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# Factors Affecting the Rate of $CaCO_3$ Precipitation in Biocementation of Heavy Metal Contaminated Soil

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**Abstract**

Ground improvement methods using physical and chemical treatments are considered adequate but costly, involving extensive engineering work and may pose serious environmental problems. Therefore, biocementation is introduced using the enzyme-induced calcite precipitation (EICP) technique. The efficiency of EICP is influenced by the production of calcite carbonate, $CaCO_3$ and is governed by multiple factors. While some preliminary studies have been done on various soil types, none have been performed on heavy-metal-contaminated soil. This paper presents the research conducted on factors affecting the $CaCO_3$ precipitation in biocementation of mining waste collected from a copper mine in Sabah, Malaysia, treated using EICP solution, cured in a leaching cell and tested using inductively coupled plasma optical emission spectroscopy and acid washing test. Results concluded that factors affecting the production of calcite carbonate content are the cementation concentration (1.0M > 0.5M), degree of compaction (70% MDD> 80% MDD) and curing temperature (25 $^0$C > 15 $^0$C > 5 $^0$C). Meanwhile, immediate production (1-day curing) indicates that curing time is not a significant factor. Hence, the results proposed that the optimum production of $CaCO_3$ for treatment of heavy metals in contaminated soils is at a cementation solution of 1.0M, compacted at 70% MDD and cured at 25 °C temperature.

## 1. Introduction

Extensive research has been carried out on methods and techniques for improving soil, particularly to improve the engineering properties of soils such as hydraulic conductivity, compressibility, shear strength and compaction. Physical, chemical and biological techniques are the major methods of improvement.

Physical remediation methods include soil replacement, which replaces soil contaminated with heavy metals on the surface and deep layers with clean soil; thermal analysis, which evaporates volatile heavy metals in contaminated soil; and electrical remediation, which removes heavy metals in contaminated soil. Although physical remediation is effective in some cases, it is considered expensive for small projects and can lead to cross-

contamination. Other methods require significant technical effort, and heavy metals may still be present in the treated soil.

Chemically, the treatment is mainly aimed at remediating contaminated soil by chemical passivation, which seeks to reduce the migration capacity of heavy metals by adding hardening agents or by reactions between heavy metals. However, the long-term effects of chemical treatment of these wastes remain controversial, as industrial byproducts can alter the pH of groundwater, thereby causing serious environmental problems and destroying ecosystems [1]. Another issue associated with the targeted control of specific heavy metal elements is that their different physicochemical properties can react depending on the elution reagent. Therefore, the selected eluents used for chemical processing are often only effective in remediating a single heavy metal element, failing to achieve the objective of remediating various heavy metal contaminants simultaneously [2].

Biological remediation methods include phytoremediation, in which plants absorb and fix heavy metals and purify the soil, and animal remediation, in which insects and animals such as earthworms and cockroaches eat and disperse heavy metal contamination. Both phytoremediation and animal-based methods have advantages in terms of cost, sustainability, and increased soil organic content, but also disadvantages such as limited heavy metal accumulation and slow and long treatment cycles. Furthermore, different heavy metals can have different effects on the biochemical functions of animals [2].

The limitations of these current practices necessitate exploring environmentally friendly and sustainable technologies methods, hence the introduction of controlled or induced calcium carbonate (calcite or $CaCO_3$) precipitation technique such as EICP. In the EICP technique, a solution composed of urease enzyme and reagents which produce calcite is utilised as a cementation material. Research has demonstrated that precipitated calcite forms the bonds between soil particles, restricts the mobility of heavy metals, enhances the strength, stiffness, and reduces the hydraulic conductivity of treated soils.

Previous studies indicate that multiple factors govern the efficiency of calcite precipitation in EICP, including concentration of the cementation solution, concentration of the dissolved inorganic carbon (soil type and chemical composition), pH and availability of nucleation sites (pore space condition) [3]. However, none of these studies were conducted on heavy-metal-contaminated soils. Most of the EICP research has focused on improving the strength of organic, clay, or sandy soils. There is limited data on how this method can be applied to waste management in mines. In Malaysia, there is no data on the effects of temperature, degree of compaction, cement solution concentration and curing time on the EICP treatment of soils contaminated with heavy metals collected at low temperatures, thus necessitating this study.

