Maximum tolerance concentration detection of microorganisms capable of bioremediation of toxic heavy metals from the ash dyke of thermal power plant

Sonali Banerjee[1], Ragini Gothalwal[2], Shweta Sao[1]

[1]Department of Life Science, Dr C.V. Raman University, Bilaspur, Chattisgarh, India;
[2]Department of Biotechnology, Barkatullah University, Bhopal, Madhya Pradesh, India,
Email: sonalibanerjee.biotech@gmail.com

ABSTRACT

Global pollution is increasing due to the variations in natural and anthropogenic activities, leading to contaminations in various aquatic and terrestrial ecosystems with heavy metals, organic and inorganic chemical compounds. Toxic metals are common contaminants of natural waters and may adversely affect the potentially important biodegradation processes naturally occurring in environment. Sources of these pollutants may include leachates from hazardous waste sites, discharges from industrial plants and effluents from wastewater treatment plants. These heavy metal residues enter in environment with different human activities and causes serious health related problems. Experiments were conducted to determine the presence of microorganisms in ash dyke sample (soil/water), having the potency to tolerate the heavy metals present in the sample. The removal and recovery of heavy metals from any heavy metal contaminated site may be done by using a mixture of multiple metal-tolerant bacterial strains. 25 bacterias isolated from the thermal power plant have been found to resist the presence of heavy metals in the media. These strains showed inherent or acquired resistance to multiple heavy metals viz. Ni, Cu, Pb, Hg, Mn, and Co and could grow upto 15mM concentrations in solid media both with and without glucose (10mM) as instant carbon source.

Keywords: bioremediation, heavy metals, tolerance, toxicity, ash dyke

INTRODUCTION

Most of the metal pollutants are primarily distributed in the atmosphere, water, soil and sediments [1-3]. Heavy metal pollution arises mainly from the mining, smelting and refining of metallic ores, the manufacturing and use of metallic products [4,5], burning of fossil fuels, controlled and uncontrolled discharge of solid and liquid waste, accidental spillage and agricultural advancement have significantly contributed heavy metal pollution [6,7]. Two categories of the heavy metal contamination are defined as; bioavailable (soluble, nonsorbed and mobile) and non-bioavailable (precipitated, complexed, sorbed and non-mobile) [8]. Bioavailable metal concentrations are highly toxic to the biological systems but some other metals are also of concern [9,10]. Major inputs of metals into the environment also occur from municipal and industrial sludges and by-products [11]. Elevated metal concentration in the environment has a wide range impact on the animal, plants and microbial species [12]. Many of the toxic heavy metals from different sources are deposited and buried in the soil and water. They also reach the water bodies when washed off from the soil. Humus is the organic material present in the soil, has high affinity for heavy metals cations and can

Research Article, Acta Biologica Indica 2014, 3(2):642-646

pISSN 2319-1244, eISSN 2279-0160

be extracted from water that passes through soil. Roots of the crops and of other plants could pick up these contaminants present in the waters. Heavy metals are also retained in the soil by adsorption on mineral particles of the soil and precipitation reactions. In water, particles with adsorbed heavy metals settle to bottom and sediments may accumulate over them. If the organisms consume these, then heavy metals enter the food web. Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc, essentially serve as micronutrients and are used for redox-processes to stabilize molecules through electrostatic interactions as components of various enzymes and for regulation of osmotic pressure. Many other metals have no biological role (e.g. silver, aluminium, cadmium, gold, lead and mercury) and are nonessential [13] and potentially toxic to microorganisms. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions [13,14]. For example, $Hg^{2+,}$ Cd^{2+} and Ag^{2+} tend to bind to SH groups, and thus inhibit the activity of sensitive enzymes [14]. In addition, at high concentrations both essential and nonessential metals can damage cell membranes, alter enzyme specificity, disrupts cellular functions and damage the structure of DNA [13].

In recent years, evidence has been presented that exposure to some metal may be more dangerous than hitherto expected [15,16]. Some of the heavy metal toxicity can result in stopping the functions of DNA and increases the risk of cancer in the human body [17]. Some of the metal ions may cause conformational changes in enzymes rendering them inactive. Toxicity of metals is also caused when the metals block the defensive protein of body which fights against the infection of microorganisms [18]. With the recent advancement in bioremediation technologies and increased interest in the studies dealing with molecular markers of health and disease expression of genes, a specific toxicant "signature" has been developed to detect the toxicity of arsenic, cadmium, mercury, chromium, lead, copper, nickel, manganese, and other heavy metals [19].

