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Isolation and Screening of Heavy Metal Resistant Bacteria from Industrial Effluent

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ABSTRACT

In recent years, pollution has been a rising at an alarming rate due to different anthropogenic activities. Heavy metals are one of the major pollutants nowadays. Heavy metals are an essential component of the atmosphere but due to disturbance in their natural geochemical cycles their concentration has increased to a safer level. These metals are introduced into the ecosystem by both natural and anthropogenic activity. Heavy metals having a property to be non-biodegradable or non-destroyable can only be converted to high toxic form to less toxic form. Removal of these metals from the environment has become very essential to make the environment healthier. Several physiochemical strategies are used to remove these metals but they are very costly and less effective. Bioremediation is a great alternative to these methods to being environmentally safe and less costly. This method uses the microbes, plants or other living organisms to remove the toxic pollutant from the contaminated areas. Majorly microorganisms are selected for this purpose which uses these toxic metals as their food sources. In present work, we isolated four heavy metal resistant strains from industrial wastewater from GIDC Vadodara (Gujarat). Initially, 10 isolates were tested on plates having different heavy metal salts Cu^{+2}, Cr^{+6}, Cd^{+2} and Ni^{+2} at concentrations of 50 ppm. Isolates were chosen for their high level of heavy metal tolerance. The results show that all the 4 isolates show the resistant to heavy metals present in industrial effluent hence these isolates have greater application in bioremediation.

KEYWORDS: -*Heavy Metal; Isolation; Screening; Bioremediation; Environmental Pollution*

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INTRODUCTION

Every year, massive amounts of organic and inorganic substances are introduced into the atmosphere as a result of anthropogenic activities. Many of these substances are poisonous as well as persistent in both terrestrial and aquatic habitats. Contamination of soil, surface, and water bodies is simply the result of harmful substances accumulating in excess of acceptable amounts [1]. Heavy metals, greenhouse gases, pesticides, and hydrocarbons are among the harmful substances that cause environmental and human concerns due to their toxicity. Out of these major pollutants, heavy metals have recently get attention worldwide [2]. The phase heavy metals are metals and metalloids with densities greater than 5 g/cm^3 such as cadmium, chromium, iron, lead, copper, mercury, arsenic, silver, zinc, and others. They are extensively concerned in activities such as mining, electroplating, dye, fossil fuel burning, pigment processing, fertilizers, and other activities, which are then discharged into the environment in massive quantities everyday via wastewater or other paths [3]. The release of these heavy metals into the atmosphere is a severe problem all over the world because they can't be transformed to non-toxic forms and hence have long-term environmental consequences. Many of them are hazardous even in little amounts; Cr, Cu, Cd, Pb, As, Hg, Ni, Zn, Ag, and others are cytotoxic as well as carcinogenic and mutagenic. [1]. Plants, humans, and microbes all rely on heavy metals for metabolic, biochemical and physiological activities. Heavy metals like Cr, Cu, Ni, Co, and Zn act as micronutrients and are required for redox reactions. They play a significant role in molecule stabilisation through electrostatic interactions, osmotic pressure management, and cofactors for a variety of enzymes and electron transport chains. Non-essential toxic metals such as As, Cd, Ag, Hg, and Pb have no biological significance and are extremely harmful when discovered in the ecosystem [5]. These heavy metals have many deleterious effects on

humans such as brain and kidney damage, allergic skin diseases, dizziness, fatigue, short-term memory loss, elevated levels result in liver cirrhosis and cancer, while in plants it causes inhibition in plant growth, interfere with cellular process, reduces biomass and shows necrotic and chlorotic symptoms. In microorganisms it affects the cellular and metabolic activities, inhibit enzymatic function and growth rates, altered cell division, transcription and translational activities. [6,7]

