



ISOLATION OF SOIL MICROBIAL POPULATION FROM HEAVY METAL CONTAMINATED SOIL AND EVALUATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF HEAVY METALS AGAINST THE ISOLATED BACTERIAL POPULATION: A REVIEW

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ABSTRACT:

Many metals are essential for life can be harmful to humans, animals, plants, and microorganisms when present at toxic levels. The occurrence of heavy metals in soil is primarily attributed to industrial, mining, and agricultural activities. This study focuses on isolating soil microbial populations from heavy metal-contaminated soil and determining the minimum inhibitory concentration (MIC) of heavy metals against bacterial populations collected from a metal industry site. Heavy metal contamination in soil poses a significant environmental challenge. The microbial community identified in the study comprised two groups: heavy metal-resistant and sensitive populations. The resistant microbial isolates included species such as *Pseudomonas*, *Bacillus*, *Corynebacterium*, and *Micrococcus*. The MIC of the isolates was evaluated for metals like cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) in soil media. The isolated heavy metal bacteria could be effective and useful for the bioremediation of heavy metal contaminated soil.

Keywords: - Contaminated soil, Heavy metals, Bioremediation, Soil microorganism.

INTRODUCTION :

Soil is a fundamental resource for human survival and remains essential even in modern society. It serves as the foundation for human production and acts as a medium that connects various beneficial relationships (Dian Chu et al., 2018). Soil plays crucial roles in supporting primary productivity, regulating nutrient cycles, and sustaining terrestrial ecosystem health. However, the accumulation of toxic substances like heavy metals in soil can harm the ecosystem by adversely affecting soil microorganisms. Soil contamination with heavy metals has emerged as a significant global environmental issue. Heavy metals are among the most hazardous pollutants in soil ecosystems (Smejkalova et al., 2003) Microorganisms, the most active component of soil, are vital for nutrient transformation, storage, mineral decomposition, organic matter breakdown, and nutrient release. These nutrients

can be absorbed by plant roots, effectively nourishing the plants (Chu, D. et al., 2018). Heavy metal contamination poses a severe threat as these metals cannot be naturally degraded like organic pollutants and tend to accumulate throughout the food chain. Heavy metals are defined as metals with a density greater than 5 g/cm³ and atomic weights ranging between 63.5 and 200.6 (Efe, D. et al., 2020). Microorganisms play a crucial role in breaking down chemical contaminants by using them as energy sources through metabolic processes. However, excessive inorganic nutrients in soil can inhibit microbial activity. Some microorganisms have the ability to detoxify, degrade, and even accumulate harmful organic and inorganic substances (Ahirwar N. K. & Gupta G., 2016). As a living component of soil organic matter, soil microorganisms are responsible for mineralization, nutrient cycling, and the breakdown or transformation of toxic

compounds. The microbial biomass, a dynamic fraction of soil organic matter, serves as an early indicator of soil changes and can reflect trends in organic matter and soil development (Algaidi A.A. et al., 2010).

The isolated heavy metal bacteria could be affective and useful for bioremediation this study will be useful because of Microorganisms can remove heavy metal from the environment through processes like bioleaching, biomineralization and biotransformation.

Review of literature

The current study focuses on isolating and characterizing heavy metal-resistant bacteria from soil impacted by industrial activity. Initially, 150 bacterial colonies were screened using nutrient agar medium supplemented with heavy metals, followed by a secondary screening that selected 25 isolates. Ultimately, five strains exhibiting strong resistance to heavy metals were chosen for further analysis. Among these, *Proteus vulgaris* (MR1), *Pseudomonas fluorescens* (SS4), and *Pseudomonas fluorescens* (SS5) were identified as Gram-negative, rod-shaped, and motile bacteria, while *Bacillus cereus* (MR2) and *Bacillus decolorationis* (MR3) were Gram-positive, rod-shaped, and motile. The optimal growth conditions for the isolates were observed at 30°C and pH 7.0. Biochemical characterization and growth studies were conducted on these bacteria in the presence of heavy metals, including Cd, Cr, As, Pb, and Ni at 200 mg/L and Hg at 100 mg/L, prepared from stock solutions. After 48 hours of incubation, the results demonstrated that the isolates showed significant resistance to most of the heavy metals, except for Cr and Hg, to which they were sensitive. These findings suggest that microorganisms with the ability to resist and transform heavy metals into non-toxic forms could play a crucial role in detoxifying heavy metal-contaminated soils. The minimum inhibitory concentration (MIC) of heavy metals for

strains MR1, MR2, MR3, SS4, and SS5 varied between 0.35 and 17.5 mM. Among the tested metals, zinc and nickel were found to be the least toxic, while chromium and mercury were the most toxic to all strains (N. K. Ahirwar and G. Gupta, et al., 2016).

M Mustaphaa, and N Halimoonb et al., (2015).

