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# Earth stabilisation via carbonate precipitation by plant-derived urease for building applications

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**ABSTRACT:** Raw (unfired) earth represents a sustainable and efficient alternative to traditional construction materials but its dissemination into building practice has been hindered by a relatively high vulnerability to water erosion. Enzyme induced carbonate precipitation (EICP) can improve the durability of earth materials without using traditional chemical binders such as cement and lime. EICP utilises the urease enzyme to catalyse the hydrolysis of urea, which produces carbonate ions that react with the calcium ions dissolved in the pore water, thus resulting in the precipitation of calcium carbonate. The calcium carbonate fills the soil voids and binds particles together, which reduces water permeability and increases material strength. The urease enzyme is a hexameric protein that is found in the tissues of many common plants. This work proposes a low-cost and simple stabilisation technology that makes use of crude urease enzyme extracted from soybeans. This technology is applied to the stabilisation of compacted earth, whose properties are then assessed via unconfined compression, moisture buffering and durability tests. The findings suggest a noticeable improvement of material strength and durability, though further investigation is necessary to increase the competitiveness of EICP stabilisation against standard techniques using cement and lime.

**Keywords:** Soil stabilisation; Biocementation; Enzyme induced carbonate precipitation (EICP); Plant-derived urease; Earth construction

## 1. INTRODUCTION

### 1.1. Context

Raw earth is a construction material consisting of a compacted mix of soil and water which is put in place with the least possible transformation.<sup>1</sup> Archaeological evidence from clay-rich regions of ancient Mesopotamia (between the Euphrates and Tigris rivers) and Afghanistan indicates that raw earth was used for the construction of buildings since 10000 BC. Earth is harmless to humans, can be locally sourced and is easily transported to the construction site. Earth is also recyclable, inexhaustible and, when properly manufactured, offers high strength, excellent hygrothermal properties and small embodied energy at very low costs. Therefore, raw earth materials can dramatically reduce exploitation of natural resources not only during construction of buildings but also during the service life by cutting heating/air conditioning needs and limiting demolition waste.<sup>2</sup>

Despite these advantages, the dissemination of earth materials into routine building has been limited mainly because of their high vulnerability to water erosion. Earth rapidly absorb any free water that it meets,<sup>3,4</sup> generally exhibiting a linear increase of moisture content with the square root of time.<sup>5,6</sup> Moisture ingress reduces the strength and stiffness of the earth while producing structural damages caused by swelling of the clay fraction. For example, erosion depths between 5 mm and 10 mm have been reported on the surface of 400 mm thick unstabilised earth wallets exposed to a wet continental climate for twenty years.<sup>7</sup> Therefore, well-preserved earth buildings, dating back hundreds of years, are relatively common in dry climates but are unusual in wet climates.

One of the main challenges lies in the improvement of earth durability by means of stabilisation methods that avoid energy intensive processes or environmentally unfriendly binders such as cement or lime. Stabilisation by cement or lime undermines the hygroscopic properties of raw earth, thus reducing the hygro-thermal inertia of building walls with a consequent increase of operational

48 energy.<sup>8,9</sup> Alternative chemical binders, such polymers or alkoxysilanes, have also been used to  
49 stabilise the superficial layer of earth walls exposed to environmental actions. These techniques have,  
50 however, encountered limited success as they do not counteract the swelling/shrinkage of clay  
51 minerals but only diminish the symptoms of the wearing process, thus being ineffective in the long-  
52 term.<sup>10</sup>

## 53 1.2. Research objectives

54 This paper describes a biomediated earth stabilisation method that relies on the hydrolysis of urea  
55 (ureolysis) to induce the precipitation of calcium carbonate with the consequent enhancement of  
56 material properties.<sup>11-16</sup> The application of microbial processes to the catalysis of ureolytic reactions  
57 inside soils has captured the attention of many scientists across the world.<sup>13,17-21</sup> Past research has  
58 shown that ureolytic reactions are catalysed by the urease enzyme, which is produced by the metabolic  
59 activity of microbes, either exogenous or endogenous to the soil.<sup>13-15,18,22-31</sup> Based on these findings,  
60 researchers have developed microbially induced carbonate precipitation (MICP) techniques, which  
61 have proven generally successful in stabilising earth materials but have also displayed several  
62 shortcomings. The main shortcoming is perhaps the unsuitability of MICP to the stabilisation of  
63 compacted fine-grained materials (i.e., clays and silts) due to the impossibility of microorganisms to  
64 migrate through very small pores.<sup>32</sup> For example, the *Sporosarcina pasteurii* bacteria have a size of the  
65 order of microns, which is larger than most pores inside fine-grained materials. Furthermore, microbial  
66 colonies remain alive inside the soil long after the stabilisation reactions have occurred with potential  
67 negative impacts on the surrounding ecosystem.

