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Article

Efficacy of Enzymatically Induced Calcium Carbonate Precipitation in the Retention of Heavy Metal Ions

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Abstract: This study evaluated the efficacy of enzyme induced calcite precipitation (EICP) in restricting the mobility of heavy metals in soils. EICP is an environmentally friendly method that has wide ranging applications in the sustainable development of civil infrastructure. The study examined the desorption of three heavy metals from treated and untreated soils using ethylene diamine tetra-acetic acid (EDTA) and citric acid ($C_6H_8O_7$) extractants under harsh conditions. Two natural soils spiked with cadmium (Cd), nickel (Ni), and lead (Pb) were studied in this research. The soils were treated with three types of enzyme solutions (ESs) to achieve EICP. A combination of urea of one molarity (M), 0.67 M calcium chloride, and urease enzyme (3 g/L) was mixed in deionized (DI) water to prepare enzyme solution 1 (ES1); non-fat milk powder (4 g/L) was added to ES1 to prepare enzyme solution 2 (ES2); and 0.37 M urea, 0.25 M calcium chloride, 0.85 g/L urease enzyme, and 4 g/L non-fat milk powder were mixed in DI water to prepare enzyme solution 3 (ES3). Ni, Cd, and Pb were added with load ratios of 50 and 100 mg/kg to both untreated and treated soils to study the effect of EICP on desorption rates of the heavy metals from soil. Desorption studies were performed after a curing period of 40 days. The curing period started after the soil samples were spiked with heavy metals. Soils treated with ESs were spiked with heavy metals after a curing period of 21 days and then further cured for 40 days. The amount of $CaCO_3$ precipitated in the soil by the ESs was quantified using a gravimetric acid digestion test, which related the desorption of heavy metals to the amount of precipitated $CaCO_3$. The order of desorption was as follows: $Cd > Ni > Pb$. It was observed that the average maximum removal efficiency of the untreated soil samples (irrespective of the load ratio and contaminants) was approximately 48% when extracted by EDTA and 46% when extracted by citric acid. The soil samples treated with ES2 exhibited average maximum removal efficiencies of 19% and 10% when extracted by EDTA and citric acid, respectively. It was observed that ES2 precipitated a maximum amount of calcium carbonate ($CaCO_3$) when compared to ES1 and ES3 and retained the maximum amount of heavy metals in the soil by forming a $CaCO_3$ shield on the heavy metals, thus decreasing their mobility. An approximate improvement of 30% in the retention of heavy metal ions was observed in soils treated with ESs when compared to untreated soil samples. Therefore, the study suggests that ESs can be an effective alternative in the remediation of soils contaminated with heavy metal ions.

Keywords: heavy metals; soil; enzyme solutions; desorption; extractant

1. Introduction

Increase in population and attempts to satisfy the ever-growing demands of the same have led to industrialization, widespread construction activity, and extensive mining. Industrial effluents contaminate land and thereby pose a threat to the environment [1,2]. The number of contaminated sites identified in the United States of America alone until 2004 was 294,000 [3], which indicates an alarming need to implement remediation and decontamination methods to maintain a balance in nature. Remediation of the sites is costly because conventional remediation methods, such as excavation and dumping on unused land, are outdated, and contaminant removal by physical methods is difficult. As a result, contaminated soils arrive in engineered landfills. Engineered landfills, which are identified as the most viable means of landfilling solid municipal wastes, have also become sources of leachates rich with high levels of toxicity, fluorides, nitrates, and heavy metals [4–6]. Hence, attempts to mitigate the adverse effects of hazardous wastes present in landfills has attracted significant research attention. Urbanization also poses a worldwide challenge for landfill management. Developing an environmentally friendly method to decontaminate soils is a priority research topic.

One of the methods adopted to decrease landfill hazards involves adding liners to landfills that act as barriers relative to leachate infiltration from the landfills to the soil beneath [7–9]. A liner is an essential part of an engineered landfill and should be durable and properly designed for the safety of the circumferential environment [10]. The use of locally available, fine, and cohesive soil as liner material is an easy and convenient option [11]. However, not all locally available material is suitable for such uses. In such cases, suitable material is imported, which adds to the overall costs of the project. Another method of decontaminating soils is by washing the soils with suitable chemicals to neutralize the contaminants. In general, these methods for decontaminating soils are costly owing to difficulties involved with physical separation or soil washing. Furthermore, due to the heterogeneous soil texture, removal of soil contaminants by soil washing solutions becomes difficult [3].

Another method to strengthen the landfill liner is to stabilize the soil using lime or cement. Stabilization of soil by cement and lime have been vastly used [12–15] in landfill liners, and these methods contribute to the emission of greenhouse gases, leading to large scale global warming. Production of cement and lime result in about 800–900 and 600–700 kg CO₂ per ton, respectively [16]. The cement industry contributes about 5% of CO₂ emissions globally [17,18]. With such an alarming situation, researchers have searched for sustainable stabilization methods for soil, wherein partial or total replacement of the cement or lime binders are tested for their reliability. These stabilizers include palm oil fuel ash [19], flyash [20], rice husk ash [21,22], residue of calcium carbide [23], alkali-activated agro-waste [24], and so forth.

As an attempt to contribute to the field of soil stabilization, bio cementation by CaCO₃ is also employed as a sustainable method of soil stabilization [25–29], which includes improvement of geotechnical properties of soil as well as contaminant remediation. This study evaluates the use of one such method, known as enzyme induced calcite precipitation (EICP), to immobilize heavy metals as a method of decontaminating soil. The main objective is to study the ability of the EICP method to retain heavy metal contaminants such as nickel, cadmium, and lead in soil and to determine the dosage of Enzyme Solution (ES) that leads to the maximum retention of heavy metals. Another aim is to identify the percentage of heavy metal contaminants retained in the soil as a result of EICP.

2. Background

The dumping of contaminated soils into landfills has made landfill management a major challenge, as these sites become major sources of leachates rich in heavy metals, fluorides, and other contaminations. Furthermore, the intrusion of leachates from landfills has been found to introduce large quantities of heavy metals, fluorides, and nitrates to ground water used for irrigation, industrial purposes, and drinking [30]. High concentrations of metals in a low-pH soil increase soil acidity, thereby making the soil vulnerable to contamination [6]. The presence of less than 1000 mg/kg of heavy metals in soil is rarely toxic, although human activities involving the disposal of them into the soil above these levels

poses a threat to the flora and fauna. Heavy metals that are naturally present in the soil cause less damage than those that accumulate due to human activity [31]. Specifically, Cd is naturally available in earth's crust with a concentration of 0.1–0.5 ppm associated with zinc, copper, and lead ores [32]. Industries play an important role in increasing heavy metal concentrations in surface soils above permissible levels by releasing toxic fumes into the atmosphere, because traces of fumes find their way to the soil surface [33,34].

Soil in its natural form exhibits different components, such as phyllosilicates, humic substances, and carbonates, which contribute to the sorption process of heavy metals [35]. Concentrations of metals in the soil are governed by anthropogenic effects, pedogenic processes, and parent material [36]. Immobilization [37], phytoremediation [38,39], and soil washing [40] methods are adopted to achieve remediation of heavy metals in soils [7,41,42]. Remediation of contaminated soils can be performed using in situ or ex situ methods based on site conditions [4,43]. Landfill management continues to constitute a complex issue and should be examined further [39].

Industries also emit toxic elements into water bodies. These elements are then absorbed by aquatic plants, making them unsafe for consumption. Sahu et al. [44] examined concentrations of different heavy metals in macrophytes and observed an average of 13 mg/kg of Pb in seven aquatic plants from the Kharun River in India. Metal contaminants also pose a threat to terrestrial plants because of the increase in anthropogenic activities, which eventually leads to their intrusion in the food chain [45]. Heavy metals that are most harmful to human health include Pb, Cd, As, Zn, Cu, Hg, Cr, and Ni. Heavy metals happen to be the most commonly found carcinogens among the pollutants; for example, Hg leads to mutations and genetic damage, and Cu and Pb can affect the brain and bones [46]. Heavy metals are generally removed from the soil via precipitation–dissolution, oxidation–reduction, and adsorption–desorption processes, among which adsorption–desorption is observed as the most effective geochemical process for contaminant remediation [47]. The removal of metals can also be performed by chemical precipitation, bio-precipitation, ion exchange, adsorption, biosorption, physical separation, electrochemical separation, solvent extraction, flotation, and cementation [48,49]. Another technique adopted to decrease the hazardous effects of heavy metals involves retaining contaminants in soil via encapsulation. Methods adopted for retaining heavy metals by encapsulation decrease the mobility of heavy metal ions in the soil. The use of nano calcium silicate in retaining Cd, Ni, and Pb was observed as an effective [50,51] approach towards contaminant remediation. However, the costs associated with production of nano compounds on a macro scale is not economically feasible.