These contaminated areas should be treated to remove the toxic metals and make them available for humans and other use. But non-biodegradable properties of these metals makes them difficult to remove from contaminated areas. Several physicochemical procedures for eliminating heavy metals from contaminated water have been used, including electrochemical treatment, filtration, chemical precipitation, membrane technology, evaporation, reverse osmosis, ion exchange and membrane technology. However, the majority of them seem to be costly, inefficient, labour-intensive operations, or lack selectivity in the treatment process [8]. An alternative to this physiochemical process is bioremediation. Bioremediation is the process in which microbes, fungi or plants are used to treat the contaminated areas. These microbes will use the toxic metals or contaminants as their food source as a result of that environment is became healthy [9]. Among these microorganism bacteria are widely used for bioremediation. *Pseudomonas, Comamonas or Acinetobacter* are the major genera of bacteria involved in this method. They all are being gram negative bacteria [10]. Bioremediation is basically a technique that uses natural biological activity to remove or render certain contaminants. As a result, it employs lesser cost, low-technology techniques that are widely accepted by the population and may frequently be performed on-site [11]. The goal of this experiment was to isolate and characterize heavy metal tolerant bacteria from industrial wastewater, which could then be used to eliminate heavy metals from the wastewater in these locations.

MATERIAL AND METHODS

Effluent Sampling

Industrial wastewater samples were obtained from the electroplating industrial outlet at Sardar Estate GIDC in Vadodara (Gujarat, India). The sampling was placed around 2:00 p.m. in the month of November, 2020. Samples were obtained in sterilised containers, transported to laboratories aseptically, and processed immediately.

Sterilization of materials

All of the glassware was thoroughly cleaned, washed, and dried using deionized water and detergent. Following that, the glassware and distilled water used in further process was sterilized by using an autoclave at 121 degrees Celsius for 15 minutes. The working area was also cleaned with 75 percent alcohol before usage [12].

Isolation of Cd, Cu, Cr and Ni resistant bacteria

For the selective isolation, the collected wastewater samples were diluted in sterilized deionized water and each dilution is plated on nutrient agar media having heavy metals such as Cd, Cu, Cr, and Ni. These heavy metals are added in the form of their salts like $CdCl_2$, $CuCl_2$, $K_2Cr_2O_7$, and $NiSO_4$ respectively at 50 ppm concentration. After Inoculation the plates were incubated at 37^o C for 24-48 h. Following the incubation, the plates were checked for any signs of media growth. To purify the colonies generated on this selective media, the isolated and heterogeneous colonies were repeatedly subcultured on the similar media. The physical characteristics of pure culture were employed to identify it. [10].

Measuring the MIC of heavy metals

The investigation of heavy metal ion tolerance was conducted by calculating the metals ions' Minimal Inhibitory Concentration (MIC) in nutritional broth media. To make the necessary stock solutions, analytical grade $CdCl_2$, $CuCl_2$, $K2Cr2O_7$, and $NiSO_4$ were dissolved in sterilised deionized water. Each isolate was inoculated in 10 mL of sterile nutritious broth containing 100, 200, 300, 400, and 500 ppm of each metal. At 37 degrees Celsius, the tubes were incubated for 24 hours. After 24 hours, bacterial growth was measured at 600 nm using nutritional broth having the same concentration of metal as a blank [10,13].

Growth studies

Isolated bacteria were grown in 250 mL flasks containing 50 mL nutritional broth medium containing 100 ppm concentrations of all metals Cd, Cu, Ni, and Cr. The media was inoculated with 100 microlitre of overnight culture of bacteria and agitated at 150 rpm on a rotary shaker. The absorbance at 600 nm was measured at certain time intervals of 2, 3, 4, 6, 24, 48, and 72 hours to investigate growth as a function of biomass [14].

RESULTS AND DISCUSSION

Isolation of Cd, Cu, Cr and Ni resistant bacteria

In the current research we isolate heavy metal tolerant bacteria from industrial wastewater. 10 colonies were screened initially which can tolerate 50ppm of different heavy metals concentration. Four of the finest colonies were chosen for further study after growing on nutrient agar supplemented with all four metals at 50ppm concentration (figure 1-4). These four strains were chosen for future research based on their heavy metal resistance properties. Isolates were kept at 4°C on Nutrient agar slant and a glycerol stock, and were given the codes HM01 through HM04. Metal binding abilities have been demonstrated in these bacteria, and they have been found to not only tolerate but even detoxify metals. These types of results also have been reported earlier in Pandit et al., (2013) studies.

