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Screening and Characterization of Copper Resistant Bacteria Isolated from Metal Contaminated Site

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A B S T R A C T

Copper is one of the heavy metals released into the environment by human beings in excessive amounts in the vicinity of copper mining and also in industrial wastewater discharge such as plating, fertilizers, pesticides, etc. According to US-EPA, in drinking water copper levels should not exceed 1300 µg/l. Such toxic metals should be cleaned up by removed from the environment. Bioremoval of copper is an environmentally friendly method. There were twenty isolates were screened as copper resistant, from the copper contaminating site. These isolates showed MIC for copper ranging from 600 - 1800 µg/ml. Three isolates TICu02, TICu05, and TICu08 showed maximum tolerance of 1800 µg/ml concentration of Cu. The effect of different parameters on the removal efficiency of three isolates was studied. Isolate TICu02 was able to remove 100µg/ml Cu (II) completely at 40°C at pH-7 in 48 hours. These isolates can be employed efficiently for the removal of copper at copper-contaminated sites.

***Key words*:** Bioremediation, Copper bio removal, Heavy metals, Copper contamination sites

Wastewater discharged from metal plating processes contains toxic substances such as cyanides, alkaline cleaning agents, degreasing solvents, oil, fat, and toxic metals such as copper. These heavy metals have a fatal effect on the human body as well as causing environmental pollution and the indigenous aquatic biota even at low levels, and heavy metal ions are known to be carcinogenic. Therefore, the toxic and hazardous effluents that emanate from the metal plating factories must be properly treated so that they do not cause more damage to the environment [1]. Environmental contamination due to copper is caused by metal plating industries, metallurgical, fibre production, pipe corrosion, mining, and printed circuits. Some other industries majorly discharging copper in their effluents are wood preserving, pulp and paper, petroleum refining, and agricultural sources such as fungicidal sprays, fertilizers, and animal wastes also lead to water pollution due to copper [2].

Copper may be found as a contaminant in various foods especially liver, mushrooms, shellfish, chocolates, and nuts. Any packaging container using copper material may contaminate the product such as food, water, and drink. Copper has been reported to cause neurotoxicity commonly known as "Wilson's disease" due to the deposition of copper in the lenticular nucleus of the brain and kidney failure. In some instances, exposure to copper has resulted in jaundice and enlarged liver. It is suspected to be responsible for one form of metal fume fever. Copper-containing sprays are linked to an increase in lung cancer among exposed workers. A higher concentration of copper also affects microbes by disrupting cell functions and cell membrane as well as nucleic acid [3].

The U. S. Environmental Protection Agency (US-EPA) has determined that copper levels in drinking water should not exceed 1300 µg/l. Some conventional methods are available for the removal of such toxic heavy metals but these are cost-effective and produce secondary pollution in the environment. Bioremediation by using microorganisms is an effective and eco-friendly method for environmental cleanup. *Bacillus* sp tolerated high heavy metal concentrations of copper either actively by bioaccumulation or passively by adsorption [4]. The role of microorganisms and plants in the biotransformation of heavy metals into nontoxic forms is well-documented, and understanding the molecular mechanism of metal accumulation has numerous biotechnological implications for the bioremediation of metal-contaminated sites. In view of this, the present study investigates the abilities of microorganisms in terms of tolerance and bio-removal of heavy metals. Also, advances in bioremediation technologies and strategies to explore these immense and valuable biological resources for bioremediation are discussed. An assessment of the current status of technology deployment and suggestions for future bioremediation research has also been included [5]. This study aimed to screen copper-resistant isolates and characterize their copper removal ability.

MATERIALS AND METHODS

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Sample collection and metal analysis

An electroplating effluent sample was collected from the plating industry in MIDC Nanded. The effluent sample was collected in sterile glass sampling bottles and transported aseptically in an ice box to the laboratory immediately for further analysis [6]. The sample was determined for the presence of copper by using Inductively Coupled Plasma-Atomic Emission Spectroscopy ICP-AES [7].

Isolation and identification of copper resistant bacteria

Samples were serially diluted up to 10-5 dilutions. Copper tolerating bacteria were isolated by spreading diluted samples on nutrient agar plates containing 100 µg/ml of copper sulphate salt. These plates were kept for incubation at 30°C for 24-48 hrs. The nutrient agar and stock solution of metal salts solution were sterilized separately before addition [8]. Identification of these isolates was done by the 16s r RNA sequencing method.