Among the approaches to encapsulate heavy metals in soils, the use of microorganisms is also adopted by researchers. This method, popularly known as bioremediation, involves processes such as microbial induced calcite precipitation (MICP) to encapsulate heavy metals inside the precipitated calcium carbonate. The MICP technique fosters metabolic activity in certain types of soil bacteria (*Sporosarcina pasteurii*), which results in the formation of inorganic compounds (such as CaCO_3) outside the cellular structure; these compounds can bind soil particles together and stabilize the soil. In addition, it is possible to encapsulate heavy metals present in the soil inside the CaCO_3 crystals. Another branch of bioremediation, known as enzyme induced calcite precipitation (EICP), uses plant-based urease enzymes to precipitate calcium carbonate. The use of enzymes is advantageous, because they are non-toxic and ecofriendly [52].

Nathan et al. [53] found the enzyme treatment effective in reducing the heavy metals in paper pulp. EICP is a biologically inspired soil improvement process designed to initiate urea hydrolysis using urease enzyme extracted from jack beans [25,54–57] or watermelon seeds [58]. The precipitation of CaCO_3 through enzyme activity is obtained by mixing urea, calcium chloride, and urease enzyme with deionized water. This solution can then be mixed with soil to prepare soil samples for testing [59]. Additionally, MICP is a process in which CaCO_3 is precipitated by the activity of bacteria, such as *Sporosarcina pasteurii*, to improve the geotechnical properties of soil [60]. Calcium carbonate precipitates are observed to fill the voids between soil grains, thereby reducing the permeability of soil and improving unconfined compressive strength (UCS) [61,62] and shear strength of the soil [63].

Furthermore, the precipitates mitigate liquefaction below the existing structures, stabilize roadways, control the flow of groundwater, immobilize contaminants [37], and remediate hazardous trace metals in soils [64]. The urease enzyme used in the study was crystallized from jack beans (*Canavalia ensiformis*) and formed the main source of urea hydrolysis [65]. Plant-based urease is optimal if obtained from jack bean and is identified as the first crystallized enzyme as well as the first nickel metalloenzyme [66]. Concerning remediation of soil contaminants, CaCO_3 precipitates are relatively easy to implement, useful in retaining the contaminants, and economic. They are readily accepted in society, based on an approximate scoring proposed by Dejong et al. [67].

3. Materials and Methods

3.1. Soil

The present study was performed on two soils, namely black cotton soil collected from Yadgir district ($16^\circ 45' 20.556''$ N, $77^\circ 9' 4.5072''$ E) and red soil from Bangalore district ($13^\circ 2' 14.1396''$ N, $77^\circ 37' 11.928''$ E) in Karnataka, India. The dominant mineral in black cotton soil was identified as montmorillonite (henceforth referred to as 'Soil M') and kaolinite in red soil (referred to as 'Soil K'). Basic tests of these soils were conducted and are listed in Table 1. Soil M was classified as clay with high plasticity (CH), whereas Soil K was classified as clay with low plasticity (CL) according to the Unified Soil Classification System.

Table 1. Engineering properties of tested soils.

| Property | Soil K | Soil M |
|---|--------|--------|
| Color | Red | Black |
| Specific gravity | 2.6 | 2.5 |
| Liquid limit (%) | 30 | 54 |
| Plastic limit (%) | 17 | 27 |
| Plasticity index (%) | 13 | 27 |
| Classification (USCS) | CL | CH |
| pH | 5.7 | 8.3 |
| Organic content % | 0.87 | 0.77 |
| Optimum water content (%) | 16.8 | 15.2 |
| Maximum dry density (kN/m^3) | 17.9 | 15.8 |
| Unconfined compressive strength (MPa) | 0.07 | 0.013 |

3.2. Heavy Metal Contaminants

Three heavy metals, namely cadmium (Cd), nickel (Ni), and lead (Pb), were spiked in the soils by preparing a stock solution with predetermined load ratios to obtain the target concentrations of contaminants in the soil as described by Mohammed and Moghal [2].

Analytical reagent grade (AR) nitrates of nickel ($\text{Ni}(\text{NO}_3)_2$), cadmium ($\text{Cd}(\text{NO}_3)_2$), and lead ($\text{Pb}(\text{NO}_3)_2$) were used to prepare the stock solutions. The target load ratio was identified as 50 and 100 mg/kg of heavy metal contaminants. Salt solutions were prepared in containers of borosilicate glass to maintain a sufficiently wet consistency to spike soils with contaminants. Oven-dried soil samples of a predetermined quantity were placed in glassware and properly washed with deionized double-distilled water (DI), and the heavy metal stock solution was added to the soil samples and mixed thoroughly to achieve the target Ni, Cd, and Pb load ratios [68]. Soil containers were covered with thin sheets of aluminum with minute perforations to ensure free airflow and to avoid dust intrusion and cross-contamination [51]. Subsequently, the samples were placed on a flat dry platform in a temperature- and humidity-controlled room where the temperature was maintained at $23\text{--}27^\circ\text{C}$ with proper ventilation and average relative humidity of 45%. The soil samples were left to cure for 40 days such that the probability of contaminants desorbing from the soil in the initial stages was minimized. After the soil samples were cured for 40 days, acid digestion was conducted, and the

actual amount of heavy metal concentration in the soils was established using an atomic absorption spectrophotometer (AAS) (PerkinElmer Model A-Analyst 400). The results are tabulated in Table 2.

Table 2. Determination of actual load ratio of metal ions via acid digestion method.

| Load Ratio Available with 50 mg/kg Initial Load Ratio | | | | | | | | |
|--|--------|--------------|--------------|--------------|--------|--------------|--------------|--------------|
| Metal Ion | Soil K | Soil K + ES1 | Soil K + ES2 | Soil K + ES3 | Soil M | Soil M + ES1 | Soil M + ES2 | Soil M + ES3 |
| Ni | 49.37 | 48.67 | 46.19 | 47.69 | 48.37 | 47.65 | 45.61 | 46.97 |
| Cd | 48.97 | 47.68 | 44.93 | 46.37 | 48.69 | 47.86 | 45.57 | 46.59 |
| Pb | 49.01 | 48.79 | 46.82 | 48.03 | 48.04 | 47.63 | 45.39 | 46.55 |
| Load Ratio Available with 100 mg/kg Initial Load Ratio | | | | | | | | |
| Metal Ion | Soil K | Soil K + ES1 | Soil K + ES2 | Soil K + ES3 | Soil M | Soil M + ES1 | Soil M + ES2 | Soil M + ES3 |
| Ni | 99.09 | 98.67 | 95.98 | 97.64 | 99.12 | 98.36 | 95.63 | 97.86 |
| Cd | 98.39 | 98.49 | 96.15 | 97.35 | 98.87 | 98.65 | 95.58 | 97.55 |
| Pb | 99.2 | 98.52 | 96.28 | 97.36 | 99.24 | 99.83 | 96.05 | 97.64 |

The same procedure was repeated for the soils treated with ESs, and the concentrations of the contaminants in the soils treated with the ESs were determined for comparison with those of untreated soils.

3.3. Enzyme Solutions

Three types of enzyme solutions were prepared to treat the soil using Analytical Reagent (AR) grade materials. Specifically, the following materials were used to prepare the ESs:

- Urea ($\text{CH}_4\text{N}_2\text{O}$)
- Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)
- Urease enzyme (*Canavalia ensiformis* (jack bean) Type III, powder, 15,000–50,000 units/g solid)
- Non-fat milk powder

The chemicals were procured from Winlab Chemical, Market Harborough, United Kingdom, and the urease enzyme was procured from Sigma-Aldrich, St. Louis, MO, USA. The composition of each of the ESs is presented in Table 3. It can be noted here that ES1 and ES2 have similar compositions, except for the use of non-fat milk powder, which aided in developing nucleation sites in the soil masses, thereby leading to the precipitation of CaCO_3 at the contact points [25]. Similarly, ES2 and ES3 differ by the concentration of the urease enzyme, used as per Almajed et al. [25].

Table 3. Composition of ESs.

| Solution | Concentration of Component 1 | Concentration of Component 2 | Concentration of Component 3 | Concentration of Component 4 |
|----------|--|--|------------------------------|------------------------------|
| | Urea ($\text{CH}_4\text{N}_2\text{O}$) | Calcium Chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) | Urease Enzyme | Non-Fat Milk Powder |
| ES1 | 1.00 M | 0.67 M | 3.00 g/L | - |
| ES2 | 1.00 M | 0.67 M | 3.00 g/L | 4.00 g/L |
| ES3 | 0.37 M | 0.25 M | 0.85 g/L | 4.00 g/L |

3.4. EICP Treatments

Enzyme-treated soil samples were prepared by compacting soil passing through a 425- μ sieve and mixed with an ES to obtain a moisture content equal to the optimum moisture content of the soil by weight. The soil was mixed with ES in a 5-cm diameter mold with a depth of 10 cm in three layers to ensure maximum density and minimal voids. Subsequently, the soil samples were cured in a desiccator for 21 days after they were sealed in an airtight seal pack to ensure proper calcite precipitation.

