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Screening of heavy metal degrading microbes from coal mine soils of Ramagundam and Sathupally

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ABSTRACT

Bacterial community has been evolving and is been utilized in many ways for human welfare. Their very adaptive nature made them good remediators of the habitat of their presence. Such bacteria presence in the coal mine dumps in particular in the rhizosphere soils are having very vital role. They are the true bioremediation army for the heavy metals present in those soils via various metabolic processes. Such heavy metal degrading microbes are been screened from the rhizosphere soils of Ramagundam and Sathupally coal mine study areas and were studied for their bioremediation property in vitro. It was found that the gram positive bacteria have the capacity of heavy metal bioremediation in a better way than the gram negative ones in the present study.

Introduction:

Heavy metals are been considered as contaminants in the natural resources for humans when they are ingested more than the permissible levels. But nature has been always surprising in showing its potential towards maximum utilization of these heavy metals by breaking them into suitable metals. The remediation process is done through microbial assistance, so microbial remediation process has been playing a vital role in reduction of such pollutants from water and soil resources (Singh and Rai. 2024). Renewable and non-renewable resources of environment are been providing the needs of humans, and indirectly or directly mankind is dependent on them for their survival. Coal mines are the non-renewable resource that was helping humans from age old in generation of fuel and electricity. These are the resources rich in heavy metals and mostly the soils are also believed to be contaminated with them due to mining of coal (Harun et al. 2014). Biological remediation through microbes include various mechanisms such as reduction, accumulation, complexation, adsorption, transportation and precipitation of heavy metals (Bai et al. 2023). The rehabilitation of mining soils in particular the Rhizosphere soils is much more in need, so that the environment there can be restored and soil wastage can be minimised. A bioconversion of coal waste into humic material which show potential support in soil fertility there by promoting flora growth has been studied (Sekhohola-

Dlamini et al. 2022). In such bioconversion, the microbial role as biocatalysts in revitalizing the soils was well documented (Lu et al.2024). The low level of heavy metal exposure can lead to growth retardation of microbes, but due to over exposure of heavy metals in coal mine rhizosphere soils made them tolerant (Baker and Dopson, 2007; Nies, 2003; Young, 2003). This observation introduced interest towards exploring heavy metals degrading microbes from the rhizosphere soils of coal mines in the present study from the selected sites and to further understand their biochemistry in doing so was attempted to explore.

Materials and methods:

Sample collection:

Rhizosphere soils of two study sites Ramagundam and Sathupally were collected using sterile shovels at 10 cm depth and poured in sterile polythene bags and stored at 4^0C before further use.

Deduction of heavy metal concentration and pH of soil samples:

Using standard acid digestion method, the concentration of heavy metals were deducted. The analysis of the samples were done using ICP-OES (Inductively coupled plasma optical emission spectrometry (Gupta et al. 2012; Yilmaz, 2003). The pH was determined using digital pH meter (Rayment and Higginson, 1992).

Microbial isolation:

After performing 10 fold serial dilutions, 0.1 mL from appropriate dilutions were transferred onto nutrient agar plates and was spread evenly using sterile L shaped rod and plates were incubated for 72 hours at 37^0C. After incubation the distinct colonies were picked up and streaked onto fresh nutrient agar plates to get isolated colonies.

Morphological identification:

The isolates were observed for their morphology microscopically for shape, size, motility, arrangement and other structures after Grams staining. Colony morphology was identified macroscopically for their color, texture, edges and surface.

Biochemical characterization:

The isolates were analysed for IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization tests), Catalase, Urease, Nitrate reduction, Oxidase and Lactose tests to understand their biochemical characterization.

Isolates showing heavy metal tolerance:

The microbial isolates were grown in 100 mg/L Iron ($FeSO_4$), 1 mg/L Cadmium ($CdCl_2$) and 1 mg/L Chromium ($CrCl_3$) solutions on LB broth until mid-log phase with O.D approximately 0.5. Then nutrient agar plates were prepared and 10 µL of culture was spot inoculated onto metal supplemented plates and incubated for 72 hours at 37^0C by adjusting the pH at 5. Isolates grown in this media were considered as metal tolerant (Kumar et al. 2011).