Determination of minimum inhibitory concentration

MIC of copper resistant isolates was determined by preparing a stock solution of copper sulphate in doubled distilled deionized water. The sterile stock solution was added in increasing concentrations from 100, 200, and 300 up to 2500 µg/ml in nutrient broth tubes. All these tubes were inoculated with an equal volume of an active culture of each isolate. All the tubes were incubated at 30°C for 48 hrs. The bacterial growth in terms of turbidity was determined by measuring optical density at 600nm by using UV-VIS Spectrophotometer (Model No-Systronics 119, UV-VIS spectrophotometer).

Copper bio-removal assay

Copper-resistant isolates were inoculated in nutrient broth with 100µg/ml of copper sulphate and without metal salt as a control. The flasks were incubated at 30°C for 48 hrs. Initial (0 hour) and final (48 hours) concentration of Copper II was determined by the Bathocuproine method [9-10]. Copper removal efficiency was calculated in percentage formula given by Shahin *et al*. [11].

Effect of various parameters on bio removal efficiency isolates

Each Copper resistant isolate was inoculated in separate flasks containing 100ml nutrient broth with copper sulphate at a concentration of 100µg/ml and without metal salt as a control. The effects of different parameters were studied on bio removal efficiency and growth of isolates. These parameters were included various temperature (10, 20, 30, 40 and 50°C), pH (5, 6, 7, 8 and 9), inoculum size (1, 2, 3, 4 and 5%), copper concentration (100, 200, 300, 400 and 500 µg/ml), incubation period (24, 48, 72, 96, 120 and 144 hours) and agitation (0, 50, 100, 150, 200 rpm). Samples were collected during the study of each parameter. The bio removal efficiency of isolates was determined by measuring the concentration of copper in each sample.

RESULTS AND DISCUSSION

Analysis of copper, screening, and identification of copper resistant isolates

ICP-AES analysis of the plating sample showed a 0.021 ppm concentration of copper whereas chromium and zinc were present in concentrations of 5.889 and 0.033 ppm respectively. Gupta *et al*. [12] determined copper, chromium, and zinc in concentrations of 0.067, 0.1416, and 0.026 ppm respectively. Twenty-one differentiated colonies of isolates were screened on copper amended nutrient agar plates. These isolates were labelled as TICu01 to TICu21. In this study, among all isolates, TICu02 could highly efficient copper remove hence this isolates TICu02 was identified by 16S rRNA sequencing as *Bacillus cereus* with 99% similarity. Velusamy *et al*. [13] isolated heavy metal resistant bacteria showing copper resistance up to 50 µg/ml was identified as *Bacillus megaterium* X4.

Determination of minimum inhibition concentration

All twenty-one Cu resistant isolates were determined for MIC of Cu as per the result shown in (Fig 1). Three isolates TICu02, TICu05, and TICu08 showed higher MIC i.e., 1800 µg/ml in nutrient broth amended with the salt of Cu II. These isolates showed MIC of Cu ranging from 600-1800 µg/ml. Mustapha and Halimoon [14] screened isolate MH 4 which showed a MIC 200 µg/ml for Cu. Baz *et al*. [15] reported that *Actinobacteria* showed 0.10 mg/ml MIC for Cu recorded.