3.4.1. UCS Tests

UCS tests were also performed for the enzyme-treated soil samples as per ASTM D2166 [69] to gain a better understanding of the effect of ESs on the strength properties of soils. Figure 1 shows the UCS results obtained for soil samples using ESs cured for 7, 14, and 21 days. It was observed that the UCS values of the soil samples increased to the maximum level when the samples were treated with ES2 and cured for 21 days. Non-fat milk powder in ES2 is the main strength booster, because it creates sites of nucleation in the soil, enhancing the precipitation of CaCO_3 . However, non-fat milk powder is also used in ES3, and the strength gain is less than that of the ES2, because the amount of urease enzyme used in ES3 is 0.85 g/L, which is less than that of ES2 [25]. The same reason potentially holds in terms of understanding the amount of CaCO_3 precipitated in the soil when ES3 is used, which constitutes a reduction in CaCO_3 precipitated when compared to precipitation given the use of ES2.

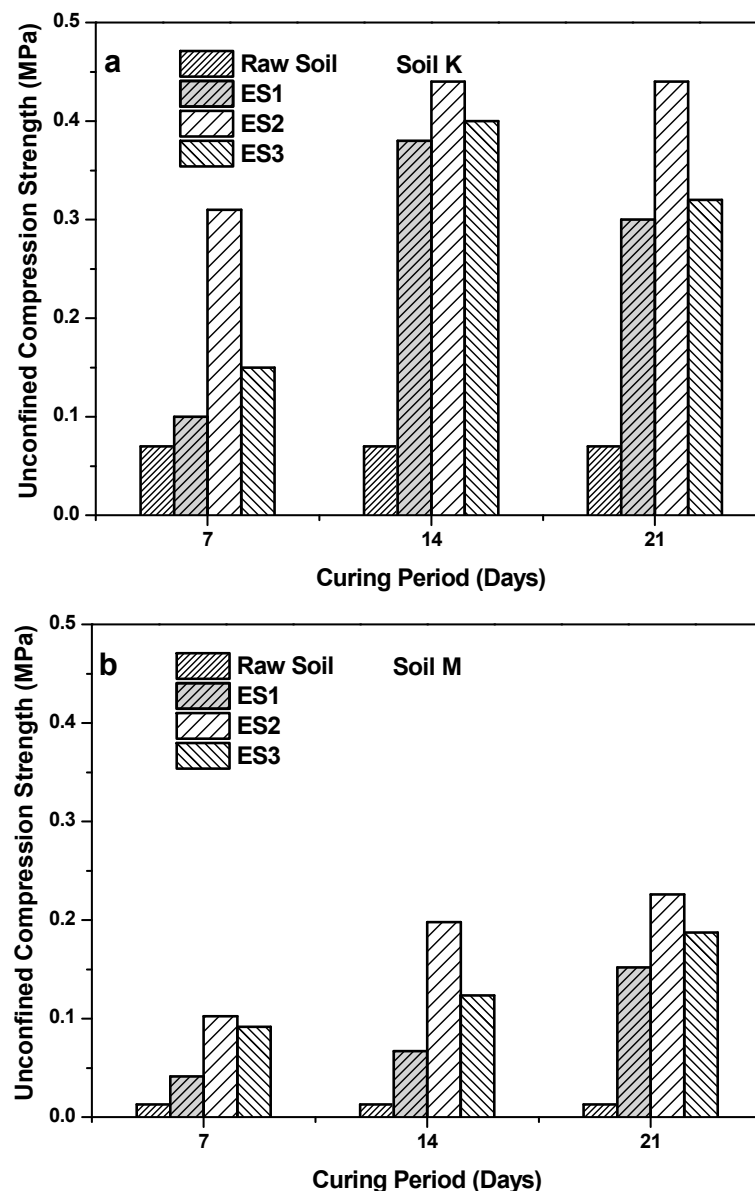


Figure 1. UCS results of EICP treated soils with different enzyme solutions: (a) Soil K (b) Soil M.

3.4.2. Measurement of Calcium Carbonate Precipitation

The ES-treated soil samples were kept in a desiccator after they were sealed in air-tight packs and were tested to identify the amount of CaCO_3 precipitated by gravimetric acid digestion. 40 to 50 g of soil

specimens was soaked in 1 M hydrochloric acid for an hour until the disappearance of the effervescence was observed after soaking (due to the dissolution of CaCO_3 in the soil). Subsequently, the samples were rinsed and placed in an oven for drying at a temperature of 105 °C. The difference in the masses of the soil samples before and after soaking in 1.0 M hydrochloric acid was determined to calculate the percentage of CaCO_3 precipitated in soil. The soil samples were tested for the amount of CaCO_3 precipitated over 7, 14, and 21 days to understand the precipitation trend. These results are presented in Table 4.

Table 4. Calcium Carbonate Precipitation (%) as obtained by gravimetric acid digestion test.

| Curing Period (Days) | Soil K | | | Soil M | | |
|----------------------|--------|-------|-------|--------|-------|-------|
| | ES1 | ES2 | ES3 | ES1 | ES2 | ES3 |
| 7 | 1.315 | 1.387 | 1.367 | 1.581 | 2.631 | 1.671 |
| 14 | 1.769 | 2.286 | 1.933 | 2.463 | 4.722 | 2.632 |
| 21 | 1.689 | 2.043 | 1.811 | 2.026 | 4.582 | 2.328 |

3.5. Extractants

The extractants used for the desorption tests included ethylene diamine tetra-acetic acid (EDTA) and citric acid in three molar concentrations (i.e., 0.1, 0.25, and 0.5 M) for the removal of heavy metals retained in the soil by acid digestion. A solid-to-liquid ratio of 1:20 was maintained. Both EDTA [70] and citric acid [71] were proven to be effective in extracting heavy metals from soil surfaces. Gu and Yeung [71] noted that citric acid dominant industrial wastewater is effective in desorbing Cd from soil surfaces in the pH range of 4 to 8.

3.6. Desorption Tests

A desorption test was conducted on the soil samples spiked with contaminants after a curing period of 40 days. Five grams of contaminated soil samples was placed in 0.1, 0.25, and 0.5 M extractants in 50-mL polytetrafluoroethylene bottles and shaken as per ASTM D3987 [72] in a mechanical shaker at 30 rpm for 24 h to ensure that the soil particles were uniformly distributed in the solution, and thereby releasing heavy metal contaminants. Subsequently, the slurry was filtered through filter papers (Whatman No. 42) to obtain solutions that were tested in AAS to identify their metal contamination levels. Each set of tests was performed with three samples, and an average of the three results was considered as the final value. The pH values of the solutions tested in AAS were determined, and their removal efficiencies were calculated as follows [73].

$$\text{Removal Efficiency (\%)} = \frac{\text{Contaminant mass from desorption}}{\text{Initial contaminant mass in soil}} \quad (1)$$

4. Results and Discussions

The data obtained from the desorption experiments were plotted (Figures 2–4) with the molar concentrations of the extractants on the abscissa and heavy metal removal efficiencies (%) on the ordinate. A lower removal efficiency percentage is favorable, as it means that heavy metal contaminants are immobilized in the soil and cannot be extracted from it. Among the three heavy metals, it was observed that the removal efficiency of Cd was minimal when compared to that of Ni or Pb.

The formation of CaCO_3 was initiated in the soil by the reaction between urea and calcium chloride; this occurs when the calcium ions and carbonate ions combine [74]. The process of precipitation of calcium carbonate is accelerated by the urease enzyme [75]. The recipe of the ESs adopted provides an environment for efficient utilization of enzyme for CaCO_3 precipitation [76]. CaCO_3 is precipitated along the nucleation sites with ES2 due to the use of non-fat milk powder [25]. The main process that leads to the formation of calcite is hydrolysis of urea by the urease enzyme into CO_2 and NH_3 , and the speciation of NH_3 leads to the development of NH_4^+ ions, which creates a suitable environment for

precipitation of CaCO_3 in calcium rich solution of CaCl_2 [26]. It is also proposed that the heavy metal ions with ion radii close to that of Ca^{2+} (e.g., Pb^{2+} , Cu^{2+} , Cd^{2+} , and Sr^{2+}) are incorporated in CaCO_3 crystal lattice by replacing Ca^{2+} ions or by creating defects on the calcium carbonate crystals, or even by penetrating the CaCO_3 interstice. This indicates that the EICP technique can be used for the remediation of heavy metals [77]. The formation of carbonates of heavy metals occurs in the microenvironment of the mineral carbonates, and the process is even applicable for radionuclides such as strontium forming strontium carbonate (SrCO_3) [74]. Metal ions in soil tend to cluster with carbonates that are already present in the soil, and heavy metal retention is expected due to the precipitation of heavy metal carbonates [78,79]. Enzymatic mechanism of bioremediation is found to be effective in issues concerning heavy metal bioaccumulation in paper pulp [53].

