IJMPR 2021, 5(2), 141-147

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

ISOLATION, IDENTIFICATION AND BIOSORPTION OF HEAVY METAL BY *E.COLI* SPP. *AND BACILLUS* SPP. FROM SOIL

Shashank Chauhan, Rupesh Kumar, Sandeep Kumar, Dinesh Kumar and Maya Datt Joshi*

Department of Biotechnology, Shobhit Institute of Engineering & Technology (Deemed to be University), Meerut.

Received on: 01/03/2021
Revised on: 19/03/2021
Accepted on: 09/04/2021

***Corresponding Author**
Dr. Maya Datt Joshi
Department of Biotechnology, Shobhit Institute of Engineering & Technology (Deemed to be University), Meerut.

ABSTRACT

Industrialization has led to introduction of heavy metals in the environment. Heavy metals are known to persist in the environment and become a risk for living beings. Micro-organisms present in industrial effluents are capable to cope up with the harmful effects of these metals. Microorganism adopt different mechanism and one such strategy is biosorption which is binding of metal ions with metal binding proteins present on the cell wall of microorganism. The present study is intended to analyze soil sample contaminated with heavy metals like Zinc (Zn), Copper (Cu), Cadmium (Cd), and Mercury (Hg) in *Bacillus* spp.and *E. coli* against different concentrations. Result showed that *Bacillus* spp. and *E. coli* has minimize the concentration of heavy metals in soil sample.

KEYWORD: Soil sample, Identification, Biochemical, Growth pattern, Biosorption.

INTRODUCTION

Heavy metals are natural elements characterized by a relatively high density, and are toxic even at low concentrations. Heavy metals are generated from both anthropogenic and natural sources and are eventually discharged into the environment(Glombitza F. *et.al* 2014). The discharge of heavy metals through various man-made activities, for example excessive application of chemical fertilizers, wood burning, coal combustion, vehicle exhaust, mining, smelting and incineration has caused a wide spread disruption of the normal biogeochemical cycles of metals causing a larger accumulation of heavy metals in the environment, especially in the soil (C. M. Davidson, A. L. Duncan, D. Littlejohn, A. M. Ure, L. M.Garden). The major heavy metals of concern include lead, cadmium, arsenic, mercury, copper and chromium because of their toxic impact on human health(Dutton and Fisher, 2011; Takahashi et al., 2012); for instance, environmental exposure to high concentrations of heavy metals has been linked with various cancers, liver, and kidney issues. (Czekalski N, Gascón Díez E, Bürgmann H, 2014) Heavy metals have also greatly affected soil microorganisms and plants growth and development. (S. Oancea, N. Foca, and A. Airinei).

The presence of heavy metals in the environment has been a source of concern over the past few decades due to their persistence, potential harm and toxicological hazards. Besides the fact that they are non-biodegradable, they may also undergo microbial or chemical transformation. However, the soil is a reservoir for some essential trace elements such as zinc and copper, which are necessary for the growth of plants and animals, but external influence can increase their concentration and consequently reduce the soil fertility and agricultural efficiency. Heavy metals can accumulate in the food chain, heavy and can have adverse effects on human and animal health.(Dutton and Fisher, 2011; Takahashi et al., 2012). There is also the risk of leaching of heavy metals, and this may contaminate underground water and in turn affect human health, especially those that consume underground water through boreholes and well water.(Hutton, M.(et.al.1986).

The increase in industrialization and urbanization offers ascend to heavy metal pollution of the environment, which might have resulted from the discharge of effluents containing metals such as lead, cadmium, chromium, nickel and mercury. The emissions of these metal pollutants have become primary concern due to their immediate and devastating effects on biological systems. A selective removal of heavy metals under varied physicochemical properties, adsorption and desorption and another advantage of microorganisms is their high surface-to-volume ratio. A wide variety of microbes have good potentials of metal absorption. Metal transport systems in microbes are of varying specificity. The present investigation is conducted to analysis the efficacy of *Bacillus* spp. and *E. coli* against various concentrations of heavy metals.

MATERIALS AND METHODS

Sample collection: Samples were collected from the industrial place from Dumas, Surat and municipal area, Surat. All the samples were collected in polythene bag

from the depth of 10 cm and then labeled properly and transported in laboratory for further analysis (Cline, M.G. 1944.).

