SHORT COMMUNICATION

Optimize Biosorption Potential of *Micrococcus luteus* on Arsenic, Lead, and Mercury obtained from Coal mine

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Abstract

The main aim of the study was to find the metal biosorption capability of arsenic, lead, and mercury- resistant bacteria *Micrococcus luteus* isolated from heavy metal-contaminated soil of Mand coalfield Raigarh. Bacteria were screened for multiple heavy metal resistance capabilities through enumeration on LB agar containing 300 ppm $Pb(NO_3)_2^2$, $NaAsO_2$, and $HgCl_2$ separately. *M. luteus* showed high biosorption ability against Pb(II) (95.21%), As(III) (90.23%), and Hg(II) (80.67%), analyzed using ICP-MS. The effect of pH, temperature, and metal concentrations on the biosorption potential was also determined. The optimum condition of lead, arsenic, and mercury at pH 4.30, 4.80 and 5.0 respectively, the temperature at 37°C and metal concentration As(III) (260mM/L), Hg(II) (240mM/L), and Pb(II) (340mM/L) by *M. luteus* was studied. The present study indicates that *M. luteus* removes arsenic, lead, and mercury competently from soil contaminated with heavy metals, and therefore further research should explore to remediation of soil contaminated with heavy metals.

Keywords: *Micrococcus luteus*, Biosorption, Heavy Metal, Resistance, Remediation.

Highlights

- *Micrococcus luteus* demonstrated resistance to lead, arsenic, and mercury.
- *Micrococcus luteus* exhibited a high biosorption capacity of 95.21% for lead (300 ppm) as measured by ICP-MS.
- The optimal pH levels for the bioremediation of lead, arsenic, and mercury were found to be 4.30, 4.80, and 5.0, respectively.
- The optimal concentrations for the bioremediation of as (III) (260 mM/L), Hg (II) (240 mM/L), and Pb (II) (340 mM/L) were determined.

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Introduction

The industrial revolution is continuously increasing human activity in mining and agriculture areas and contaminating air, soil, and water with heavy metals. The toxicity of these substances can affect humans as well as the high density of heavy metals, which are difficult to remediate (Puyen *et al.,* 2012). For example, a geochemical study presented a very high concentration of lead isotopic ratio and heavy metals like Cr, Cu, V, Zn, and Mn in the Gevra opencast coal mine in Korba, Chhattisgarh (Das *et al.,* 2018).

Heavy metals are a collection of factors with metallic residences that encompass transition metals (Mafi and Greiner, 2021). A number of them are critical cofactors for various enzymes, while others are not crucial. The primary group includes hint elements whose concentration is regulated by interaction with binding proteins because they represent capacity threads to cellular characteristics. The second group is non-critical metals that make potent toxins and affect the properties of cells. (González and Ghneim, 2021) Mercury, cadmium, arsenic, and lead are highly toxic heavy metals for living beings present in the environment, as per the Environmental Protection Agency and the United States Agency for Toxic Substances and Disease Registry (Goyer, 2004). Four of these metals, arsenic (As), are ranked 1 due to acute poisoning, lead (Pb) is ranked 2, and cadmium is ranked 7 among the top 20 heavy metals listed by the US Agency for Toxic Substances and Disease Registry. (Flora *et al.,* 2011) Previous findings are significant and demand immediate measures. Health and Environmental Impact of Coal Mining in Chhattisgarh reveals that large-scale mining, coal-fired power plants and associated industries have inflicted lasting negative impacts on the population living for generations in the Raigarh region of Chhattisgarh. Their environmental physical and mental health have been compromised, as revealed in the study of village populations, through exposure to worrisomely high levels of Stoxic heavy metals found in air, water, soil and sediment samples. Therefore, bioremediation of mercury, lead, and arsenic is a major goal of the study in the paper. Heavy metals such as lead (323 ppm/kg), arsenic (15 ppm/kg), and mercury (14 ppm/kg) were analyzed in a soil sample from Mand Coalfield Raigarh (Rinchin *et al.,* 2017). The permissible limit of heavy metal in the soil (mg/kg) is Hg (30), Cd (6), Zn (600), As (3) Mn (20), Pb (500), Cu (270), Ni (75), Cr (50) (Shirkhanloo *et al.,* 2015).

