

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.509.040>

Bacterial Tolerance to Chromium and Cadmium in Different Textural Soils

Tanjila Jesmin^{1*} and Sirajul Hoque²

¹Department of Crop Science, University of Illinois at Urbana-Champaign, USA

²Department of Soil, Water and Environment, University of Dhaka, Bangladesh

*Corresponding author

ABSTRACT

This study intends to quantify the tolerance of six bacterial strains to chromium (Cr^{6+}) and cadmium (Cd^{2+}) in three different soils (sandy loam, silt loam, silty clay loam). In this experiment, chromium and cadmium were selected as the heavy metals since these metals are generally discharged from industries like tannery, textiles, dyeing factories etc. in the city of Dhaka. The six bacterial strains, named as A1, A2, A3 were collected from agricultural soil and C1, C2, C3 were procured from industrial contaminated soil which were acquired from the stock of soil microbiology laboratory at University of Dhaka, and identified at molecular level by Tanu and Hoque (2013). Among the six bacterial strains, five samples were belonged to the *Bacillus* species; whereas, the remaining one fell into *Micrococcus* species. Three representative agricultural soil samples which were tested for heavy metal tolerance under different intervals of incubation, were collected from Dhamrai, Khustia and Gopalganj. Bacterial tolerance to Cr^{6+} and Cd^{2+} in different soils exhibited different trends with varying incubation periods. Test results indicate that both *Bacillus* (A1), and *Micrococcus*(C3) showed the highest tolerance to heavy metals in silty clay loam soil.

Keywords

Bacterial strain,
Heavy metals,
Contamination,
Incubation,
Tolerance.

Article Info

Accepted:

15 August 2016

Available Online:

10 September 2016

Introduction

Every particular soil type hosts an incredible diversity of organisms like bacteria that constitute a major portion of our ecosystem. Bacteria carry out wide range of functions including natural decomposition, maintaining soil aggregation and porosity which improve soil quality as well as fertility (Bache, 1979). The ingress of heavy metals due to industrial contamination as well as environmental pollution, in various forms can lead to significant change into the microbial communities and affect their activities (Hassen *et al.*, 1998).

Such Industrial operations like leather tanning, electroplating, paints, pigment production, steel manufacture etc. contribute to widespread soil pollution with Chromium compounds (Wang and Shen, 1995). On a similar note, Cadmium is another toxic industrial pollutant that is widely used for electroplating, plastic stabilization, corrosion protection, pesticides. Any soil enriched with heavy concentrations of such contaminants can inhibit the bacterial growth process and thereby, result in endanger the entire soil ecosystem (Das *et al.*, 1997).

To this end, the ability of bacteria to survive under toxic concentrations of heavy metal should be examined thoroughly so that a scientific insight to the detoxification and other resistant mechanisms can properly be identified (Winge *et al.*, 1989). Previous studies reveal that a particular group of bacteria in artificial media supplemented with heavy metal can exhibit high tolerance level; whereas, tolerance level may be totally different with natural soil medium by contraries (Ahmad *et al.*, 2001; Hayat *et al.*, 2002). Many bacterial isolates were found to be resistant to very high concentrations of heavy metals regardless of the degree of contamination in their environment. The dominant isolates at high concentrations of metal ions include *Bacillus* spp, *Pseudomonas* spp, *Corynebacterium* spp., *Micrococcus* spp., and *Flavobacterium* spp. *Proteus*, *Citrobacter*, *Alcaligenes*, *Enterobacter*, etc. (Anyanwu and Nwachukwu, 2011).

Besides the bacteria types and incubation medium, the effects of heavy metal toxicity are also dependent on soil properties as well as time. Several soil variables control trace element contents and behavior in soil. The rates of trace element migration in the soil profiles are affected by associated chemical, physical, and biological properties.

Kabata-Pendias *et al.*, (1992) established empirical correlations by relating soil properties like pH, clay fraction content, cation-exchange capacity to resistance against metal toxicity. Since heavy metal cations are most mobile under acidic (pH < 7) condition, an increase in pH value usually reduces their bioavailability. In addition, most heavy metals exist mainly as cations in the soil solution, and their adsorption therefore depends on the density of negative charges on the surfaces of the soil colloids. Moreover, colloidal soil organic matter has a

major influence on the chemical properties of soils. In this perspective many approaches have been used to assess the ability of different textural soils to reduce the effect of contaminating metals for specific bacterial strain under certain conditions.

