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FROM WASTEWATER TO WONDER BUGS FINDING BACTERIA TO CLEAN UP POLLUTION

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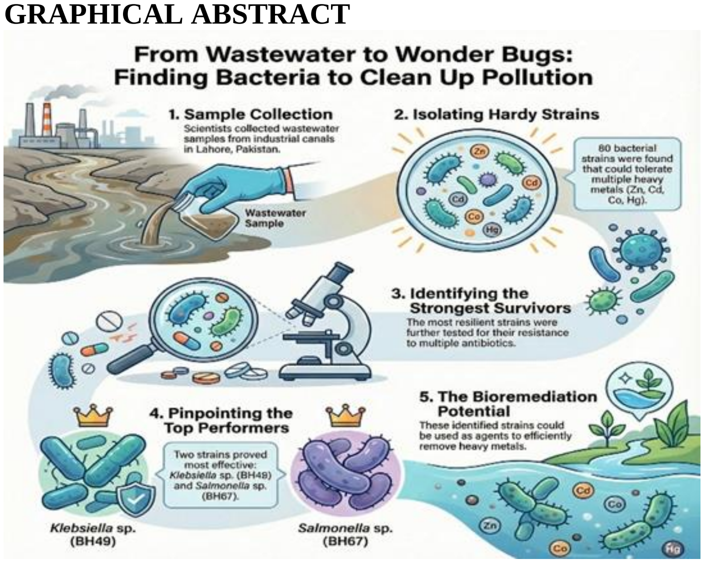
Abstract: The level of heavy metal like Cr, Zn, Co, and Cu by the industrial effluents have brought the environmental degradation and damage to human health to an alarming scale. Nevertheless, 1) lead (Pb), 2) cadmium (Cd) and 3) chromium Cr do not naturally break down; the amount of them in the soil and water bodies can be described as long-term pollution. The chemical precipitation treatment, filtration method or ion exchange method of treatment will turn out to be costly on an average and can pose other environmental risks. Nonetheless, microbiological treatment of metal contaminated wastewater through microbes is emerging as an economic and environment friendly technology. This paper has a purpose of expressing potential efficacy of various microorganisms in bioremediation as well as biodegradation of Chromium, Zinc, Cobalt and copper contained in industrial effluents. Certain bacteria, fungi, and algae were used because they are known to have the high capacity to absorb or store heavy metals or light metals using their natural biological processes-biosorption, bioaccumulation, and biotransformation thereby limiting toxicity as well as movement in the environmental structure. Industrial wastes were isolated and strains obtained which produced a high concentration of metals to pollute the environment.

Key words: Heavy Metals, Industrial Wastewater, Microbial Bioremediation, Bioaccumulation, Metal Detoxification.

GRAPHICAL ABSTRACT



INTRODUCTION

The concept of heavy metals neutralization can be defined as the decrease or total inhibition of toxicity, mobility, and bioavailability of heavy metals in any particular matrix due to the use of chemical, physical and biological technology (Abd Elnabi *et al*. 2023). Pollution should be controlled to preserve prevention on the environment and human beings (Khanam, Sultana, and Mushtaq, 2023). The heavy metal is inorganic compounds of Pb, Hg, Cd, and As in soils or waters which will be harmful to the organisms. Neutralization presents a wide range of physical, chemical and biological techniques which are aimed at reducing their dangerous toxicity and also spreading muscularly. Heavy metals neutralization is usually done through three methods (Fenglian and Wang, 2011).