## 2. Literature Review

Unlike the microbially-induced calcite precipitation (MICP), which uses bacteria, in the EICP technique, a urease enzyme solution and reagents that produce calcite are utilised as cementation material. Studies have indicated that precipitated calcite is attributed to forming bonds between soil particles, impeding the movement of heavy metals, and improving the strength, stiffness, and reducing the hydraulic conductivity of treated soils. The mechanisms of both techniques are depicted in Fig. 1.

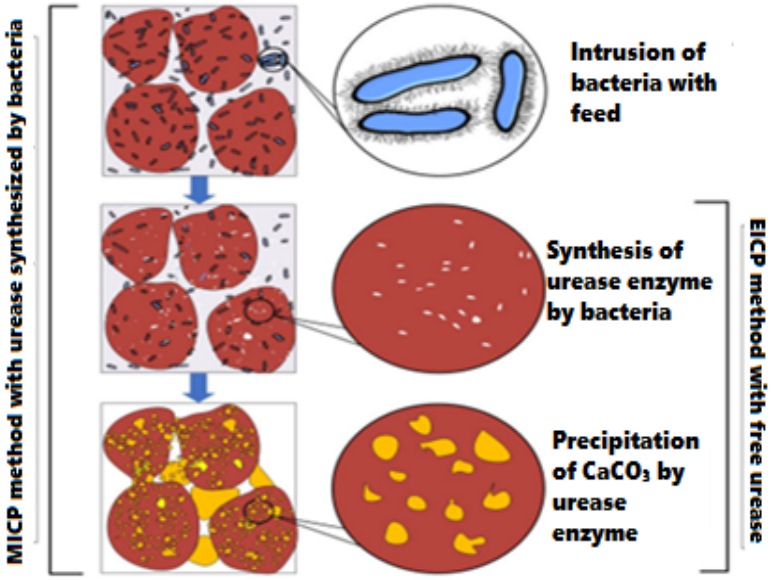


**Fig. 1** *Precipitation of $CaCO_3$ by urease enzyme in MICP and EICP [4]*

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In terms of cementation solution, high concentrations 0.5–1.0 Molar (M) of urea and calcium chloride treatment solution can generate a substantial amount of calcite [5]. However, the calcite production at lower concentrations (0.05- 0.25M) is more efficient [6]. This implies that the lower concentration is more economically beneficial and acceptable for performance. Studies using sandy and silty clay comparing the formation of calcite in the different concentrations of cementation reagent (0.15M to 0.45M) and curing conditions show that they have a significant impact with the optimal treatment is a combination of 0.25 M cementing reagent and the presence of bacteria and nutrients, which increased the formation of calcite by 61.3% [7].

Furthermore, it was shown that the presence of organic substances affected the pH, the development and efficacy of calcite, especially in soil with heavy metal compositions [8]. Regarding pore space conditions, it is noted that bigger and more available pore spaces can accommodate more calcite precipitation. Research found that calcite precipitated in residual specimens is 1.080-1.889%, while in sand 2.661-6.102% [9]. Comparing the calcium carbonate content between soils, it was concluded that soil particle size plays a vital role in calcite formation. Their study concluded that the limited pore space affects calcite formation because some soil particles are smaller than 2 μm.

While previous research concluded that urease concentration increases the precipitation of $CaCO_3$ proportionally, the studies were conducted at a temperature range of 20-50°C [10]. Similarly, the effect of external environmental parameters such as soil compositions, relative densities and EICP geometric compatibility on the amount of calcite precipitation in mine waste soils should be investigated. By observing the increase in the MICP-treated residual soil compacted to 85, 90, and 95% of MDD, the carbonate content was reported to be 1.750%, 2.559%, and 2.381%, respectively. The increase in calcite content after treatment was 1.080%, 1.889%, and 1.711%, respectively. Meanwhile, the carbonate content of sand compacted to 85, 90, and 95% of MDD was reported to be 6.376%, 5.943%, and 2.945%, respectively, and the calcite content after treatment was 6.102%, 5.669%, and 2.671% [9].