This experiment opts to evaluate the resistance of six different bacterial strain to two most common contaminating metals that are widely being discharged from industries within the proximity of Dhaka city. Bacterial strains were selected to represent microorganisms found in both agricultural and industrial soils. Three different soil textures were used as the culture medium to avoid any probable synthetic tolerance. Physico-chemical properties of corresponding soil types were determined followed by the examination of metal tolerance with varying incubation periods.

Methodology

Study Area and Collection of Soil Samples

Representative soil samples were collected from agricultural land of Dhamrai (23.9083° N, 90.2167° E), Gopalganj (23.2000° N, 89.8000° E), and Khustia (23.9199° N, 89.2200° E) areas in Bangladesh. These lands are generally cultivated with various types of cereal crops and vegetables. Based on the study these soils are considered to be devoid of any harmful effluent.

Samples were scraped from the A-horizon (typical soil zone enriched with organic matter) up to a depth of 7 cm. The samples were then transported in marked polyethylene bags to the laboratory. After that, the soils were airdried and preserved for future use.

Measurement of Physical and Chemical Properties of the Soil Samples

Soil pH was determined electrochemically by using a glass electrode pH meter (JENWAY 3305) with a soil to water ratio of 1:2.5. The particle size analysis of soils was done by hydrometer method as described by Gee and Bauder (1986). Soil organic carbon was determined by Walkley and Black's (1934) wet oxidation method. Soil organic matter was calculated by multiplying the value of organic carbon with a conversion factor of 1.724.

Cation Exchange Capacity (CEC) was determined as followed by Jackson (1973). Chromium (Cr) and Cadmium (Cd) contents of heavy metal treated soils were determined directly by Atomic Absorption Spectrophotometer (Varian AA240) after digestion with HNO₃.

Collection of Bacterial Strains

Tanu and Hoque (2013) isolated and identified six bacterial strains named as *Bacillus pocheonensis* strain TR2-6, *Bacillus amyloliquefaciens* strain SCSAAB0007, *Bacillus megaterium* strain H2, *Bacillus cereus* isolate PGBw4, *Micrococcus luteus* strain P4-3, 1320, and *Bacillus subtilis* strain DP14 through DNA polymerase chain reaction. These bacterial strains were used in this study as well.

Three of these bacterial strains were isolated from contaminated soil of industrial area and rests were isolated from agricultural soil of different location (Table 1).

All of the bacterial isolates were cultured in nutrient agar plate from preserved nutrient agar slant followed by streak plate technique. Each strain were collected from pure cultured plate and suspended in sterilized water before incubation (Hiroki, 1992).

Determining Tolerance Limit of Indigenous Bacterial Strains in Heavy Metal Treated Soil

Two gram soil was taken in test tube (10mL) for incubation experiment. After plugging with cottons, incubation tubes were sterilized in autoclave repeatedly two times for 20 minutes at 121 psi. Stock solutions of different concentrations were prepared for Cadmium (Cd²⁺) from CdCl₂.2.5 H₂O and Chromium (Cr⁶⁺) from K₂Cr₂O₇ which were sterilized in autoclave machine before mixing into soils. Metal salts solution were mixed in different soil samples at the concentrations ranging from 1000 to 15000 µg/g. Metal salts solutions were added maintaining 30% water content (Giller et al., 1998). All of the incubation tubes were kept in incubator at 37°C. After 24 h, 72 h and 7 days of incubation, the soils were taken with sterilized loop and diluted in sterile distilled water. After dilution, 0.1 ml of diluted sample was spreaded over the surface of nutrient agar plate following spread plate technique (Cappucino and Sherman, 2005). The plates were then kept for (24-72) h in an incubator at 37°C for observation. A control experiment was run simultaneously using all the reagents except bacterial strains. Growth appearance in the plate was a sign of resistance of that bacterial strain and growth absence in plate warned the tolerance limit of that strain.

Results and Discussion

Soil Characteristics

The characteristics and heavy metal contents of associated soils are presented in Table 2. All the parameters of the soils are below the permissible limit, recommended to use for incubation as described and used by Krishna Murti and Vishwanathan (1991).

Bacterial Tolerance to Chromium in Soils

Six bacterial species tolerant to Cr^{6+} after 24 h of incubation ranged from 8000 to 12000 $\mu\text{g/g}$ in sandy soil, from 9000 to 13000 $\mu\text{g/g}$ in silt loam soil, from 10000 to 14000 $\mu\text{g/g}$ in silty clay loam soil. After 72 h of incubation, tolerance to Cr^{6+} varies from 7000 to 12000 $\mu\text{g/g}$ in sandy soil, 8000 to 12000 $\mu\text{g/g}$ in silt loam soil, from 10000 to 14000 $\mu\text{g/g}$ in silty clay loam soil. Tolerance to Cr^{6+} after 7 days of incubation ranged from 9000 to 11000 $\mu\text{g/g}$, from 8000 to 11000 $\mu\text{g/g}$, from 9000 to 13000 $\mu\text{g/g}$ in sandy soil, silt loam soil, and silty clay loam soil, respectively (Figure 1, 2 and 3).