Most commonly these processes are chemical neutralization such as ion exchange, adsorption and precipitation (Parmar and Patel (2025). Microbial bioremediation is a biochemical technique which is subject to wide research due to its efficiency and environmental friendliness. Physical technologies are aimed at the elimination or fixation of heavy metals without alteration in their chemical state (Abo-Alkasem *et al*.2023). Filtration and sedimentation are some of these methods because the metals are physically eliminated in the water. Activated carbon is one of the materials used in the process of adsorption to trap metals. Electrokinetic remediation involves the application of potential difference to relocate metals in polluted soils and solidification of metals with other materials such as cement that makes them immobile (Gunatilake, 2015). This paper seeks to neutralize various heavy metals such as Zinc (Zn^{+2}), Copper (Cu^{+2}), Chromium (Cr^{+2}), and Cobalt (Co^{+2}) (Virkutyte *et al.*, 2002). Mining activities, agricultural run-offs, and industrial effluents are the key contributors to the contamination of the copper. Precipitation, adsorption and bioremediation are some of the methods of neutralization. Literatures that connect metal plating, mining and industrial wastes with Zinc contamination (Leitão, 2009). The industries of tanning and electroplating release hexavalent chromium (Cr^{+}) which is highly perilous. Reduction processes involve the transformation of Cr^{+} to significantly less toxic Cr^{+3} with the help of sulfur compounds or iron salts (Tripathi *et al*., 2021). Cobalt pollution is primarily in mining, battery production, and industrial wastes. The tanning and electroplating companies make huge distributions of extremely toxic hexavalent chromium (Cr^{+}). One of the processes is the reduction process whereby Cr^{+} is reduced to a lot less toxic Cr^{+3} aided by any sulphur compound or iron salts (Arshi, and Singh, 2021).The entry into the environment of cobalt is largely due to mining, battery manufacturing and other industrial activities. The microorganisms in the waste or its treatment procedure bioremediation by decomposing or changing form toxic agents. The microbes can aid in the degradation of heavy metals and other toxins of petroleum, textile, pharmaceutical, and food processing companies Coetzee *et al.*, 2020). These metals in combination with hydrocarbons are digested by bacteria including Pseudomonas and Bacillus, in some cases complex organic compounds are digested by fungi including Aspergillus and Penicillium (Girma, 2015). The microbes are involved in biosystems which involve biofilters, constructed wetlands, and activated sludge. The development of genetic engineering has provided an opportunity to increase the ability of higher pollutant degradation of these microbes, and problems with microbial adaptability, scale-up and control impede useful applications (Aguirre-Sierra, 2020). The microorganisms are crucial to the treatment of wastewater since they both decompose organic matter and remove the contamination of the sewage, industrial waste, and agricultural runoffs. Important microbial groups are *Nitrosomonas* and *Nitrobacter* for bacteria and fungi *Aspergillus* and *Penicillium*. The other microbial species that help in the process of metal detoxification by biomineralization include *Bacillus cereus*, *Lactobacillus* and *Pseudomonas stutzeri*. This is mostly through the precipitation of carbonate or phosphate or sulfide formation of insoluble complexes (Gadd, 2010).

MATERIALS AND METHODS

Collection of Wastewater Samples

The industrial wastewater was supplied by manufacturing plants and sewerage systems that are known to discharge chemicals. The samples were collected in sterile and sealed containers before being taken to the lab to ensure that there was no contamination (Sanders, 2012).

Preparation of Growth Media

Nutrient Broth and Luria Bertani (LB) broth culture media were prepared aseptically to enhance the growth of microbes. These were autoclaved prior to use in order to eliminate unwanted organisms (Wang, *et al.*, 2023).

Wastewater Innoculation

The obtained wastewater was further put into the sterile growth media and native microorganisms developed and settled in a controlled environment.

First Incubation Period

The inoculated media was placed in a shaking incubator whose temperature was kept to 30degC to 37° C. To enhance the growth and adaptation of the microbes, this procedure was done within a period of seven days.

Microbial proliferation should be monitored.

After incubation, the growth patterns on solid agar plates, turbidity in broth cultures and microscopic analyses were employed to confirm the presence of microorganisms.

Introduction of Heavy Metals

In order to test the resistance of the organisms and their possible ability to detoxify, different heavy metals containing lead (Pb), cadmium (Cd), and chromium (Cr) were added to the microbial cultures in different amounts.

Second Incubation Period

To promote the interaction of the microbes with the toxic elements, these cultures that were supplemented with heavy metals were incubated under identical conditions during an extra seven days.

Bioremediation Efficiency Evaluation

The level of neutralization of heavy metals by the activity of microorganisms was evaluated in terms of turbidity, coloration, and the surviving rate of the microbial colonies.

Spectrophotometric Analysis

Optical density at 600 nm (OD600) was measured in a spectrophotometer to quantitatively determine the bacterial growth as well as test the toxicity of heavy metals (Rathnayake, *et al*., 2013).

RESULTS AND DISCUSSION

The bacteria cultures showed a high resistance and persistence of viability at all the test concentrations, a strategy that suggests a high degree of resistance and metabolism. The concept of active biosorption and detoxification was justified using spectroscopic analysis, whereby the absorbance levels were lower in most of the test cases. Although there was a lot of metal stress, PCR analysis showed that there was the formation of a distinct band in all the treatments, demonstrating the presence of genomic integrity. The biological stability of the microbial strains was verified by the fact that no degradation or mutation was detected.

Spectrophotometric Data:

Spectroscopy was done using 6 levels of concentration of 1000, 750, 550, and 100 uL of 5000uL of culture. The values of absorbance of zinc (Zn), copper (Cu), chromium (Cr), and cobalt (Co).