Arsenic (As) is a poisonous metalloid present in varied concentrations in the environment. (Bhattacharya P, 2009) Arsenic accumulation in field soils and in the food chain through the rice plant can cause kidney cancer, lung cancer, skin cancer, hyperkeratosis, neurological disorders, and muscular weakness. The toxicity of arsenic depends on oxidation states (+5, +3, +2, +1, and −3) capable of mobilizing under various environmental

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Fig. 1: Study place Mand Coalfield Raigarh

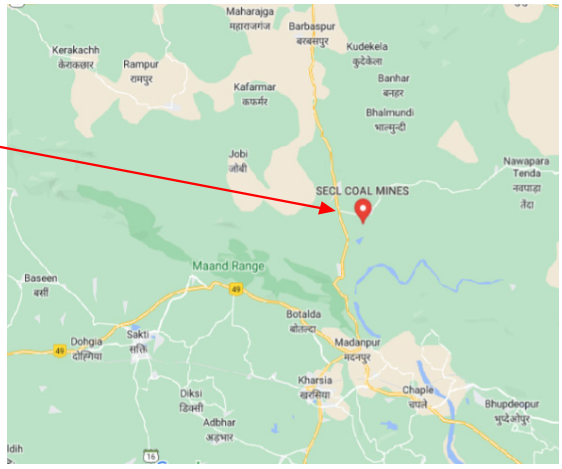


Fig. 2: Sampling site

conditions. Pentavalent arsenate and trivalent arsenite inorganic forms of As are the majority in nature. (Vrajan Vijay *et al.,* 2017) Mercury present in the environment, in the form of organic mercury and inorganic mercury, consists of Hg^0, $Hg^{2+,}$ or mercuric (Hg^{2+}) salts. The mer operon of bacteria helps the enzymes mercuric reductase and NAD(P)H-dependent flavin oxidoreductase to reduce and resist metallic mercury. (Kotwal *et al.,* 2018) Lead is naturally present in nature through mining, fuel burning, and manufacturing. (Tchounwou *et al.,* 2012) It is a non-essential toxic heavy metal accumulation in high quantity in soil and water that affects physiological and biochemical levels in living organisms. (González and Ghneim, 2021)

In the present study, *Micrococcus luteus* was used for bioremediation studies in the presence of lead, arsenic, and mercury. The assessment of ability regarding lead, arsenic, and mercury removal from the coal mine soil was found. *M. luteus* can be a potential agent for decontamination of metal-polluted sites.

Materials and Methodology

Bacterial Strain and Screening

Micrococcus luteus (GenBank accession No. OQ691646) was isolated from a soil sample of Mand coalfield Raigarh (Figs 1 and 2), and culture was maintained and screened for heavy metal resistance in LB agar media containing lead nitrate (Himedia). Multiple heavy metal resistances were tested against $Pb(NO_3)_2$, $HgCl_2$, and $NaAsO_2$.

Stock Heavy Metal Solution Preparation

Stock solutions of arsenic, lead, or mercury were prepared as lead nitrate, sodium arsenite, and mercury chloride (Merck, India). The 300 ppm $HgCl_2$, $NaAsO_2$, and $Pb(NO_3)_2$ solution was made by mixing the accurate amount of $Pb(NO_3)_2$, $HgCl_2$, and $NaAsO_2$ in double-distilled water. The stock solution was filtered through a 0.2-µm filter (Sigma Aldrich) and stored at 4°C in the dark.

Exposure of M. luteus to various concentration of As(III), Hg(II) and Pb(II)

Twelve hour old 100 µL *M. luteus* culture was inoculated into 10 ml Luria bertani broth with various concentrations of $Pb(NO_3)_2$, $HgCl_2$ and $NaAsO_2$, pH 7, and maintained and incubated in a shaking incubator (120 rpm) at 37°C for 24 hours.