Bacterial Tolerance to Cadmium in Soils

After 24 h of incubation bacterial tolerance to Cd^{2+} ranged from 7000 to 10000 $\mu\text{g/g}$ in sandy soil, from 7000 to 11000 $\mu\text{g/g}$ in silt loam soil, from 8000 to 11000 $\mu\text{g/g}$ in silty clay loam soil. Tolerance to Cd^{2+} after 72 h of incubation ranged from 7000 to 10000 $\mu\text{g/g}$ in sandy soil, 7000 to 11000 $\mu\text{g/g}$ in silt loam soil, from 10000 to 14000 $\mu\text{g/g}$ in silty clay loam soil. After 7 days of incubation tolerance to Cd^{2+} ranged from 6000 to 9000 $\mu\text{g/g}$, from 6000 to 10000 $\mu\text{g/g}$, from 7000 to 10000 $\mu\text{g/g}$ in sandy soil, silt loam soil, and silty clay loam soil, respectively (Figure 5, 6 and 7).

Comparison of Heavy Metal Tolerance

According to the results showed in Figure 1 to 8, *Micrococcus luteus* (C3) isolated from contaminated soil could tolerate Cr up to 13000 $\mu\text{g/g}$ but Cd up to 10000 $\mu\text{g/g}$, whereas *B. pocheonensis* (C2) tolerated up to 11000 $\mu\text{g/g}$ of Cr and 10000 $\mu\text{g/g}$ of Cd; *B. megaterium* (C1) isolated from contaminated soil could also tolerate up to 13000 $\mu\text{g/g}$ of Cr but 8000 $\mu\text{g/g}$ of Cd. On the other hand, *Bacillus amyloliquefaciens* (A3) isolated from agricultural soil tolerated

up to 9000 $\mu\text{g/g}$ of Cr but 7000 $\mu\text{g/g}$ of Cd, *B. subtilis* (A2) isolated from agricultural soil tolerated up to 11000 $\mu\text{g/g}$ of Cr but up to 10000 $\mu\text{g/g}$ of Cd, *B. cereus* (A1) isolated from agricultural soil tolerated 12000 $\mu\text{g/g}$ of Cr but up to 9000 $\mu\text{g/g}$ of Cd after 7 days of incubation in silty clay loam soil (Figure 4)

The order of tolerance to Cr and Cd of identified bacterial strain are as follows:

Chromium: *Micrococcus luteus* = *B. megaterium* > *B. cereus* > *B. pocheonensis* > *B. subtilis* > *Bacillus amyloliquefaciens*

Cadmium: *B. pocheonensis* = *Micrococcus luteus* > *B. pocheonensis* > *B. cereus* > *B. subtilis* > *Bacillus megaterium* > *B. amyloliquefaciens*

The most Cr tolerant species are the *Micrococcus luteus* and *Bacillus megaterium* which were isolated from contaminated soil of Savar EPZ area and the least Cr tolerant species is the *Bacillus amyloliquefaciens* isolated from an agricultural soil of Dhamrai area whereas the most Cd tolerant species were *Bacillus pocheonensis* and *Micrococcus luteus* isolated from EPZ area (Figure 8) and the least Cd tolerant species was *Bacillus amyloliquefaciens* isolated from an agricultural field of Dhamrai.

The toxicity exerted by heavy metals may suppress or even kill sensitive parts of the microbial community and may lead to a shift in community structure (Fliegbach *et al.*, 1994). Similar study using soils of different levels of metal pollution and control should be studied to draw a conclusion on long-term impact of heavy metal pollution on genetic diversity of soil microbial populations to explore the possible metal-microbe interaction and their possible impact on soil health. The most striking

feature of the data set is that the response of microbial community to the heavy metal pollution is apparently dependent of the soil type. Cluster analysis underlines the similarity of enzyme activity pattern among the controls and among the polluted soils (Kandeler *et al.*, 2000).

Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanisms. To survive under metal-stressed conditions, microorganisms have evolved several types of mechanisms to tolerate the uptake of heavy metal ions and resist the heavy metal stress (Bruins *et al.*, 2000). There is no general mechanism for resistances to all heavy metal ions. Bacteria have adapted to metals through a variety of chromosomal, transposon, and plasmid-mediated resistance systems.

