils with a subsequent hazardous impacts on both ecological and human health (Kotrba *et al.*, 2009; Wang and Chen, 2006). The metal concentration accumulated in soil is dependent upon the level of industrial discharge laden with metal species, the transportation of metals from the source to the disposing site and the retention of the metal once it is reached into the soils (Matyar *et al.*, 2010; Rani and

Goel, 2009). In fact, heavy metals cannot be degraded chemically or biologically and are eventually indestructible. Therefore, they are not easily eliminated from the polluted soils (Ahemad, 2012). Although, some of the heavy metals are required by organisms at low concentration and are essential for various biochemical reactions in microbial cell metabolism (Adriano, 2001). For example, zinc is the component of a variety of metalloenzymes or it may act as cofactor for several enzymes (dehydrogenases, proteinases, peptidases, oxidase) (Hewitt, 1983). Besides, zinc is also essential to mediate the biochemical reactions related to various sugars and protein metabolism (Shier, 1994). In the same way, many enzymes catalyzing the carbohydrate biosynthesis, energy releasing processes and nitrogen metabolism require copper in trace amount as co-factor (Kabata-Pendias and Pendias, 2001). However, the elevated concentration of such metals above threshold levels in soils negatively affects the microbial communities belonging to several genera both quantitatively and qualitatively (Wani *et al.*, 2008; Pajuelo *et al.*, 2008; Rajkumar *et al.*, 2006). Beyond the threshold levels, heavy metal ions form highly toxic unspecific complexes in the microbial cells which adversely affect the physiological functions of microbial cells (Nies, 1999) by either entirely arresting the microbial growth or organisms may develop tolerance/resistance to metals (Khan *et al.*, 2009).

Soil is a complex ecological system where different microorganisms play important roles in maintaining the soil fertility and plant productivity through the interactions with both biological and physico-chemical components (Ahemad *et al.*, 2009). Under metal stress, soil microorganisms have developed many strategies to escape the toxicological effects generated by the various metals. These mechanisms include the expulsion and biosorption of metal species on bacterial cell surface, bioaccumulation of the metal ions inside the cell, biotransformation of toxic metals to less toxic forms (Ahemad, 2012; Ryan *et al.*, 2005). Therefore, microorganisms from metal stressed environment are more tolerant to different heavy metals compared to those found in the unpolluted one (Rajkumar *et al.*, 2010). Further, heavy metal resistant microorganisms also contribute to the maintenance of antibiotic resistance genes (Baker-Austin *et al.*, 2006; Habi and Daba, 2009). There is a correlation between metal tolerance and antibiotic resistance owing to the probability that genes responsible for both antibiotics and heavy metals may be present intimately in concert on the same plasmid (Ahemad, 2012; Spain and Alm, 2003). Therefore, this study was designed to assess the incidence of heavy metal and antibiotic resistant bacteria in agricultural soils irrigated with wastewater which received effluents from both metal industries and domestic sewage.

MATERIALS AND METHODS

Bacterial isolation: Three surface soil samples were collected from agricultural fields on Mathura road, 7 km far away from Aligarh city (27°29' latitude and 72°29' longitude), Uttar Pradesh, India. This agricultural soil was an alluvial soils (sand, silt, clay and organic matter ($g\ kg^{-1}$): 667, 190, 143 and 6.2, respectively; Kjeldahl N and Olsen P: 0.75 $g\ kg^{-1}$ and 16 $mg\ kg^{-1}$, respectively; WHC, CEC and AEC: 0.44 $mL\ g^{-1}$, 11.7 $cmoL\ kg^{-1}$ and 5.1 $cmoL\ kg^{-1}$, respectively; pH: 7.2) and had been irrigated consistently with wastewater which contained both the industrial effluents released from the metal factories and the domestic sewage. Actually, there are a number of lock manufacturing, steel and electroplating, metal surface treating, fertilizer and pesticide industries in Aligarh city. Remarkably, perfect partition for industrial effluents and domestic wastes is completely absent due to house-based small scale industries and lack of proper compartmentalization of different sewages owing to carelessness of municipal laws. Hence, the two types of waters get mixed. Soil samples were collected in sterile polythene bags and maintained at 4°C to guarantee minimal biological activity. They were immediately transported to the laboratory and were mixed well as described by Reddy *et al.* (1986) and pH of soil samples was measured (Alef and Nannipieri, 1995). Heavy metals in soil samples were determined with flame atomic absorption spectrophotometer (McGrath and Cunliffe, 1985). Bacteria were isolated according to Reddy *et al.* (1986) and identified following Holt *et al.* (1994).