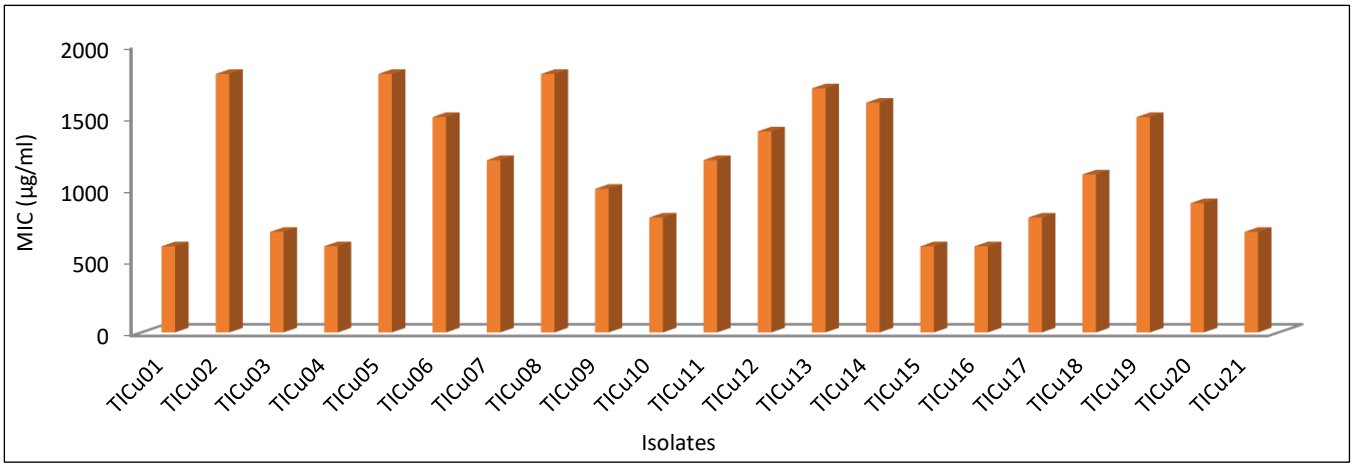

Fig 1 MIC of Cu II for copper resistant isolates

Copper removal assay of isolates

Three isolates (TICu02, TICu05, and TICu08) that showed a higher MIC of Cu were selected for copper removal assay. Bacterial isolate isolates TICu02, TICu05 and TICu08 were showed 100%, 73% and 84% respectively. TICu08 showed complete removal of Cu II in 48 hrs. Nanda *et al*. [16] showed that *Bacillus* sp. and *Pseudomonas* sp. could remove 62% and 34% of initial copper after 48 hours at 35±2°C and 80rpm.

Effect of different parameters on copper removal efficiency of isolates

As shown in (Fig 2), Cu-resistant isolates showed most Cu II removal in 48 to 72 hours in nutrient broth with 100ug/ml

of Cu II. Isolate TICu02 was achieved complete Cu removal in 48 hrs. The optimum temperature for maximum Cu removal of all isolates was 40°C (Fig 3). Oves *et al*. [17] investigated that *Bacillus thuringiensis* OSM29 showed the optimum temperature for copper biosorption was 32±2°C at pH 6. As shown in (Fig 4), isolates were able to remove Cu ranging from pH-5 to pH-9. At pH-7, maximum Cu removal was observed. Isolate TICu02 showed complete Cu removal at pH-7. Benmalek and Fardeau [18] reported that at pH 6-8.5, the living cells of *Micrococcus* showed the largest uptake of 83.90% capacity for copper. The optimum pH for copper biosorption by *Micrococcus* and *Pseudomonas* was 5.0 [19]. As shown in (Fig 5), Isolates TICu02, TICu05 and TICu08 achieved optimum Cu removal by adding 4% initial inoculum in size. The effect of different initial Cu concentrations amended was as shown in (Fig 6). In this case, Cu removal efficiency and concentration of Cu salt were reciprocal hence as the initial Cu concentration was increased from 100 to 500µg/ml, the bacterial efficiency of Cu removal was decreased. *Bacillus* sp. showed more growth at 1 µg/ml and a decrease in growth at the 3 µg/ml level of Cu [20]. Cu-resistant isolates showed optimum efficiency of Cu removal at 150 rpm (Fig 7).