Media preparation: Nutrient Broth medium was used for pre-enrichment process and for isolation, Chromogenic Coliform Agar(CCA) based medium for ***E.coli*** and on the other hand for ***Bacillus*** Spp. used same Nutrient Broth medium for pre-enrichment process and this media was prepare according to manufacturer's instruction and it was autoclaved at 121 °C for 15 min and was allowed to come at room temperature and then aseptically transferred into Laminar Air Flow for further used in petri dishes and allowed to solidify.

Pre-Enrichment and Isolation of microorganism: Firstly the microorganisms were identified by the inoculation of the sample into the Nutrient Broth Medium which shows a turbidity and for further process used isolation technique by the help of pour plate method. Chromogenic Coliform Agar(CCA) for ***E.coli*** and MYP Agar based medium for *Bacillus*.Spp. which was aseptically incubated at ambient temperature for 30°C, 35°C and 37 °C for 24 hr for bacteria.(IS 5887 part-1 & IS 5887 part-6).

Screening and isolation of the organisms: Different soil samples were treated for bacterial isolation by serial dilution technique using nutrient agar medium. The inoculated agar plates were incubated at 37^0C for 24hrs(Williams ST, Cross T.1971.).

Morphological identification of Microorganism: The morphological identification of microorganism were studied using nutrient agar plate. The physiological characteristics of all the obtained isolates were studied (Holt *et al.*,1994,).

Biochemical analysis: The bacterial isolates were characterized by colonial morphology and biochemical analysis were done using standard protocol of Indian standard 5887 part-1 for ***E.coli*** and Indian standard 5887 part-6 for ***Bacillus*** spp . The test for motility, catalase, oxidase, coagulase, indole, methyl-red test (MR-VP), urease, Simmons citrate utilization, triple sugar iron agar as well as Gram staining were performed. (Holt et al., 1994).

Growth pattern of microorganisms: Growth pattern analysis of microorganism were done at different concentration viz. 0.2ppm,0.4ppm, 0.6ppm, 0.8ppm, 1ppm, 2ppm, 3ppm, 4ppm, 5ppm of zinc sulphate ($ZnSo_4.7H_2O$), Copper sulphate($CuSo_4.5H_2O$), Mercuric Chloride($HgCl_2.7H_2O$) in 100ml Nutrient Broth and then optical density were measured in UV-VIS spectrophotometer at different time interval (2day, 4days, 6 days & 8 days). (Abioye OP, Yusuf GB, Aransiola SA, Oyewole OA, Bala JD 2017).

Uptake of the heavy metal by *E.coli* spp. & *Bacillus* spp. Quantification of the heavy metals weredone by using inductively coupled Plasma-Optical Emission Spectroscopy (Rajneet Kour Soodan, Yogesh B.Pakade, Avinash 2014). Determination of the uptake of heavy metals by microorganism were done in different concentration (0.2ppm, 0.4ppm 0.6ppm,0.8ppm, 1.0ppm,2.0ppm,3.0ppm,4.0ppm,5.0ppm)at different time interval (2day, 4days, 6days, 8days) (Wang Yuqing, Huang Yifan, Huang Wenqi, Chen Guiyu, Ou Chaolian, Li Daoning.)

RESULTS

Pre-Enrichment and Isolation of microorganism: Here 10 well isolated colony observed on to the Nutrient Agar(N.A) based medium, which sample has been collected from the Industrial region and Municipal Sample from Surat. In this result we isolate this characteristic colony for further Biochemical process.

Morphological identification of Microorganism: The total number of isolates proceeded for their morphological character. The colony characteristics of the obtained isolates were studied on nutrient agar plate, which give circular/rhizoid, white/pink, small/large colonies with entire margin after 24hr incubation. In them 6 were gram negative, and 4 were gram positive isolates and perform the biochemical test.(shown in table.1).

Biochemical analysis: In this analysis we performed the different kind of the biochemical analysis were conducted such as: Indole, MR, VP etc. for the both of the organism which mentioned below in the (Table 1).

Growth pattern of microorganisms: In this analysis growth pattern were analyze using UV-Vis spectrophotometer (Shimadzu 1800) at optical density 600 nm for *E.coli spp.* & optical density 600 nm for *Baciilus spp.* by using $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ & $HgCl_2$ $.7H_2O$. The growth of *Bacillus* spp. and *E.coli* were found intense at 3 ppm concentration in comparison with other concentration.