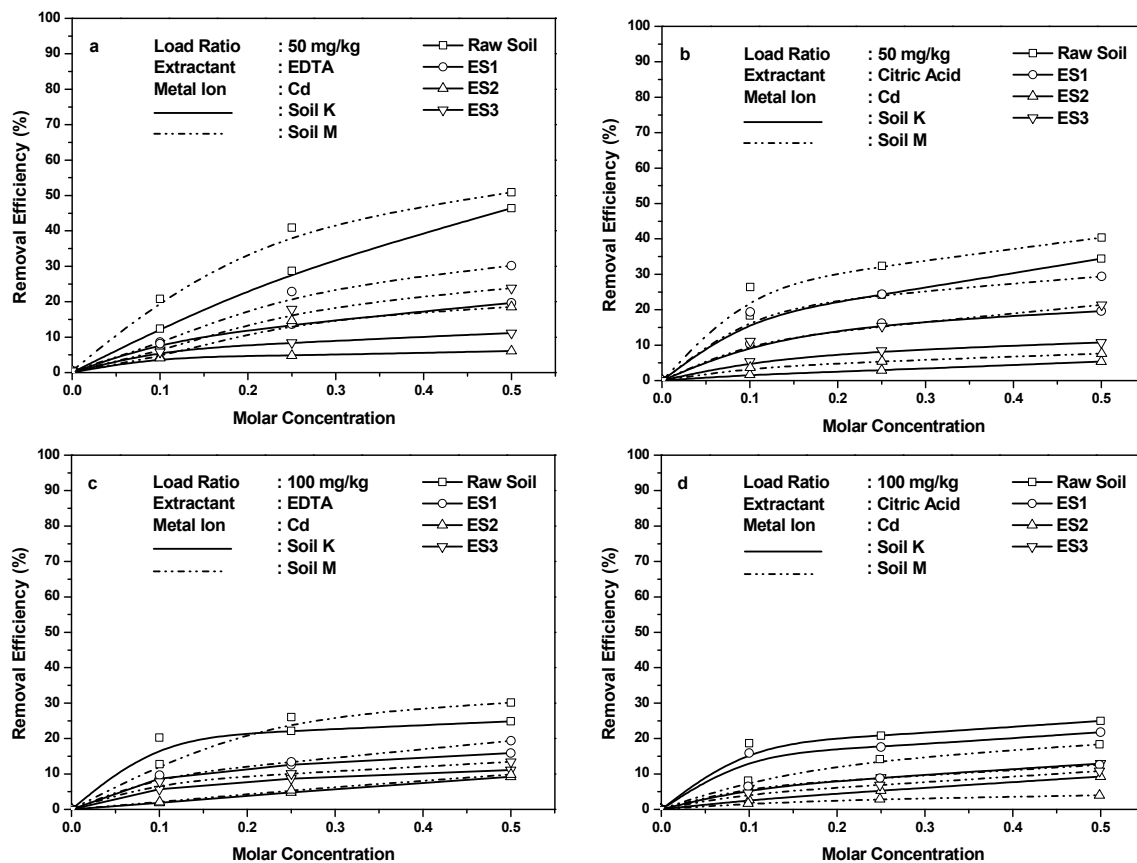


Figure 2. Effect of extractants on 'Cd' removal efficiencies of soils treated with different enzymatic solutions with (a) EDTA extractant and load ratio of 50 mg/kg, (b) Citric acid extractant and load ratio of 50 mg/kg, (c) EDTA extractant and load ratio of 100 mg/kg, (d) Citric acid extractant and load ratio of 100 mg/kg.

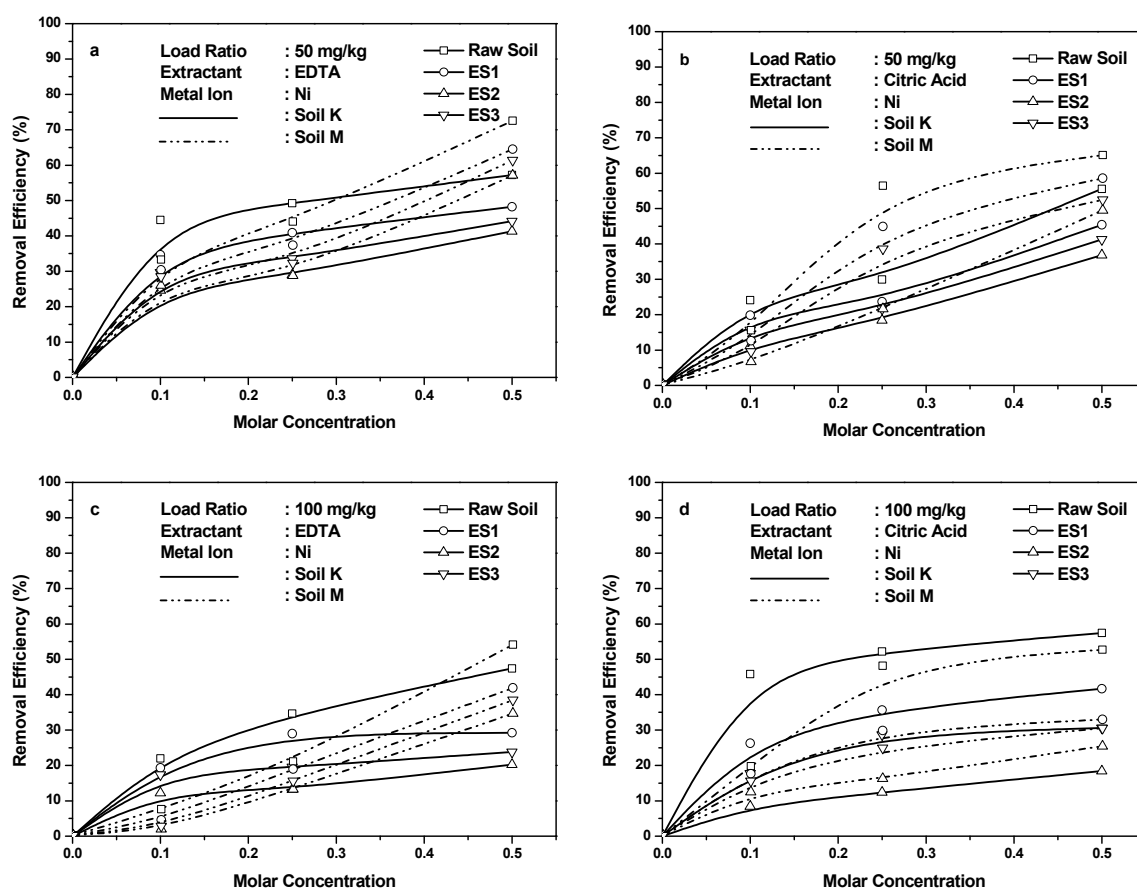


Figure 3. Effect of extractants on ‘Ni’ removal efficiencies of soils treated with different enzymatic solutions with (a) EDTA extractant and load ratio of 50 mg/kg, (b) Citric acid extractant and load ratio of 50 mg/kg, (c) EDTA extractant and load ratio of 100 mg/kg, (d) Citric acid extractant and load ratio of 100 mg/kg.

4.1. Cd Retention in Soils

The highest removal efficiency of Cd was observed as 50.39% for untreated Soil M and 46.37% for untreated Soil K (see Figure 2). The lowest removal efficiency for Cd when extracted by 0.5 M citric acid was observed as 3.97% for Soil M and 5.39% for Soil K treated with ES2 (see Figure 2). An increase in clay or silt particles in the soil decreases the release of Cd due to strong deposits of heavy metal traces that occur in the finer fractions of clay [80]. Hence, it was observed that higher silt or clay content expanded soil capacity for heavy metal retention [81,82]. Our results are in line with these observations.

The Cd removal efficiency was also low due to the precipitation of a comparatively large amount of CaCO_3 in soil treated with ES2 (see Table 4). Wang et al. [83] found that the presence of CaCO_3 played a major role in immobilizing heavy metals, as observed in their study, when treating soils with CaCO_3 . The EICP method used in this study for the retention of heavy metals is comparatively more effective in immobilizing Cd. Therefore, the EICP treatment can be termed an adsorbent selective to Cd contamination.

The role of pH is also important in the retention of Cd in the soil, and the desorption of Cd is observed to increase when pH decreases. Additionally, increase in the pH of soil results in immobilization of Cd ions [32]. Citric acid is observed to desorb Cd to a lesser extent at lower molar concentrations and to a greater extent at higher molar concentrations (see Figure 2). This can be attributed to the formation of Cd-citric acid complexes in an aqueous state, which detach from soil surfaces. EDTA is observed to retain greater amounts of Cd, even at a pH of 5.05 [84]. The range of

pH was between 5–8 with citric acid as chelant with 0.1 M for extraction of Cd. This pH reduced (within range of 3–6) at 0.5 M molarity, leading to comparatively higher removal of Cd. The removal efficiencies and associated standard deviation values are provided in the Supplementary Materials (Tables S1 and S2 respectively).