Assessment of Minimum Inhibitory Concentration (MIC) and antibiotic resistance pattern and isolation of plasmid DNA: The resistance levels of bacterial strains to the increasing concentrations (3.12-2400 μg mL^{-1}) of heavy metals [Zn^{2+} ($ZnCl_2$), Cu^{2+} ($CuSO_4$), Cr^{6+} ($K_2Cr_2O_7$), Cr^{3+} ($CrCl_3.6H_2O$), Cd^{2+} ($CdCl_2$), Hg^{2+} ($HgCl_2$), Ni^{2+} ($NiCl_2$) and Pb^{2+} ($Pb(CH_3COOH)_2$)] was evaluated by the plate dilution assay (Summers and Silver, 1972) using *E. coli* K-12 as a control. The lowest concentration of the metals, which inhibited the bacterial growth, was referred to as MIC. Antibiotic resistance pattern of bacterial isolates was determined following disc diffusion method (Bauer *et al.*, 1966) using *E. coli* B as a sensitive bacterial strain. Plasmids were isolated as described by Kado and Liu (1981) and Sambrook *et al.* (1989).

Statistical analysis: Experiments were replicated three times on different time intervals and Sigma Plot 10.0 was used to drive mean values of the replicates.

RESULTS

In this study, heavy metals analysis of soil sample from the metal contaminated agricultural field revealed that concentrations of various heavy metals in polluted soils were as follows (mg kg^{-1} soil): Zn 4890, Cu 669.1, Cd 11.5, Cr 67.5, Ni 290.1 and Pb 195. Further, a total of 34 bacterial isolates differing in colony characteristics and pigmentations were selected from nutrient agar plates. All isolates further characterized morphologically as well as biochemically. Thus, 23 bacterial isolates were identified as *Pseudomonas*, 5 isolates as *Bacillus* and 6 isolates as *Staphylococcus*.

Further, bacterial isolates were assessed for resistance levels against heavy metals. Of the total bacterial isolates, 8.8% of isolates exhibited resistance to mercury by 50 μg mL^{-1}, 2.9% isolates to cadmium by 200 μg mL^{-1}, 8.8% isolates to copper by 1600 μg mL^{-1}, 5.9% isolates to zinc by 1600 μg mL^{-1}. In addition, 11.8% isolates demonstrated resistance to nickel by 1600 μg mL^{-1}. In contrast, 91.1, 8.8 and 2.9% bacterial isolates displayed resistance to lead by 2400 μg mL^{-1}, chromium (3^+) by 2400 μg mL^{-1} and chromium (6^+) by 800 μg mL^{-1}, respectively. Moreover, 14.7% of the isolates were resistant to mercury while 2.9% of the isolates to cadmium, 91.2% to copper, 79.5% to zinc, 91.2% to nickel, 97% to lead, 94.1% to chromium (3^+) and 79.4% to chromium (6^+). The following decreasing trend of bacterial resistance to the selected heavy metals was found: $Pb^{2+}>Cr^{3+}>Cu^{2+} = Ni^{2+}>Zn^{2+} = Cr^{6+}>Hg^{2+}>Cd^{2+}$. Remarkably, bacterial strains exhibited lower MIC values to Cr^{6+} compared to Cr^{3+} (Table 1).

In this study, majority of the soil isolates showed Multiple Metal Resistance (MMR). For instance, 8.8% of the isolates showed resistance to four heavy metals in two different combinations while 26.5% of the isolates displayed resistance to five metals in four different combinations. Moreover, 38.2% of the isolates exhibited resistance to six heavy metals in four different combinations whereas 17.6% of the isolates were resistant to seven heavy metals in two different combinations. However, 8.8% of the isolates showed resistance to eight heavy metals at a time (Table 2).

All the bacterial isolates were also tested for antibiotic susceptibility to eight different antibiotics (neomycin, methicillin, ciprofloxacin, nalidixic acid, polymixin B, tetracyclin, cloxacillin, amoxicillin).

