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IMMOBILIZATION OF LEAD-CONTAMINATED MINE WASTE BY *PARARHODOBACTER* SP

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Presentation outline

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- Objectives
- Methodology
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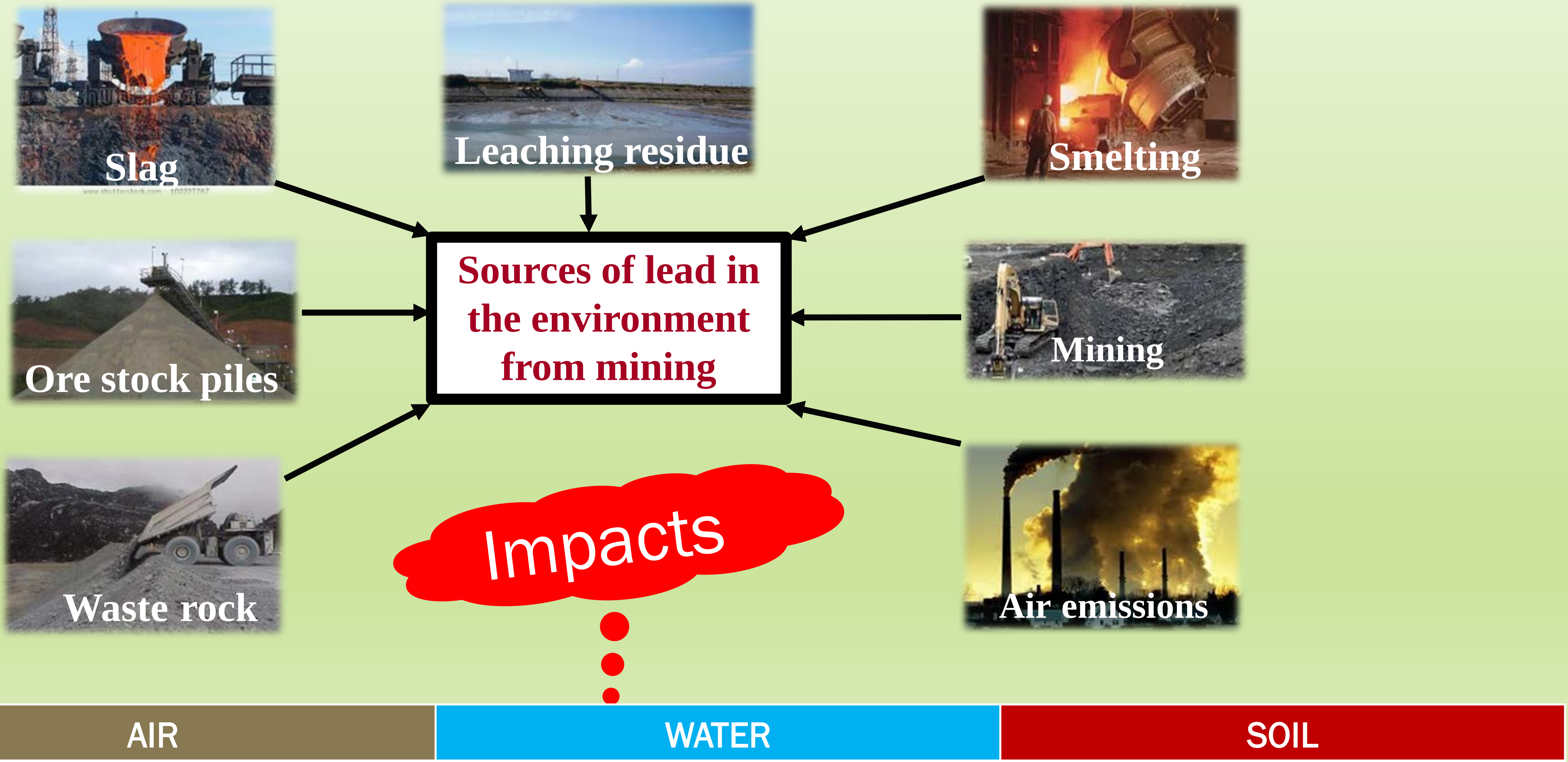
Introduction

Rapid urbanization and industrialization has resulted in extensive exploitation of the mineral resource has resulted in environmental pollution by toxic metals (Cd, Cu, Pb, Hg, Cr, and Fe) which pose a threat to the environment and to human health.



Techniques for removing lead from soil

REMEDIATION TECHNIQUE	UNIT	COST ($)	ADDITIONAL FACTORS/EXPENSES
Vitrification	Tonne	75 - 425	Long term monitoring
Capping	Tonne	100 - 500	Transport/excavation/Monitoring
Chemical treatment	Tonne	100 - 500	Recycling of contaminants
Electrokinetic	Tonne	20 - 200	Long term monitoring
Thermal treatment	Tonne	750 - 1200	Long term monitoring
Phytoextraction	Tonne	5 - 40	Long term monitoring

Sources: http://www.building.co.uk

Physico-chemical methods take longer and have high costs associated with energy and chemical consumption in addition to possible emission of secondary pollutants. Therefore there is a the need to develop bioremediation methods. One method is to immobilize heavy metals based on microbially induced calcium carbonate precipitation (MICP):