Heavy metal-tolerant bacteria were isolated from an electroplating industry, which is a significant contributor to heavy metal pollution. A total of 21 bacterial colonies were tested and isolated in the presence of various metals. Among them, five highly resistant isolates—MH15, MH1, MH6, MH4, and MH21—were selected after screening. The results revealed that all five isolates could tolerate 50 mg/L of lead (Pb), chromium (Cr), cadmium (Cd), and copper (Cu). Notably, isolate MH4 exhibited a high level of resistance to copper, while isolates MH1 and MH21 were capable of tolerating up to 200 mg/L of cadmium. These findings suggest that these isolates can be effectively utilized for the removal of heavy metals from contaminated industrial effluents. The copper-resistant isolate MH4 was round in shape, milky in color, and displayed an entire raised margin. Similarly, the cadmium-resistant isolate MH1 shared the same colony morphology as MH4. On the other hand, the lead-resistant isolate MH6 and the chromium-resistant isolate MH21 exhibited identical colony characteristics, including a milky white color, convex elevation, and undulate margins. However, MH21 was irregular in shape, whereas MH6 was round. The isolate MH15 displayed unique properties, characterized by a yellowish color, round shape, entire margin, and flat elevation.

L dinu, and L anghel et al., (2011) Researchers isolated microbial strains from soil polluted by battery manufacturing activities and tested their tolerance to heavy metals, including cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn), and nickel (Ni). During primary screening, 24 aerobic bacterial strains were identified that could

tolerate high concentrations of Cd, Pb, Zn, and Ni (up to 10 mM), which are commonly used hazardous chemicals in the battery industry. Notably, intracellular lead accumulation caused a color change in bacterial colonies grown on lead-containing media. Eight selected strains were further characterized for their morphological and physicochemical properties, and their plasmid profiles were analyzed. The findings suggest that these bacteria have significant potential for heavy metal bioremediation.

L Smejkalova and O Mikanova et al., (2003) Six samples were collected from the alluvial soils of the Litavka River between 1999 and 2001, during both spring and autumn seasons. The sampling locations included: (i) soil above the contamination source for control purposes, (ii) soil directly below the contamination source, and (iii) soils downstream at distances of 5.0 km, 6.7 km, 10.7 km, and 17.8 km. The soils were classified as fluvisols. Basic chemical properties of the soils and the vegetation cover were analyzed, with detailed transect and soil descriptions available in Borůvka (1997). Samples were collected from a depth of 0–20 cm, sieved through a 2-mm mesh, and analyzed in triplicate, with average values reported. Statistical analysis was conducted using Statgraphics Plus for Windows.

N. k Ahirwar and G. Gupta, et al., (2016) A total of 175 isolates were screened from industrially contaminated soil, and five isolates were selected based on their tolerance to high levels of heavy metal contamination. Biochemical and morphological analyses identified these isolates as *Pseudomonas fluorescens* (SS4), *Pseudomonas fluorescens* (SS5), *Proteus vulgaris* (MR1), *Bacillus cereus* (MR2), and *Bacillus decolorationis* (MR3). The Isolates exhibited optimal growth at 30°C and a pH of 7.0. They demonstrated resistance to heavy metals such as arsenic (As), nickel (Ni), cadmium (Cd), lead (Pb), and chromium (Cr). The minimal inhibitory

concentrations (MICs) for Cr, Cd, Pb, and Ni were determined on solid media. These heavy metal-resistant bacteria show potential for application in the bioremediation of heavy metal-contaminated soil.

W. peng and SHI LIN Qi, et al., (2007). The maximum concentration of metals in soil is determined by their total concentration. The potential toxicity of heavy metals in soil depends on their speciation and bioavailability. A study was conducted to investigate the effects of heavy metal availability and speciation on soil microbial activity under increasing Cu and Zn contamination. The study examined enzyme activities and microbial biomass in soil contaminated with Cu and Zn. The findings showed that elevated metal levels negatively impacted microbial biomass. A negative correlation was observed between NH₄NO₃-extractable metals, phosphatase activity, and soil microbial biomass. Soil microbial activity can be predicted using empirical models based on the availability of Zn and Cu. The results indicated that 72% of the variation in phosphatase activity could be explained by the NH₄NO₃-extractable and total heavy metal concentrations.

CONCLUSION :

The bioremediation process is influenced by various factors, including soil type, pH, temperature, nutrients, amendments, and oxygen availability. The ability of microbial strains to thrive in the presence of heavy metals is particularly advantageous for treating contaminated soils, as microorganisms play a key role in the decomposition of organic matter during biological soil treatment. In this study, a high level of resistance to various heavy metals was observed in bacteria isolated from industrially affected soils. Among the heavy metals tested, zinc and nickel were found to be the least toxic, while chromium and mercury were highly toxic to all bacterial isolates.

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