Table 1: Incidence of metal resistance in bacterial isolates[†] from wastewater irrigated agricultural soil

Metals	Sensitive range			Resistant range					Total (%)
Mercury									
MIC ($\mu g\ mL^{-1}$)	3.12	6.25	12.5[a]	-	25	50	100		
No. of isolates inhibited	4 (11.8)	22 (64.7)	2 (5.9)	-	2 (5.9)	3 (8.8)	-[b]		14.7
Cadmium									
MIC ($\mu g\ mL^{-1}$)	25	50	100[a]	200	400	800	1600		
No. of isolates inhibited	9 (26.5)	10 (29.4)	14 (41.2)	1 (2.9)	-	-	-		2.9
Copper									
MIC ($\mu g\ mL^{-1}$)	50	100	200[a]	400	800	1200	1600		
No. of isolates inhibited	-	-	3 (8.8)	11 (32.4)	14 (41.2)	3 (8.8)	3 (8.8)		91.2
Zinc									
MIC ($\mu g\ mL^{-1}$)	50	100	200[a]	400	800	1200	1600		
No. of isolates inhibited	-	1 (2.9)	6 (17.6)	11 (32.4)	14 (41.2)	-	2 (5.9)		79.5
Nickel									
MIC ($\mu g\ mL^{-1}$)	50	-	100[a]	200	400	800	1200	1600	
No. of isolates inhibited	2 (5.9)	-	1 (2.9)	1 (2.9)	11 (32.4)	4 (11.8)	11 (32.4)	4 (11.8)	91.2
Lead									
MIC ($\mu g\ mL^{-1}$)	50	-	100[a]	200	400	800	1600	2400	
No. of isolates inhibited	-	-	1 (2.9)	-	-	-	2 (5.9)	31 (91.1)	97
Chromium (3^+)									
MIC ($\mu g\ mL^{-1}$)	50	100	200[a]	400	800	1600	2400		
No. of isolates inhibited	-	2 (5.9)	-	-	-	29 (85.3)	3 (8.8)		94.1
Chromium (6^+)									
MIC ($\mu g\ mL^{-1}$)	50	-	100[a]	200	400	800	1600		
No. of isolates inhibited	2 (5.9)	-	5 (14.7)	7 (20.6)	19 (55.9)	1 (2.9)	-		79.4

[†]Total No. of isolates = 34, Values in parenthesis indicate percentage of the total isolates, MIC: Minimum inhibitory concentration, [a]MIC of the standard strain, [b]Not detected

Table 2: Pattern of heavy metal resistance in bacterial isolates[†] from wastewater irrigated agricultural soil

No. of heavy metals	No. resistant isolates (%)	Resistance pattern
1	nd	-
2	nd	-
3	nd	-
4	3 (8.8)	Cu^{2+}, Cr^{3+}, Cr^{6+}, Pb^{2+} (2)
		Cr^{3+}, Hg^{2+}, Ni^{2+}, Pb^{2+} (1)
5	9 (26.5)	Cu^{2+}, Zn^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+} (3)
		Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Pb^{2+} (1)
		Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (1)
		Cu^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+} (2)
		Zn^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (2)
6	13 (38.2)	Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+} (7)
		Cu^{2+}, Zn^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (2)
		Cu^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (2)
		Cu^{2+}, Zn^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (1)
		Zn^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (1)
7	6 (17.6)	Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, Cd^{2+}(5)
		Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Hg^{2+}, Ni^{2+}, Pb^{2+} (1)
8	3 (8.8)	Cu^{2+}, Zn^{2+}, Cr^{3+}, Cr^{6+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+} (3)

[†]Total No. of isolates = 34, nd: Not detected, Values in parenthesis indicate percentage of the total isolates

Table 3: Percent resistance of in bacterial isolates[†] from wastewater irrigated agricultural soil to antibiotics

Antibiotics	Concentration (µg disc^{-1})	No. of isolates	Percent resistance
Neomycin (N)	30	29	85.3
Methicillin (M)	30	11	32.2
Ciprofloxacin (C_f)	30	6	17.6
Nalidixic acid (N_a)	30	12	35.3
Polymyxin B (P_b)	30	14	41.2
Tetracyclin (T)	30	12	35.3
Cloxacillin (C_x)	30	28	82.4
Amoxycillin (A_m)	25	28	82.4

[†] Total No. of isolates = 34

Table 4: Pattern of antibiotic resistance in bacterial isolates[†] from wastewater irrigated agricultural soil