Therefore, Soon et al. [9] concluded that soil particle size influences the amount of calcite precipitated. The amount of calcite in sand (2.661% to 6.102%) was generally higher than in residual soil (1.750% to 2.559%). This is because the larger pore spaces in the sand can accommodate more treatment solutions and, therefore, more calcite precipitation. It was also found that although a higher precipitated calcite content is observed in the sand, the improvement is still much smaller than that of the residual soil. Given the large pore size of the sand, this may be due to the lack of calcite formation at the contact points between particles. The researchers found that finer-grained soils may be effectively amended even with lower calcite content, as contact points per unit volume play a dominant role. Nevertheless, the study by Soon et al. [11] found a good agreement between the amount of calcite and the reduced shear strength and hydraulic conductivity of treated sand samples, as precipitated calcite leads to pore space blockage and shrinkage of soil particles. However, the observed lines do not represent a linear relationship on a semi-logarithmic scale as reported by Yasuhara [12].

It is, however, essential to study whether the EICP efficiency factors affecting non-mine waste media could be observed in heavy-metal contaminated soil or to what extent they are effective. Most EICP studies have focused on uncontaminated organic, clay, or sandy soils. This study is necessary because there is limited data on how this method can be applied to mine waste treatment.

## 3. Research Methodology

### 3.1 Materials

The sampling point was located at 6° 0' 46'' N and 116° 44' 20'' E. This is near the abandoned Mamut copper mine in Sabah, Malaysia, as shown in Fig. 2. The wastes from this mine were dumped at Lohan Dam and nearby locations. A 0.5 m to 3.0 m trial pit was dug to collect the soil samples. The EICP solution used in this study consists of urea, anhydrous calcium chloride, and plant-sourced urease enzyme (Fig. 3) and is supplied at 99% purity by Fisher Scientific Company, USA.

### 3.2 Soil Classification

Geotechnical and geochemical properties, including particle size distribution, Atterberg's limits, specific gravity, natural moisture content, pH, and organic content, were studied, followed by hydraulic conductivity and the Standard Proctor compaction test. The purpose of the tests was to pre-assess the soil for EICP compatibility. The tests were performed according to BS EN ISO 17892-12:2018, BS 1377: Part 2: 1990 and reported extensively in Jodin et al. [13], [14].

### 3.3 EICP Treatment and Curing

The cementing solution was mixed with the soil sample and compressed into three layers according to the degree of compaction in a standard-size permeability cell. The concentration of the solution 1.0 and 0.5 M was prepared

by mixing urea ($CH_4N_2O$) and calcium chloride anhydrous ($CaCl_2$) with distilled and deionised water according to the molar concentration until it was completely dissolved. The urease enzyme of 3g/L is added just before adding to the soil sample to avoid early precipitation. For each sample, the amount of dry soil is estimated at 1010 g. The amount of water added corresponds to 70% and 80% of the MDD. Minor adjustments were made to each mixture layer to achieve the final set height. The cells are then cured at various curing temperatures (°C, Celsius) and times (days) according to the treatment series shown in Table 1. For each series, a triplication was performed to obtain the average results for a total of 39 samples.

**Fig. 2** *Abandoned Mamut mine, Sabah*


**Fig. 3** *Plant-based urease enzyme*

**Table 1** *EICP treatment series*

| Series Name | EICP Cementation (M) | Compaction (% ) | Temperature ($^0$C) |
|---|---|---|---|
| Raw1 | - | 70 | Room |
| 0.5M-70%-5C | 0.5 | 70 | 5 |
| 0.5M-70%-15C | 0.5 | 70 | 15 |
| 0.5M-70%-25C | 0.5 | 70 | 25 |
| 0.5M-80%-5C | 0.5 | 80 | 5 |
| 0.5M-80%-15C | 0.5 | 80 | 15 |
| 0.5M-80%-25C | 0.5 | 80 | 25 |
| 1.0M-70%-5C | 1.0 | 70 | 5 |
| 1.0M-70%-15C | 1.0 | 70 | 15 |
| 1.0M-70%-25C | 1.0 | 70 | 25 |
| 1.0M-80%-5C | 1.0 | 80 | 5 |
| 1.0M-80%-15C | 1.0 | 80 | 15 |
| 1.0M-80%-25C | 1.0 | 80 | 25 |

## 3.4 $CaCO_3$ Content Determination

A gravimetric acid wash method was used to determine the amount of precipitated calcite. The procedure involved soaking the EICP-treated soil specimen in acid hydrochloric, HCL of 1M concentration until the sample stopped seething. The purpose of the washing process is to remove all soluble calcium from soil particles. After the process, the sample is filtered on filter paper with a coarse pore sieve (#200). The sample is then rinsed with distilled water for 10 minutes to remove residual chemical reagents before drying. The weight of soil samples before and after was taken, and the difference was recorded as the $CaCO_3$ content (CCC, in percentage) [15]. To determine the retention behaviour of CCC, the acid washing test was conducted both before (pre) and after (post) the hydraulic conductivity test. For the post test, the average value of CCC is taken from sampling at the sample's bottom, middle and top.

