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## **Isolation of heavy metal resistant bacteria from industrial effluent and perform biosorption**

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### **Abstract**

Industries release waste effluents to the environment, it contains toxic heavy metals and cause health hazards to plants, animal, aquatic life and human beings. In biological system metals play an important role and as a living cell can't exist without metal ions, but essential metals can be toxic to the organism when their concentration are either too high or too low. The main source of heavy metal contaminants are industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber, plastics and wood products producing industries. Heavy metal resistant bacteria isolated by serial dilution method and pour plate method. MIC was noted when the colonies failed to grow on the medium and concentration of heavy metal was measured by using atomic absorption spectrophotometer. In this review article discussed the information about screening of heavy metal resistant bacteria, to determination of MIC and removal of heavy metals by biosorption method.

**Keywords:** heavy metals, toxicity, biosorption, minimum inhibitory concentration, industrial wastewater

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### **Introduction**

Air, water and land which are the fundamental components of life are contaminated continually because of expanding population, rapid urbanization and industrialization. Industries release waste effluents to the environment, it contains toxic heavy metals and cause health hazards to plants, animals, aquatic life and human beings <sup>[1]</sup>. Heavy metals like Hg, Cr, Cu, Zn, Pb and Cd are essential trace elements at low concentrations and such metals can serve as important components in life processes but above certain high concentrations these heavy metals can become toxic to many species. Heavy metals are not biodegradable but rather can be changed through sorption, methylation and complexation and changes in valance state <sup>[2][3]</sup>. Out of 17 most significant heavy metals Fe, Cu, Mo, Mo are classified under low toxicity, Zn, Ni, V, CO, W, Cr are categorized under the average toxicity and s, Ag, Sb, Cd, Hg, Pd, U are grouped under highly toxic heavy metals <sup>[5]</sup>. Common sources of heavy metals contamination incorporate release from industries like electroplating, plastic manufacturing, fertilizer production and wastes leftover after mining and metallurgical processes <sup>[4]</sup>.

The presence of heavy metals into the environment induce changes in morphological and physiological characteristics of microbes <sup>[6]</sup>. To survive in heavy metal contaminated environment numerous microorganisms created resistance to toxic metal ions, these mechanisms incorporate: metal prohibition by permeability, active transport of the metal away from the cell living being, intracellular sequestration, enzymatic detoxification of the metal to a less toxic and decrease in metal affectability of cell targets <sup>[4]</sup>. Microorganisms can adjust and develop under different extreme conditions like pH, temperature and availability of nutrients as well as high metal concentration

<sup>[6]</sup>. Microorganisms and microbial products can be exceptionally effective bioaccumulators of dissolvable and particulate types of heavy metals and microbes related technologies may gave another option or expansion to conventional method of metal expulsion <sup>[5]</sup>. Hence microorganisms have created mechanisms to endure the metals either by presence of heavy metals through efflux, complexation or reduction of metal particles or to utilize them as terminal electron acceptors in anaerobic respiration. Microorganisms that are resistance from and develop on metals play a significant role in the biogeochemical cycling of those metal particles <sup>[7]</sup>. The detoxifying capacity of microorganisms have important role in bioremediation of heavy metals in wastewater treatment. Effluents having heavy metals can be treated with these microorganisms by the processes like biosorption, bioaccumulation and bioprecipitation <sup>[8]</sup>.

### **Industrial wastewater**

The arrival of untreated wastewater from the industries is the significant source of the water contamination and the effluent generating from the industries which contain an alternate concentration of pollutants are released into the streams or other water sources. The foreign substances from the industrial wastewater that seek after in the environment are pesticides, dyes, aromatic hydrocarbons, oils, heavy metals, etc., and these toxins are a significant hazard to the individual's health and encompassing <sup>[2,6]</sup>. The majority of the industries release wastewater and their effluents containing poisonous materials into waterways without adequate treatment. The greater part of the point sources of heavy metal contaminants are industrial wastewater from mining, metal processing, tanneries,

pharmaceuticals, pesticides, organic chemicals, pigment and wood products production [27]. The indirect release of this wastewater into municipal sewage treatment plants may majorly affect the activated sludge and block the proficiency of the plants. When they enter into the food chain, high concentrations of heavy metals may accumulate in the human body and if the metals are ingested beyond the allowed concentration, they can cause serious chronic health diseases [25].

### Toxicity of Heavy metals

In biological system metals play an important role and as a living cell can't exist without metal ions, generally in minimum concentration many metals are significant to normal physiological function like iron in oxygen transport, manganese and selenium in antioxidant system and zinc in metabolic process. But essential metals can be toxic to the organism when their concentration are either too high or too low, in the other hand some heavy metals like arsenic and mercury are not detected physiological concentration so such heavy metals can possibly cause toxicity to the cell [29][30].