From the toxicity studies, it was evident that the bacterial tolerance was dependent on the type of soil used for incubation. Theoretically, if a significant proportion of the bacterial population is resistant to high concentrations of the metal contaminant, then the judgment is made that the soil is negatively affected by the presence of the metal (Olson and Thornton, 1982). It was observed that the tolerance level were higher in silty clay loam and silt loam soil than that in sandy loam soil. Bacterial tolerances to supplied metals were decreasing with increasing time of incubation. In clay rich soil, availability of metal concentrations may become lower due to metal complexation, precipitation, antagonistic interactions with other metals or other reactions with organic ingredients present in soils.

Table.1 Bacterial strains used to determine tolerance limit in different soils

Soil	Bacterial Strains No.	Identified Isolates
Agricultural (Uncontaminated soil)	A1	<i>Bacillus cereus</i>
	A2	<i>Bacillus subtilis</i>
	A3	<i>Bacillus amyloliquefaciens</i>
Industrial (Contaminated Soil)	C1	<i>Bacillus megaterium</i>
	C2	<i>Bacillus pocheonensis</i>
	C3	<i>Micrococcus luteus</i>

Table.2 Physical and chemical characteristics status of three soils used for incubation

Parameters	Soil Sample 1	Soil Sample 2	Soil Sample 3
<i>pH</i>	7.53	7.85	5.98
<i>%Sand</i>	53.57	6.65	4.95
<i>%Silt</i>	39.41	81.85	57.98
<i>%Clay</i>	7.02	11.50	37.07
<i>Texture</i>	Sandy loam	Silt loam	Silty clay loam
<i>Organic Carbon (%)</i>	0.12	0.84	0.98
<i>Organic Matter</i>	0.2	1.45	1.67
<i>CEC (meq/100g)</i>	9.62	30.9	35.43
<i>Chromium (Cr) (µg/g)</i>	0.0402	0.0449	0.0789
<i>Cadmium (Cd) (µg/g)</i>	0.0008	0.0009	0.0007

Fig.1 Tolerance of bacterial isolates Cr⁶⁺ in three soils (after 24 h of incubation)

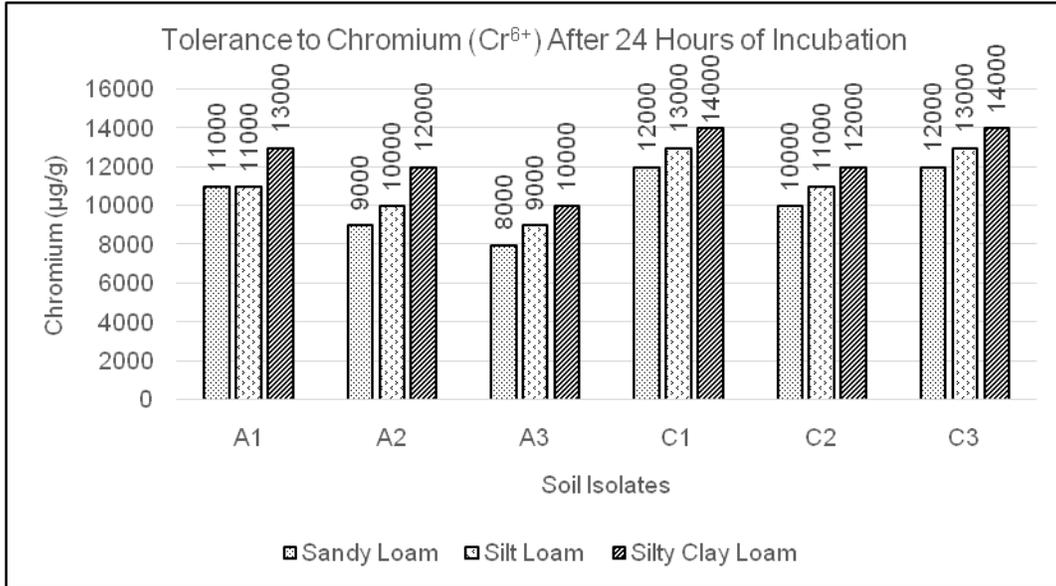


Fig.2 Tolerance of bacterial isolates Cr⁶⁺ in three soils (after 72 h of incubation)