Relative effects of microbial growth and heavy metal consumption

The optimum growth situation of *M. luteus* against different concentrations of As(III), Pb(II), and Hg(II) was determined. The bacterial suspension was inoculated in LB broth media supplemented with As(III), Pb(II), and Hg(II) (100–1000 µg/mL) and incubated in a rotary shaker at 120 rpm at 37°C for 24 hours while pH 7.0 was adjusted separately. The optical density was measured (600 nm) using a UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). After 12 hours of incubation, the residual effects of heavy metals on their growth were observed. (Marzan *et al.,* 2017)

Visualization of M. luteus cell structure by SEM

M. luteus cultures were fixed using 2.5% glutaraldehyde for 2 hours, washed several times in the phosphate buffer, dehydrated via acetone in increasing concentrations (30% to 100%), and dried. Dried cells were stained with metal and covered with gold. Using a scanning electron microscope (JSM-IT200, Joel), images was generated.

Bioremediation of Pb(II), As(III), and Hg(II)

A hundred microliters of *M. luteus* overnight culture were inoculated into 20 mL of LB medium containing 300 ppm of $Pb(NO_3)_2$, $HgCl_2$, and $NaAsO_2$ in a 100-ml conical flask. The pH was maintained at 7.0 (neutral) with one N hydrochloric acid or one molar sodium hydroxide and incubated in a shaking incubator (120 rpm) at 37°C for 24 hours. Control sets were also incubated with tests. After 24 hours of incubation, cultures were centrifuged at 5000g for 10 min. Concentrations of $Pb(NO_3)_2$, $HgCl_2$, and $NaAsO_2$ were calculated by an inductively coupled plasma mass spectrophotometer (Agilent, 7800 ICP-MS), and the percentage of remediation was calculated by diversity among initial as well as final concentrations of $Pb(NO_3)_2$, $HgCl_2$ and $NaAsO_2$ in the centrifuged supernatant (Puyen *et al.,* 2012).

$$\% \text{ of HMs utilized} = \frac{\text{Heavy metal used by microbes (ppm)}}{\text{Initial Level of Heavy metal (ppm)}} \text{ X } 100$$

Heavy metal used by heavy metal (ppm) = Initial level of heavy metal (ppm) – Final level of heavy metal in culture (ppm)

Optimization of pH, Temperature and Metal Concentration for heavy metal removal

M. luteus luteus was inoculated into 250 mL conical flasks at different pH, temperatures, and various concentrations of As(III), Pb(II), and Hg(II), and separately incubated at 37°C. After 96 hours of incubation, heavy metal removal and biomass were measured, according to Gupta *et al. (2012*).

Result and Discussion

Screening and Multiple Metal Resistant Capacity

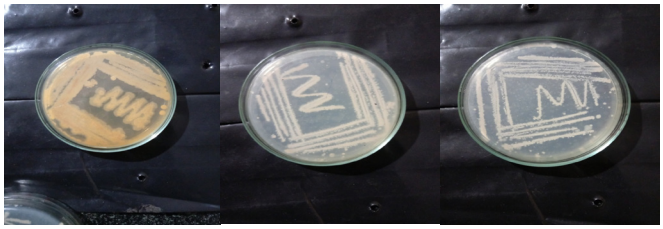
M. luteus obtainable visual growth after 24 hours of incubation in LB Agar media containing heavy metal supplemented (300 ppm) indicated that *M. luteus* has metal remediation ability. To establish the potential multiple HMs degrading ability to bacteria, growth curve analysis was conducted in Luria bertani broth medium, including Pb(II), As(III), and Hg(II), separately. In this experiment, *M. luteus* indicated a high resistance capacity against arsenic and also showed resistance against Hg(II), and Pb(II) (Fig. 3)(Table 1).