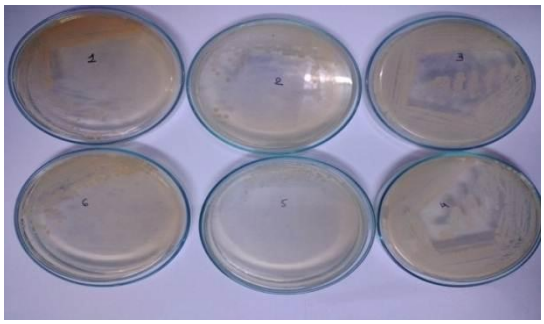

Fig. 1: Uptake ofheavy metals by *E.coli.*spp and *Bacillus* spp.

Total number of 10 isolates were screened from 2 different soil samples and identified by different biochemical tests.

Table 1: Biochemical testing of 2 different soil samples.

Test	Escherichia spp.	Bacillus spp.
Indole	+ve	-ve
MR	+ve	-ve
VP	-ve	+ve
Simmon citrate	-ve	-ve
Motility	+ve	+ve
Nitrate	+ve	-ve
TSI	Butt- acidic Slant-alkaline	Butt-alkaline Slant-alkaline
Urease	+ve	+ve
lactose	+ve	+ve
Glucose	+ve	+ve
Mannitol	+ve	-ve
sucrose	+ve	+ve

The morphological and biochemical characteristics. ***E.coli*** *spp.* and ***Bacillus*** *spp.* Were taken for further process. In this biochemical analysis we seeked and differentiate between ***E.coli*** *spp.* and ***Bacillus*** *spp.* With the help of Indole test gives po TSI slant, Organism growth pattern were done by UV-VIS Spectrophotometer.

Table 2: Optical Density at 600nm of *E.coli spp.*

	Concentration (ppm)	After 2 Days(nm)	After 4 Days(nm)	After 6 Days(nm)	After 8 Days(nm)
Zinc(Zn)	0.2	0.690	0.870	0.680	0.680
	0.4	0.760	0.919	0.876	0.840
	0.6	0.843	0.970	0.903	0.870
	0.8	0.990	1.025	0.992	0.884
	1.0	1.241	1.521	1.430	0.893
	2.0	1.896	1.971	1.801	0.917
	3.0	2.021	2.340	1.970	0.980
	4.0	1.011	1.212	0.996	0.840
	5.0	0.970	1.010	0.870	0.790
Copper(Cu)	0.2	0.770	0.890	0.720	0.690
	0.4	0.890	0.910	0.820	0.800
	0.6	0.927	0.938	0.857	0.790
	0.8	0.969	1.027	0.958	0.885
	1.0	1.035	1.245	0.996	0.896
	2.0	1.320	1.980	1.740	0.956
	3.0	1.993	2.026	1.946	0.993
	4.0	0.980	0.990	0.970	0.840
	5.0	0.910	0.970	0.939	0.710
Mercury(Hg)	0.2	0.540	0.480	0.210	0.180
	0.4	0.671	0.590	0.430	0.370
	0.6	0.720	0.696	0.590	0.525
	0.8	0.881	0.780	0.670	0.590
	1.0	0.920	0.885	0.690	0.573
	2.0	1.230	1.010	0.870	0.866
	3.0	0.970	0.930	0.720	0.700
	4.0	0.890	0.826	0.630	0.611
	5.0	0.730	0.699	0.551	0.549

E.coli. spp. at 600nm for different concentration with 0.2ppm,0.4ppm, 0.6ppm,0.8ppm, 1.0ppm,2.0ppm, 3.0ppm, 4.0ppm, 5.0ppm, gave growth by using $ZnSo_4.7H_2O$, concentration(nm) and consumption after 2 days was 0.690nm, 0.760nm,0.843nm, 0.990nm,1.241nm,1.896nm,2.021nm, 1.01nm, 0.970nm,

after 4 days was 0.870nm, 0.919nm, 0.970nm,1.025nm, 1.521nm,1.971nm,2.340nm,1.212nm,1.010nm.