Morphological characteristics of isolates

The colony shape and characteristics of Cadmium resistant bacteria (HM01) and Copper resistant bacteria (HM02) are the same, with the colony border, colony elevation, and colony surface being Entire, Convex, and Smooth, respectively. The colonies are distinct by their colour. Where (HM01) was yellow white, (HM02) was Yellow. Similar type of bacteria was also found earlier in the study of Gupta & Kumar, (2012). Chromium resistant bacteria (HM03) and Nickel resistant bacteria (HM04) were found to be round and medium in shape and size. While off white and Shiny orange in colour respectively, whereas the elevation and surface of the colony were convex and smooth. (Table 1.)

Determination of MIC

Four isolated bacteria show a high level of resistance to all heavy metals (figure 5,6). Isolates were found to be resistant to four heavy metals in concentrations ranging from 100 to 300 parts per million. The graphs show how bacteria develop in the presence of Cd, Cu, Cr, and Ni. Except for HM01, which was resistant to Ni+2 and HM02, which was resistant to Cd+2 up to 100 ppm, all four isolates were resistant to Cd+2, Cu+2, Cr+6, and Ni+2 up to 200 ppm. While HM03 and HM04 show the maximum resistant to Ni^{+2} and Cu^{+2} upto 300ppm respectively (Table 2). The results clearly show that the isolates have a high level of metal resistance.

Growth studies

In the presence of all the four metals, the growth curves of each isolated colonies were investigated. The graphs reveal that the presence of Cd, Cu, Cr and Ni at 100ppm concentrations was ineffective for the isolate's growth (figure 7). This also suggests that the isolates are finely tolerant to the measured metals and in the presence of metal, isolates grew at a somewhat slower rate than controls.

Table 1. Grams and colony characteristics of isolates

Isolate	Colonies colour	Staining (Gram's)	Shape/size	Elevation	Surface
HM01	Yellow white	Gram-ve, rod	Round- Small	Convex	Smooth
HM02	Yellow	Gram-ve, rod	Round- Small	Convex	Smooth
HM03	Off white	Gram-ve, rod	Round- medium	Convex	Smooth
HM04	Orange (shiny)	Gram-ve, rod	Round- medium	Convex	Smooth

Table 2. MIC data of 4 isolates

Isolates	MIC (ppm)			
	Cd^{+2}	Cu^{+2}	Cr^{+6}	Ni^{+2}
HM01	200	200	200	100
HM02	100	200	200	200
HM03	200	200	200	300
HM04	200	300	200	200

Figure 1. Pure Culture of Isolate HM01 (Cd resistant bacteria)

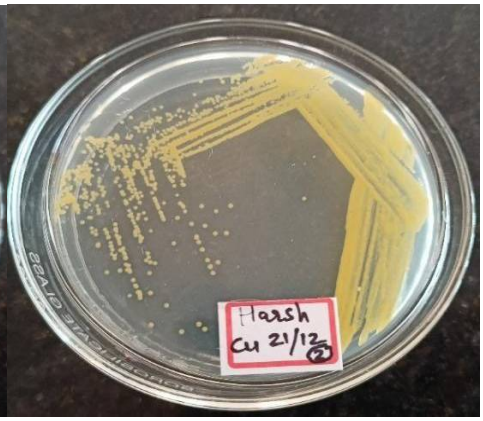

Figure 2. Pure Culture of Isolate HM02 (Cu resistant bacteria)

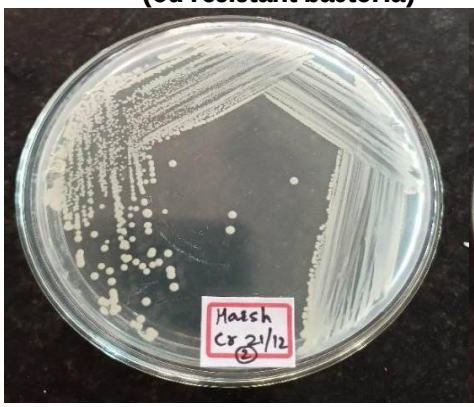

Figure 3 Pure Culture of Isolate HM03 (Cr resistant bacteria)