Arsenic is known to be exceptionally harmful to all living things and has been ordered by World Health Organization as a group 1 human cancer-causing substance. The chronic intake of inorganic arsenic in drinking water can cause various types of skin problem and cancer which are collectively known as a arsenicosis [17]. Copper is the most widely utilized heavy metal, is predominantly used in industries like electrical and electroplating and in large quantity it is very toxic to living organisms. The presence of copper (II) ions can cause severe toxicological concerns, it is normally deposit in brain, skin, liver, pancreas and myocardium [18]. Mercury has no known physiological use in the human body and Mercury is known to be harmful in micrograms per liter concentration even at nanograms per liter concentration dosages. It can cause chromosomal damage and abnormalities in the foetus of an exposed mother, being lipid dissolvable it can amass in tissues and furthermore cross the blood brain barrier they harming the sensory system [19].

Cadmium is known to be a widely spread environmental pollutant and a potential to cause toxic effect in human health and kidney is the primary organ affected by the chronic cadmium. The complex form of metallothionein bound cadmium (Cd – MT) is degraded in endosomes and lysosomes and releasing free Cd<sup>2+</sup> into the cytosol, where it can generate reactive oxygen species (ROS) and activated the cell death pathways [20]. Chromium is broadly utilized industries like wood preservation, electroplating, metallurgy, paints and pigments production, chemical production, tanning and pulp and paper industry. The hexavalent types of chromium is the most harmful types of chromium it can cause anemia, irritations and ulcers in the small intestine and stomach, damage sperm and male reproductive system [24].

### Screening of Heavy metal resistant bacteria

This strategy was applied to isolate some bacterial strains able to grow in the presence of heavy metals. To isolate the ideal bacterial strains that have to more resistance to high concentration of heavy metals [9]. The screening of heavy metal resistant bacteria was completed from the industrial effluent utilizing nutrient agar as basal medium. The method used was based on the serial dilution method, 1ml of effluent was added to a tube containing 9ml of sterile double distilled water and mixed well,

after that to set up the second dilution 1ml of the first one was transferred to second tube containing 9ml of water, the dilution process was continued until tenfold ten concentration [14]. Then pour plate technique was carried in nutrient agar medium and plates were incubated at 37°C for 24 to 48 hours [11]. After incubation individual colonies of bacterial strains was isolated based on the different color and shape and streaked that bacterial colonies on selective media with the help of inoculation loop and incubated at 37°C for 24 hours. Biochemical and morphological characteristics of the prevalent bacterial genera confined were studied and finally characterized and identified by standard identification methods [10].

### Minimum Inhibitory Concentration (MIC):

Minimum inhibitory concentration (MIC) of the heavy metal resistant bacteria was grow on heavy metals supplemented media against respective heavy metal salts were determined by gradually increasing the concentration of metal salts on nutrient agar plates until the complete inhibition of the growth of bacterial colonies on the nutrient agar plates. MIC was noted when the bacterial strains failed to grow on the plates even after 48 hours of incubation [13]. MIC was carried out using nutrient agar plates and LB broth medium.

In nutrient agar plate method, MIC of the heavy metal resistant bacteria was grown on plates containing heavy metal incorporated medium, against respective heavy metal was determined by continuously increasing the concentration of the heavy metal. The isolate growing on the last concentration was transferred to the higher concentration by streak plate method, MIC was noted when the colonies failed to grow on the plates [15]. LB broth media was prepared in tubes for every metallic concentration, then inoculated with 200µl of an 18hour old culture of the studied bacterial isolates. A positive control contains a metal deficient medium inoculated with the microorganism and a negative control contains without the microorganism [16]. Then finally incubated the tubes (test sample, positive control and negative control) at 37C for 24hour and the growth was measured as optical density at 600nm [12].

### Biosorption

Biosorption can be characterized as the capacity of the biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake. The biosorption process consists of two phases: solid phase (sorbent or biosorbent, adsorbent, biological material) and a liquid phase (solvent, normally a water) containing a dissolved species to be sorbent (adsorbate, metal) [21]. These biosorbents incorporate some agricultural wastes, fungi, algae and bacteria studies utilizing bioadsorbents have shown that both living and dead microbial cells can adsorb metal ions and offer likely inexpensive option in contrast to conventional adsorbents [22]. Biosorption includes mechanisms like ion exchange, chelation and complexation. The intake process is complex and various mechanisms are operative, depending on the species used the biomass and its processing [23].

LB broth was prepared and amended with different concentrations of heavy metals went from 1 ppm to 10 ppm at pH 7.0 and inoculated with 200µl of pure culture was placed in rotatory shaker at 150rpm for 37°C for 48 hours and heavy metal was added after 3 hours of incubating and estimated at different

time intervals. Cell was centrifuged at 10000 rpm for 10 min, then the supernatant was sifted through Whatmann No.1 filter paper and then the decrease concentration of heavy metal was measured by using atomic absorption spectrophotometer [28].

### Conclusion

Rapid urbanization and industrialization results the contamination of environments. Every day industries release wastewater effluents contain toxic heavy metals and these heavy metal cause health hazards to all living organisms. Microorganisms to live in the environment polluted by the heavy metal, to develop resistance against toxic metal ions. These microorganisms have important role in bioremediation of heavy metals. This review concluded the method for isolation of heavy metal resistant bacteria from industrial effluent and using that bacteria to determine the minimum inhibitory concentration and finally using the method of biosorption for decrease the concentration of heavy metals.

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