Table 3: Optical Density at 600nm of *Baciilus spp.*

	Concentration (ppm)	After 2 Days(nm)	After 4 Days(nm)	After 6 Days(nm)	After 8 Days(nm)
Zinc(Zn)	0.2	0.789	0.891	0.693	0.665
	0.4	0.821	0.960	0.701	0.605
	0.6	0.976	1.801	0.899	0.725
	0.8	0.993	1.996	0.970	0.839
	1.0	1.026	2.010	1.904	0.961
	2.0	1.657	2.156	1.506	0.832
	3.0	1.987	2.569	1.401	0.702
	4.0	1.001	1.996	0.996	0.665
	5.0	1.013	1.876	0.703	0.573
Copper(Cu)	0.2	0.890	0.880	0.730	0.553
	0.4	0.980	0.940	0.851	0.673
	0.6	1.028	0.980	0.880	0.680
	0.8	1.238	1.210	0.990	0.881
	1.0	1.548	1.410	0.945	0.846
	2.0	1.946	1.944	0.922	0.726
	3.0	2.510	2.002	1.180	0.920
	4.0	2.000	1.993	1.560	0.430
	5.0	1.881	1.760	1.320	0.246
Mercury(Hg)	0.2	0.670	0.230	0.220	0.224
	0.4	0.765	0.420	0.410	0.400
	0.6	0.820	0.630	0.520	0.501
	0.8	0.980	0.758	0.430	0.396
	1.0	1.230	0.890	0.670	0.590
	2.0	0.290	0.130	0.090	0.086
	3.0	0.140	0.089	0.079	0.072
	4.0	0.090	0.087	0.083	0.079
	5.0	0.079	0.069	0.070	0.066

Table 4: Effect of heavy metals on *E.coli spp.* through ICP-OES.

	Concentration (ppm)	After 2 Days Absorption (%)	After 8 Days A bsorption (%)
Zinc(Zn)	0.2	25.6	5.68
	0.4	29.3	7.56
	0.6	28.1	4.99
	0.8	30.2	5.93
	1.0	35.1	6.18
	2.0	33.6	5.93
	3.0	30.8	7.33
	4.0	20.3	3.81
	5.0	17.4	2.98
Copper(Cu)	0.2	29.2	4.53
	0.4	32.1	5.90
	0.6	31.8	5.96
	0.8	33.6	4.31
	1.0	37.2	5.38
	2.0	35.1	6.21
	3.0	21.8	2.81
	4.0	17.3	4.19
	5.0	13.6	2.11
Mercury(Hg)	0.2	20.8	1.09
	0.4	5.70	0.13
	0.6	0.11	0.05
	0.8	0.05	0.00

	1.0	0.01	0.00
	2.0	0.00	0.00
	3.0	0.00	0.00
	4.0	0.00	0.00
	5.0	0.00	0.00

Table 5: Effect of heavy metals on *Bacillus Spp.* through ICP-OES.

	Concentration (ppm)	After 2 Days Absorption %	After 8 Days Absorption %
Zinc(Zn)	0.2	20.3	3.08
	0.4	23.1	3.11
	0.6	15.3	1.83
	0.8	17.1	2.10
	1.0	12.8	1.80
	2.0	10.3	0.96
	3.0	3.11	0.00
	4.0	1.13	0.00
	5.0	0.00	0.00
Copper(Cu)	0.2	43.8	2.88
	0.4	41.7	1.98
	0.6	42.3	2.11
	0.8	43.1	0.96
	1.0	41.2	0.99
	2.0	19.1	1.21
	3.0	12.3	0.80
	4.0	0.50	0.00
	5.0	0.50	0.00
Mercury(Hg)	0.2	15.0	0.87
	0.4	3.96	0.08
	0.6	0.13	0.00
	0.8	0.03	0.00
	1.0	0.01	0.00
	2.0	0.00	0.00
	3.0	0.00	0.00
	4.0	0.00	0.00
	5.0	0.00	0.00

DISCUSSION

*E.coli spp.*absorb zinc salt, copper salt and mercury salt by 35.1 %, 37.2 % and 5.70% after 2 days and bacillus spp. absorb zinc salt, copper salt and zinc salt 17.1%, 43.1% and 3.96% after 2days.Similarly, the absorption of these heavy metals by E.coli. after 8 days were ,7.56%,6.21% and 0.13%.In the same manner, Bacillus spp. absorbed these heavy metals by,3.11%,2.88% and 0.87% after 8 days.

CONCLUSION

The preliminary work of screening of *E.coli spp.* and *Bacillus* spp. from chosen sampling site and biochemical identification of isolates under study has been successfully carried out. By ICP spectroscopy maximum metal consumption 43.1 % was done by *Bacillus* spp. at 0.8ppm concentration.