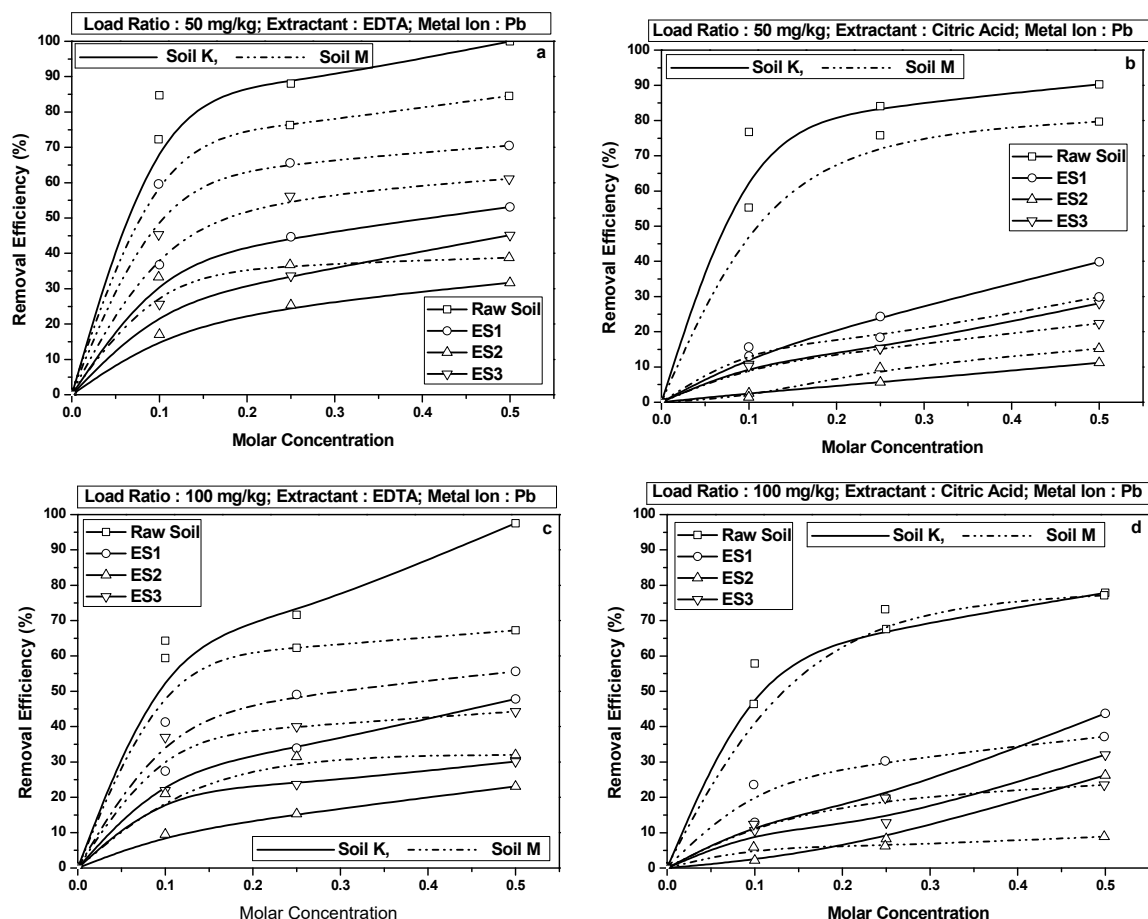


Figure 4. Effect of extractants on Pb removal efficiencies of soils treated with different enzymatic solutions with (a) EDTA extractant and load ratio of 50 mg/kg, (b) Citric acid extractant and load ratio of 50 mg/kg, (c) EDTA extractant and load ratio of 100 mg/kg, (d) citric acid extractant and load ratio of 100 mg/kg.

4.2. Ni Retention in Soils

Figure 3 shows the plots for Ni extracted from both soils using EDTA and citric acid. Figure 3a,b shows the removal efficiency for load ratios of 50 mg/kg. Figure 3c,d shows the removal efficiency for load ratios of 100 mg/kg. The plots indicate that the encapsulation of Ni in the soils with ES2 exceeded that in the soils with ES1 and ES3. The difference in removal efficiency was not significant between the two load ratios (50 and 100 mg/kg). Both load ratios yielded removal efficiency values of approximately 15–20%.

The maximum range of Ni desorption was observed as approximately 72.36–57.2% for soils M and K, respectively, and the minimum amounts of Ni desorption were 34.48% for Soil M and 20.26% for Soil K when extracted by 0.5 M EDTA. Nickel is one of the most commonly found contaminants in the brownfields, and nickel contamination originates from the discharge of industries involving metal plating, nickel refinement, and mining sites. Nickel precipitates into a stable compound in the form of nickel hydroxide $[\text{Ni}(\text{OH})_2]$ in slightly alkaline and neutral solutions. Nickel can be effectively removed by citric acid and EDTA chelating agents [70], and nickel retention can be understood from the relationship between the sorptive surface and ion concentration that decreases metal ion removal.

When metal ion concentrations are low, the number of binding sites for heavy metals is initially high and results in the immobilization of heavy metals [85]. The mechanism of Ni retention can also be expressed as a mechanism in which metal binds to a carboxylic group, given the competition between protons, metals, and ion exchange interactions in the solution, and leads to the immobility of Ni ions [86]. Precipitation of heavy metals occurs when a solution is saturated with a specific element through a homogeneous or heterogeneous aggregation processes. The former aggregation process is an outcome of nucleation of the supersaturated phase in the soil solution, whereas the latter process involves precipitation formed by the nucleation of other materials (i.e., soil particles), which hold metals as sorbents on the surface [87]. The urease enzyme used in the study is reportedly active and stable when the EDTA solution exhibits a neutral pH. The enzymatic activity plays a major role in already present nickel ions in the urease enzyme. It is also established that nickel ions are active in strengthening the enzyme activity completely; thus, attempts to remove nickel from the urease enzyme were not successful [66]. Thus, it can be assumed that preexisting nickel in urease and added nickel potentially cluster and this behavior leads to the retention of Ni in the soil. This can explain the results shown in Figure 3.

4.3. Pb Retention in Soils

Figure 4 shows the Pb removal efficiency. As shown in the figure, Pb retention was minimal when compared to Ni and Cd retention. Raw soil K contaminated with Pb demonstrated very high removal efficiency, which ranges from 99.96% to 97.52% for soil treated with 0.5 M EDTA extractant and with load ratios of 50 and 100 mg/kg, respectively. The removal efficiencies after acid digestion by citric acid decreased to 11.2% and 26.26% for the same soil with 50 and 100 mg/kg load ratios, respectively, when Soil K was treated with ES2.

Pb and Cd get adsorbed on CaCO_3 precipitates, limiting their mobility. The retention of Pb and Cd was observed as predominant by polydopamine CaCO_3 when compared to natural CaCO_3 [88]. Thus, it was inferred that decreases in Cd and Pb removal efficiency observed in the present study occurred due to the presence of CaCO_3 in the soil. Pb retention can also occur via diffusion of solid-state and precipitation reactions resulting in PbSO_4 and PbCO_3 precipitates when the metal contamination levels exceed solubility levels of the carbonates and hydroxides at a given pH [89,90]. Kumpiene et al. [91] performed Pb immobilization studies via phosphorus-containing chemicals, such as apatites and hydroxyapatites, in synthetic and natural forms. This is because precipitation and ionic exchange of pyromorphite-type minerals decrease Pb mobility, and because compounds of Ca are generally efficient in performing Pb immobilization as soil pH increases within the range 8–9, thereby leading to the retention of Pb. Almost 99% of Pb retention was obtained by Wang et al. [83] using reagent-grade stabilizers, namely $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and CaCO_3 in a dumpsite in Taiwan.

The heavy metal desorption process is affected by the pH of the pore fluid solution [42]. Even waste soils with neutral pH values exhibit significant levels of heavy metal sorption [92]. Harter [93] examined the effects of soil pH on heavy metal adsorption and stated that the degree to which metal ions hydrolyze at a specific extractant pH (leading to their release from the host soil) is unknown. In a previous study, Harter [93] suggested that retention of univalent Pb hydroxides (when compared to divalent ions) can increase by 60% when pH increases from 6 to 8 and forms precipitates of metals at high pH levels. The same study revealed that Ni was retained due to a reaction that formed precipitates at high pH levels and concluded that the validity of pH, as a deciding factor in ascertaining the quantity of heavy metal that can be safely retained in the soil, is uncertain, although the pH of the extractants used for Pb retention was within the range 3–5.

Gupta and Lataye [94] indicated that the pH of a solution affects the charge and process of ionization of the sorbent in the solution. Therefore, it is presumed that pH increases the duration for which metal contaminants are released by extractants. The process of heavy metal removal was performed in the study by conducting separate desorption tests on soils spiked with individual

contaminants, because the simultaneous removal of heavy metals using a single extraction method is difficult [95].

5. Conclusions

The aim of this study was to examine the efficacy of EICP in fixing Cd, Ni, and Pb that were spiked in Soil M and Soil K in addition to improving their strength characteristics. The following conclusions were made from the study:

- Soil grains adhered to each other due to CaCO_3 precipitation initiated by the urease enzyme, and there exists a high probability that metal ions are encapsulated between the soil grains and CaCO_3 precipitates. This leads to the effective retention of heavy metals in the soil matrix.
- Heavy metal retention in the soil occurred in the following order: $\text{Cd} > \text{Ni} > \text{Pb}$. The EICP-treated soil retained the maximum quantity of Cd among all the heavy metals. Additionally, Cd retention exceeded Ni or Pb retention even after treatment with chelants EDTA and citric acid. This was potentially due to the formation of CdCO_3 in the soil matrix.
- EICP treatment using ES2 was observed to be better in terms of retaining heavy metals in the soil when compared to ES1 and ES3.
- The use of non-fat milk powder in the preparation of ES2 played a major role in boosting the UCS strength of the soil and also in retaining heavy metals in the soil due to the precipitation of CaCO_3 . Overall, the effects of CaCO_3 precipitation due to the ESs consisted of improvements in heavy metal retention and UCS strength when compared to those of raw soils. Hence, it was concluded that the precipitates of CaCO_3 hold heavy metals and improve UCS strength irrespective of whether the quantity of precipitation varies for different ESs.
- Based on these results, it is clear that EICP has sufficient ability to immobilize heavy metals in contaminated soil. This study portrayed appreciable outcomes for Cd when compared to Ni and Pb; this technique can be employed to immobilize specific contaminants by identifying its effectiveness on other heavy metals also. However, further testing in the field is necessary before drawing this conclusion.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/12/17/7019/s1>, Table S1: Removal efficiencies (%) for different contaminants for EDTA and Citric Acid extractants, Table S2: Standard deviation values obtained for the removal efficiencies.