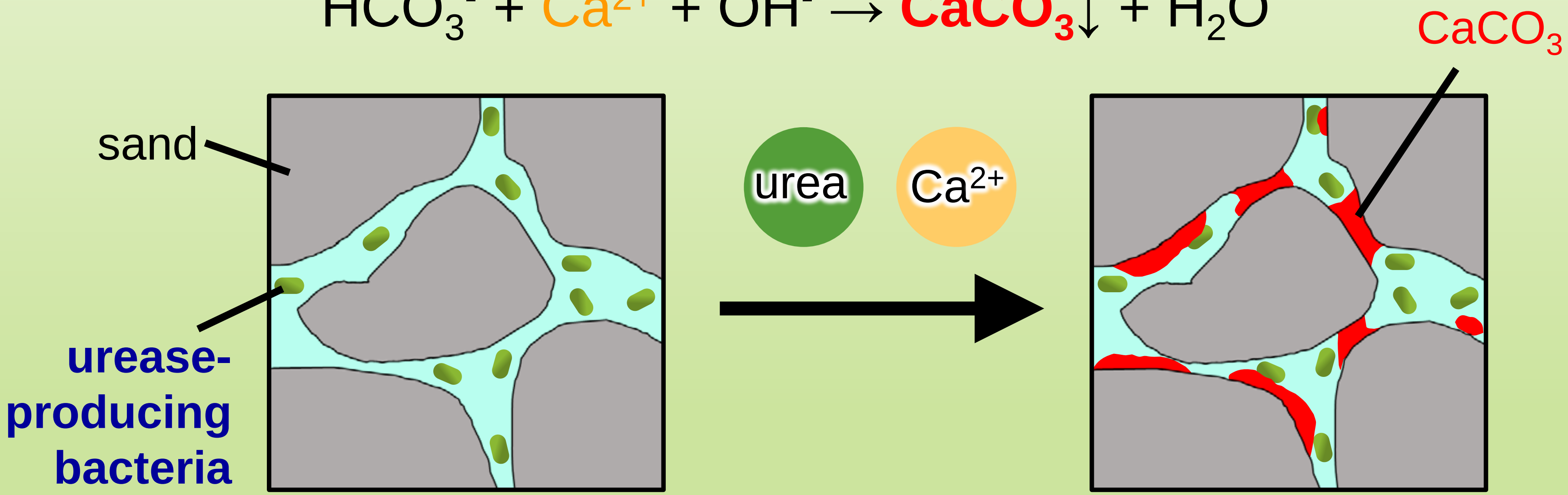
- ✓ Eco-friendly
- ✓ Easily controlled
- ✓ Low cost

MICP mechanism

MICP involves the hydrolysis of urea into ammonium and carbarmate by urease catalysis which results in CaCO3 formation in the presence of Ca ions.

$$\overset{\text{urea}}{CO(NH_2)_2} + 2H_2O \xrightarrow{\textit{urease}} 2NH_3 + HCO_3^- + H^+$$

$$HCO_3^- + Ca^{2+} + OH^- \rightarrow \mathbf{CaCO_3}\downarrow + H_2O$$



Pararhodobacter sp. was selected for investigation because it has shown high urease activity and can maintain the enzyme activity for a long time.

Objectives

1. Investigate the effects of lead on microbial growth;

2. Determine the effectiveness of lead removal by *Pararhodobacter* sp. in bioprecipitation experiment;

3. Determine the effects of varying the injection interval of the bacteria on unconfined compressive strength (UCS) for fine and coarse-grained sand;

METHODOLOGY

Materials

Sand and mine waste

Sand type	Mean diameter (D_{50}) (µm)
Misunami sand	1200
Toyora sand	200
Leach plant residue	75
Kiln Slag	>2500

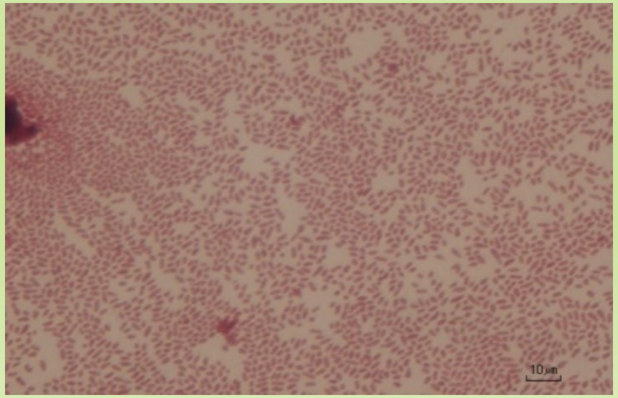
Nutrient and cementation solution

Chemical	Chemical concentration (g/L)
Nutrient Broth (g)	3
NH_4Cl (g)	10
$NaHCO_3$ (g)	2.12
$(NH_2)_2CO$ (g)	30.03
$CaCl_2$ (g)	55.49

Other conditions

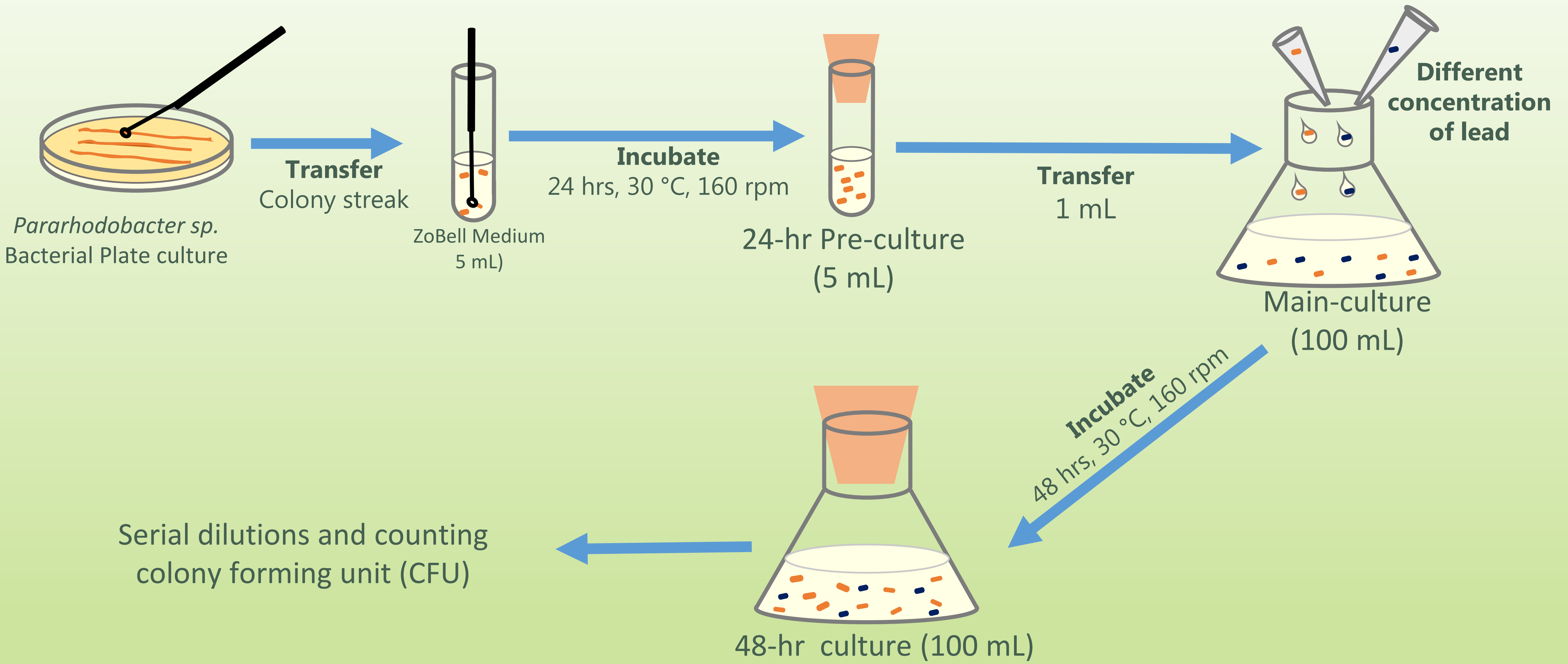
Parameters	Conditions
Temperature	30°C
Bacteria OD_{600}	1.0
Injection intervals	1,2,4,7
Medium	ZoBell2216 media