Assessment of Exposure against various heavy metals

The *M. luteus* was examined for Pb(II) ranging from 50 to 1800 µg/mL. The growth of *M. luteus* was high in the control experiment without any heavy metal supplementation, but in the test experiments containing heavy metals, growth decreased simultaneously with increasing concentrations of heavy metals. In lead residual cultures, no significant changes in growth were observed until the concentration of Pb(II) was above 2500 µg/mL, while differences were observed in Hg(II), and As(III). $HgCl_2$ and $NaAsO_2$ were examined, ranging from 05 to 100 µg/mL. Similarly, the growth of bacteria decreased as the concentrations of Pb(II) and Hg(II) increased. In As(III), and Hg(II), the growth of bacteria was not observed after 95 µg/mL and 15 µg/mL, respectively. A decrease in biovolume and cell number was calculated as the $HgCl_2$, and $NaAsO_2$ residual increased, which is satisfactory with the result obtained from

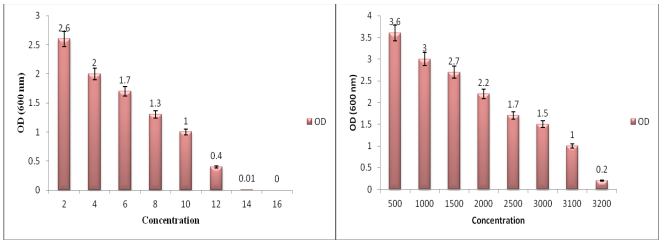
Table 1: Multiple Heavy Metal ability by *Micrococcus luteus*

M. luteus	*As*	*Hg*	*Pb*
Multiple heavy metal assessment	+++	++	++
Exposure of heavy metal (MIC)	93 ±1.2	12.5 ± 0.5	3150 ± 3.5

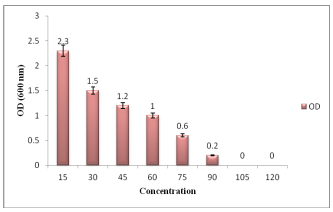


A B C

Fig. 3: Multiple heavy metal assessment of *M. luteus* (a) Arsenic (b) Mercury (c) Lead



(a) Pb (II) Concentration (b) Hg (II) Concentration



(c) As (III) Concentration

Fig. 4: Total Growth of *M. luteus* at various concentration of (a) Pb(II) (b) Hg(II), and (c) As(III)

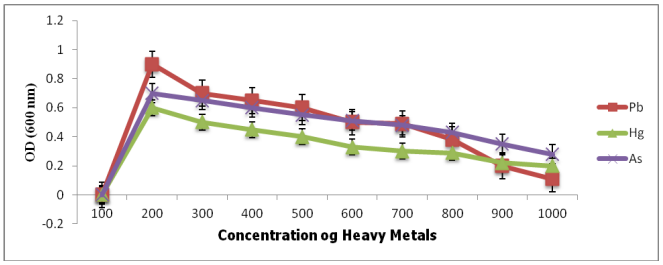


Fig. 5: Optical density (λ= 600 nm) was measured after 12 hours incubation in luria bertani broth containing heavy metals Pb(II), Hg(II), and As(III) to examine relative HMs utilization rate on the bacterial growth

the spectrophotometer (UV-1800, Shimadzu, Japan). Puyen *et al.* (2012) also reported the consequence of these metals on *M. luteus* that a conversion relation was there between the metal concentration and cell numbers (Fig. 4).

Analysis of Relative effects on bacterial growth against heavy metal

Relative effects in different concentrations (100–1000 µg/mL) of $Pb(NO_3)_2$, $HgCl_2$ and $NaAsO_2$ on bacterial growth were studied. In this experiment, we observed that bacterial growth concentrations were dependent on the concentration of heavy metal. It was indicated that decreasing absorbance (λ=600 nm) increased the concentration of HMs (Fig. 5).

Visualization of the cell structure

A scanning electron microscope was used to determine the morphological structure of *Micrococcus luteus*. The obtained images indicated that the bacterium structure is tetrads, without flagella, irregular clusters, and regular packets. *Micrococcus luteus* treated with 300 ppm Pb(II) was analyzed, and no significant differences were obtained in the structure. The ultra-slim sections of the cells show the differences after exposure to Pb (II). Similarly, testes were made in *M. luteus* treated with Hg(II) and As(III), and their structure was compared