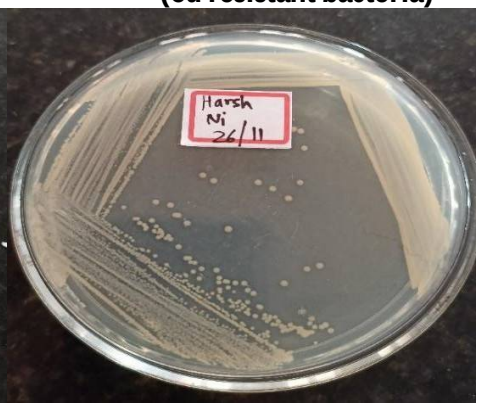

Figure 4. Pure Culture of Isolate HM04 (Ni resistant bacteria)

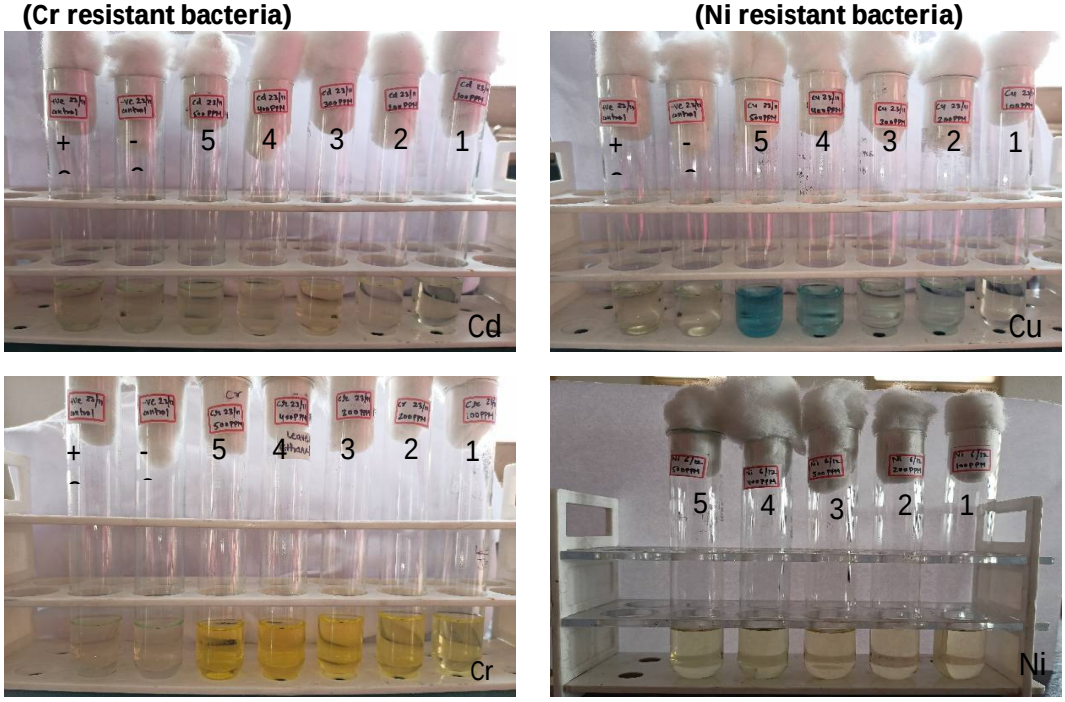

Figure 5. HM01, MIC Before Experiment (Note: 1-100,2-200,3-300,4-400,5-500 in ppm, +C- Positive Control, -C- Negative Control)