Bacteria



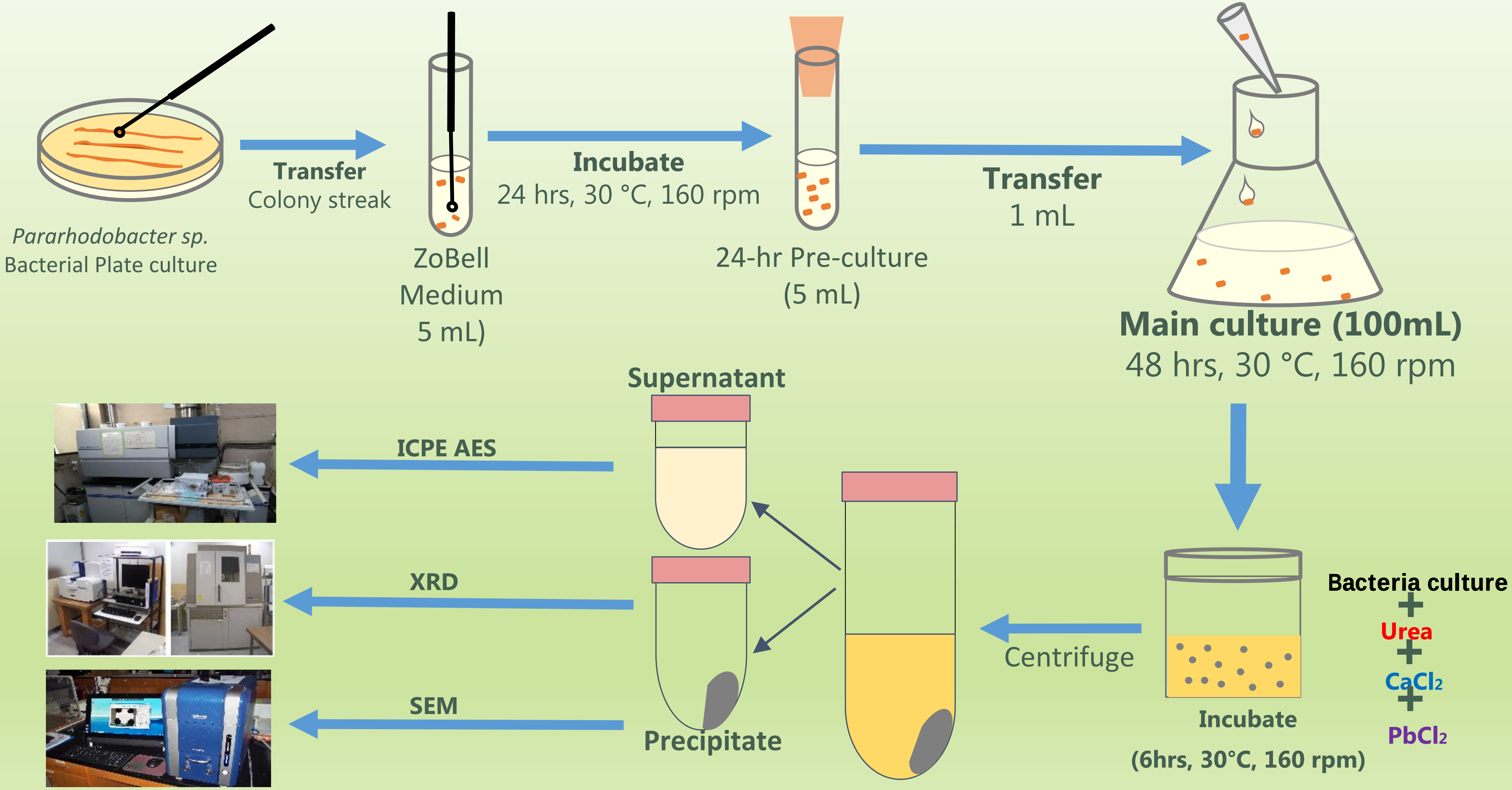
Pararhodobacter sp. isolated from Okinawa, Japan.