REFERENCES

1. Hutton, M., & Symon, C. The quantities of cadmium, lead, mercury and arsenic entering the UK environment from human activities. *Science of the total environment*, 1986; *57*: 129-150.
2. Nriagu, J. O., & Pacyna, J. M. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, 1988; *333*(6169): 134-139.
3. Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. Heavy metal pollution and human biotoxic effects. *International Journal of physical sciences*, 2007; *2*(5): 112-118.
4. Hawkes, S. J. What is a" heavy metal"?. *Journal of Chemical Education*, 1997; *74*(11): 1374.
5. Farlex, I. Definition: environment, the free dictionary. *Farlex Inc. Publishing, USA (http://www. thefreedictionary. com/)*, 2005.
6. McCarthy, G., Puce, A., Gore, J. C., & Allison, T. Face-specific processing in the human fusiform gyrus. *Journal of cognitive neuroscience*, 1997; *9*(5): 605-610.
7. Zhylyak, G. A., Dzyadevich, S. V., Korpan, Y. I., Soldatkin, A. P., & El'Skaya, A. V. Application of urease conductometric biosensor for heavy-metal

ion determination. *Sensors and Actuators B: Chemical*, 1995; *24*(1-3): 145-148.
8. De Smet, P. A., Keller, K., Hänsel, R., & Chandler, R. F. (Eds.). *Adverse effects of herbal drugs* (pp. 1-13). Berlin/Heidelberg: Springer, 1992.
9. Musico, Y. L. F., Santos, C. M., Dalida, M. L. P., & Rodrigues, D. F. Improved removal of lead (II) from water using a polymer-based graphene oxide nanocomposite. *Journal of Materials Chemistry A*, 2013; *1*(11): 3789-3796.
10. Ozdemir, G., Ozturk, T., Ceyhan, N., Isler, R., & Cosar, T. Heavy metal biosorption by biomass of Ochrobactrum anthropi producing exopolysaccharide in activated sludge. *Bioresource technology*, 2003; *90*(1): 71-74.
11. Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., & Sastry, M. Synthesis of gold nanotriangles and silver nanoparticles using Aloevera plant extract. *Biotechnology progress*, 2006; *22*(2): 577-583.
12. Celik, A., & Demirbaş, A. Removal of heavy metal ions from aqueous solutions via adsorption onto modified lignin from pulping wastes. *Energy sources*, 2005; *27*(12): 1167-1177.
13. Faisal, M., & Hasnain, S. Microbial conversion of Cr (VI) in to Cr (III) in industrial effluent. *African journal of Biotechnology*, 2004; *3*(11): 610-617.
14. Deng, L., Su, Y., Su, H., Wang, X., & Zhu, X. Sorption and desorption of lead (II) from wastewater by green algae Cladophora fascicularis. *Journal of Hazardous Materials*, 2007; *143*(1-2): 220-225.
15. Holan, Z. R., & Volesky, B. Biosorption of lead and nickel by biomass of marine algae. *Biotechnology and bioengineering*, 1994; *43*(11): 1001-1009.
16. Davis, T. A., Volesky, B., & Mucci, A. A review of the biochemistry of heavy metal biosorption by brown algae. *Water research*, 2003; *37*(18): 4311-4330.
17. Sud, D., Mahajan, G., & Kaur, M. P. Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions–A review. *Bioresource technology*, 2008; *99*(14): 6017-6027.
18. Fu, F., & Wang, Q. Removal of heavy metal ions from wastewaters: a review. *Journal of environmental management*, 2011; *92*(3): 407-418.
19. Kaczala, F., Marques, M., & Hogland, W. Lead and vanadium removal from a real industrial wastewater by gravitational settling/sedimentation and sorption onto Pinus sylvestris sawdust. *Bioresource technology*, 2009; *100*(1): 235-243.
20. Park, H. J., Jeong, S. W., Yang, J. K., Kim, B. G., & Lee, S. M. Removal of heavy metals using waste eggshell. *Journal of environmental sciences*, 2007; *19*(12): 1436-1441.
21. Romera, E., González, F., Ballester, A., Blázquez, M. L., & Munoz, J. A. Comparative study of biosorption of heavy metals using different types of algae. *Bioresource technology*, 2007; *98*(17): 3344-3353.
22. Ajjabi, L. C., & Chouba, L. Biosorption of Cu2+ and Zn2+ from aqueous solutions by dried marine green macroalga Chaetomorpha linum. *Journal of environmental management*, 2009; *90*(11): 3485-3489.
23. Townsley, C. C. Biorecovery of metallic residues from various industrial effluents using filamentous fungi. *Process Metal*, 1986; *4*: 279-289.
24. Salah Azab, M., & Peterson, P. J. The removal of cadmium from water by the use of biological sorbents. *Water Science and Technology*, 1989; *21*(12): 1705-1706.
25. Veglio, F., & Beolchini, F. Removal of metals by biosorption: a review. *Hydrometallurgy*, 1997; *44*(3): 301-316.
26. Valls, M., & De Lorenzo, V. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS microbiology Reviews*, 2002; *26*(4): 327-338.
27. PAN, J. H., LIU, R. X., & TANG, H. X. Surface reaction of Bacillus cereus biomass and its biosorption for lead and copper ions. *Journal of Environmental Sciences*, 2007; *19*(4): 403-408.
28. Muñoz, A. J., Ruiz, E., Abriouel, H., Gálvez, A., Ezzouhri, L., Lairini, K., & Espínola, F. Heavy metal tolerance of microorganisms isolated from wastewaters: Identification and evaluation of its potential for biosorption. *Chemical Engineering Journal*, 2012; *210*: 325-332.
29. Tuomanen, E., Cozens, R., Tosch, W., Zak, O., & Tomasz, A. The rate of killing of Escherichia coli byβ-lactam antibiotics is strictly proportional to the rate of bacterial growth. *Microbiology*, 1986; *132*(5): 1297-1304.
30. Volesky, B. Biosorption and me. *Water research*, 2007; *41*(18): 4017-4029.
31. Wehrheim, B., & Wettern, M. Biosorption of cadmium, copper and lead by isolated mother cell walls and whole cells of Chlorella fusca. *Applied microbiology and Biotechnology*, 1994; *41*(6): 725-728.
32. Wang Yuqing, Huang Yifan, Huang Wenqi, Chen Guiyu, Ou Chaolian, Li Daoning. Graphite Microwave Digestion- Determination of Heavy Metals in Soil of Guangxi Cassava Producing Area by ICP-Mass Spectrometry [J]. Volkswagen Technology, 2016; 08: 50-52.
33. Pacyna JM, Pacyna EG An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. Environmental Reviews, 2001; 9: 269-298.
34. Czekalski N, Gascón Díez E, Bürgmann H Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. ISME J, 2014; 8: 1381-1390.
35. 8. Duruibe J, Ogwuegbu M, Egwurugwu J Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences, 2007; 2: 112-118.