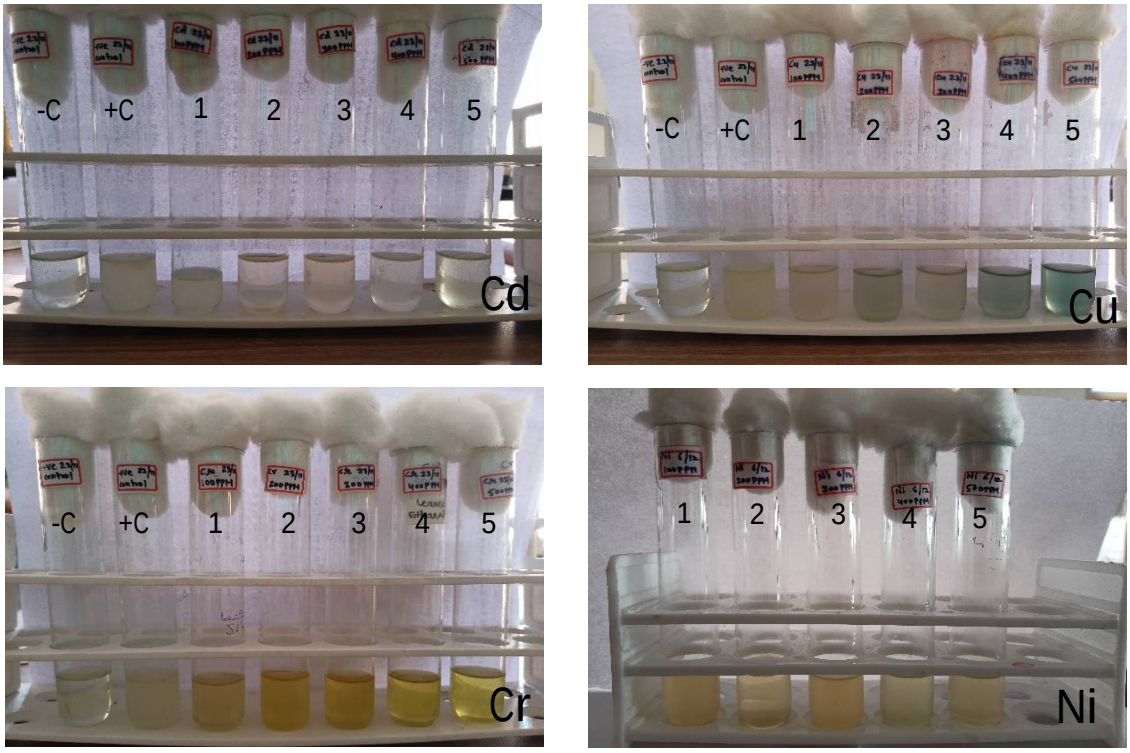

Figure 6 HM01, MIC After Experiment (Note: 1-100,2-200,3-300,4-400,5-500 in ppm, +C- Positive Control, -C- Negative Control)