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References

1. Chalermyanont, T.; Arrykul, S.; Charoenthaisong, N. Potential use of lateritic and marine clay soils as landfill liners to retain heavy metals. *Waste Manag.* **2009**, *29*, 117–127. [[CrossRef](#)] [[PubMed](#)]
2. Mohammed, S.A.S.; Moghal, A.A.B. Soils Amended with Admixtures as Stabilizing Agent to Retain Heavy Metals. In Proceedings of the Geo-Congress, Atlanta, Georgia, 23–26 February 2014; pp. 2216–2225. [[CrossRef](#)]
3. Tsang, D.C.W.; Lo, I.M.C.; Surampalli, R.Y. *Chelating Agents for Land Decontamination Technologies*; American Society of Civil Engineers: Reston, VA, USA, 2012; ISBN 978-0-7844-1218-3.

4. Reddy, K.R.; Kumar, G.; Giri, R.K. System Effects on Bioreactor Landfill Performance Based on Coupled Hydro-Bio-Mechanical Modeling. *J. Hazard. Toxic Radioact. Waste* **2018**, *22*, 04017024. [[CrossRef](#)]
5. Zhang, Q.; Lu, H.; Liu, J.; Wang, W.; Zhang, X. Hydraulic and mechanical behavior of landfill clay liner containing SSA in contact with leachate. *Environ. Technol.* **2018**, *39*, 1307–1315. [[CrossRef](#)] [[PubMed](#)]
6. Srivastava, S.K.; Ramanathan, A.L. Geochemical assessment of groundwater quality in vicinity of Bhalswa landfill, Delhi, India, using graphical and multivariate statistical methods. *Environ. Geol.* **2008**, *53*, 1509–1528. [[CrossRef](#)]
7. Wuana, R.A.; Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecol.* **2011**, *2011*. [[CrossRef](#)]
8. de Galvão, T.C.B.; Kaya, A.; Ören, A.H.; Yükselen, Y. Geomechanics of Landfills—Innovative Technology for Liners. *Soil Sediment Contam. Int. J.* **2008**, *17*, 411–424. [[CrossRef](#)]
9. Lo, I.M.-C. Innovative Waste Containment Barriers for Subsurface Pollution Control. *Pract. Period. Hazard. Toxic Radioact. Waste Manag.* **2003**, *7*, 37–45. [[CrossRef](#)]
10. Tuncan, A.; Tuncan, M.; Koyuncu, H.; Guney, Y. Use of natural zeolites as a landfill liner. *Waste Manag. Res. J. Int. Solid Wastes Public Clean. Assoc. ISWA* **2003**, *21*, 54–61. [[CrossRef](#)]
11. Naeini, S.A.; Gholampoor, N.; Jahanfar, M.A. Effect of leachate's components on undrained shear strength of clay-bentonite liners. *Eur. J. Environ. Civ. Eng.* **2019**, *23*, 395–408. [[CrossRef](#)]
12. Sariosseiri, F.; Muhunthan, B. Effect of cement treatment on geotechnical properties of some Washington State soils. *Eng. Geol.* **2009**, *104*, 119–125. [[CrossRef](#)]
13. Celaya, M.; Veisi, M.; Nazarian, S.; Puppala, A. Accelerated Design Process of Lime-Stabilized Clays. In Proceedings of the Geo-Frontiers, Dallas, TX, USA, 13–16 March 2011; pp. 4468–4478. [[CrossRef](#)]
14. Horpibulsuk, S.; Rachan, R.; Suddepong, A. Assessment of strength development in blended cement admixed Bangkok clay. *Constr. Build. Mater.* **2011**, *25*, 1521–1531. [[CrossRef](#)]
15. Dash, S.K.; Hussain, M. Lime Stabilization of Soils: Reappraisal. *J. Mater. Civ. Eng.* **2012**, *24*, 707–714. [[CrossRef](#)]
16. Fasihnikoutalab, M.H.; Asadi, A.; Kim Huat, B.; Westgate, P.; Ball, R.J.; Pourakbar, S. Laboratory-scale model of carbon dioxide deposition for soil stabilisation. *J. Rock Mech. Geotech. Eng.* **2016**, *8*, 178–186. [[CrossRef](#)]
17. Sabine, C.L.; Feely, R.A.; Gruber, N.; Key, R.M.; Lee, K.; Bullister, J.L.; Wanninkhof, R.; Wong, C.S.; Wallace, D.W.R.; Tilbrook, B.; et al. The Oceanic Sink for Anthropogenic CO₂. *Science* **2004**, *305*, 367–371. [[CrossRef](#)]
18. Feely, R.A.; Sabine, C.L.; Lee, K.; Berelson, W.; Kleypas, J.; Fabry, V.J.; Millero, F.J. Impact of Anthropogenic CO₂ on the CaCO₃ System in the Oceans. *Science* **2004**, *305*, 362–366. [[CrossRef](#)]
19. Yin, C.Y.; Wan Ali, W.S.; Lim, Y.P. Oil palm ash as partial replacement of cement for solidification/stabilization of nickel hydroxide sludge. *J. Hazard. Mater.* **2008**, *150*, 413–418. [[CrossRef](#)]
20. Horpibulsuk, S.; Rachan, R.; Raksachon, Y. Role of Fly Ash on Strength and Microstructure Development in Blended Cement Stabilized Silty Clay. *Soils Found.* **2009**, *49*, 85–98. [[CrossRef](#)]
21. Anupam, A.K.; Kumar, P.; Ransingchung, R.N.G.D. Performance evaluation of structural properties for soil stabilised using rice husk ash. *Road Mater. Pavement Des.* **2014**, *15*, 539–553. [[CrossRef](#)]
22. Ali, F.H.; Adnan, A.; Choy, C.K. Geotechnical properties of a chemically stabilized soil from Malaysia with rice husk ash as an additive. *Geotech. Geol. Eng.* **1992**, *10*, 117–134. [[CrossRef](#)]
23. Kampala, A.; Horpibulsuk, S. Engineering Properties of Silty Clay Stabilized with Calcium Carbide Residue. *J. Mater. Civ. Eng.* **2013**, *25*, 632–644. [[CrossRef](#)]
24. Pourakbar, S.; Asadi, A.; Huat, B.B.K.; Fasihnikoutalab, M.H. Soil stabilisation with alkali-activated agro-waste. *Environ. Geotech.* **2015**, *2*, 359–370. [[CrossRef](#)]
25. Almajed, A.; Tirkolaei, H.K.; Kavazanjian, E.; Hamdan, N. Enzyme Induced Biocemented Sand with High Strength at Low Carbonate Content. *Sci. Rep.* **2019**, *9*, 1135. [[CrossRef](#)] [[PubMed](#)]
26. Kavazanjian, E.; Almajed, A.; Hamdan, N. Bio-Inspired Soil Improvement Using EICP Soil Columns and Soil Nails. In Proceedings of the Grouting, Honolulu, HI, USA, 9–12 July 2017; pp. 13–22. [[CrossRef](#)]
27. Arab, M.; Omar, M.; Aljassmi, R.; Nasef, R.; Nassar, L.; Miro, S. EICP Cemented Sand Modified with Biopolymer. In *International Congress and Exhibition "Sustainable Civil Infrastructures"*; Springer: Cham, Switzerland, 2019; pp. 74–85.