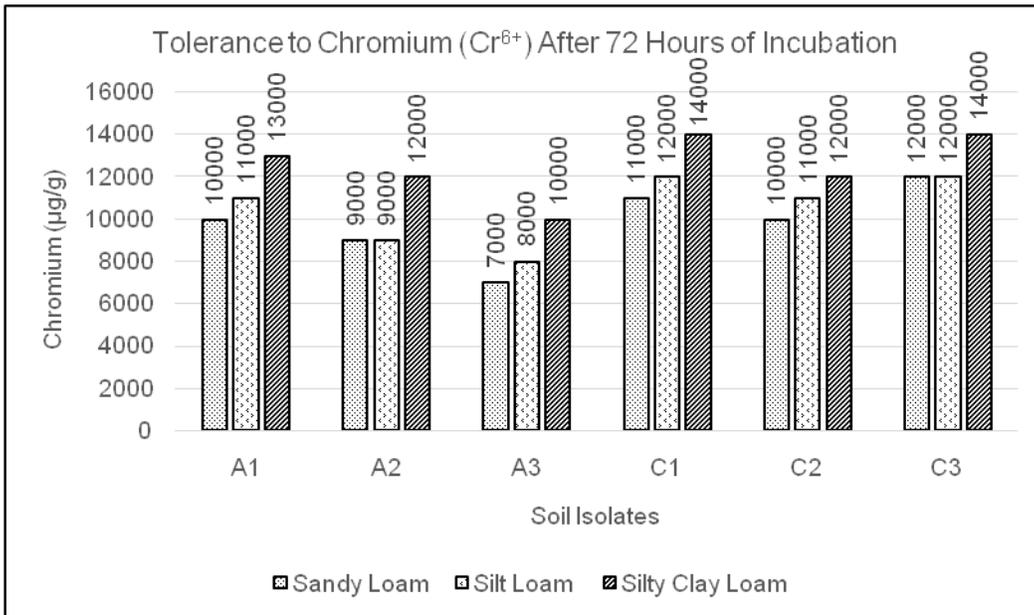


Fig.3 Tolerance of bacterial isolates Cr6+ in three soils (after 7 days of incubation)

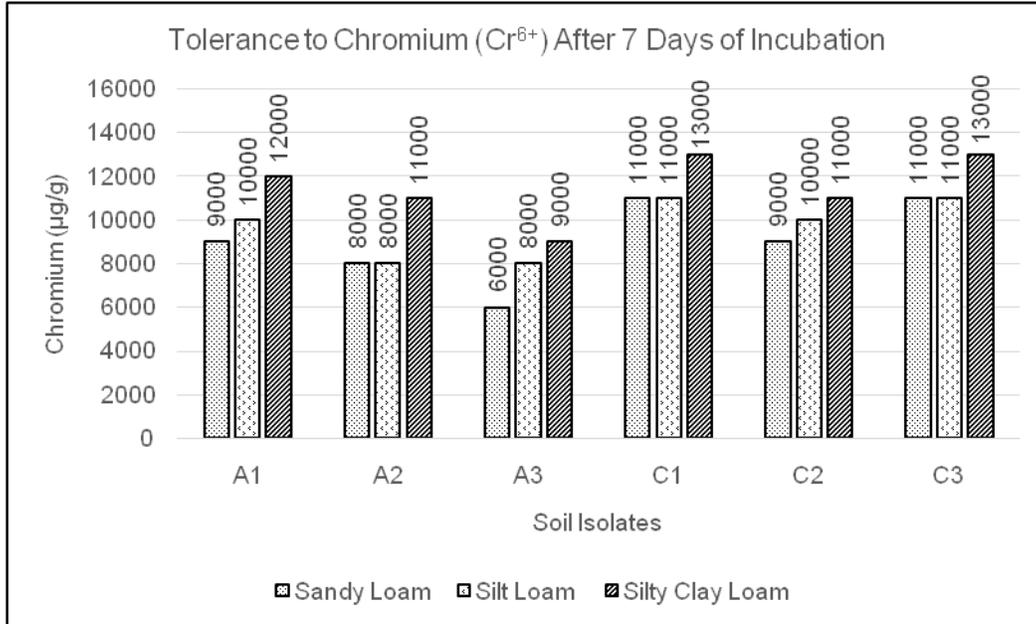


Fig.4 Growth of A1 isolates (after 24 h of incubation) at 5000 µg/g, 6000 µg/g, 7000 µg/g, 8000 µg/g, 9000 µg/g and 10000 µg/g of Cr6+ in sandy loam soil



Fig.5 Tolerance of bacterial isolates Cd²⁺ in three soils (after 24 h of incubation)