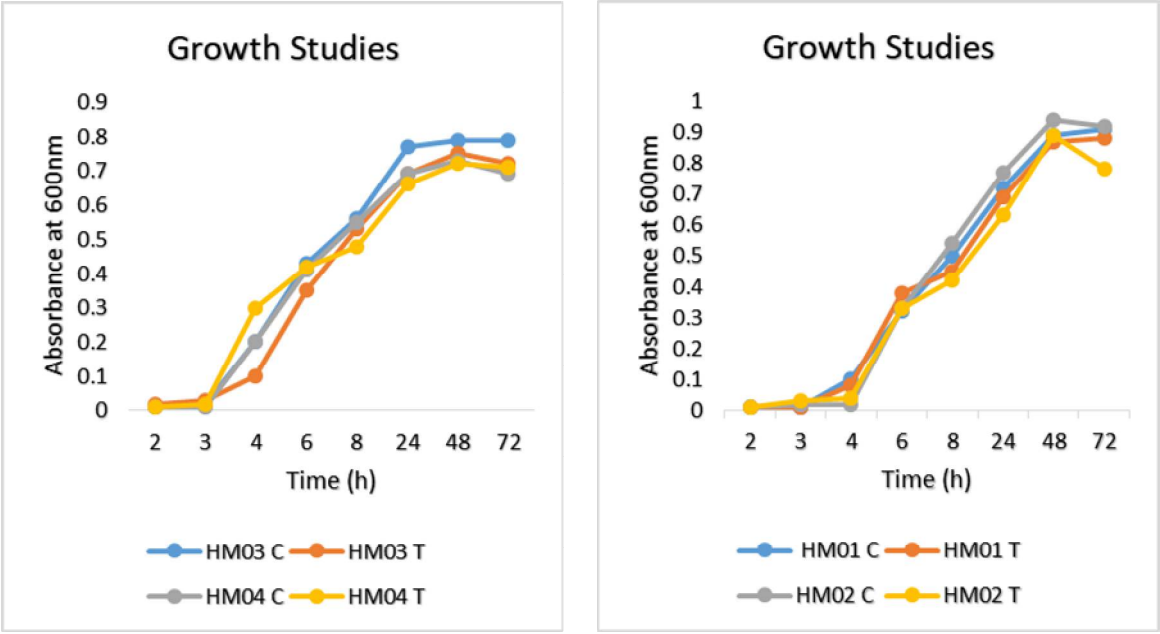

Figure 7 Growth studies of isolates in presence of all four metals. C= Control, T= Test.

CONCLUSION

The ability of bacteria to resist several heavy metals may be an important tool for monitoring multiple toxins and pollutants in the atmosphere. It is obvious that the toxic effects of household and industrial wastes are the cause of bacterial resistance mechanisms, as well as a threat to people's health and the atmosphere. The purpose of this study was to extract and screen four heavy metal-resistant bacteria (Ni, Cu, Cd and Cr) from industrial wastewater polluted sites in Sardar Estate GIDC, Vadodara (Gujarat, India). All of the isolates are highly tolerant to (Ni, Cu, Cr and Cd). A kinetic examination of growth in the presence of heavy metals demonstrates that metals have no significant effect on the rate of growth of isolates. So based on the above characteristics we can predict that four isolates mostly belong to *bacillus*

or *pseudomonas* species. This demonstrates that the isolated bacterial strains can be employed as a bioremediation technique to treat heavy metal-containing industrial wastewater.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES
1. Kensa VM. (2011). Bioremediation-an overview. I Control Pollution. ;27(2):161-8.
2. Nyoyoko VF, Anyanwu CU. Isolation and screening of heavy metal resistant ammonia oxidizing bacteria from soil and waste dump: a potential candidates for bioremediation of heavy metals. bioRxiv. 2019 Jan 1:836890.
3. Leong YK, Chang JS. Bioremediation of heavy metals using microalgae: recent advances and mechanisms. Bioresource technology. 2020 May 1;303:122886.
4. Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability. 2015 Feb;7(2):2189-212.
5. Fashola MO, Ngole-Jeme VM, Babalola OO. (2016). Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. International journal of environmental research and public health.13(11):1047.
6. Yadav KK, Gupta N, Kumar V, Singh JK. (2017). Bioremediation of heavy metals from contaminated sites using potential species: a review. Indian J. Environ. Prot. 37(1):65.
7. Igiri BE, Okoduwa SI, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. Journal of toxicology. 20-23.
8. Guo H, Luo S, Chen L, Xiao X, Xi Q, Wei W, Zeng G, Liu C, Wan Y, Chen J, He Y. (2010). Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium Bacillus sp. L14. Bioresource technology. 101(22):8599-605.
9. Mani D, Kumar C. (2014). Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. International journal of environmental science and technology. ;11(3):843-72.
10. Pandit RJ, Patel B, Kunjadia PD, Nagee A. (2013). Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi-Ankleshwar, Gujarat. International Journal of Environmental Sciences. 3(5):1689-99.
11. Vidali M. (2001). Bioremediation. An overview. Pure and applied chemistry. ;73(7):1163-72.
12. Kumar A, Murthy LN, Jeyakumari A, Laly SJ. (2011). Sterilization technique used in microbiology. Mumbai Research Centre of ICAR-Central Institute of Fisheries Technology, Vashi, India.
13. Sridevi V, Raghuram M, Ravisankar D. (2018). Isolation and Screening of Heavy Metal Resistant Microorganisms from Industrial Soil. Journal of Pure and Applied Microbiology.1;12(3):1667-74.
14. Raja CE, Selvam GS, Omine KI. (2009). Isolation, identification and characterization of heavy metal resistant bacteria from sewage. InInt Joint Symp on Geodisaster Prevention and Geoenvironment in Asia. (pp. 205-211).
15. Gupta N, Kumar V. (2012). Identification and isolation of heavy metal (copper) resistant bacteria. Archives of Applied Science Research. ;4(1):577-83.

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