28. Almajed, A.; Abbas, H.; Arab, M.; Alsabhan, A.; Hamid, W.; Al-Salloum, Y. Enzyme-Induced Carbonate Precipitation (EICP)-Based methods for ecofriendly stabilization of different types of natural sands. *J. Clean. Prod.* **2020**, *274*, 122627. [[CrossRef](#)]
29. Zhao, Z.; Hamdan, N.; Shen, L.; Nan, H.; Almajed, A.; Kavazanjian, E.; He, X. Biomimetic Hydrogel Composites for Soil Stabilization and Contaminant Mitigation. *Environ. Sci. Technol.* **2016**, *50*, 12401–12410. [[CrossRef](#)]
30. Singh, R.K.; Datta, M.; Nema, A.K.; Pérez, I.V. Evaluating Groundwater Contamination Hazard Rating of Municipal Solid Waste Landfills in India and Europe Using a New System. *J. Hazard. Toxic Radioact. Waste* **2013**, *17*, 62–73. [[CrossRef](#)]
31. Kumar, M.; Gogoi, A.; Kumari, D.; Borah, R.; Das, P.; Mazumder, P.; Tyagi, V.K. Review of Perspective, Problems, Challenges, and Future Scenario of Metal Contamination in the Urban Environment. *J. Hazard. Toxic Radioact. Waste* **2017**, *21*, 04017007. [[CrossRef](#)]
32. Mohammed, S.A.S.; Sanaulla, P.F.; Moghal, A.A.B. Sustainable Use of Locally Available Red Earth and Black Cotton Soils in Retaining Cd²⁺ and Ni²⁺ from Aqueous Solutions. *Int. J. Civ. Eng.* **2016**, *14*, 491–505. [[CrossRef](#)]
33. Steinnes, E.; Lierhagen, S. Geographical distribution of trace elements in natural surface soils: Atmospheric influence from natural and anthropogenic sources. *Appl. Geochem.* **2018**, *88*, 2–9. [[CrossRef](#)]
34. Srinivasa Gowd, S.; Ramakrishna Reddy, M.; Govil, P.K. Assessment of heavy metal contamination in soils at Jajmau (Kanpur) and Unnao industrial areas of the Ganga Plain, Uttar Pradesh, India. *J. Hazard. Mater.* **2010**, *174*, 113–121. [[CrossRef](#)]
35. Violante, A.; Pigna, M. Sorption-Desorption Processes of Metals and Metalloids in Soil Environments. *Rev. Cienc. Suelo Nutr. Veg.* **2008**, *8*, 95–101. [[CrossRef](#)]
36. dos Santos, N.M.; do Nascimento, C.W.A.; de Aguiar Accioly, A.M. Guideline Values and Metal Contamination in Soils of an Environmentally Impacted Bay. *Water Air Soil Pollut.* **2017**, *228*, 88. [[CrossRef](#)]
37. DeJong, J.T.; Kavazanjian, E. Bio-mediated and Bio-inspired Geotechnics. In *Geotechnical Fundamentals for Addressing New World Challenges*; Lu, N., Mitchell, J.K., Eds.; Springer Series in Geomechanics and Geoenvironmental Engineering; Springer International Publishing: Cham, Switzerland, 2019; pp. 193–207, ISBN 978-3-030-06249-1.
38. Salt, D.E.; Blaylock, M.; Kumar, N.P.B.A.; Dushenkov, V.; Ensley, B.D.; Chet, I.; Raskin, I. Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants. *Bio/Technology* **1995**, *13*, 468–474. [[CrossRef](#)] [[PubMed](#)]
39. Ciomasu, I.M.; Costica, M.; Costica, N.; Neamtu, M.; Dirtu, A.C.; de Alencastro, L.F.; Buzdugan, L.; Andriesa, R.; Iconomu, L.; Stratu, A.; et al. Complex Risks from Old Urban Waste Landfills: Sustainability Perspective from Iasi, Romania. *J. Hazard. Toxic Radioact. Waste* **2012**, *16*, 158–168. [[CrossRef](#)]
40. Rojas, L.A.; Yáñez, C.; González, M.; Lobos, S.; Smalla, K.; Seeger, M. Characterization of the metabolically modified heavy metal-resistant *Cupriavidus metallidurans* strain MSR33 generated for mercury bioremediation. *PLoS ONE* **2011**, *6*, e17555. [[CrossRef](#)]
41. Mulligan, C.N.; Yong, R.N.; Gibbs, B.F. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. *Eng. Geol.* **2001**, *60*, 193–207. [[CrossRef](#)]
42. Moghal, A.A.B.; Mohammed, S.A.S.; Almajed, A.; Al-Shamrani, M.A. Desorption of Heavy Metals from Lime-Stabilized Arid-Soils using Different Extractants. *Int. J. Civ. Eng.* **2020**, *18*, 449–461. [[CrossRef](#)]
43. Dermont, G.; Bergeron, M.; Mercier, G.; Richer-Lafleche, M. Metal-Contaminated Soils: Remediation Practices and Treatment Technologies. *Pract. Period. Hazard. Toxic Radioact. Waste Manag.* **2008**, *12*, 188–209. [[CrossRef](#)]
44. Sahu, Y.K.; Deb, M.K.; Patel, K.S.; Martín-Ramos, P.; Towett, E.K.; Tarkowska-Kukuryk, M. Bioaccumulation of Nutrients and Toxic Elements with Macrophytes. *J. Hazard. Toxic Radioact. Waste* **2020**, *24*, 05019007. [[CrossRef](#)]
45. Muhammad, D.; Chen, F.; Zhao, J.; Zhang, G.; Wu, F. Comparison of EDTA- and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. *Int. J. Phytoremediat.* **2009**, *11*, 558–574. [[CrossRef](#)]
46. Mishra, S.P. Adsorption-desorption of heavy metal ions. *Curr. Sci.* **2014**, *107*, 12.
47. Goldberg, S.; Criscenti, L.J.; Turner, D.R.; Davis, J.; Cantrell, K.J. Adsorption-Desorption Processes in Subsurface Reactive Transport Modeling. *Vadose Zone J.* **2007**, *6*, 407–435. [[CrossRef](#)]
48. Blais, J.F.; Djedidi, Z.; Cheikh, R.B.; Tyagi, R.D.; Mercier, G. Metals Precipitation from Effluents: Review. *Pract. Period. Hazard. Toxic Radioact. Waste Manag.* **2008**, *12*, 135–149. [[CrossRef](#)]

49. Moghal, A.A.B.; Reddy, K.R.; Mohammed, S.A.S.; Al-Shamrani, M.A.; Zahid, W.M. Lime-Amended Semi-arid Soils in Retaining Copper, Lead, and Zinc from Aqueous Solutions. *Water Air Soil Pollut.* **2016**, *227*, 372. [[CrossRef](#)]
50. Mohammed, S.A.S.; Moghal, A.A.B. Efficacy of nano calcium silicate (NCS) treatment on tropical soils in encapsulating heavy metal ions: Leaching studies validation. *Innov. Infrastruct. Solut.* **2016**, *1*, 21. [[CrossRef](#)]
51. Moghal, A.A.B.; Reddy, K.R.; Mohammed, S.A.S.; Al-Shamrani, M.A.; Zahid, W.M. Sorptive Response of Chromium (Cr^{+6}) and Mercury (Hg^{+2}) From Aqueous Solutions Using Chemically Modified Soils. *J. Test. Eval.* **2017**, *45*, 105–119. [[CrossRef](#)]
52. Madhu, A.; Chakraborty, J.N. Developments in application of enzymes for textile processing. *J. Clean. Prod.* **2017**, *145*, 114–133. [[CrossRef](#)]
53. Nathan, V.K.; Rani, M.E.; Gunaseeli, R.; Kannan, N.D. Enhanced biobleaching efficacy and heavy metal remediation through enzyme mediated lab-scale paper pulp deinking process. *J. Clean. Prod.* **2018**, *203*, 926–932. [[CrossRef](#)]
54. Almajed, A.A. Enzyme Induced Carbonate Precipitation (EICP) for Soil Improvement. Ph.D. Thesis, Arizona State University, Tempe, AZ, USA, 2017.
55. Almajed, A. Enzyme induced cementation of biochar-intercalated soil: Fabrication and characterization. *Arab. J. Geosci.* **2019**, *12*, 403. [[CrossRef](#)]
56. Almajed, A.; Khodadadi, H.; Kavazanjian, E. Sisal Fiber Reinforcement of EICP-Treated Soil. In Proceedings of the IFCEE, Orlando, FL, USA, 5–10 March 2018; pp. 29–36. [[CrossRef](#)]
57. Pasillas, J.N.; Khodadadi, H.; Martin, K.; Bandini, P.; Newton, C.M.; Kavazanjian, E. Viscosity-Enhanced EICP Treatment of Soil. In Proceedings of the IFCEE, Orlando, FL, USA, 5–10 March 2018; pp. 145–154. [[CrossRef](#)]
58. Javadi, N.; Khodadadi, H.; Hamdan, N.; Kavazanjian, E. EICP Treatment of Soil by Using Urease Enzyme Extracted from Watermelon Seeds. In Proceedings of the IFCEE, Orlando, FL, USA, 5–10 March 2018; pp. 115–124. [[CrossRef](#)]
59. Oliveira, P.J.V.; Freitas, L.D.; Carmona, J.P.S.F. Effect of Soil Type on the Enzymatic Calcium Carbonate Precipitation Process Used for Soil Improvement. *J. Mater. Civ. Eng.* **2017**, *29*, 04016263. [[CrossRef](#)]
60. Zhao, Q.; Li, L.; Li, C.; Li, M.; Amini, F.; Zhang, H. Factors Affecting Improvement of Engineering Properties of MICP-Treated Soil Catalyzed by Bacteria and Urease. *J. Mater. Civ. Eng.* **2014**, *26*, 04014094. [[CrossRef](#)]
61. Venda Oliveira, P.J.; da Costa, M.S.; Costa, J.N.P.; Nobre, M.F. Comparison of the Ability of Two Bacteria to Improve the Behavior of Sandy Soil. *J. Mater. Civ. Eng.* **2015**, *27*, 06014025. [[CrossRef](#)]
62. Hommel, J.; Akyel, A.; Frieling, Z.; Phillips, A.J.; Gerlach, R.; Cunningham, A.B.; Class, H. A Numerical Model for Enzymatically Induced Calcium Carbonate Precipitation. *Appl. Sci.* **2020**, *10*, 4538. [[CrossRef](#)]
63. Chandra, A.; Ravi, K. Application of Enzyme Induced Carbonate Precipitation (EICP) to improve the shear strength of different type of soils. In Proceedings of the Indian Geotechnical Conference, Bengaluru, India, 13–15 December 2018; p. 8.
64. Handley-Sidhu, S.; Sham, E.; Cuthbert, M.O.; Nougazol, S.; Mantle, M.; Johns, M.L.; Macaskie, L.E.; Renshaw, J.C. Kinetics of urease mediated calcite precipitation and permeability reduction of porous media evidenced by magnetic resonance imaging. *Int. J. Environ. Sci. Technol.* **2013**, *10*, 881–890. [[CrossRef](#)]
65. Dharmakeerthi, R.S.; Thenabadu, M.W. Urease activity in soils: A review. *J. Natl. Sci. Found. Sri Lanka* **1996**, *24*, 159–195. [[CrossRef](#)]
66. Blakeley, R.L.; Zerner, B. Jack bean urease: The first nickel enzyme. *J. Mol. Catal.* **1984**, *23*, 263–292. [[CrossRef](#)]
67. Dejong, J.T.; Soga, K.; Kavazanjian, E.; Burns, S.; Van Paassen, L.A.; Al Qabany, A.; Aydilek, A.; Bang, S.S.; Burbank, M.; Caslake, L.F.; et al. Biogeochemical processes and geotechnical applications: Progress, opportunities and challenges. *Géotechnique* **2013**, *63*, 287–301. [[CrossRef](#)]
68. Mohammed, S.A.S.; Moghal, A.A.B.; Sanaulla, P.F.; Kotresha, K.; Reddy, H.P. Cadmium Fixation Studies on Contaminated Soils Using Nano Calcium Silicate—Treatment Strategy. In Proceedings of the Geotechnical Frontiers, Orlando, FL, USA, 12–15 March 2017; pp. 434–442. [[CrossRef](#)]
69. ASTM D2166/D2166M. *Test Method for Unconfined Compressive Strength of Cohesive Soil*; ASTM International: West Conshohocken, PA, USA, 2016.
70. Khodadoust, A.P.; Reddy, K.R.; Maturi, K. Removal of Nickel and Phenanthrene from Kaolin Soil Using Different Extractants. *Environ. Eng. Sci.* **2004**, *21*, 691–704. [[CrossRef](#)]