Effect of lead on microbial growth-viable plate count

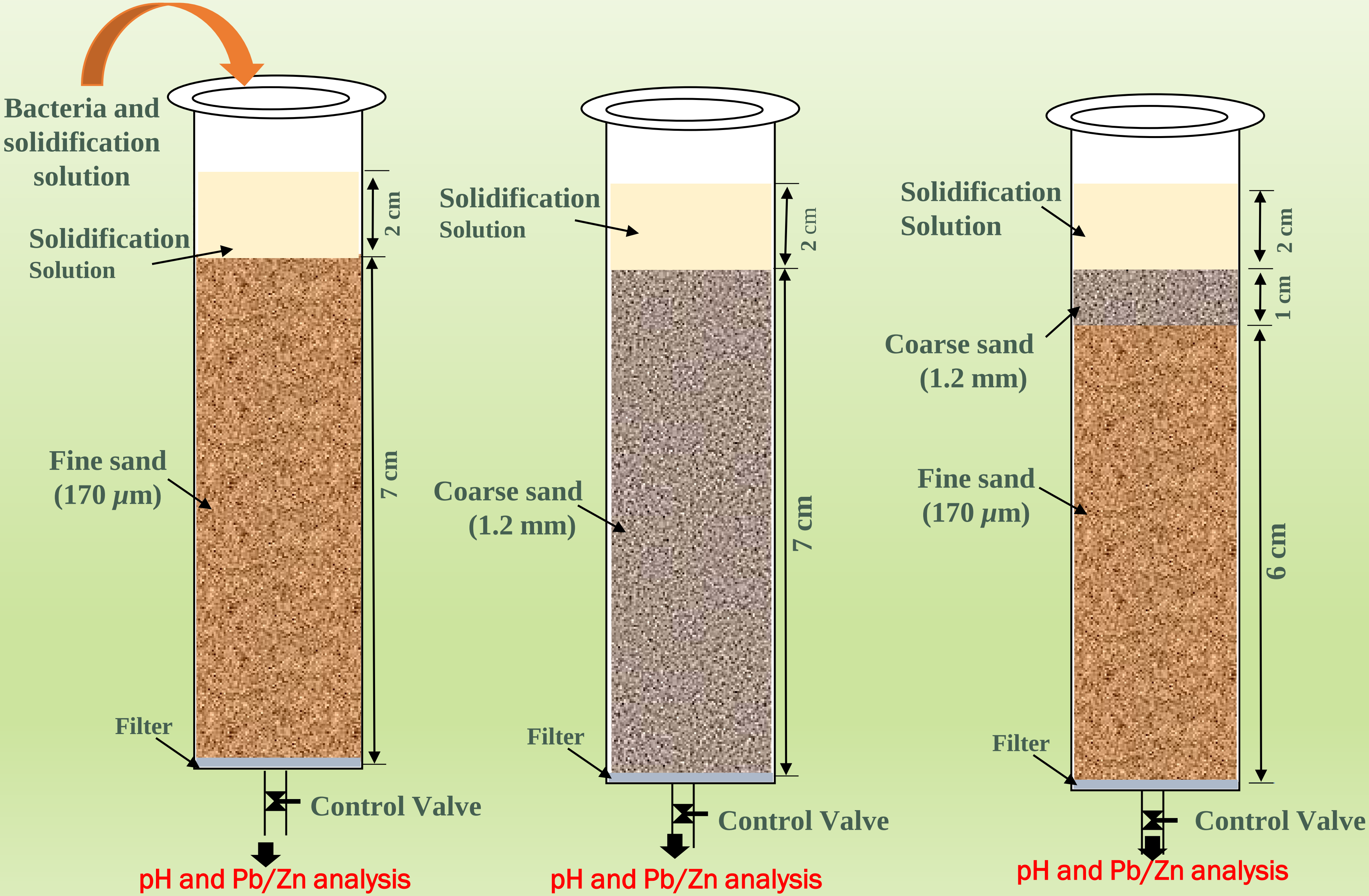


$$\frac{CFU}{mL} = \frac{(\text{Number of colonies } \times \text{ Dilution factor})}{\text{Volume of liquid culture sample plated}}$$ (Miles And Misra, 1938)