No. of antibiotics	No. of resistant isolates (%)	Resistance pattern
1	2 (5.9)	N (1)
		C_f (1)
2	3 (8.8)	C_f, P_b (1)
		C_x, A_m (1)
		N, N_a (1)
3	7 (20.6)	M, T, A_m (1)
		N, C_x, A_m (3)
		N, M, C_x (1)
		P_b, C_x, A_m (1)
		N, P_b, C_x (1)
4	11 (32.4)	N, M, C_x, A_m (1)
		N, P_b, C_x, A_m (6)
		N, N_a, C_x, A_m (1)
		N, N_a, T, A_m (1)
		N, T, C_x, A_m (2)
5	3 (8.8)	N, M, N_a, C_x, A_m (1)
		N, M, T, C_x, A_m (1)
		N, M, C_f, C_x, A_m (1)
6	5 (14.7)	N, M, N_a, P_b, C_x, A_m (5)
7	2 (5.9)	N, C_f, N_a, Pb, T, C_x, A_m (2)
8	1 (2.9)	N, M, C_f, N_a, Pb, T, C_x, A_m (1)

[†] Total No. of isolates = 34, Values in parenthesis indicate percentage of the total isolates

The present study indicated that 85.3% of the isolates were resistant to neomycin while 82.4% isolates showed resistance to both cloxacillin and amoxicillin. In contrast, the bacterial sensitivity was the maximum to methicillin, ciprofloxacin, nalidixic acid and tetracycline (Table 3). Further, a total of eight combinations of the selected antibiotic resistant patterns were observed. For example, 20.6% of the isolates were resistant to three antibiotics in five different combinations whereas 32.4% of the isolates exhibited resistance to four antibiotics in five different combinations. However, only 2.9% of the isolates were resistant to eight antibiotics (Table 4).

The most promising multiple metal resistant bacterial isolates (SN7, SN28, SN29, SN30, SN34 and SN18) were screened for the presence of plasmid DNA. Figure 1 shows the agarose gel electrophoretic pattern of plasmid DNA isolated from bacterial isolates SN7, SN28, SN29, SN30, SN34 and SN18 in lanes b, c, d, e, f and g, respectively. In the present study, the most resistant bacterial isolates were found to be positive for the presence of plasmid DNA.

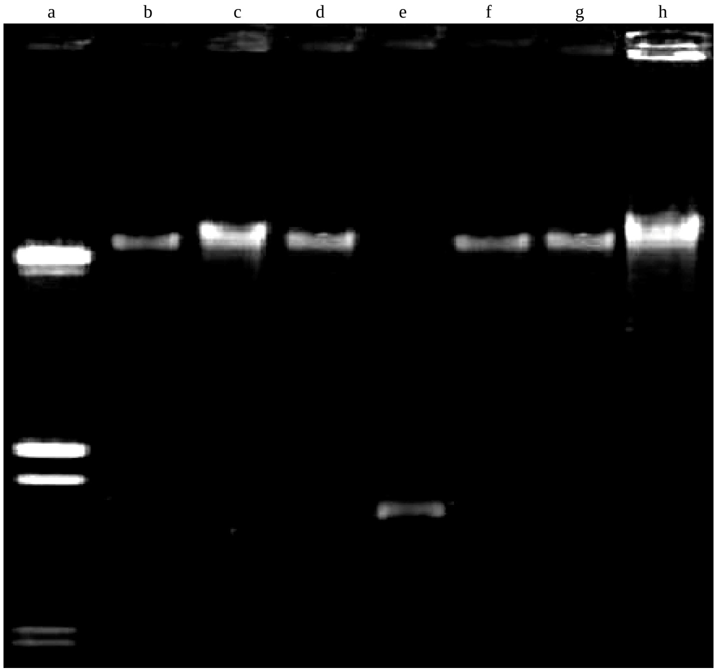

Fig. 1(a-h): Agarose gel electrophoresis profiles of plasmid DNA from bacterial isolates from waste water irrigated agricultural soil, (a)Undigested lamda DNA, (b) Plasmid DNA isolated from SN7, (c) plasmid DNA isolated from SN28, (d) Plasmid DNA isolated from SN29, (e) Plasmid DNA isolated from SN30, (f) Plasmid DNA isolated from SN34, (g) Plasmid DNA isolated from SN18, (h) Lamda DNA digested with EcoRI