71. Gu, Y.-Y.; Yeung, A.T. Desorption of cadmium from a natural Shanghai clay using citric acid industrial wastewater. *J. Hazard. Mater.* **2011**, *191*, 144–149. [[CrossRef](#)]
72. ASTM D3987. *Standard Test Method for Shake Extraction of Solid Waste with Water*; ASTM International: West Conshohocken, PA, USA, 2012.
73. Moghal, A.A.B.; Ashfaq, M.; Al-Shamrani, M.A.; Al-Mahbashi, A. Effect of Heavy Metal Contamination on the Compressibility and Strength Characteristics of Chemically Modified Semi-arid Soils. *J. Hazard. Toxic Radioact. Waste* **2020**, *24*, 04020029. [[CrossRef](#)]
74. Dhami, N.K.; Reddy, M.S.; Mukherjee, A. Biomineralization of calcium carbonates and their engineered applications: A review. *Front. Microbiol.* **2013**, *4*, 314. [[CrossRef](#)]
75. Krajewska, B. Urease-aided calcium carbonate mineralization for engineering applications: A review. *J. Adv. Res.* **2018**, *13*, 59–67. [[CrossRef](#)] [[PubMed](#)]
76. Almajed, A.; Khodadadi Tirkolaei, H.; Kavazanjian, E. Baseline Investigation on Enzyme-Induced Calcium Carbonate Precipitation. *J. Geotech. Geoenviron. Eng.* **2018**, *144*, 04018081. [[CrossRef](#)]
77. Ran, D.; Kawasaki, S. Effective Use of Plant-Derived Urease in the Field of Geoenvironmental/Geotechnical Engineering. *J. Civ. Environ. Eng.* **2016**, *6*, 1–13. [[CrossRef](#)]
78. Madrid, L.; Diaz-Barrientos, E. Influence of carbonate on the reaction of heavy metals in soils. *J. Soil Sci.* **1992**, *43*, 709–721. [[CrossRef](#)]
79. Torres-Aravena, Á.E.; Duarte-Nass, C.; Azócar, L.; Mella-Herrera, R.; Rivas, M.; Jeison, D. Can Microbially Induced Calcite Precipitation (MICP) through a Ureolytic Pathway Be Successfully Applied for Removing Heavy Metals from Wastewaters? *Crystals* **2018**, *8*, 438. [[CrossRef](#)]
80. Vega, F.A.; Covelo, E.F.; Andrade, M.L. A versatile parameter for comparing the capacities of soils for sorption and retention of heavy metals dumped individually or together: Results for cadmium, copper and lead in twenty soil horizons. *J. Colloid Interface Sci.* **2008**, *327*, 275–286. [[CrossRef](#)] [[PubMed](#)]
81. Acosta, J.A.; Jansen, B.; Kalbitz, K.; Faz, A.; Martínez-Martínez, S. Salinity increases mobility of heavy metals in soils. *Chemosphere* **2011**, *85*, 1318–1324. [[CrossRef](#)]
82. González Costa, J.J.; Reigosa, M.J.; Matías, J.M.; Covelo, E.F. Soil Cd, Cr, Cu, Ni, Pb and Zn sorption and retention models using SVM: Variable selection and competitive model. *Sci. Total Environ.* **2017**, *593–594*, 508–522. [[CrossRef](#)]
83. Wang, Y.M.; Chen, T.C.; Yeh, K.J.; Shue, M.F. Stabilization of an elevated heavy metal contaminated site. *J. Hazard. Mater.* **2001**, *88*, 63–74. [[CrossRef](#)]
84. Zhou, D.M.; Wang, S.Q.; Chen, H.M. Interaction of Cd and citric acid, EDTA in red soil. *J. Environ. Sci.* **2001**, *13*, 153–156.
85. Manjeet, B.; Diwan, S.; Garg, V.K.; Pawan, R. Use of Agricultural Waste for the Removal of Nickel Ions from Aqueous Solutions: Equilibrium and Kinetics Studies. *Int. J. Environ. Sci. Eng.* **2009**, *1*, 108–114. [[CrossRef](#)]
86. Attar, K.; Demey, H.; Bouazza, D.; Sastre, A.M. Sorption and Desorption Studies of Pb(II) and Ni(II) from Aqueous Solutions by a New Composite Based on Alginate and Magadiite Materials. *Polymers* **2019**, *11*, 340. [[CrossRef](#)] [[PubMed](#)]
87. Karna, R.R.; Luxton, T.; Bronstein, K.E.; Redmon, J.H.; Scheckel, K.G. State of the science review: Potential for beneficial use of waste by-products for in situ remediation of metal-contaminated soil and sediment. *Crit. Rev. Environ. Sci. Technol.* **2017**, *47*, 65–129. [[CrossRef](#)] [[PubMed](#)]
88. Li, C.; Qian, Z.; Zhou, C.; Su, W.; Hong, P.; Liu, S.; He, L.; Chen, Z.; Ji, H. Mussel-inspired synthesis of polydopamine-functionalized calcium carbonate as reusable adsorbents for heavy metal ions. *RSC Adv.* **2014**, *4*, 47848–47852. [[CrossRef](#)]
89. Peters, R.W. Chelant extraction of heavy metals from contaminated soils. *J. Hazard. Mater.* **1999**, *66*, 151–210. [[CrossRef](#)]
90. Cline, S.R.; Reed, B.E. Lead Removal from Soils via Bench-Scale Soil Washing Techniques. *J. Environ. Eng.* **1995**, *121*, 700–705. [[CrossRef](#)]
91. Kumpiene, J.; Lagerkvist, A.; Maurice, C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—A review. *Waste Manag.* **2008**, *28*, 215–225. [[CrossRef](#)]
92. Tian, K.; Benson, C.H.; Tinjum, J.M. Chemical Characteristics of Leachate in Low-Level Radioactive Waste Disposal Facilities. *J. Hazard. Toxic Radioact. Waste* **2017**, *21*, 04017010. [[CrossRef](#)]
93. Harter, R.D. Effect of Soil pH on Adsorption of Lead, Copper, Zinc, and Nickel. *Soil Sci. Soc. Am. J.* **1983**, *47*, 47–51. [[CrossRef](#)]

94. Gupta, T.B.; Lataye, D.H. Adsorption of Indigo Carmine Dye onto *Acacia Nilotica* (Babool) Sawdust Activated Carbon. *J. Hazard. Toxic Radioact. Waste* **2017**, *21*, 04017013. [[CrossRef](#)]
95. Maturi, K.; Reddy, K.R. Extractants for the Removal of Mixed Contaminants from Soils. *Soil Sediment Contam. Int. J.* **2008**, *17*, 586–608. [[CrossRef](#)]



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