Chhattisgarh is one of the leading producers of electricity in our country having two National Thermal Power Corporations (NTPC) established in Korba and Sipat. Private plants are also established for producing electricity. Approximately more than 50 Million Tonnes per annum (MTPA) of coal is utilized for production of electricity and releases various toxic effluents in the aquatic reservoirs [20].

MATERIALS AND METHODS

Sampling and Isolation of Microorganisms

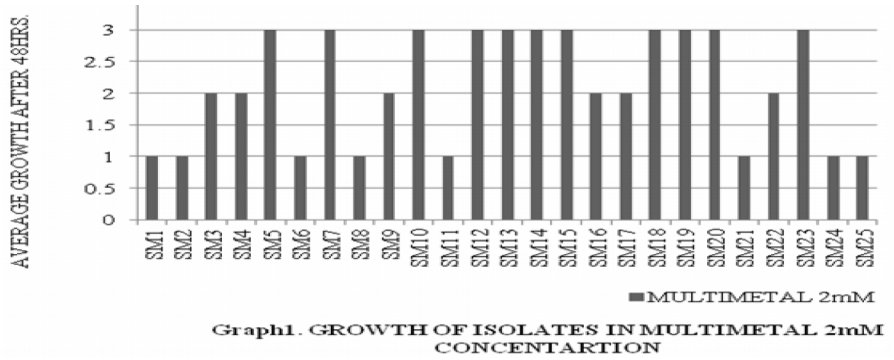
Soil samples were obtained from Ash Dyke area of Thermal Power Plants of Chhattisgarh. The samples were collected in sterilized flasks and beakers and after sealing with parafilm, they were stored at 4°C in a refrigerator. Different bacteria were isolated from the Ash Dyke area (soil/water) using serial dilution technique in nutrient medium. Following and consulting the available literatures, Nutrient medium was selected as the best medium for the isolation of bacteria [21-23].1g of the soil sample was put in a test tube with 9ml saline. From this sample then, their respective serial dilution series was prepared. Out of which, $10^{-3}$and 10^{-4} dilutions were selected and were spread onto the sterilized Petri plates containing the nutrient medium [24,25]. Then the 25 possibly different isolates were selected randomly from the four plates and were streaked by pick and patch method onto the separate Petri plates containing 1mM heavy metals each viz. Ni, Cu, Pb, Hg, Mn and Co and 10mM glucose. Isolates were named as SM1 to SM25. Biochemical tests formed the basis for the detection of the 25 isolates. Bacterial isolates were identified as per the standard methods following Bergey's Manual of Determinative Bacteriology.

Bacterial Maximum Tolerance Concentration (MTC) for Heavy Metals

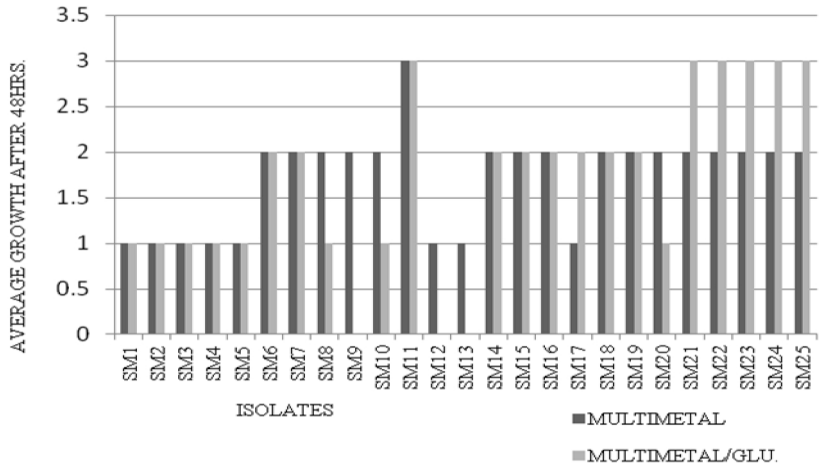
The maximum tolerance concentration (MTC) has formed the basis for the selection of the potent isolates, and MTC of these selected isolates towards different selected heavy metals has been checked till 15mM heavy metal concentration in multiple metals together with or without 10mM glucose and sucrose concentration in the media.