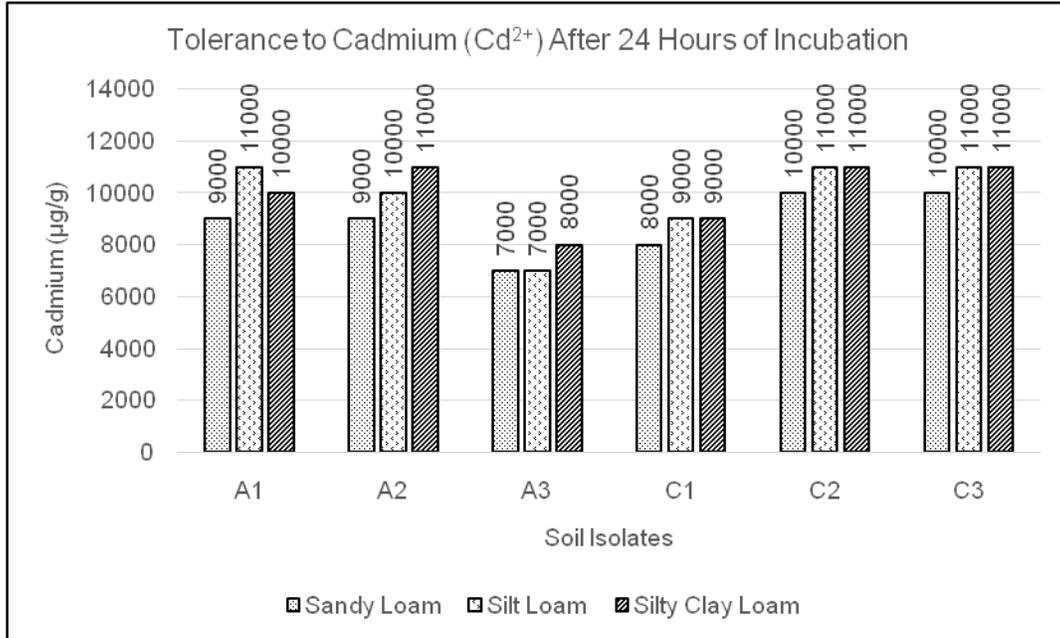


Fig.6 Tolerance of bacterial isolates Cd²⁺ in three soils (after 72 h of incubation)

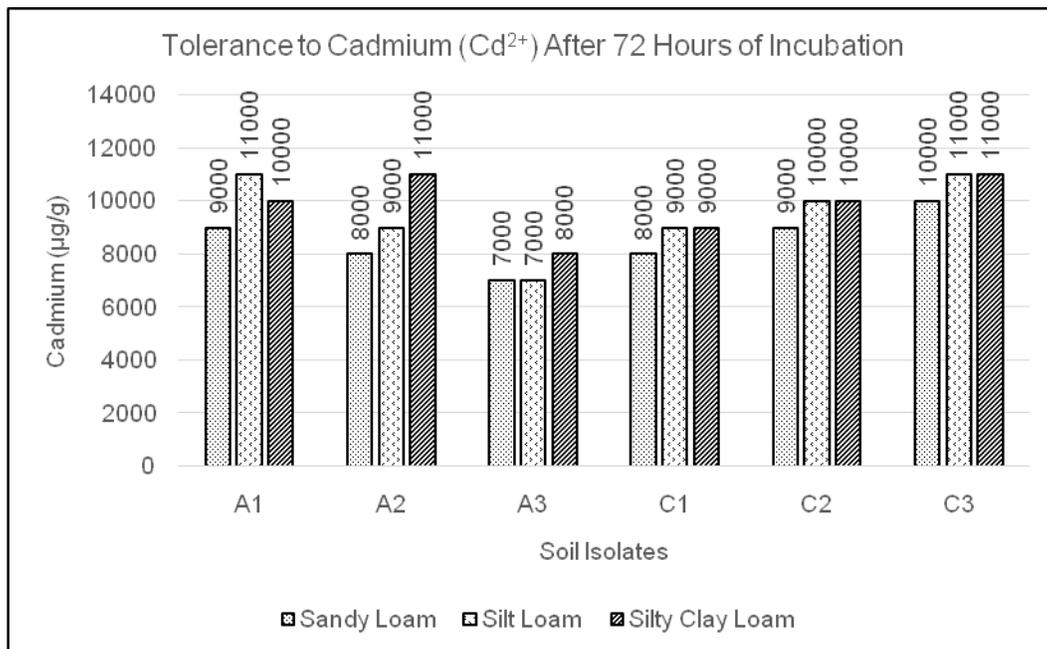


Fig.7 Tolerance of bacterial isolates Cd²⁺ in three soils (after 7 days of incubation)