DISCUSSION

Long term metal deposition into soils from various sources results into metal accumulation at high concentration which affects the soil microflora negatively (Singh *et al.*, 2010). Metal-microbe interaction being a complex process is influenced by several edaphic factors, such as pH or organic matter content (Saeki *et al.*, 2002; Rani and Goel, 2009). At low concentrations, some metals (e.g., zinc, copper and cobalt etc.) are essential for microbes, since they are the part of co-factors for metalloproteins and different enzymes (Nies, 1999; Li *et al.*, 2004). Notwithstanding, metal concentration higher than the threshold level exerts inhibitory action on microorganisms by altering essential functional groups or modifying the active conformations of biological molecules, the ability to grow even at high metal concentration is nevertheless, may be the result of intrinsic or induced mechanisms (Giller *et al.*, 1998). It is also reported that these metals exert a selective pressure on microorganisms, resulting in their greater tolerance to metals, but with lower microbial diversity (Baath *et al.*, 1998). In this study, bacterial isolates from agricultural soils irrigated with wastewater showed varying degree of heavy metal resistance. For instance, the highest level of resistance (2400 μg mL^{-1}) was observed to both lead and chromium (3^{+}) and the maximum number of isolates (97%) showed resistance to lead. The reason for the varying levels of resistance levels to different metals in this study may be that different microbes follow different modes to overcome the metal toxicity. For instance, they subside or alleviate the toxicity of metallic compounds through intra or extracellular sequestration, operating efflux pumps, enzymatic reduction, methylation, precipitation and biosorption and bioaccumulation processes (Sarma *et al.*, 2010). In a study, Iutynska and Petrusha (1999) observed a high degree of resistance to heavy metals in bacterial isolates of grey podozolized soils. They inferred that 40% of the total isolates resisted a mixture of metal ions (Zn^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+}). They also reported that humus helped in developing metal resistance by decreasing heavy metal toxicity to soil microflora. In the study conducted by Wani *et al.* (2007), three *Bacillus* isolates PSB1, PSB7 and PSB10 tolerated Cr^{6+} ($K_2Cr_2O_7$) 550, 400 and 550 μg mL^{-1}, respectively. Similarly, metal resistance by 112 *Pseudomonas* strains was also reported by Anisimova *et al.* (1993) as 1-10 mM for Ni^{2+} and 1-6 mM for Zn^{2+}. In addition, these results also substantiated that the hexavalent chromium (Cr^{6+}) is more toxic to bacterial growth compared to trivalent chromium (Cr^{3+}) form. Among the different forms of chromium, Cr^{6+} is more toxic species and is also carcinogenic and mutagenic (Kamaludeen *et al.*, 2003; Ortegel *et al.*, 2002) attributable to rapid permeability across the membranes, greater solubility in aqueous environment, and interactions with both proteins and nucleic acids in cells (Losi *et al.*, 1994). Even though, the reduction of Cr^{6+} into Cr^{3+} causes the chromate toxicity, the reduced form (Cr^{3+}) is comparatively stable, sparingly soluble, and less toxic (Michel *et al.*, 2001). Contradictory reports are nevertheless, available with respect to the resistance levels of bacteria against different heavy metals, which could probably be on account of different media and growth conditions (Rajkumar *et al.*, 2005). However, the present study showed that the resistance levels of the selected bacterial strains were significantly high.

The resistance traits to heavy metals in bacteria are encoded in extrachromosomal plasmid DNA facilitating the transfer of metal resistance factor among different bacterial genera. Moreover, the bacterial strains occurring in the metal stressed environment may carry plasmid not only in terms of the frequency but also in size (Nies, 1999). There is a correlation between metal resistance and antibiotic resistance characters in metal resistant bacteria. Mostly, the resistance genes to both antibiotics and heavy metals may be intimately together on the same plasmid and consequently, the probability is higher that they may be transferred horizontally together from one bacterium to another bacterium (Spain and Alm, 2003). Moreover, the collective expressions of heavy metal and antibiotic resistance traits are not by chance rather than are due to selection pressure imposed by heavy metals. Similarly, Lazer *et al.* (2002) studied the multiple antibiotic and metal resistant bacterial isolates from both soils and water and concluded that the genes for heavy metals and antibiotics are clustered on plasmids. However, bacterial isolates in this study also exhibited substantial resistance to different antibiotics. In general, bacterial resistance to antibiotics is achieved by decline in membrane permeability or rapid efflux of antibiotics and enzymatic inactivation (Krulwich *et al.*, 2005). Generally, degree of bacterial resistance to various antibiotics generally, reflects history of antibiotic application. According to Qureshi and Qureshi (1992), sewage disposal is also a factor in promoting antibiotic resistance in bacterial communities of the contaminated environments.

