I J R B A T, Issue (XIII) Volume (I) Jan 2025: 123-126
A Double-Blind Peer Reviewed & Refereed Journal
OPEN ACCESS
e-ISSN 2347 – 517X
Review Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

ISOLATION OF SITE- SPECIFIC NITROGEN FIXING BACTERIA FROM HEAVY METAL CONTAMINATED SOIL WITH SENSITIVITY TO COPPER - A REVIEW

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Communicated :20.11.2024 | Revision: 25.11.2024 & 19.12.2024 Accepted: 27.12.2024 | Published: 30.01.2025

ABSTRACT:
Heavy metal pollution of soil is a critical problem for the environment as it limits the growth of plants and agriculture crops. Biological Nitrogen (N2) fixation has an important role for the growth of plants. Modern industrialization is the main cause of heavy metal contamination present in the soil in the form of aero soils, residues, strong waste, particulate issue and effluents which is directly dumped into the atmosphere and finally disposed into the soil causing pollution to a great extent. Copper is a micronutrient and an essential trace element required for growth of microorganisms since it is a cofactor for numerous enzymes and an important electron transfer carrier. Increasing levels of copper can be harmful for soil microbiota. This review examines copper concentrations present in the soil by determining the minimal inhibitory concentration (MIC) of soil isolates against Cu. The present review was conducted to potentially isolate N2-fixing bacteria from heavy metal contaminated soil samples as well as examine copper concentrations present in the soil by determining the minimal inhibitory concentration (MIC) of soil isolates against Cu.

Keywords: - Nitrogen fixing bacteria, Heavy metals, MIC determination of copper.

INTRODUCTION :

Heavy metal contamination of soil is a major environmental issue. Heavy metals such as Cu, Pb, Zi, As, etc present naturally in the soil as a consequence of anthropogenic activities. Other activities such as mining, overuse of fertilizers and pesticides, residue release from chemical industries leads to the increase in concentrations of heavy metal in soil. Human beings, plants as well as microorganisms require a small amount of heavy metals for their survival. Consequently, an increase in concentration of heavy metal becomes toxic for all organisms (D. Efe, 2020).

Nitrogen is a vital element as it promotes growth and development of plants. Presence of nitrogen is abundant in nature but plants can not absorb it as they depend upon combined or fixed forms of nitrogen such as ammonia, fertilizers, soil manure and organic matter decomposition. Prokaryotic bacteria such as *Azotobacter sp.,Azospirillum sp.*, Cyanobacteria fixes nitrogen about 50 % in nature. Rhizobium sp. and Bradyrhizobium sp. forms interaction between plants and bacteria from symbiotic relationship with leguminous plants (Shomi et al.,2021). Soil encountered many challenges as chemical and industrial pollution is a threat to property of soil. Heavy metals like Cu show negative effects on ecosystems whereas increasing levels disturbs the N-cycling process. Study of how the N- cycle is affected by heavy metal tolerance is still not understood (Rijk et al.,2023). The damaged ecosystems and environmental problems of N2-fixation could be dealt with by biotechnology (Ferreira et al., 2018). Changes in soil diversity polluted with heavy metals depend upon the soil properties. Disposal of municipal solid waste, dumping of domestic and industrial sludges lead to loading of Cu in soil Industrial effluents as irrigation source, discharge, dumping and leaching into aquatic environments causes deposition of heavy metals such as As, Pb, Cr in soil.

Underground mining also increases the concentration of Cu, in soil. Spraying of metal containing insecticides and fungicides and application of excess fertilizers lead to Cu, Pb, As, Cd contamination in soil (N. Hamsa et al.,2017). Heavy metals when present in excess have also been found to delay the nodulation process in some legume crops (E. Ahmad et al.,2012). The present review was carried out to evaluate the status of waste done in the area of isolation of site specific nitrogen fixing bacteria from heavy metal contaminated soil.

Rising pollution and industrialization are the main causes of contamination of the environment with heavy metals.. It is estimated that around 40% deaths are caused by soil pollution. It was stated that copper at increasing levels causes serious health issues in human beings. Hence bioremediation is an important aspect as heavy metal is not degradable by the environment. The effects of heavy metal contamination is quite disturbing as it affects the organisms and ecological stability . Nitrogen fixation contains a number of enzymes and Microorganisms which can be inhibited by heavy metals. Tolerance level can be dependent upon the properties of soil as well as concentration of heavy metal. Therefore present studies of the isolated Nitrogen fixing bacteria will be reviewed.

D. Efe (2020) studied how Plant Growth-Promoting (PGP) bacteria can help plants grow in heavy metal contaminated soils. Bacteria were isolated based on their resistance to two heavy metals that are zinc and lead. Bacterial isolates were identified using classical and molecular techniques. Isolates were evaluated for their plant growth-promoting characteristics and response to three heavy metals (lead, zinc, copper). The MIC of heavy metals was determined using the plate dilution method. The study suggests that heavy metal resistant PGP bacteria can be used to improve plant growth in contaminated soils, offering a potential solution for environmental remediation.