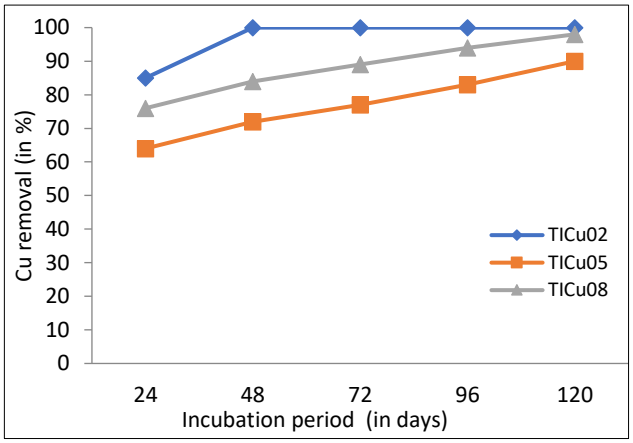

Fig 2 Effect of incubation period on Cu II removal

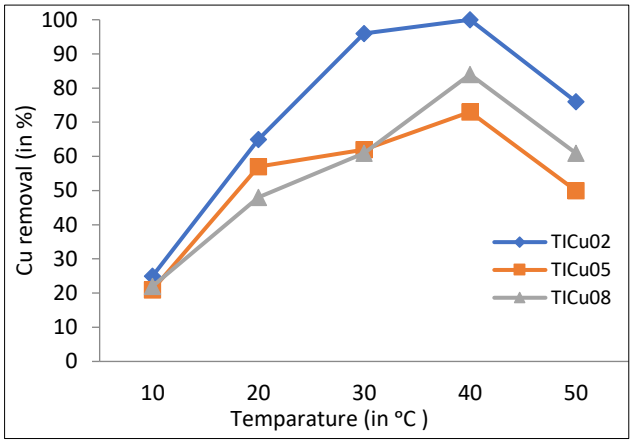

Fig 3 Effect of temperature on Cu II removal

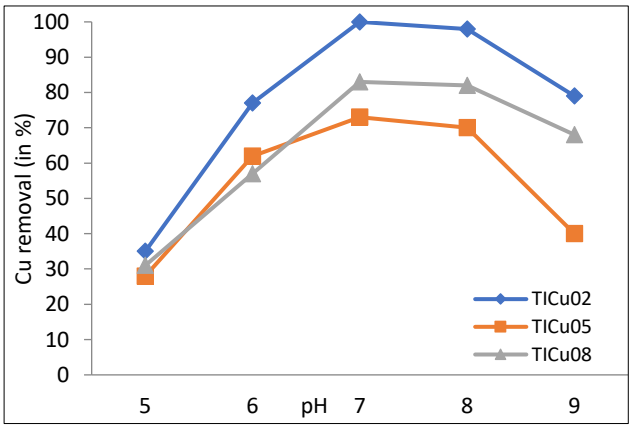

Fig 4 Effect of pH on Cu II removal

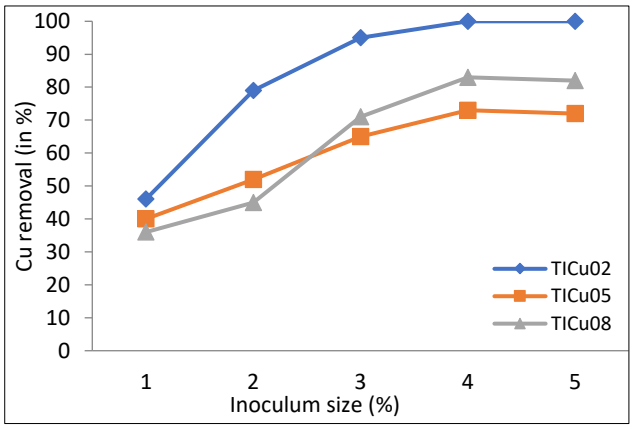

Fig 5 Effect of inoculum size on Cu II removal

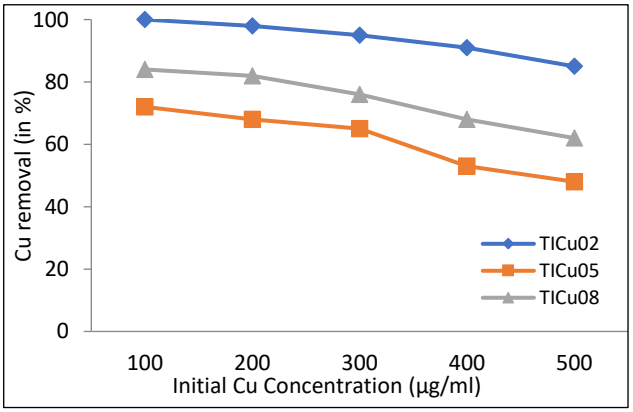

Fig 6 Effect of initial Cu concentration on Cu II removal

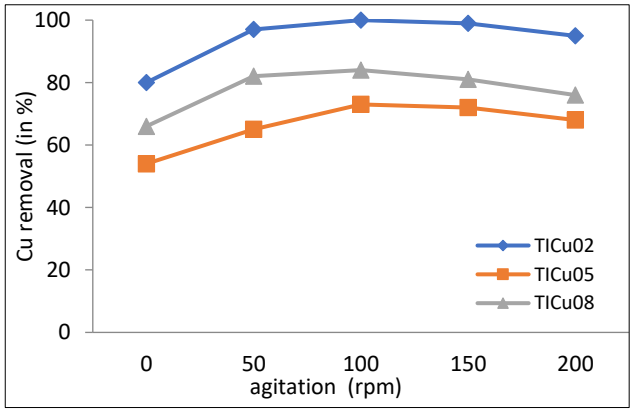

Fig 7 Effect of agitation on Cu II removal

CONCLUSION

Twenty-one Cu II tolerating bacteria were isolated from industrial effluent samples. In these isolates, three isolates showed maximum tolerance at a concentration of 1800 µg/ml of Cu (II). Effects of various parameters on removal efficiency of three isolates showed, that isolate TICu0$_2$ was able to remove Cu (II) 100% within 48 hours at pH 7, temperature 40°C at

100µg/ml Cu (II) salt by using 4% inoculum. These isolates can be employed efficiently for the removal of copper present in environmental conditions. All of these three isolates have potential use in the bioremediation of toxic Cu (II) at normally present conditions. This method of bioremediation is eco-friendly cost-effective and easily applicable.