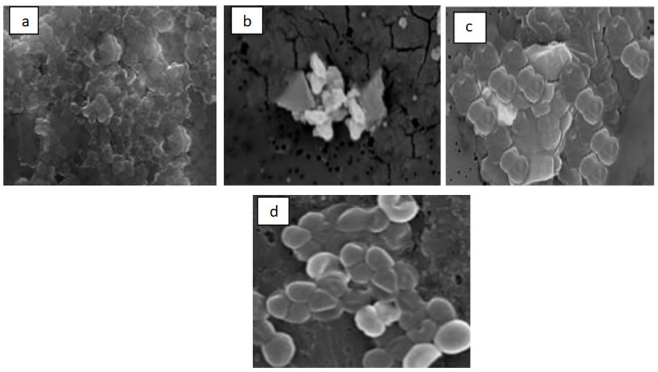


Fig. 6: Scanning Electron microscope image of *M. luteus* culture (a) treated with (b) Pb $(NO_3)_2$ (c) $NaAsO_2$ (d) $HgCl_2$

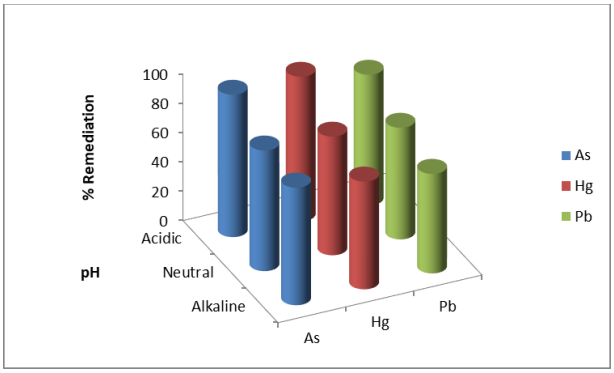


Fig. 8: Optimization of As(III), Hg(II), and Pb(II) metal ions remediation at pH (4.30, 4.80 & 5.0) at 37°C after 96 h through *M. luteus*

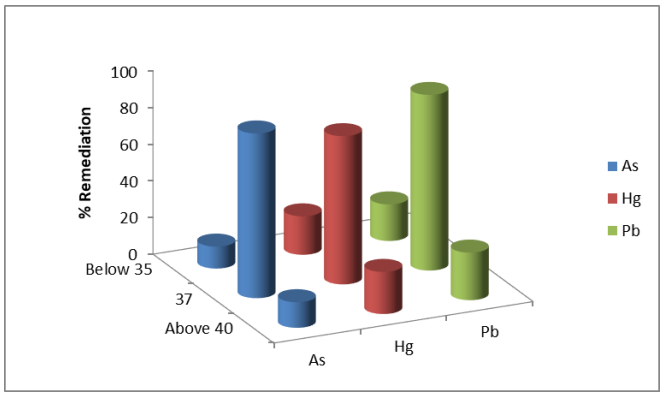


Fig. 7: Optimization of As(III), Hg(II), and Pb(II) metal ions remediation at 37°C through *M. luteus* after 96 hours of incubation

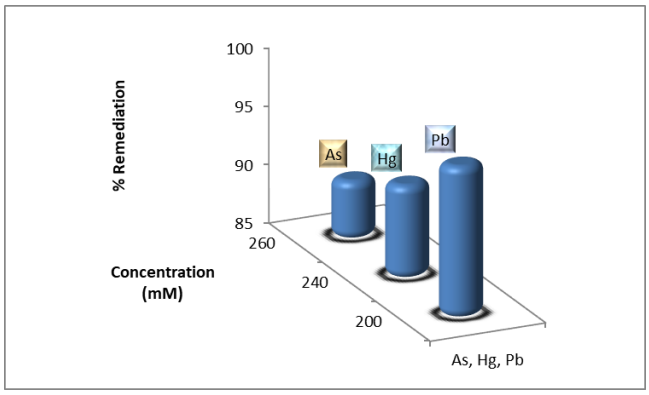


Fig. 9: Optimization of Concentration As(III), Hg(II), and Pb(II) metal ions remediation at pH 7 at 37°C after 96 h through *M. luteus*