F. Shomi et al. (2021) studied that fixation of biological Nitrogen is an important aspect for limiting the growth of plants. They isolate bacteria from garden soil by inoculating the isolate in nitrogen free Jensen's media incorporating bromothymol blue. They identified different colonies from soil samples. Ammonification test confirms the nitrogen fixing ability by using Nessler reagent. The isolate was found to be *Azotobacter sp.* will be used for production of crops by fixing nitrogen.

I. Rijk et al. (2023) concluded that the Nitrogen cycle is affected by Cu present in the soil. They were using grassland soil to check the effect of copper in the abundance and activity of nitrogen cycling and plant responses. Soils were treated with different concentrations of copper . After some time they have found that the extensive amount of Cu present in soil affects abundance of ammonia oxidizing bacteria, potential ammonia oxidizing archaea ,Nitrospira nitrate oxidizing bacteria, potential ammonia oxidizing rates and plant biomass in soils.Furthermore, effects of increasing Cu on microbial N-cycling guilds became more apparent with longer incubation time, emphasizing the importance of long-term studies to evaluate important ecosystem effects of Cu contamination. Taken together, they conclude that a combination of plant and microbial responses can give better insights on how Cu is affecting the N cycle in polluted soils.

V. Stan et al. (2011) studied that heavy metals have negative effects on microorganisms. Heavy metals disturbs their growth, genetic diversity, efficacy, nodulation ability and abundance of microbes . For the isolation and characterization of free living Rhizobia , the soil is artificially polluted with copper as well as a mixture of metals and then cultivated with red clover (*Trifolium pratense L.*) and Comparing them with

bacterial isolates with the same type of unpolluted soil .The Isolation is carried out on YEMA medium. The isolates were characterized morphologically and physiologically. The result suggested that the metal concentration is higher than the presence of Rhizobia.

P. Ferreira et al. (2018) studied that Nitrogen fixing bacteria associated with leguminous plants can recover the area affected by coal mining .They isolate and characterize bacteria using molecular techniques.The nitrogen fixing bacteria was characterized as Rhizobium sp., Bradyrhizobium sp.and Burkholderia sp. . Heavy metal tolerance against Cu as well as other heavy metals were evaluated.Most of the isolates were tolerant to a wide range of pH as well as heavy metal content. The result suggested that the isolated bacteria as inoculants and biofertilizers with pH as well as heavy metal stress can degrade coal mining area.

C. Chihching et al. (2008) isolate bacteria from heavy metal contaminated soil of chemical factories. Bacteria were identified using molecular techniques . Strains of *Acinetobacter sp. and Enterobacter sp.* were identified. The result suggested that the activities of heavy metal tolerance were quite different in both the isolates obtained from enrichment culture as well as actual isolates.

F. Altimira et al. (2012) studied that agricultural soil gets polluted with Cu due to copper mining. They reported that the bacteria isolated from Cu-polluted and non-polluted soil were characterized by DGGE. Isolated strains showed copper tolerance between 3.1 to 4.7 mM and also showed high resistance to other heavy metals.

N. Hamsa et al. (2017) studied that soil fertility and productivity get influenced by nitrogen. Heavy metal toxicity shows negative effects on soil biological process, soil microbial biomass, N-cycling and soil ecosystems. Hence , the study and monitoring of heavy metal concentrations is needed. The effects of heavy metal are quite disturbing as it affects the ecological balance and health of microorganisms, plants as well as human beings. Elevated concentrations of heavy metal inhibit enzymatic activity of nitrogen transformation. Hence the study of heavy metal for soil is an important aspect.

E. Ahmad et al. (2012) studied that the nutrition provided to plants is depend upon the interaction between Rhizobia and legumes which increases soil fertility, promoting plant growth and restore damaged ecosystems.Lower concentrations of heavy metal is necessary for metabolic activities of microbes including Rhizobia, legumes and their symbiosis. They suggested that the free living Rhizobia gets affected by varying metal concentrations and how nitrogen fixing nodule bacteria " Rhizobia" promotes plant growth. They concluded that the Rhizobia - legumes symbiosis probably helps to manage legume cultivation in heavy metal contaminated soil.

CONCLUSION :

In conclusion, Nitrogen fixing bacteria is considered to be more sensitive to heavy metal contamination in soil. The presence of Nitrogen-fixing bacteria in heavy metal contaminated soils suggests that these microorganisms can play a crucial role in maintaining soil health and ecosystem function. The sensitivity of these bacteria to copper highlights the need to monitor and mitigate heavy metal pollution in soils to protect microbial communities and ecosystem services. Further research is needed to understand the mechanism of copper tolerance in these bacteria and how they can be exploited for bioremediation and other applications.

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