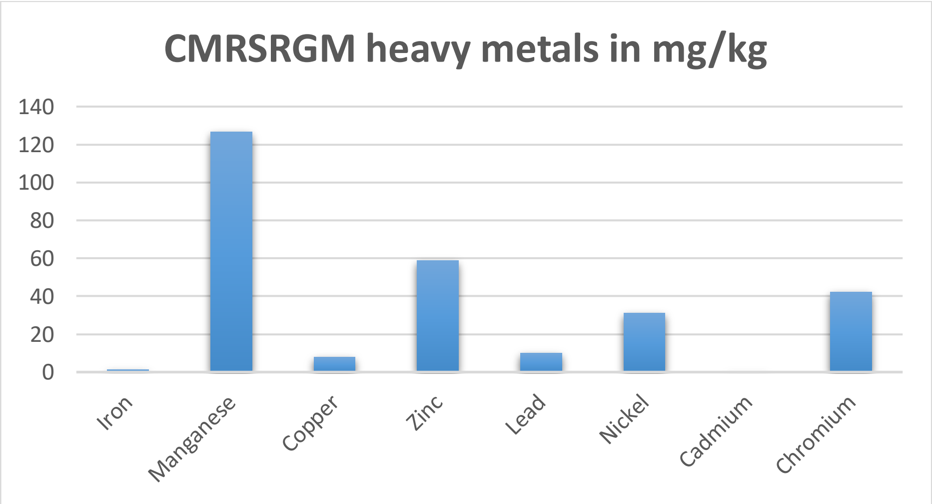
Results:

pH and heavy metal concentration of soils:

The collected Rhizosphere soil samples from Ramagundam and Sathupally were analysed for pH and heavy metal concentration, where the Ramagundam sample CMRSRG has shown a pH of 6.14 and Sathupally sample CMRSSPL has shown a pH of 7.60 respectively. The heavy metals present in the Ramagundam soil samples are iron, manganese, copper, zinc, lead, nickel, cadmium and chromium as shown in table 1. The heavy metals present in Sathupally rhizosphere soil samples are iron, manganese, copper, zinc, lead, nickel, cadmium and chromium as shown in table 2.

Table 1: Heavy metals found in coal mine rhizosphere soils Ramagundam (CMRSRG)

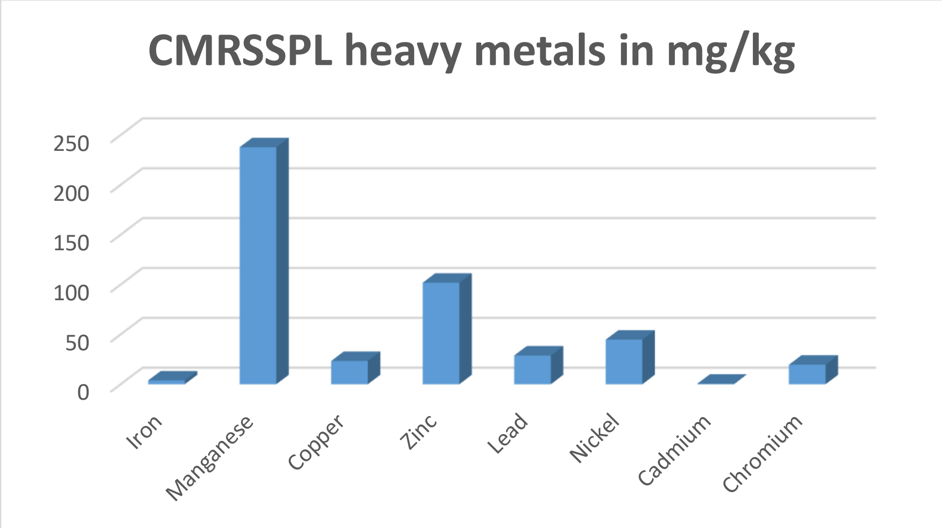
S.No.	Heavy metals detected	Result in mg/kg
1.	Iron	1.4
2.	Manganese	126.7
3.	Copper	8.0
4.	Zinc	58.9
5.	Lead	10.1
6.	Nickel	31.2
7.	Cadmium	0.3
8.	Chromium	42.3