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### 3.5 Hydraulic Conductivity and Inductively Coupled Plasma-Optical Emission Spectrometer

Before the CCC determination, a hydraulic conductivity test was conducted according to BS 1377: Part 2: 1990 and an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) test was performed using an Agilent Technologies brand. The ICP-OES test was used to detect the level of three heavy metals: nickel (Ni), copper (Cu), and lead (Pb), which were reported in percentage retained in comparison to the control sample. The hydraulic conductivity and ICP-OES tests aim to determine the correlation between the amount of CCC, soil permeability, and heavy metal retention, respectively.

## 4. Results and Discussion

### 4.1 Properties of Mine Waste Soil

Table 2 shows the geotechnical parameters of mine waste, indicating a USCS classification of SM (silty clay). Due to this, the mine waste has a medium to high degree of permeability ($3.77\times10^{-4}$ m/s). ICP-OES result shows more than ten (10) heavy metal elements, of which nickel (0.376 mg/L), copper (4.337 mg/L) and lead (0.535 mg/L) was used for the study on the impact of EICP towards pollutant retention. Meanwhile, Fig. 4 shows the SEM images of the mine wastes, indicating soil particles of angular and sub-angular shapes.

**Table 2** *Mine waste properties*

| Properties | Values |
|---|---|
| Gravel, % | 14.7 |
| Sand, % | 55.1 |
| Silt, % | 19.2 |
| Clay, % | 11.0 |
| USCS Classification | SM |
| Liquid Limit, LL (%) | 27.0 |
| Plastic Limit, PL (%) | 23.5 |
| Plasticity Index, PI (%) | 3.5 |
| Specific Gravity, Gs | 2.70 |
| Maximum Dry Density, MDD (kg/m$^3$) | 1640 |
| Optimum Water Content, % | 16 |
| Permeability, m/s | $3.77 \times 10^{-4}$ |
| Nickel, mg/L | 0.376 |
| Copper, mg/L | 4.337 |
| Lead, mg/L | 0.535 |

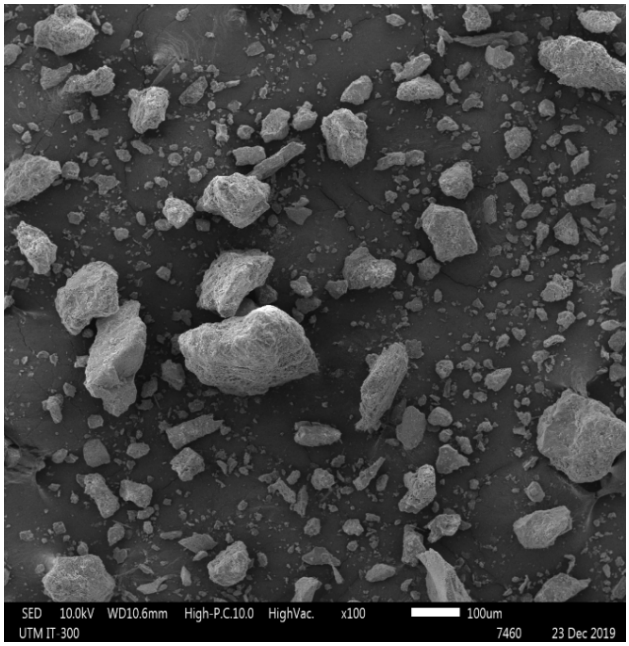


**Fig. 4** *SEM images of mine waste*

## 4.2 $CaCO_3$ Content (CCC)

The effects of cementation concentration on EICP treatment of mine waste soil are shown in Fig. 5 and Fig. 6 for $CaCO_3$ content of mine waste soil treated with 0.5M and 1.0M concentration of EICP, respectively.