Bioprecipitation experiment

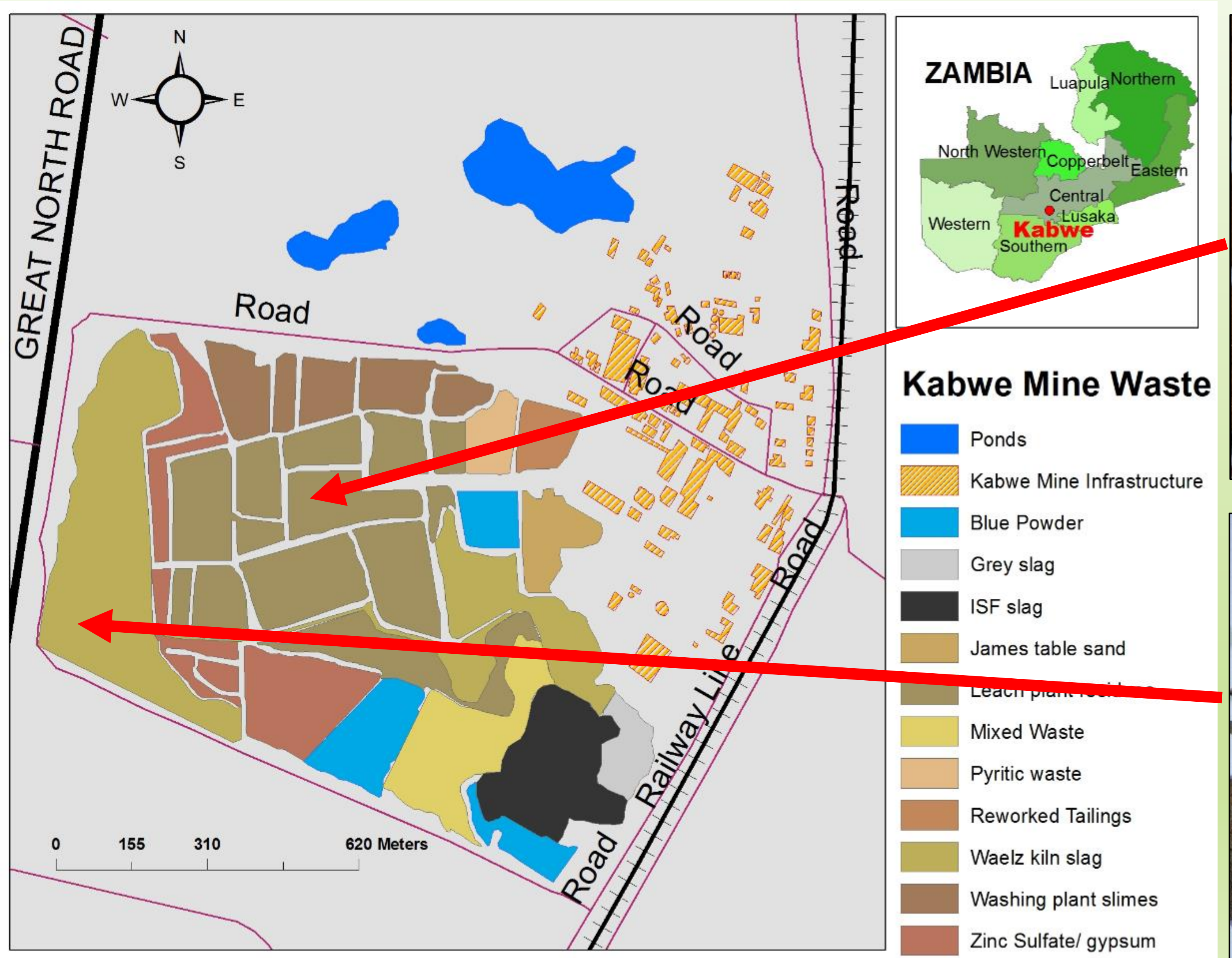


Conceptual model of experimental setup



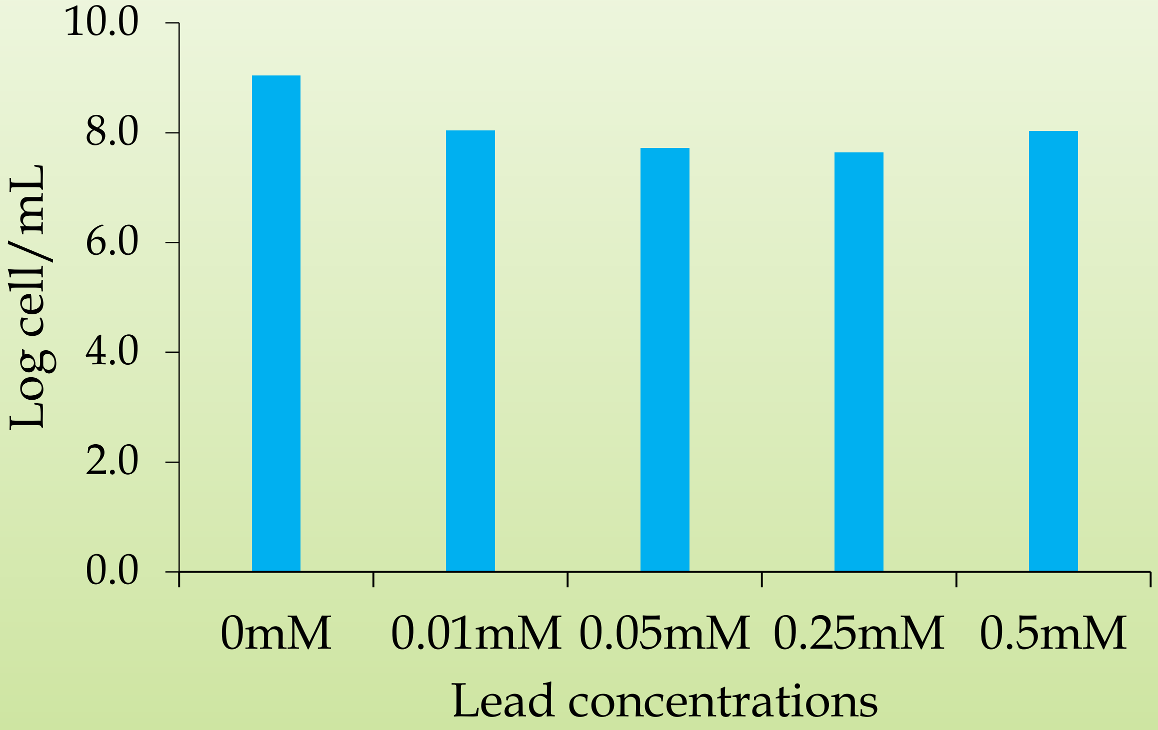
EXPERIMENTAL CONDITIONS	
Urea	0.5M
Calcium Chloride	0.5M
Nutrient Broth	3 g/L
Sodium Hydrogen Carbonate	2 g/L
Ammonium Chloride	10 g/L
Temperature	30°C
Experimental Time	14 Days
Bacteria OD_{60}	1.0
Fine sand (Toyora sand)	170µm
Course sand (Misunami sand)	1.2mm
Injection intervals	1,2,4,7