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LITERATURE CITED

1. Nevzat Beyazit. 2014. Copper (II), Chromium (VI), and Nickel (II) removal from metal plating effluent by electrocoagulation. *Int. Jr. Electrochem. Sciences* 9: 4315-4330.
2. Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D. 2015. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability* 7(2): 2189-2212.
3. Kim JH, Kang JC. 2016. The toxic effects on the stress and immune responses in juvenile rockfish, *Sebastes schlegelii* exposed to hexavalent chromium. *Environmental Toxicology and Pharmacology* 43: 128-133.
4. Tiku DR, Asikong BE, Bassey IU. 2016. Heavy metal tolerance profile among bacteria from auto-mechanic workshop and pristine soil. *British Microbiology Research Journal* 12(6): 1-10.
5. Ahluwalia SS, Goyal D. 2007. Microbial and plant derived biomass for removal of heavy metals from waste water. *Bioresource Technology* 98: 2243-2257.
6. Mahalingam PU, Ranjithkumar M, Anchana DMR. 2016. Isolation and characterization of nickel tolerant bacterial strains from electroplating effluent sediments. *International Journal of Environmental and Agriculture Research* 2(6): 130-134.
7. Jusufi K, Stafilov T, Vasjari M, Korca B, Halili J, Berisha A. 2016. Determination of heavy metals by ICP-AES in the agricultural soils surrounding Kosovo's power plants. *Fresenius Environmental Bulletin* 25(5): 1312-1320.
8. Das MP, Neha K. 2016. A microbial bioremediation approach: Removal of heavy metal using isolated bacterial strains from industrial effluent disposal site. *Int. Jr. Pharm. Sci. Rev. Research* 38(1): 111-114.
9. Chen D, Darabedian N, Li Z, Kai T, Jiang D, Zhou F. 2016. An improved bathocuproine assay for accurate valence identification and quantification of copper bound by biomolecules. *Anal. Biochemistry* 497: 27-35.
10. American Public Health Association. APHA. 2005, Standard Methods for the Examination of Water and Waste Water, American Water Works Association and water pollution control Federation, Water Environment Federation, Washington DC, USA.
11. Shahin SA, Mossad M, Fouad M. 2019. Evaluation of copper removal efficiency using water treatment sludge. *Water Science and Engineering* 12(1): 37-44.
12. Gupta R, Sundarrajan S, Mohana PK, Mohana PA, Palanichamy V, Nancy VD. 2015. Isolation and characterization of heavy metal tolerant bacterial isolates VITNJ12 and VITNJ13 from paper mill effluent, Erode district, Tamil Nadu, India. *Int. Jr. Drug Dev. and Research* 7(1): 146-148.
13. Velusamy P, Awad YM, Abd El-Azeem SAM, Ok YS. 2011. Screening of heavy metal resistant bacteria isolated from hydrocarbon contaminated soil in Korea. *Journal of Agricultural, Life and Environmental Sciences* 23(1): 40-43.
14. Mustapha MU, Halimoon N. 2015. Screening and isolation of heavy metal tolerant bacteria in industrial effluent. *Procedia Environmental Sciences* 30: 33-37.
15. Baz SE, Baz M, Barakate M, Hassani L, El-Gharmali A, Imziln B. 2015. Resistance to and accumulation of heavy metals by actinobacteria isolated from abandoned mining areas. *The Scientific World Journal*. 2015: 1-14. Article ID 761834.
16. Nanda M, Sharma D, Kumar A. 2011. Removal of heavy metals from industrial effluent using bacteria. *Int. Jr. of Environmental Sciences* 2(2): 789-795.
17. Oves M, Khan MS, Zaidi A. 2013. Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences* 20: 121-129.
18. Benmalek Y, Fardeau ML. 2016. Isolation and characterization of metal-resistant bacterial strain from wastewater and evaluation of its capacity in metal-ions removal using living and dry bacterial cells. *Int. Jr. Environ. Science and Technology* 13(9): 2153-2162.
19. Leung WC, Wong WF, Chau H, Lo W, Yu PHF, Leung CK. 2000. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water Science Technology* 41: 233-240.
20. Vijayadeep C, Sastry PS. 2014. Effect of heavy metal uptake by *E. coli* and *Bacillus* sp. *Journal of Bioremediation and Biodegradation* 5(5): 238.