### 4.2.1 Effects of Cementation Concentration on CCC

Fig. 5 and Fig. 6 indicate that the CCC is higher at higher EICP concentration. For soil treated under 0.5M EICP, the CCC percentage is in the range of 1.9-3.1%, compared to the CCC percentage of 1.0M EICP in the range of 3.80-5.10%. The higher CCC at 1.0M is due to the higher supply of biocementation materials. The significant amount of calcite generated has been previously observed in a study on the different permeability of porous media, and it was reported that cementation concentrations have a higher impact than the porosity of the media in $CaCO_3$ quantity [5], [16]. This supports the previous study on uncontaminated soil, which indicates that when enzyme concentration is increased from 0.01 to 0.1 g $l^{-1}$, it enhances the rate of $CaCO_3$ production. At the same time, increases in reactant concentrations (urea and $CaCl_2 \cdot 2H_2O$) also increase the quantity of produced $CaCO_3$ [17].

### 4.2.2 Effects of Degree of Compaction on CCC

The effects of the degree of cementation on EICP treatment of mine waste soil, as shown in Fig. 5 and Fig. 6, indicate that the CCC is higher at a lower degree of compaction. The CCC range for 70% compaction is 1.9-3.1% (0.5M) and 3.9-5.1% (1.0M). The CCC range for 80% compaction is 1.9-3.0% (0.5M) and 3.8-5.06% (1.0M). The higher CCC at 70% compaction is due to the larger space for calcite formation. The differences observed between the two compaction degrees could be attributed to the differences in the void ratio of the two compaction energies. Despite the compaction efforts, calcite formation is still possible because urease is small in size and highly soluble; therefore, it allows the cementation solution to penetrate through the pore throat of soils, even for samples containing finer particles [18].

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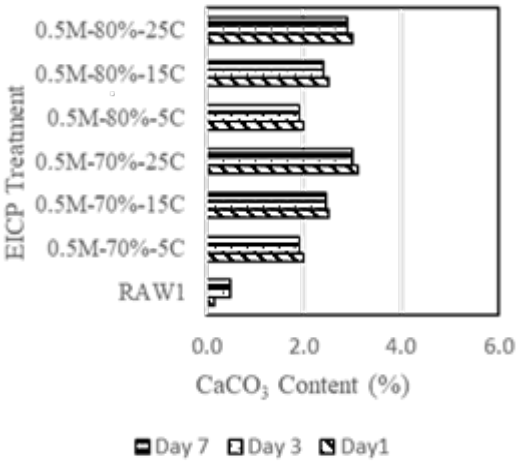


**Fig. 5** *$CaCO_3$ content of soil treated with 0.5M EICP*

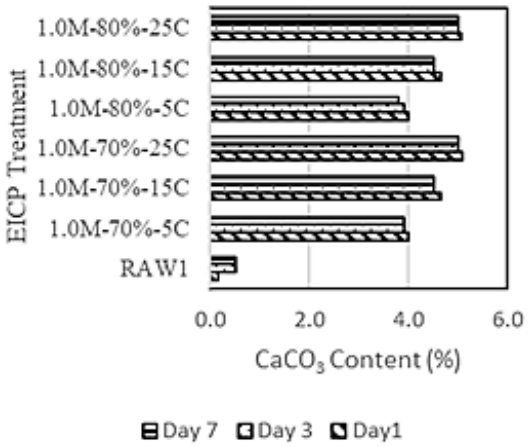


**Fig. 6** *$CaCO_3$ content of soil treated with 1.0M EICP*

### 4.2.3 Effects of Curing Duration on CCC

The effects of curing duration on EICP treatment of mine waste soil, as shown in Fig. 5 and Fig. 6, indicated that the CCC dropped slightly after 1-day curing but remained at 3-day and 7-day curing. Soil treated under all 0.5M series shows average CCC drops of 3.8% from 1-day to 3-day and maintained from this point thereafter. The highest drops for samples with 70% and 80% compaction are observed at the lowest temperatures (5 $^0$C), with 5%. Meanwhile, the lowest drop in 70% compaction is observed in samples cured in a room at 15 $^0$C at 2.0%. For 80%, the lowest drop is observed at room temperature with a 3.3% reduction. After 3 to 7 curing days, the percentages of CCC remain unchanged for all samples. Observation of soils treated under the 1.0M series shows similar trends. The findings supported the previous study on the immediate reaction of EICP [17], which was almost completed after 1-day of curing. The constant value of CCC thereafter indicates the lack of additional calcite precipitation in soils [19]. Therefore, it can be concluded that the formation of CCC in all mine waste soil treated with EICP occurred immediately during the first 24-day with a slight drop in CCC on 1 to 3-day but maintained thereafter.