RESULTS AND DISCUSSION

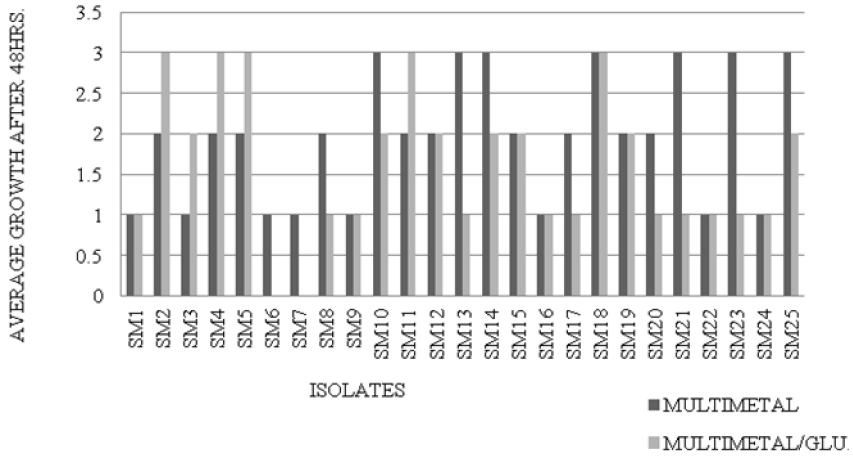
The isolates SM 2, 3, 4, 5, 7, 8, 11, 12, 14, 18, 20, 22 were found to belong to *Bacillales.*, SM 1, 6, 9, 13, 15 belongs to *Pseudomonadales*, SM 10, 16, 19, 21 belongs to *Archaeobacteria* and SM 17, 23, 24, 25 found to be of *Staphylococcales*. In the presence of 2mM heavy metal concentration in the medium, isolate no. SM 3, 4, 5, 7, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23 showed proper growth (Graph 1) whereas SM6 to SM11, SM14 to SM16, SM18, to SM25 could tolerate 5mM heavy metal concentrations (Graph 2). When comparing the growth of the isolates in 10 mM heavy metals both with/without 10mM glucose individually it was found that SM2, 4, 5, 8, 10 to SM15, SM17 to 21, 23 and 25 resist 10mM multiple heavy metal concentration in 48hrs (Graph 3).



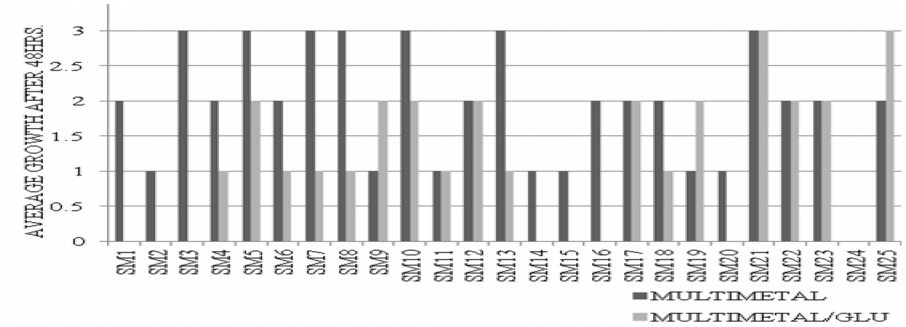
Graph1. GROWTH OF ISOLATES IN MULTIMETAL 2mM CONCENTARTION



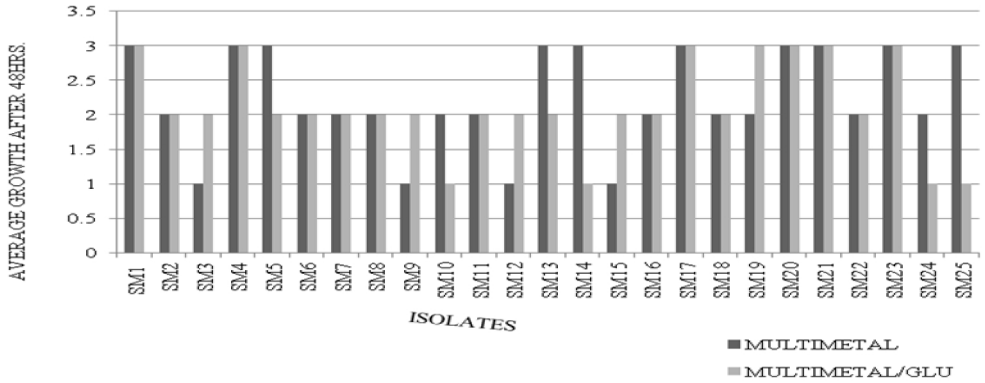
Graph 2. GROWTH OF ISOLATES IN 5mM MULTIPLE METAL MEDIA WITH/WITHOUT 10mM GLUCOSE AFTER 48HRS