CONCLUSION

The bacterial isolates from agricultural soils irrigated with wastewater, showed a wide range of resistance to different metals and antibiotics. The degree of metal resistance in soil bacterial isolates is a potential indicator of metals toxicity to other life forms in the contaminated sites. In addition, agricultural soils irrigated with wastewater may be exploited to isolate multiple metal and antibiotic resistant bacteria.

REFERENCES

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of metals. 2nd Edn., Springer, New York.

Ahemad, M., A. Zaidi, M.S. Khan and M. Oves, 2009. Factors Affecting the Variation of Microbial Communities in Different Agro-Ecosystems. In: Microbial Strategies for Crop Improvement, Khan, M.S., A. Zaidi and J. Musarrat (Eds.). Springer, Berlin, Heidelberg, pp: 301-324.

Ahemad, M., 2012. Implications of bacterial resistance against heavy metals in bioremediation: A review. IIOABJ, 3: 39-46.

Alef, K. and P. Nannipieri, 1995. Methods in Applied Soil Microbiology and Biochemistry. Academic Press Ltd., London.

Anisimova, L.A., T.V. Siunova and A.M. Boronin, 1993. Metal resistance of gram-negative bacteria isolated from soil and waste waters of industrial regions. Mikrobiologiia, 62: 843-848.

Baath, E., M.D. Ravina, A.A. Frostegand and C.D. Campball, 1998. Effect of metal-rich sludge amendments on the soil microbioal community. Applied Environ. Microbiol., 64: 238-245.

Baker-Austin, C., M.S. Wright, R. Stepanauskas and J.V. McArthur, 2006. Coselection of antibiotic and metal resistance. Trends Microbiol., 14: 176-182.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Giller, K.E., E. Witter and S.P. McGrath, 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil Biol. Biochem., 30: 1389-1414.

Habi, S. and H. Daba, 2009. Plasmid incidence, antibiotic and metal resistance among enterobacteriaceae isolated from algerian streams. Pak. J. Biol. Sci., 12: 1474-1482.

Hewitt, E.J., 1983. Essential and Functional Methods in Plants. In: Metals and Micronutrients: Uptake and Utilization by Plants, Robb, D.A. and E.S. Pierpoint (Eds.). Academic Press, London, pp: 277-300.

Holt, J.G., N.R. Krieg, P.H. Sneath, J.J. Stanley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimere, USA.

Iutynska, H.O. and Z.V. Petrusha, 1999. The resistance of soil microorganisms to soil pollution by heavy metals. Mikrobiol. Z., 61: 72-77.

Kabata-Pendias, A. and H. Pendias, 2001. Trace Elements in Soils and Plants. CRC Press, Boca Raton.

Kado, C.I. and S.T. Liu, 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol., 145: 1365-1373.

Kamaludeen, S.P., M. Megharaj, A.L. Juhasz, N. Sethunathan and R. Naidu, 2003. Chromium microorganism interactions in soils: Remediation implications. Rev. Environ. Contaim. Toxicol., 178: 93-164.

Khan, M.S., A. Zaidi, P.A. Wani and M. Oves, 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ. Chem. Lett., 7: 1-19.

Kotrba, P., J. Najmanova, T. Macek, T. Ruml and M. Mackova, 2009. Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnol. Adv., 27: 799-810.

Krulwich, T.A., O. Lewinson, E. Padan and E. Bibi 2005. Do physiological roles foster persistence of drug/multidrug-efflux transporters? A case study. Nature Rev. Microbiol., 3: 566-572.

Lazer, V., R. Cernat, C. Balotescu, A. Cotar, E. Coipan and C. Cojocaru, 2002. Correlation between multiple antibiotic resistance and heavy metal tolerance among some E. coli strains isolated from polluted waters. Bacteriol. Virusol. Parazitol. Epidemiol., 47: 155-160.

Li, Q., S. Wu, G. Liu, X. Liao, X. Deng, D. Sun, Y. Hu and Y. Huang, 2004. Simultaneous biosorption of cadmium (II) and lead (II) ions by pretreated biomass of *Phanerochaete chrysosporium*. Sep. Purif. Technol., 34: 135-142.

Losi, M.E., C. Amrhein and W.T. jr. Frankenberger, 1994. Environmental biochemistry of chromium. Rev. Environ. Contam. Toxicol., 136: 91-121.