Waste types at Kabwe Mine Site

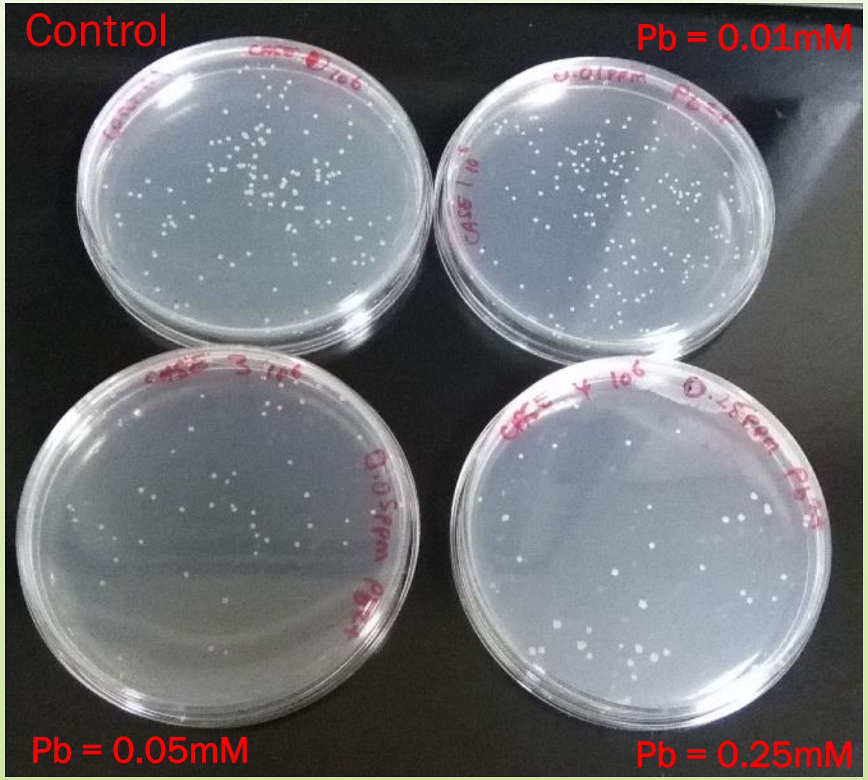


RESULTS AND DISCUSSION

Effect of lead on microbial growth and urease

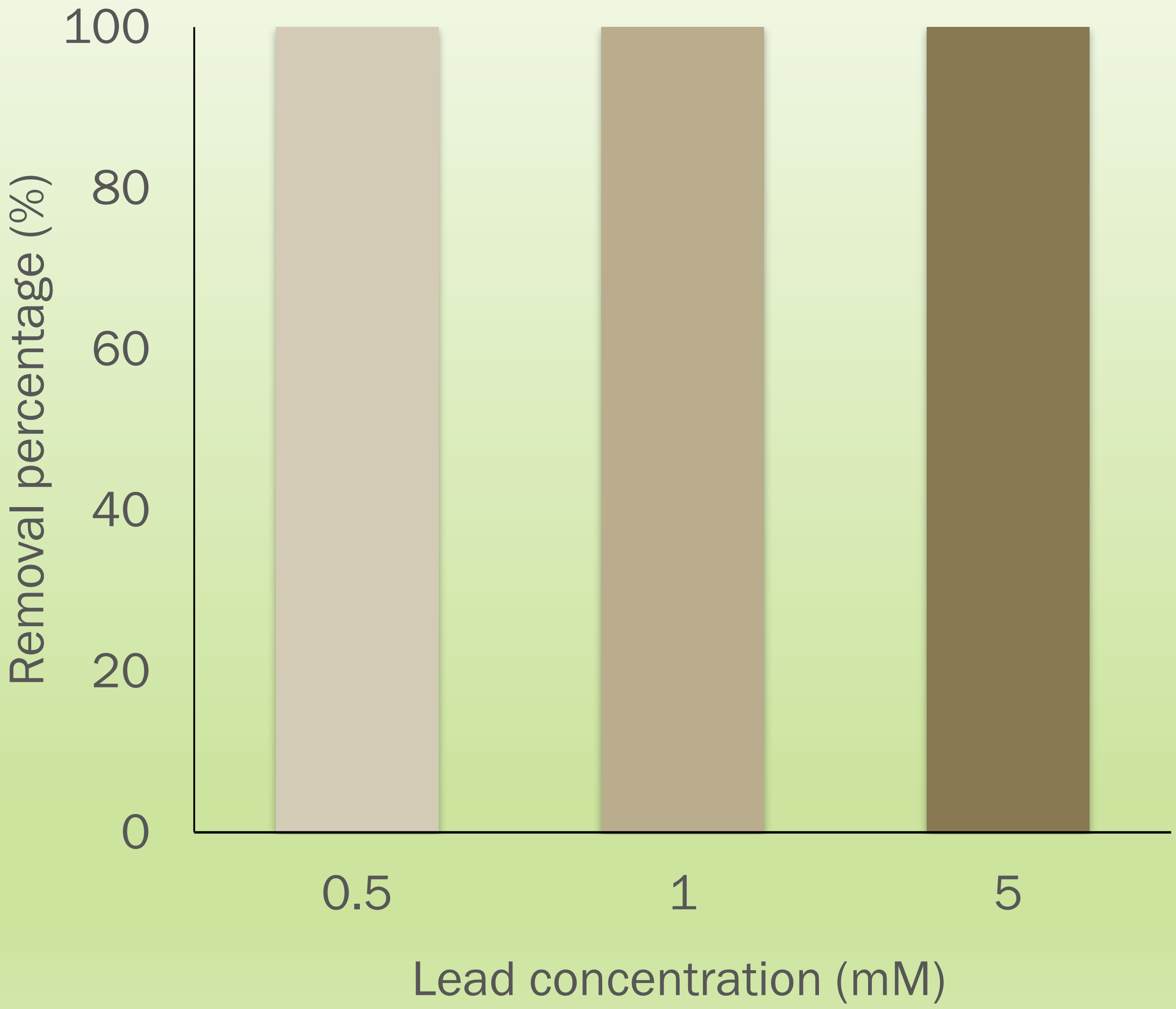