Table 2: Biosorption of lead, arsenic, and mercury by *Micrococcus luteus*

Heavy Metal	*Initial concentration (µg/L)*	*Final Metal Concentration (µg/L)*	*Metal Absorbed (µg/L)*	*% Biosorption*
$NaAsO_2$	300	29.31±1.32	270.69±0.35	90.23
$Pb(NO_3)_2$	300	14.37±2.53	285.63±3.13	95.21
$HgCl_2$	300	57.99±1.12	242.01±2.14	80.67

with that of Pb(II)-treated cells no effects were observed on cell structure (Fig. 6). Energy scattering spectra analysis, coupled with the SE microscope, stated that *M. luteus* was proficient at gathering heavy metals in the membrane but not in the interior of the cells.

Bioremediation Assay

The quantitative analysis of Pb (II), As (III), and Hg (II) bioremediation ability was measured by the inductively coupled plasma mass spectrometer compared with the control result. The higher the initial metal concentration in media, the higher the percentage of biosorption by microbes. In the current experiment, *M. luteus* was incubated in media containing arsenic, lead, or mercury and showed great affinity for removing As (III), Pb (II), and Hg (II) percentage biosorption. *Pseudomonas* sp. (Bojórquez C and Voltolina D, 2016), *Bacillus* sp. (Wierzba S, 2015), and *Streptomyces* sp.(Anderl *et al.,* 2020) showed similar results against heavy metals, indicating that the *M. luteus* can absorb Pb(II), As(III), and Hg(II) binding through the membrane (Table 2).

Effect of Factors *on Heavy Metal Bioremediation*

The *M. luteus* was studied for lead, arsenic, and mercury bioremediation ability at various pH, temperatures, pH, and Metal concentrations for 48 and 96 hours of incubation.

Effect of Temperature

M. luteus was inoculated into heavy metal-containing media at various temperatures. It showed optimum remediation ability at 37°C. Arsenic, mercury, and lead remediated 90%, 81%, and 96% after 96 hours of incubation, respectively. At below 35, the remediation ability of *M. luteus* was 12%, 21.36%, and 20.23% after 96 hours, respectively. However, at temperatures above 40°C remediation was obtained at 14.5%, 23%, and 26.3% after 96 hours of incubation, respectively (Fig. 7).

Effect of pH

The heavy metals (Pb(II), As(III), and Hg(II)) were inoculated with *Micrococcus luteus* with various pH values ranging from 2 to 10. The effect of pH on yield (%) biosorption of arsenic, lead, and mercury by *M. luteus* was 97.21%, 89.23%, and 98.65% at pH 4.30, 4.80, and 5.0, respectively (Fig. 8). The surface of the cell

increased negative charge as a result of low pH, which supports the adsorption of metals. (Abioye et al., 2015)

Effect of heavy metal concentration

It was observed that maximum arsenic, lead, and mercury biosorption ability was determined at 260 mM/L (90%), 200 mM/L (98%) and 240 mM/L (93%) under optimum conditions of pH 7 at 37°C for 96 h of incubation, while the minimum biosorption was studied at 1300 mM/L (39%), 2100 mM/L (42%) or 900 mM/L (46%) after 96 h, respectively. (Fig. 9)

Conclusion

The present study was investigated to determine the heavy metal biosorption ability of *M. luteus* isolated from a mand coalfield in Raigarh. The isolate was screened for heavy metal resistance and multiple heavy metal tolerance abilities against Pb(II), As(III), and Hg(II). *M. luteus* recorded good remediation ability, which was measured by ICP-MS. The biosorption ability was optimized based on pH, temperature, and metal concentration. The study indicated that *M. luteus* at pH 4 to 5 and temperature 37°C showed great biosorption ability. As this microorganism has great metal absorption capacity, it may be used in the future for metal bioremediation from contaminated soil.

Acknowledgment

Mr. Virendra Vaishnav did all the experiments ,and the data collection included in this work along with paper drafting. Dr. Tanushree Chatterjee being a PhD guide of Mr. Virendra Vaishnav helped him in conducting experiments and drafting papers and Dr. Manisha Agrawal also helped in drafting the paper and helped in designing the experiment.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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