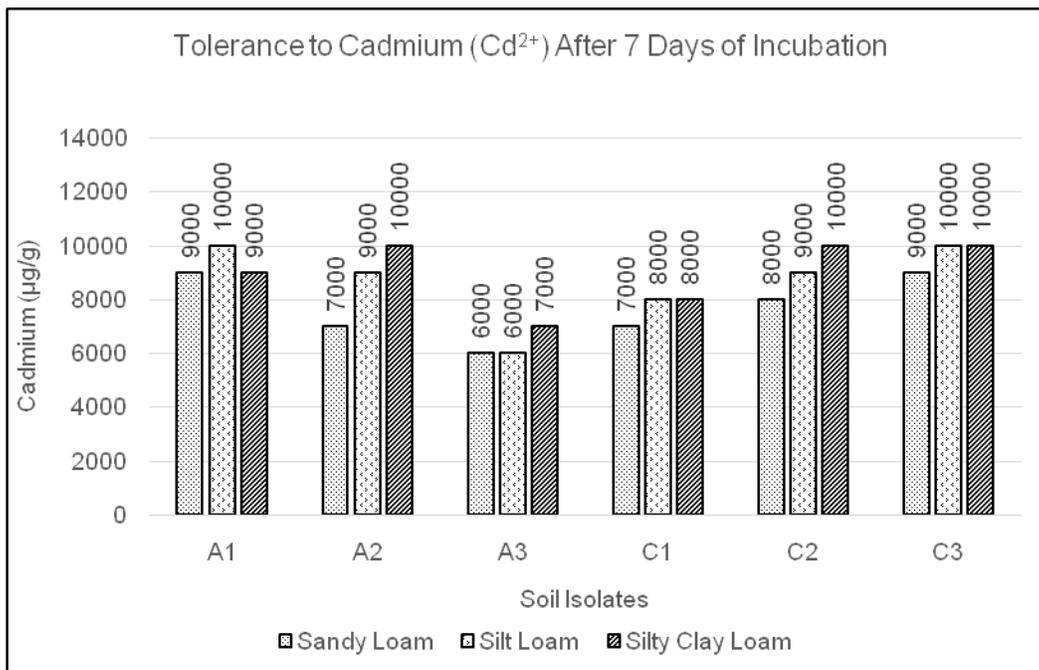
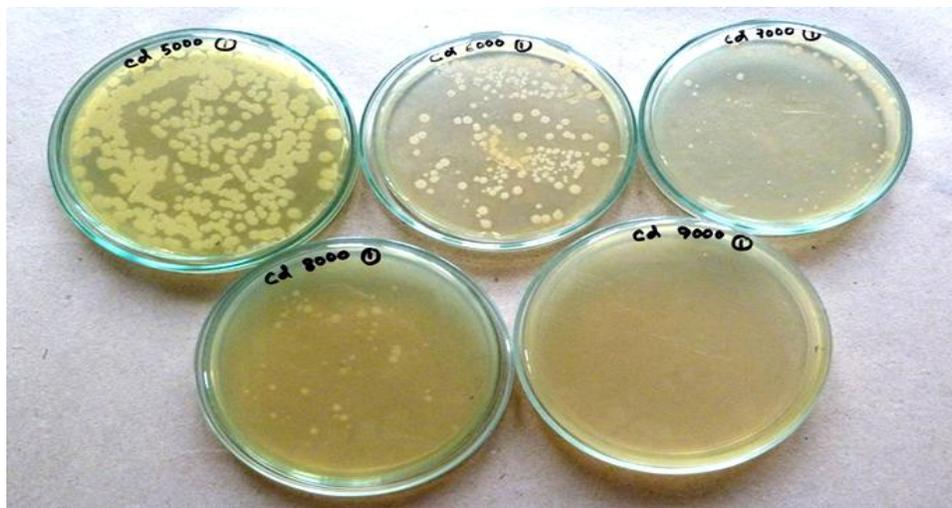


Fig.8 Growth of C3 (after 7 days of incubation) isolates at different concentration of Cd²⁺ in sandy loam soil



On the other hand, bacterial growths were inhibited compatibly at low level of metal content in sandy loam soil due to availability of metal in soil solution. Hassen *et al.* (1998) also tested the levels of tolerance of environmental bacteria to the different divalent metal ions including Cr⁶⁺, Co²⁺,

Cd²⁺, and Zn²⁺ in sandy soil and reported that the test in sandy soil was sensitive at concentrations 2 or 3 times lower than those obtained in silty or clayey soil.

As the behavior and bioavailability of metals in soil is affected by many chemical

processes, different mechanisms like adsorption, co-precipitation and organic complexation, transformation, biological methylation, and several interaction between metals and other elements present in soil, metal ions may not be available for microbes as well as bacteria. Soils have the unique system to sequester the chemicals into different fractions. So, the metals those are added from different sources are accommodated in soil. It may take long time to excrete any disastrous effect of heavy metals on soil bacteria.