Colonies of *Pararhodobacter* in lead



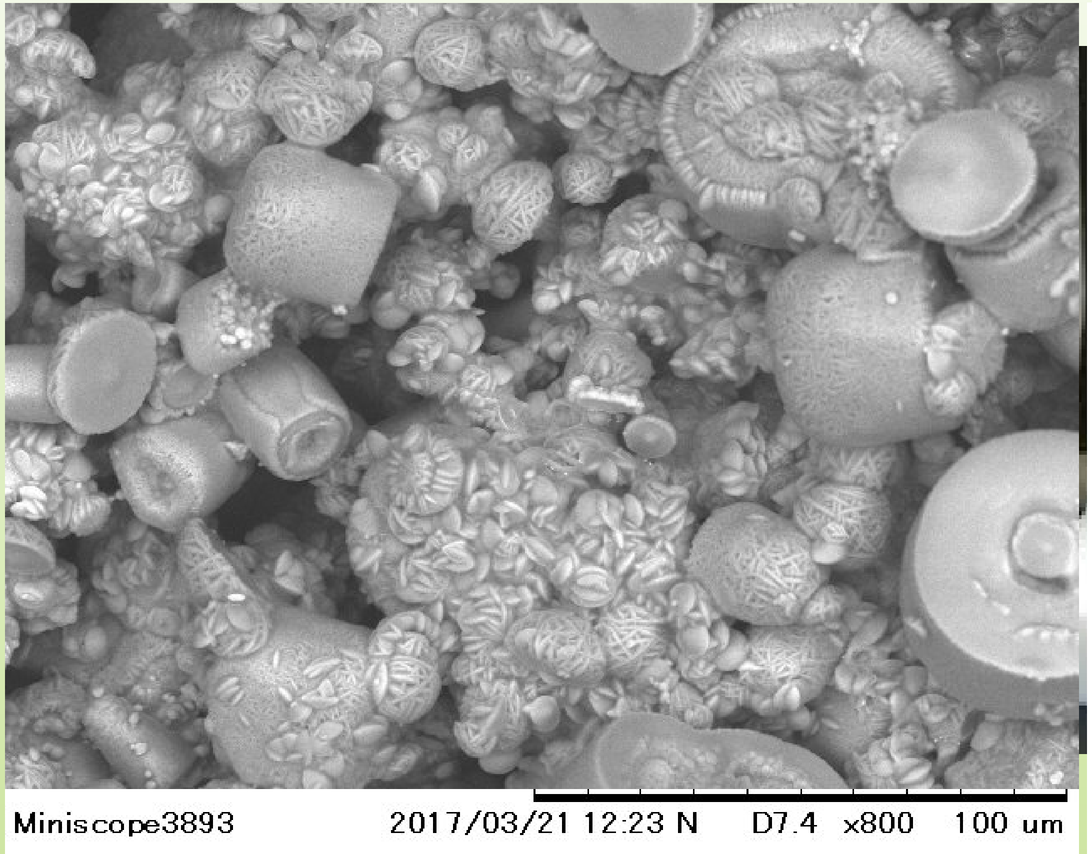
The bacterium can be used for bioremediation, as the effect of lead on the bacteria is negligible for the concentration evaluated.