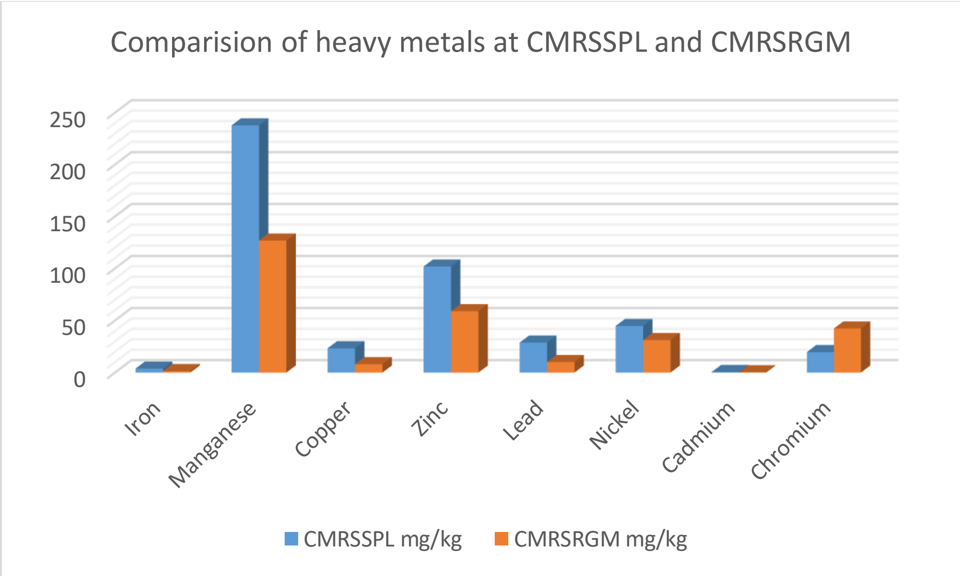
Graph 1: CMRSRGM heavy metals in mg/kg

Table 2: Heavy metals found in coal mines rhizosphere soils Sathupally (CMRSSPL)

SL.No	Heavy metals observed	Quantity in mg/kg
1.	Iron	3.8
2.	Manganese	237.7
3.	Copper	23.3
4.	Zinc	101.8
5.	Lead	28.8
6.	Nickel	44.7
7.	Cadmium	0.5
8.	Chromium	19.6



Graph 2: CMRSSPL heavy metals in mg/kg



Graph 3: Comparison of heavy metals at CMRSSPL and CMRSRGM

Isolation of microbes:

The serial diluted and spread samples on the agar plates were when streaked onto fresh agar plates showed many distinct colonies. The CMRSRG samples showed a 2.2 X 10^7 colonies and CMRSSPL showed 6.9 X 10^6 colonies as shown in Figure 1, 2.

Figure 1: CMRSRG showing 2.2X 10^7 colonies

Figure 2: CMRSSPL showing 6.9X10^6 colonies

From the obtained colonies as shown in figure 1 and 2, onto fresh nutrient agar, loopful of colonies were streaked to observe isolated colonies. Isolated colonies were observed in figure 3 and 4.

Figure 3: *Bacillus species*

Figure 4: *Enterobacter species*

Morphomacroscopic studies:

The isolates were observed for the morphological features, and as shown in figure 3, rod shaped single and some pairs Gram positive motile bacteria were seen. The colony morphology was white to slightly yellow, smooth, undulated, opaque colonies were observed. The morphomacroscopic studies of figure 4 showed that short rod shaped, gram negative, motile, non-sporing, circular clumped forms. The two types of bacterial colonies observed in figure 3 and figure 4 were seen in the samples of rhizosphere soils from Sathupally, and only figure 3 colonies were found in Ramagundam rhizosphere soils so single plate photos were taken for the present representation.