Matyar, F., T. Akkan, Y. Ucak and B. Eraslan, 2010. *Aeromonas* and *Pseudomonas*: Antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (Northeast Mediterranean Sea). Environ. Monit. Assess., 167: 309-320.

McGrath, S.P. and C.H. Cunliffe, 1985. A simplified method for the extraction of metals Fe, Zn, Cu, Ni, Cd, Pb, Cr and Mn from soils and sewage sludge. J. Sci. Food Agri., 36: 794-798.

Michel, C., M. Brugna, C. Aubert, A. Bernadac and M. Bruschi, 2001. Enzymatic reduction of chromate: Comparative studies using sulfate-reducing bacteria. Key role of polyheme cytochrome c and hydrogenases. Applied Microbiol. Biotechnol., 55: 95-100.

Nies, D.H., 1999. Microbial heavy-metal resistance. Applied Microbiol. Biotechnol., 51: 730-750.

Ortegel, J.W., E.D. Staren, L.P. Faber, W.H. Warren and D.P. Braun, 2002. Modulation of tumor infiltrating lymphocyte cytolytic activity against human non small cell lung cancer. Lung Cancer, 36: 17-25.

Pajuelo, E., I.D. Rodriguez-Llorente, M. Dary and A.J. Palomares, 2008. Toxic effects of arsenic on *Sinorhizobium-Medicago sativa* symbiotic interaction. Environ. Pollut., 154: 203-211.

Qureshi, A.A. and M.A. Qureshi, 1992. Multiple antibiotic resistant fecal coliforms in raw sewage. Water Air Soil Pollut., 61: 47-56.

Rajkumar, M., N. Ae, M.N.V. Prasad and H. Freitas, 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol., 28: 142-149.

Rajkumar, M., R. Nagendran, K.J. Lee and W.H. Lee, 2005. Characterization of a Novel Cr^{6+}reducing *Pseudomonas* sp. with plant growth promoting potential. Curr. Microbiol., 50: 266-271.

Rajkumar, M., R. Nagendran,K.J. Lee, W.H. Lee and S.Z. Kim, 2006. Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemosphere, 62: 741-748.

Rani, A. and R. Goel, 2009. Strategies for Crop Improvement in Contaminated Soils Using Metal-Tolerant Bioinoculants. In: Microbial Strategies for Crop Improvement, Khan, M.S., A. Zaidi and J. Musarrat (Eds.). Springer, Berlin, pp: 105-132.

Reddy, G.B., E. Ford and D. Aldridge, 1986. Seasonal changes in bacterial numbers and plant nutrients from point and non-point source ponds. Environ. Pollut., 40: 359-367.

Ryan, R., D. Ryan and D. Dowling, 2005. Multiple metal resistant transferable phenotypes in bacteria as indicators of soil contamination with heavy metals. J. Soils Sediments, 5: 95-100.

Saeki, K., T. Kunito, H. Oyaizu and S. Matsumoto, 2002. Relationships between bacterial tolerance levels and forms of copper and zinc in soils. J. Environ. Qual., 31: 1570-1575.

Sambrook, J., E.F. Fritsh and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York, USA.

Sarma, B., C. Acharya and S.R. Joshi, 2010. Pseudomonads: A versatile bacterial group exhibiting dual resistance to metals and antibiotics. Afr. J. Microbiol. Res., 4: 2828-2835.

Shier, W.T., 1994. Metals as toxins in plants. J. Toxicol. Toxin Rev., 13: 205-216.

Singh, V., P.K. Chauhan, R. Kanta, T. Dhewa and V. Kumar, 2010. Isolation and characterization of *Pseudomonas resistant* to heavy metals contaminants. Int. J. Pharm. Sci. Rev. Res., 3: 164-167.

Spain, A. and E. Alm, 2003. Implications of microbial heavy metal tolerance in the environment. Rev. Undergraduate Res., 2: 1-6.

Summers, A.O. and S. Silver, 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J. Bacteriol., 112: 1228-1236.

Wang, J. and C. Chen, 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotech. Adv., 24: 427-451.

Wani, P.A., M.S. Khan and A. Zaidi, 2007. Chromium reduction, plant growth promoting potentials and metal solubilization by *Bacillus* sp. isolated from alluvial soil. Curr. Microbiol., 54: 237-243.

Wani, P.A., M.S. Khan and A. Zaidi, 2008. Chromium reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium amended soil. Biotechnol. Lett., 30: 159-163.