Lead biopprecipitation - ICP-AES

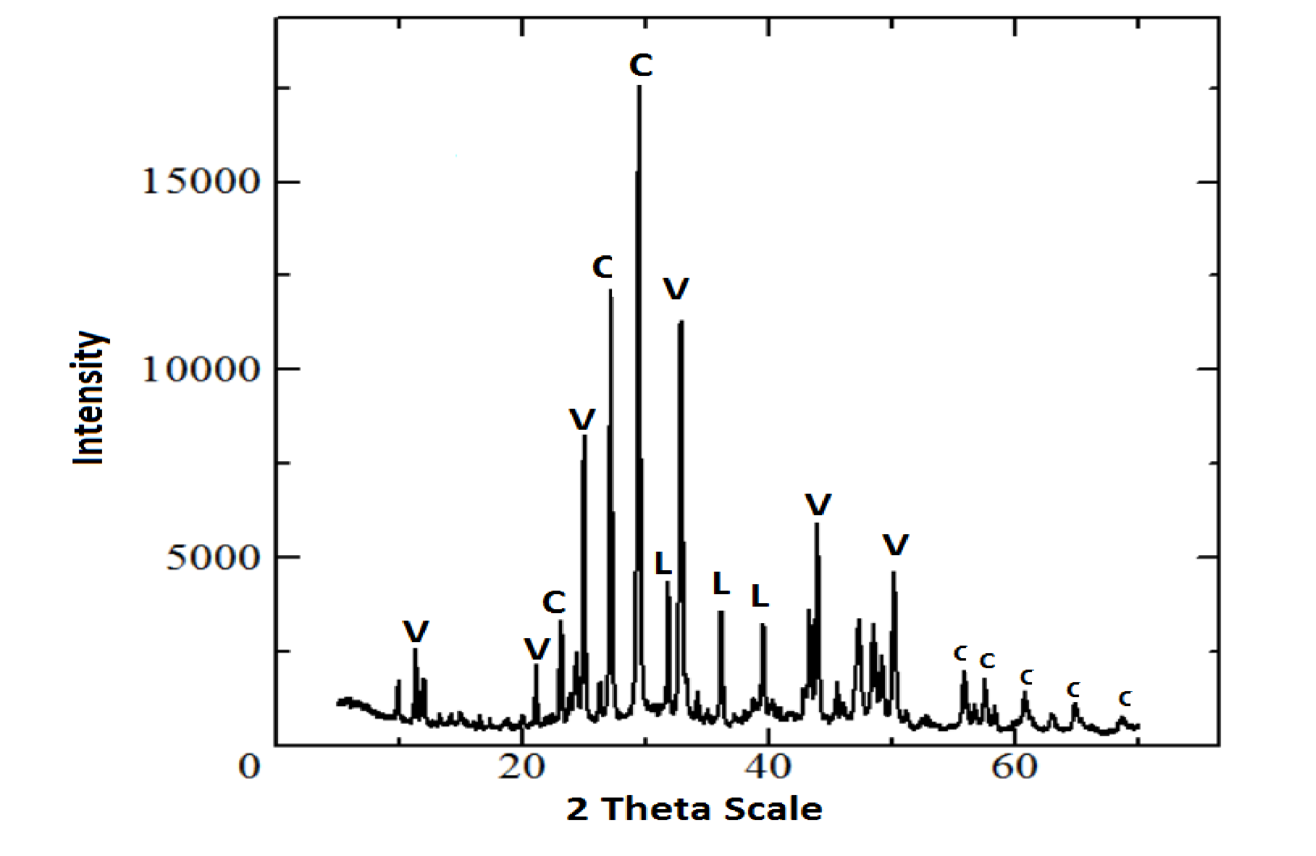


- Lead occurred with a removal percentage of 100%.
- toxic Pb^{2+} ions from soluble form to insoluble forms hence detoxifying the toxic lead ions
- Comparison between this study and previous studies involving ureolytic bacteria: *Rhodobacter spharoides* (90.31%)(Li et al., 2016); *Sporosarcina pasteurii* (100%) (Mugwar and Harbottle, 2016); *Enterobacter cloacae* (68.1%)(Kang et al., 2015); and *Terrabacter tumescens* (100%)(Li et al., 2015).

Lead bioprecipitation – SEM and XRD



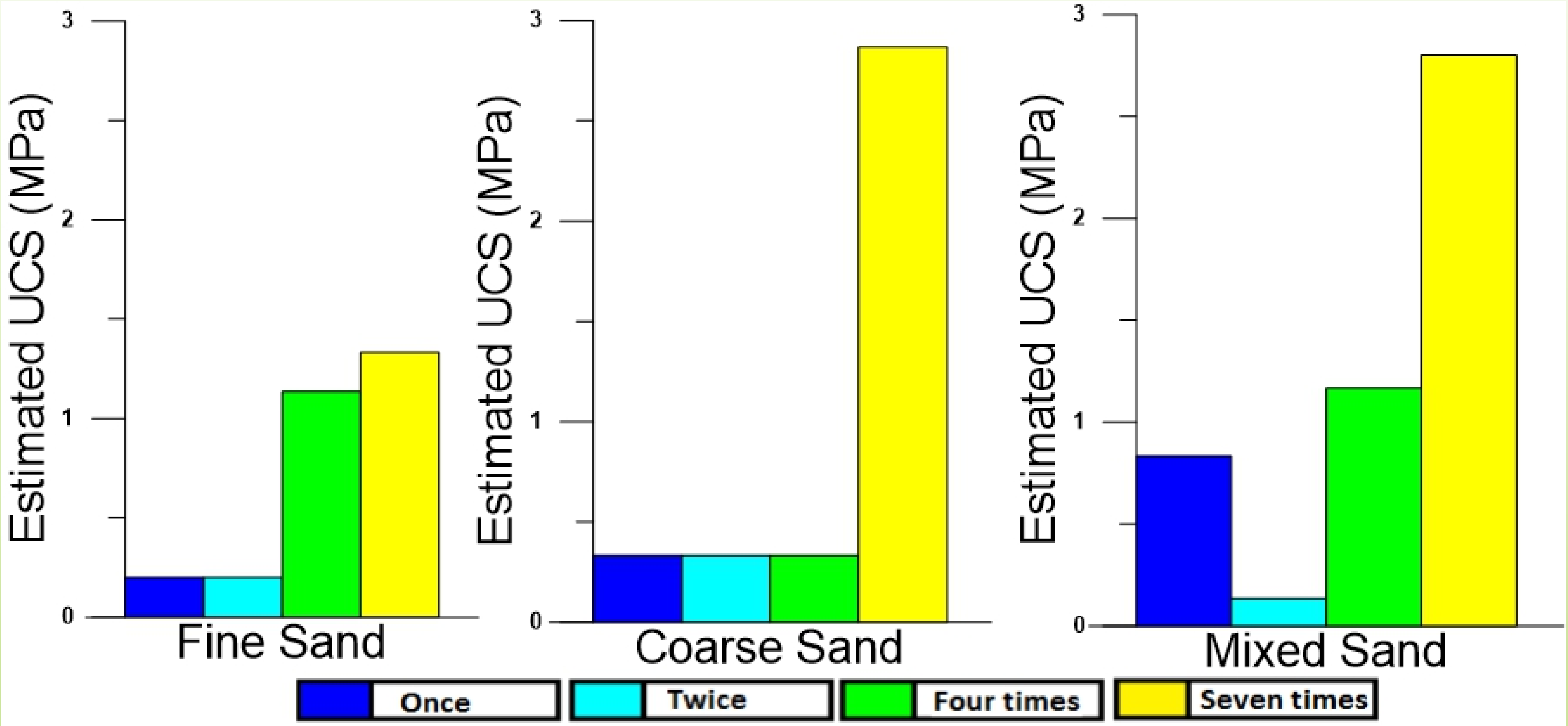
SEM image precipitate



XRD of precipitate

- SEM-framboidal aggregates were identified as vaterite, whereas spherical and rhombohedral shaped precipitates were identified as calcite.
- XRD - confirm calcium carbonate formation induced by Pararhodobacter. The free lead ions in solution are immobilized in the matrix of calcium carbonate or formation of $PbCO_3$. (C = Calcite V = Vaterite; L = Lead Oxide (PbO)).

Estimated UCS and optimal injection interval



The more the bacterial injection increased, the more the increase in UCS. This is due to the number of nucleation sites available for microbial precipitation.

Sand solidification samples

Pictorial images of the results of all syringe tests after 14 days while varying the bacterial injection interval to (a) one (b) two (c) four and (d) seven times. Left, fine sand; center, coarse sand; right, mixture of course and fine sand.

Conclusions

1. Lead has negligible effect on microbial growth and urease activity of *Pararhodobacter* and therefore, *Pararhodobacter sp.* can be used for remediation purposes.

2. *Pararhodobacter* was effective in complete removal of lead by changing toxic Pb^{2+} ions from soluble form to other forms hence detoxifying the toxic lead ions ;

3. These results will facilitate the possible bioremediation of lead in both fine and coarse materials as an eco-friendly and sustainable method of heavy metal remediation.

References

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Thank you for your attention