36. S. Oancea, N. Foca, and A. Airinei, "Effects of heavy metals on plant growth and photosynthetic activity," *Analele Ştiinţifice ale Universităţii "AL. I. CUZA1 IAŞI, Tomul I, s. Biofizică, Fizică medicală şi Fizica mediului*, 2005; 107–110.
37. Williams ST, Cross T. Actinomycetes, methods in microbiology. New York: Academic Press, 1971.
38. Glombitza F, Reichel S Metal-containing residues from industry and in the environment: Geobiotechnological urban mining. Adv Biochem Eng Biotechnol, 2014; 141: 49-107.
39. Cline, M.G. Principles of soil sampling. Soil Sci., 1944; 58: 275–288.
40. C. M. Davidson, A. L. Duncan, D. Littlejohn, A. M. Ure, L. M.Garden, A critical leval- uation of the three stage BCR sequential extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially-contaminated land, *Anal. Chim. Acta*, 1998; 363: 45 – 55.
41. Bergey's Manual of Determinative Bacteriology (Holt et al.), 1994.
42. Abioye OP, Yusuf GB, Aransiola SA, Oyewole OA, Bala JD Biosorption of chromium and zinc by *Micrococcus virians* and *Staphylococcus aureus* isolated from soil. Int Res J Environ Sci., 2017; 6(6): 1–7.
43. Rajneet Kour Soodan, Yogesh B.Pakade, Avinash Nagpal. Analytical techniquesfor estimation of heavy metals in soil ecosystem, Talanta, 2014; 125(11): 405.
44. IS 5887 (Part 1): Methods for Detection of Bacteria Responsible for Food Poisoning, Part 1: Isolation, Identification and Enumeration of Escherichia Coli.
45. IS 5887-6: Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Presumptive Bacillus Cereus, Part 6 Colony-count Technique at 30o C.