Table 1. Absorbance of culture in the presence of heavy metals at different concentration:

	Concentration of Heavy Metals					
METALS	**0.20 M**	**0.15 M**	**0.11 M**	**0.02 M**	**0.01 M**	**0.011 M**
Cu	0.393	0.320	0.331	0.487	0.537	0.678
Cr	0.414	0.258	0.211	0.191	0.205	0.343
Zn	0.366	0.268	0.162	0.246	0.242	0.206
Co	0.380	0.310	0.215	0.260	0.220	0.190

Copper

Microbial growth declines as copper concentration rises. This indicates that the growth was reduced as the copper concentration rose. The highest growth was observed at a $CuSO_4$ concentration of 0.011M.

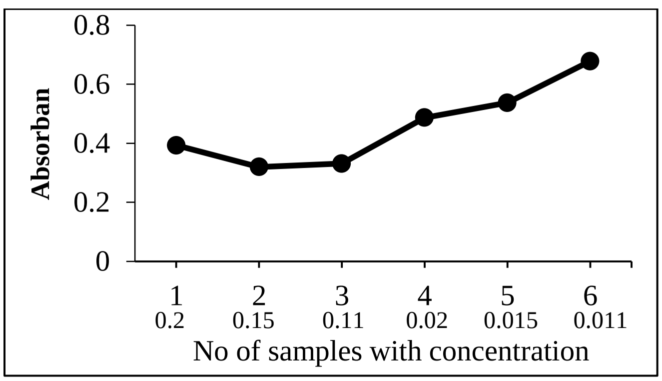


Figure 1. Absorbance of microbial growth at 600nm in the presence of copper

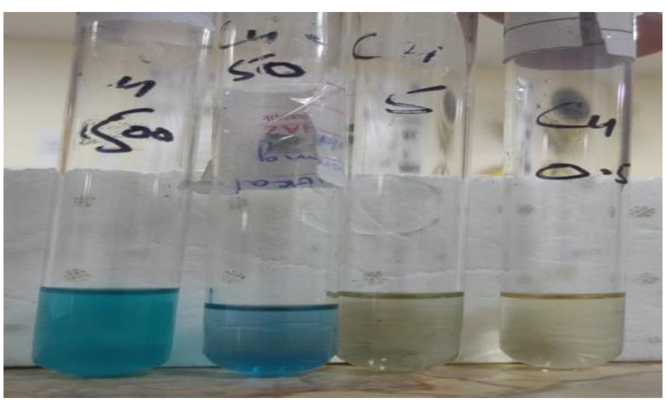


Figure 2: Growth of microbes with the higher concentration of copper. Bacteria showed the growth in all concentration of Copper.

Chromium

In the presence of chromium in media, the growth of bacteria slows down with the increase in concentration of chromium. Maximum bacterial growth was found at 0.011M (Johnson, *et al*., 1992).

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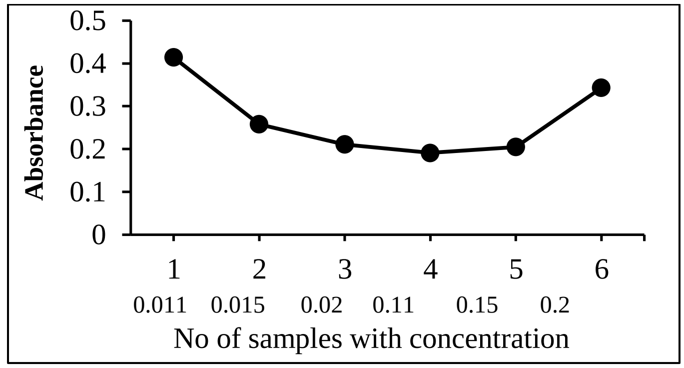

Figure 3. Absorbance of microbial growth at 600nm in the presence of chromium

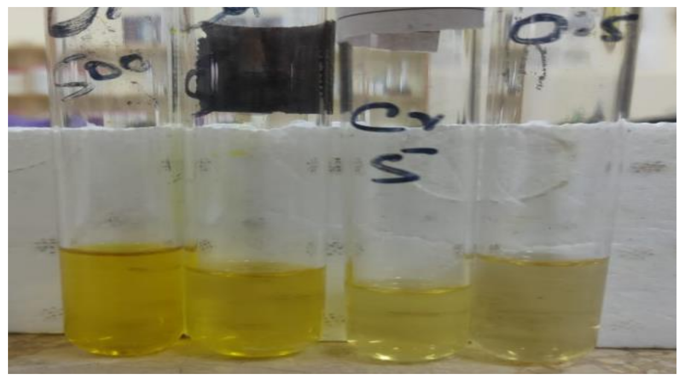

Figure 4: Growth of microbes decrease initially *with* the increase of chromium concentration and then started to increase in media.