### 4.2.4 Effects of Curing Temperature on CCC

The effects of temperature on EICP treatment indicate that the CCC increases with temperature. The highest CCC content is observed for samples treated under room temperature (25 $^0$C), regardless of compaction energy and curing durations. Samples cured under 15$^0$C and 5$^0$C were observed to have lower CCC. This indicated that, amongst the working parameters investigated, temperature is the most influential factor in the amount of calcite precipitated. The CCC is highest at room temperature, indicating that the cementation reaction is optimum at 25 $^0$C compared to 15°C and 5°C. This result on mine waste soil parallels the previous study on uncontaminated sand. At curing temperature, results showed that increasing up to 40°C has led to more calcite and dense aragonite than vaterite, which means the efficiency of the enzyme is greater than at lower temperatures [20].

## 4.3 Correlation Between Hydraulic Conductivity and CCC

Fig. 7 displays the correlation between the hydraulic conductivity and CCC for 1.0M EICP treatment. While all series showed $R^2 > 0.55$, series 1.0M-70%-25C ($R^2 = 0.709$) and 1.0M-80%-25C ($R^2 = 0.899$) are the highest in 70% MDD and 80% MDD degree of compaction respectively, indicating that in 1.0M treatment series, good correlation is observed between the hydraulic conductivity and calcite content in all series and but correlation is the highest at room temperature compared to curing at 15°C and 5°C. The effectiveness of the treatment on contaminated soil could be a result of $CaCO_3$ crystals that fill the space between soil particles, changing the soil pore volume and hence reducing soil hydraulic conductivity, similar to the observation in uncontaminated sandy soil by Nemati et al. [5] and on Toyura sand by Neupane et al. [21].

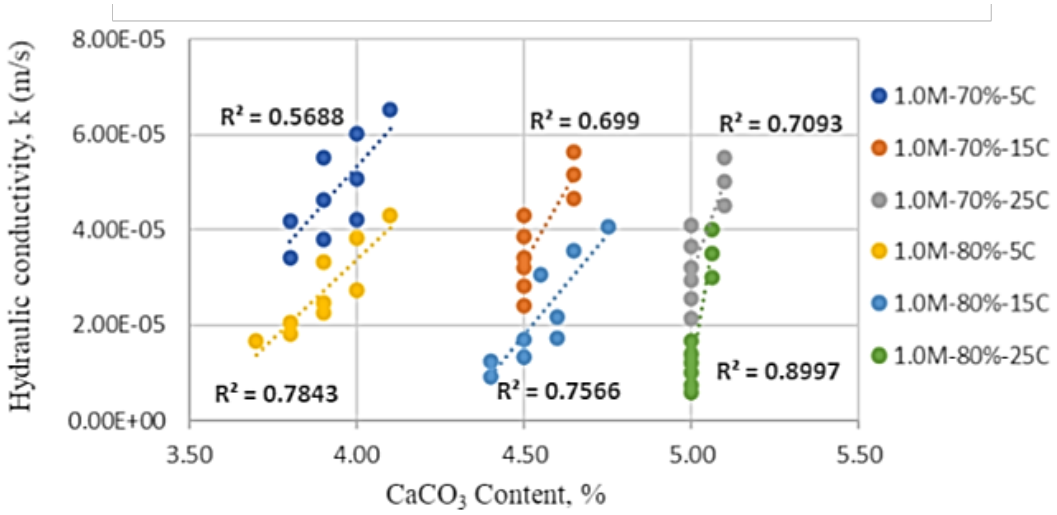


**Fig. 7** *Correlation between hydraulic conductivity and $CaCO_3$*

## 4.4 Correlation Between Heavy-Metal Retention and CCC

Fig. 8 displays the correlation between the retention percentage and $CaCO_3$ for 1.0M EICP treatment. The retained percentage of nickel, copper and lead was calculated at the end of the test to indicate the effectiveness of EICP treatment in immobilising the heavy metal contaminants.

