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

Mwandira W$^1$, Nakashima K$^2$, Kawasaki S$^2$, Chirwa M$^3$, Banda K.E.$^3$, and Nyambe I.A.$^4$

$^1$Graduate School of Engineering, Hokkaido University, Japan  
$^2$Faculty of Engineering, Hokkaido University, Japan  
$^3$Geology Department, University of Zambia, Zambia  
$^4$Integrated Water Resources Management Center, University of Zambia, Zambia
Presentation outline

■ Introduction
■ Objectives
■ Methodology
■ Results and discussion
■ Conclusions
■ References
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

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

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 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

Sources: [http://www.building.co.uk](http://www.building.co.uk)
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.

\[
\text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{HCO}_3^- + \text{H}^+
\]

\[
\text{HCO}_3^- + \text{Ca}^{2+} + \text{OH}^- \rightarrow \text{CaCO}_3\downarrow + \text{H}_2\text{O}
\]

*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

<table>
<thead>
<tr>
<th>Sand type</th>
<th>Mean diameter ($D_{50}$) (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misunami sand</td>
<td>1200</td>
</tr>
<tr>
<td>Toyora sand</td>
<td>200</td>
</tr>
<tr>
<td>Leach plant residue</td>
<td>75</td>
</tr>
<tr>
<td>Kiln Slag</td>
<td>&gt;2500</td>
</tr>
</tbody>
</table>

### Nutrient and cementation solution

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Chemical concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient Broth (g)</td>
<td>3</td>
</tr>
<tr>
<td>$\text{NH}_4\text{Cl}$ (g)</td>
<td>10</td>
</tr>
<tr>
<td>$\text{NaHCO}_3$ (g)</td>
<td>2.12</td>
</tr>
<tr>
<td>$(\text{NH}_2)_2\text{CO}$ (g)</td>
<td>30.03</td>
</tr>
<tr>
<td>$\text{CaCl}_2$ (g)</td>
<td>55.49</td>
</tr>
</tbody>
</table>

### Other conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Bacteria $\text{OD}_{600}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Injection intervals</td>
<td>1,2,4,7</td>
</tr>
<tr>
<td>Medium</td>
<td>ZoBell2216 media</td>
</tr>
</tbody>
</table>

### Bacteria

*Pararhodobacter* sp. isolated from Okinawa, Japan.
Effect of lead on microbial growth-viable plate count

**Pararhodobacter sp.**
Bacterial Plate culture

**Transfer**
Colony streak

**Incubate**
24 hrs, 30°C, 160 rpm

**Transfer**
1 mL

**ZoBell Medium**
5 mL

**24-hr Pre-culture**
(5 mL)

**Main-culture**
(100 mL)

Serial dilutions and counting colony forming unit (CFU)

\[
\frac{CFU}{mL} = \frac{\text{(Number of colonies } \times \text{ Dilution factor)}}{\text{Volume of liquid culture sample plated}}
\]

(Miles And Misra, 1938)
Bioprecipitation experiment

Pararhodobacter sp.
Bacterial Plate culture

Transfer
Colony streak

Transfer
1 mL

Incubate
ZoBell Medium
24 hrs, 30 °C, 160 rpm

Incubate
5 mL

Transfer
(6hrs, 30°C, 160 rpm)

ICPE AES

XRD

SEM

Supernatant

Precipitate

Bacteria culture
+ Urea
+ CaCl₂
+ PbCl₂

Main culture (100mL)
48 hrs, 30 °C, 160 rpm

24-hr Pre-culture
(5 mL)

Transfer
1 mL

Centrifuge

Precipitate

Urea

CaCl₂

PbCl₂
Conceptual model of experimental setup

EXPERIMENTAL CONDITIONS

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0.5M</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>0.5M</td>
</tr>
<tr>
<td>Nutrient Broth</td>
<td>3 g/L</td>
</tr>
<tr>
<td>Sodium Hydrogen Carbonate</td>
<td>2 g/L</td>
</tr>
<tr>
<td>Ammonium Chloride</td>
<td>10 g/L</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Experimental Time</td>
<td>14 Days</td>
</tr>
<tr>
<td>Bacteria OD₆₀</td>
<td>1.0</td>
</tr>
<tr>
<td>Fine sand (Toyora sand)</td>
<td>170µm</td>
</tr>
<tr>
<td>Course sand (Misunami sand)</td>
<td>1.2mm</td>
</tr>
<tr>
<td>Injection intervals</td>
<td>1, 2, 4, 7</td>
</tr>
</tbody>
</table>
Waste types at Kabwe Mine Site

- Kiln slag
- Leach plant residue (LPR)

Map showing Kabwe Mine Waste:
- Ponds
- Kabwe Mine Infrastructure
- Blue Powder
- Grey slag
- ISF slag
- James table sand
- Leach plant residue
- Mixed Waste
- Pyritic waste
- Reworked Tailings
- Waelz kiln slag
- Washing plant slimes
- Zinc Sulfate/ gypsum
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 bioprecipitation - 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 sphaeroides* (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).
 SEM image 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 PbCO₃. (C = Calcite V = Vaterite; L = Lead Oxide (PbO)).

XRD of precipitate
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