Zinc

Presence of zinc showed a consistent decline in absorbance, it showed that high concentration of zinc decreased the growth. However, growth of microbes was reported in all the concentrations of zinc.

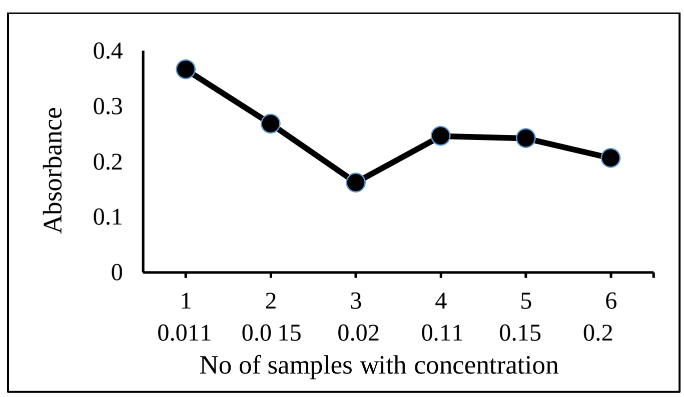

Figure 5: Absorbance of microbial growth at 600nm in the presence of Zinc. As the concentration of zinc increase the growth decreases.

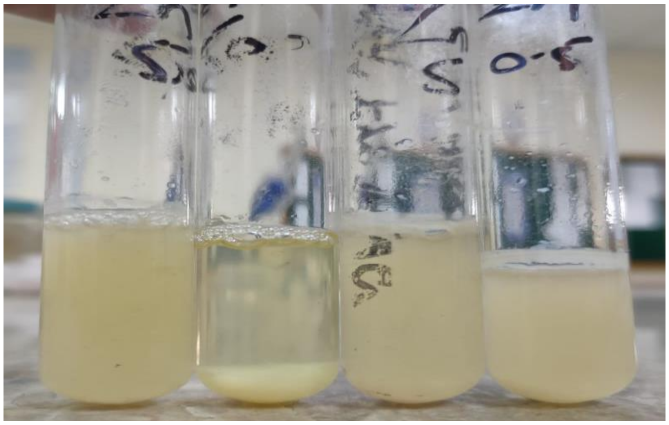

Figure 6: Growth of microbes with the higher concentration of Zinc

Cobalt

In the presence of chromium the growth decreases with the increases of cobalt concentration. The maximum growth was observed at 0.011M (Coetzee, *et al*., 2020).

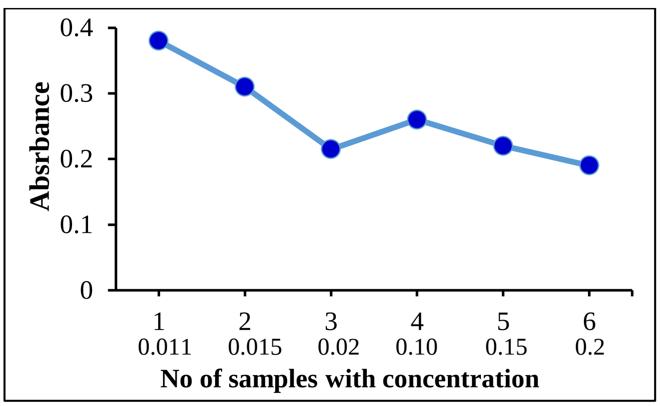

Figure 07: Bacterial growth increases as the concentration of cobalt decreases

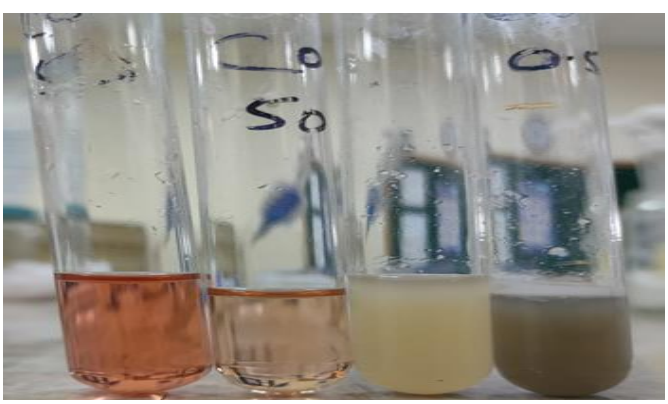

Figure 8: Bacterial growth with the higher concentration of cobalt

Conflict of interest

Authors declare no conflict of interest.

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