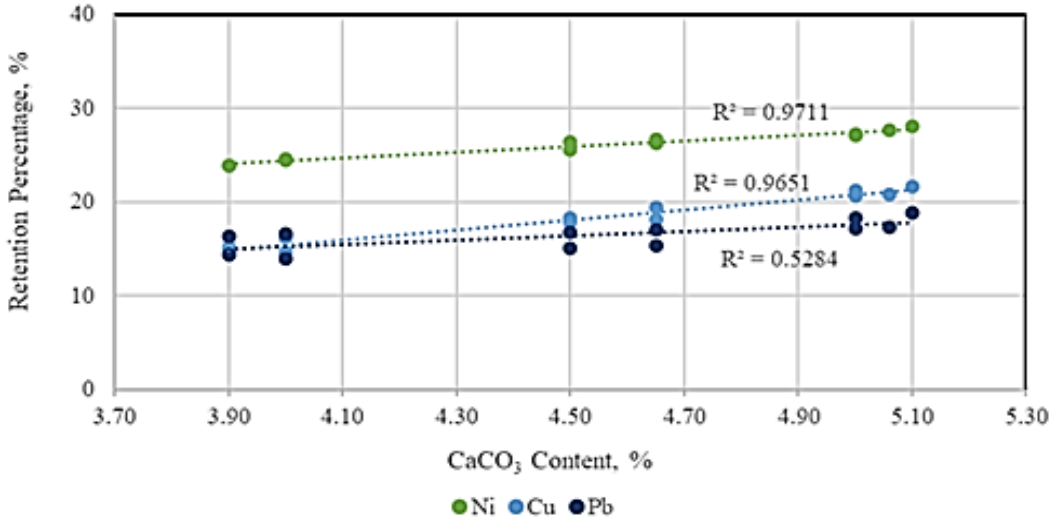


**Fig. 8** *Correlation between heavy metal retention and $CaCO_3$*

The retention of heavy metals in mine waste soil has a strong positive correlation with the amount of $CaCO_3$ produced, especially for nickel ($R^2$ = 0.971) and copper ($R^2$ = 0.965), and for lead ($R^2$ = 0.528). This indicates that when the $CaCO_3$ content in the soil increases, the retention of the heavy metals also increases due to the pollutant immobilisation. As observed in spiked-up soil, the formation of inorganic compounds ($CaCO_3$) outside the cellular structure binds and stabilises the soil particles together and encapsulates the heavy metals inside, thus immobilising them [22]. Previous studies linked the mobility and retention of heavy metals in contaminated soil to several factors, including: association of the solid phase they are bound to (sorption, desorption, precipitation, dissolution), soil pH, cation exchange capacity, salt content and soil fractions [23].

## 5. Conclusion

This research reported the impacts of several factors on the formation of $CaCO_3$ in the treatment of heavy-metal contaminated soil. It was found that cementation concentration is a significant influence as it provides the supply of biocementation materials and is the highest at room temperature, indicating that the cementation reaction is optimum at 25 $^0$C. Lower compaction has a slight edge over higher densely compacted soil because the cementation solution can penetrate through the pore throat of soils. Meanwhile, due to the immediate formation

of $CaCO_3$, curing duration is not a significant factor. Additionally, the precipitation rate is found to affect the effectiveness of EICP in reducing hydraulic conductivity and increasing the retention of heavy-metal contaminants.

In conclusion, this study contributes positively to understanding the properties of mine wastes and subsequently promotes using EICP as a sustainable measure in soil contamination mitigation. Thus, the reported effect of variable working parameters provides a better approach to attaining optimal performance in reducing the hydraulic conductivity and immobilising the migration of heavy metal elements.

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## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

## Author Contribution

*The authors confirm contribution to the paper as follows:* ***study conception and design:*** *Jodin Makinda, Khairul Anuar Kassim;* ***data collection:*** *Jodin Makinda, Abubakar Sadiq Muhammed, Muttaqa Uba Zango;* ***analysis and interpretation of results:*** *Jodin Makinda, Khairul Anuar Kassim;* ***draft manuscript preparation:*** *Jodin Makinda, Lillian Gungat, Nelly Majain. All authors reviewed the results and approved the final version of the manuscript.*

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