Graph 3. GROWTH OF ISOLATES IN 10mM MULTIPLE METAL MEDIA WITH/WITHOUT 10Mm GLUCOSE



Graph4. GROWTH OF ISOLATES IN 15mM MULTIPLE METAL MEDIA WITH/WITHOUT 10mM GLUCOSE AFTER 48HRS



Graph 5. GROWTH OF ISOLATES IN 25mM MULTIPLE METAL MEDIA WITH/WITHOUT 10mM GLUCOSE AFTER 48HRS

At higher concentrations (15mM), it was observed that the isolates behaved differently for different metals when plated separately with Mn, Ni, Cu, Co, Hg, Pb and for multiple metal containing media both with/without 10mM glucose i.e. SM 1, 3 to 8, 10, 12, 13, 16 to 18, 21 to 23 and 25 showed moderate growth in the absence of glucose whereas SM5, 9, 10, 12, 17, 19, 21, 22,

23 and 25 showed moderate growth in the presence of glucose as well. Similarly, when the process repeated for 25mM concentration of metals, SM1, 4, 5, 13, 14, 17, 20, 21, 23, 25 showed moderate growth in the absence of glucose and SM1 ,3, 17, 19, 20, 21, 23 showed moderate growth in the presence of glucose (10mM concentration) (Graph 5) Our foremost objective is to maintain and protect the microbial diversity of the culturable form of the microorganisms obtained from the contaminated soil that represents new varieties or species. The work may lead to an understanding of microbial diversity of the state of Chhattisgarh which has not been yet explored. Many changes could be developed in the organisms against any sort of pollution and which forms a basis for evolution and natural selection. Thus, the advanced culture independent techniques of the microbial community analysis, including their biases and limitations become suitable for the determination of the virtual changes exhibited by the microbes in their culture environment and community structure exposed to the impact of pollutants. The most important and the ultimate goal behind this study are to predict accurately the microbes assisted natural attenuation of the contaminants or the likely outcome of the engineered strategies to accelerate the bioremediation process.

REFERENCES

[1] Chen XB, Wright JV, Conca JL, Peurrung LM. Water, Air and Soil Pollution. 1997, 98: 57-78.
[2] Pichtel J, Pichtel TM. Environ. Eng. Sci. 1997, 14:97-104.
[3] Armitage PD, Bowes MJ, Vincent HM. River Res. and Appl. 2007, 23(9): 997-1015.
[4] Kabata-Pendias A, Pendias H. In: Soils and Plants. CRC Press, BocaRaton, FL. 1984, 3:315.
[5] Dudka S, Adriano DC. J. Environ. Qual. 1997, 26:590–602.
[6] Brantley AS, Townsend TG. Env. Eng. Sci. 1999, 16:105-116.
[7] Romic M, Romic D. Environ. Geol. 2003, 43: 795–805.
[8] Jonnalagadda SB, Prasada Rao PVV. Comp. Biochem. Physiol. 1993, 106C(3):585–595.
[9] James M, Okolo PO. Pakistan J. Sci. Industrial Res. 2003, 46(6): 439-442.
[10] Neto MC, Itavo RV, Moraes LES. Environ. Pollution. 2003, 123: 319-324.
[11] Kabata-Pendias A, Adriano DC. In: Soil Amendments and Environmental Quality. Rechcigl JE. (ed). Lewis Publishers, Boca Raton, FL., 1995, 139–167.
[12] Roane TM, Pepper IL, Miller RM. In: Bioremediation: Principles and Application. Crawford D.L. (eds). Cambridge University Press, U.K. 1996, 312-339.
[13] Bruins MR, Kapil S, Oehmei FW. Ecotoxicology Environ. Saf. 2000, 45:198–207.
[14] Nies DH. Appl. Microbiol Biotechnol. 1999, 51: 730-750.
[15] Brulport M, Schormann W, Bauer A, et al. Hepatology. 2007, 46:861–870.
[16] Bolt HM, Hengstler JG. Arch. Toxicol. 2008, 82:1–3.
[17] Beyersmann D, Hartwig A. Arch. Toxicol. 2008, 82:493–512.
[18] Sobolev D, Begonia MFT. Int. J. Environ. Res. Public Health. 2008, 5(5):450-456.
[19] Kawata K, Yokoo H, Shimazaki R, Okabe S. Environ Sci Technol. 2007, 41, 3769-3774.
[20] NTPC Performance (http://www.ntpc.co.in/companyperformance/CMD_press_release.pdf)
[21] Unaldi Coral MN, Korkmaz H, Arikan B et al. Ann .of Microbiol. 2005, 55(3):175-179.
[22] Zolgharnein H, Mohd Azmi ML, Saad MZ, et al. Iran. J. Biotechnol. 2007, 51(4): 232-239.
[23] Bahig AE, Aly EA, Khaled AA et al. Mal. J. of Microbiol. 2008, 4(2): 42-50.
[24] Jiang C, Sheng X, Qian M et al. Chemosphere. 2008, 72(2):157-164.
[25] Kamala-Kannan S and Lee KJ. Biotechnol. 2008, 7(1):149-152.