Acknowledgment

We thank F.Z. Tanu for assistance with the bacterial strains. Major experimental facility was provided by Department of Botany and Department of Microbiology at University of Dhaka.

References

- Ahmad, I., S. Hayat, A. Ahmad, A. Inam, and Samiullah. 2001. Metal and antibiotic resistance traits in *Bradyrhizobium* sp. (cajanus) isolated from soil receiving oil refinery wastewater. *World J. Microbiol. Biotechnol.*, 17: 379-384.
- Anyanwu, C.U., and Nwachukwu, O.N. 2011. Heavy metal resistance in bacteria isolated from contaminated and uncontaminated soils. *Int. J. Res. Chem. and Env.*, 1: 173 - 178.
- Bache, B.W. 1979. The Encyclopedia of Soil Science. Springer. Pp. 487-492.
- Bruins, M.R., S. Kapil, and Oehme, F.W. 2000. Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Safe*, 45: 198-207.
- Cappuccino, J.G., and Sherman, N. 2005. Microbiology: A Laboratory manual (7th ed.). Dorling Kindersley (India). Pvt. Ltd. New Delhi, India. Pp.1 – 453.
- Das, P., S. Samantaray, and Rout, G.R. 1997. Studies on cadmium toxicity in plants: a review. *Environ. Pollut.*, 98(1): 29.
- Fliessbach, A., R. Martens, and Reber, H.H. 1994. Soil microbial biomass and microbial activity in soil treated with heavy metal contaminated sewage sludge. *Soil. Biol. Biochem.*, 26: 1201-1205.
- Gee, G.W. and Bauder, J.W. 1986. Particle size analysis. In: A. Klute (ed.) Methods of soil analysis. Part 1. (2nd ed.) *Agron. Monogr.*, 9. ASA, Madison, WI.
- Giller, K.E., E. Witter, and McGrath, S.P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil. Biol. Biochem.*, 30: 1389-1414.
- Hassen, A., Saidi, N., Cherif, M. and Boudabous, A. 1998. Resistance of environmental bacteria to heavy metals. *Biores. Technol.*, 64(1): Pp.7-15.
- Hayat, S., I. Ahmad, Z.M. Azam, A. Ahmad, A. Inam, and Samiullah. 2002. Effect of long term application of oil refinery wastewater on soil health with special reference to microbiological characteristics. *Biores. Technol.*, 84: 159 - 163.
- Hiroki, M. 1992. Effects of heavy metal contamination on soil microbial population. *Soil Sci. Plant Nutri.*, 38: 141-147.
- Jackson, M.L. 1973. Soil Chemical Analysis. Prentice Hall of India (Pvt.). Ltd. New Delhi. Pp. 326-337.
- Kabata-Pendias, A., S. Dudka, A. Chipecka, and Gawinsowska, T. 1992. Biogeochem. Trac. Metal. Adriano D.C (ed.). Lewis Publishers, Boca Raton.

- Kandeler, E., D. Tscherko, K.D., Bruce, M. Stemmer, P.J., Hobbs, R.D., Bardgett, and Amelung, W. 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. *Biol. Fertil. Soils*, 32: 390 - 400.
- Krishna Murti, C.R., and Vishwanathan, P. 1991. Toxic metals in the Indian Environment. Tata McGraw Hill Publishing Company Ltd., New Delhi.
- Olson, B.H., and Thornton, I. 1982. The resistance patterns of metals of bacterial population of contaminated land. *J. Soil Sci.*, 23: 271-277.
- Tanu, F.Z., and Hoque, S. 2013. Comparison of bacterial population in industrial and agricultural soils. *Int. J. Curr. Microbiol. App. Sci.*, 2(4): 155-165.
- Wang, Y.T., and Shen, H. 1995. Bacterial reduction of hexavalent chromium. *J. Ind. Microbiol.*, 14: 159 - 163.
- Winge, D.R., R.N. Reese, K., Mehra, E.B. Tabet, A.K., Hughes, and Daeron, C.T. 1989. Structural aspects of metal-r-glutamyl peptides. In: Ion Homeostasis: Molecular Biology and Chemistry. D. H. Hamer, and D. R Winge (eds.). Alan R. Liss, New York, Pp. 301 - 311.

How to cite this article:

Tanjila Jesmin and Sirajul Hoque. 2016. Bacterial Tolerance to Chromium and Cadmium in Different Textural Soils. *Int.J.Curr.Microbiol.App.Sci*. 5(9): 369-379.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.509.040>