Isolates biochemical characterization:

Biochemical characterization was done for the isolates to understand their genus as per the morphology observed. The isolate of figure 3 showed catalase positive, voges-proskauer positive, methyl red negative, indole negative, citrate utilization negative, urease negative and oxidase negative so identified as *Bacillus species*. The isolate of figure 4 showed catalase positive, oxidase negative, lactose fermentation positive and nitrate reduction positive was observed, so identified as *Enterobacter species*.

Heavy metal tolerance:

All the isolates showed heavy metal tolerance as shown in figures 1 and 2 as the media was supplied with heavy metals iron, cadmium and chromium. Despite the heavy metal constituents in the media, the bacterial growth was observed.

Discussion:

Though coal is considered as a potent source of fossil fuel for energy in the rapid growing industrialization process, there are problems concerned with the improper handling of the dumps and spoils near coal mines. These dumps and spoils make the rhizosphere soils to be contaminated with heavy metals making the soils overwhelmed and losing their fertility. The role of microbes in remediating soils have been proven from decades onwards. These microbes are capable of living in harsh conditions and evolved in such a way that they started utilizing the contaminants and growing and remediating the environment in their habitat.

In the present study it was evident by the growth of microbes in particular bacteria which shown tolerance to the heavy metals from the study areas of coal mine rhizosphere soils of Ramagundam and Sathupally. The in vitro evidence was seen in the heavy metal tolerance test as shown in the figures 1 and 2. The bacteria were further identified upto their genus level as *Bacillus* as shown in figure 3 and *Enterobacter* species as shown in figure 4 through morphological and biochemical characterizations.

Through various mechanisms such as biosorption, bioaccumulation, bioremediation by EPS, bioprecipitation, bioremoval using PGPBs are been utilized by various *Bacillus species* (Wrobel et al.2023). Bacillus species are proven to be most supporters as plant growth promoting bacteria helping in phytoremediation (Wang et al.2021; Zaidi et al.2006; Glick, 2010; Sessitsch et al.2013). This was also evident in the present study through the heavy metals detection in the rhizosphere soils of Ramagundam and Sathupally study areas where a significant low level of heavy metals such as iron, cadmium, chromium, nickel, lead can be seen when compared to copper, zinc and manganese. The copper, zinc and manganese were in more concentration showing that the absence of manganese, zinc metal bioremediating bacterial groups such as *Proteobacteria*, *Actinobacteria* and *Firmicutes* through bioleaching process (Das et al.2015).

The *Enterobacter species* found along with *Bacillus species* in the Sathupally rhizosphere soils was earlier reported to be a potent bioaccumulator of zinc and manganese (Gandhi et al. 2015). In the present study in the Sathupally soil samples in comparison with Ramagundam samples the heavy metal content were higher as shown in graph 3. This is may be due to the development of resistance or hindrance of biochemical activity dominant by other microbial species. The findings in the present study reveals that *Bacillus species* are good bioremediators of the heavy metals than *Enterobacter species*. It was also noteworthy that Ramagundam sampling area has shown good vegetation in comparison with Sathupally sampling area making it a better evidence of bioremediation of soils through heavy metal bioremediation bacteria.

Conclusion:

The Ramagundam and Sathupally coal mines rhizosphere soils were studied for their heavy metal content and a comparison was made to understand about effect of bacteria in heavy metal bioremediation. Bacterial isolates were analysed upto genus level through morphobiochemical characterization and potent *Bacillus* and *Enterobacter* species were identified in those samples. It was understood that the *Bacillus species* are more capable of bioremediation than *Enterobacter species* from the present study and *Bacillus* phytoremediation properties also helped in more vegetation presence in the Ramagundam sampling area than Sathupally area. Further molecular investigation of the bacterial community may open new lines in the field of microbial remediation of these rhizosphere soils and may help them to be utilized for agriculture sector.

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