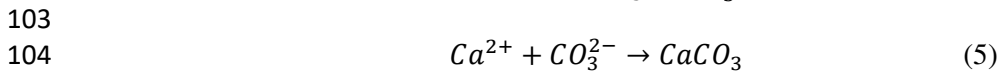
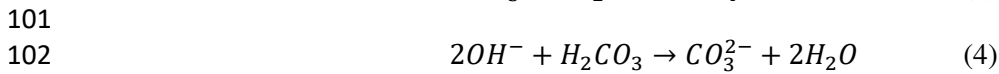
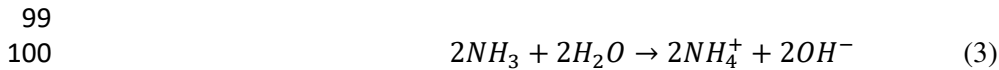
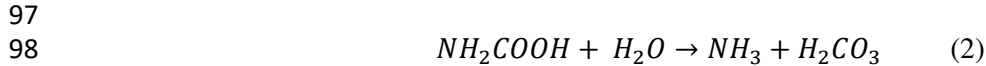
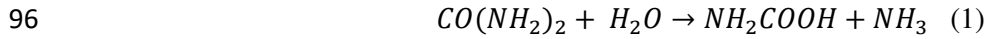
68 Because of the above limitations, some authors have recently proposed the direct use of the urease  
69 enzyme as an alternative to the cultivation of microbes inside the soil, which has led to a new category  
70 of enzyme induced carbonate precipitation (EICP) techniques for the stabilisation of earth  
71 materials.<sup>16,33-40</sup> The molecule of the urease enzyme has a size of around 12 nm and can fit in very  
72 small pores, thus overcoming the main disadvantage of MICP. Furthermore, unlike microbial colonies,  
73 the urease enzyme has a relatively short life span with an activity that naturally degrades with time,  
74 thus avoiding any significant impact on the surrounding ecosystem.<sup>41</sup> Traditional EICP techniques  
75 make use of pure reagent-grade urease enzyme that is purchased from chemical suppliers at relatively  
76 high cost. Conversely, the present work promotes the use of crude plant-derived urease enzyme via  
77 simple and cost-effective processes that can be easily adopted by building practitioners.<sup>36,42-45</sup>

78 The paper starts with an overview of the key aspects that govern the kinetics of urea hydrolysis. This  
79 provides the basis for optimising the catalyst action of the urease enzyme and, therefore, maximising  
80 calcium carbonate precipitation. A larger precipitation of calcium carbonate is here assumed to  
81 produce a greater improvement of the mechanical characteristics of the earth material. An optimised  
82 EICP method is devised and applied to the stabilisation of compacted silty clay samples, which are  
83 subjected to unconfined compression, moisture buffering and durability tests to assess the  
84 improvement of hygro-mechanical properties.

## 85 2. ENZYME INDUCED CARBONATE PRECIPITATION (EICP)

### 86 2.1. Chemical reactions leading to calcium carbonate precipitation

87 Equations 1-5 illustrate the EICP reactions that are catalysed by the free urease enzyme in an aqueous  
88 solution of urea and calcium chloride. The urease enzyme hydrolyses one mole of urea ( $\text{CO}(\text{NH}_2)_2$ )  
89 into one mole of ammonia ( $\text{NH}_3$ ) and one mole of carbamic acid ( $\text{NH}_2\text{COOH}$ ) (Equation 1).<sup>16</sup> One  
90 mole of  $\text{NH}_2\text{COOH}$  is then hydrolysed into another mole of  $\text{NH}_3$  plus one mole of carbonic acid  
91 ( $\text{H}_2\text{CO}_3$ ) (Equation 2). Carbonate ions ( $\text{CO}_3^{2-}$ ), ammonium ions ( $\text{NH}_4^+$ ) and hydroxide ions ( $\text{OH}^-$ )  
92 equilibrate in water as the result of the above reactions (Equations 3 and 4). Hydroxide ions ( $\text{OH}^-$ ), in  
93 particular, lead to an increase of the alkalinity of the solution. In the presence of calcium ions ( $\text{Ca}^{2+}$ ),  
94 under supersaturated conditions, one mole of  $\text{CO}_3^{2-}$  then precipitates into one mole of calcium  
95 carbonate ( $\text{CaCO}_3$ ) (Equation 5), which is the ultimate objective of the proposed stabilisation method.



105 The above reactions indicate that precipitation of calcium carbonate is a rather straightforward  
106 chemical process that is governed by the following factors: the source of calcium ions, the  
107 concentration of urea and calcium ions<sup>21,46</sup> and, finally, the pH and temperature of the solution.<sup>47,48</sup> The  
108 next sections briefly describe the influence of each one of these factors on the precipitation of calcium  
109 carbonate.

## 110 2.2 Source of urease enzyme

111 The urease enzyme was the first nickel metalloenzyme ever purified and crystallised from jack beans  
112 (*Canavalia ensiformis*), an achievement that yielded the Nobel Prize in Chemistry to James B.  
113 Summer in 1946. Since then, different types of nickel dependent ureases have been isolated from  
114 bacteria, fungi and plants. Chemical suppliers commercialise pure reagent-grade urease enzyme,  
115 which is effective for catalysing the hydrolysis of urea, but it is also very expensive and, therefore, not  
116 suitable for earth construction. Only a few studies have explored the use of urease extracted from  
117 plants by means of simple and cost-effective techniques, referred to herein as crude extracts.<sup>36,42-45</sup>  
118 Among these studies, those making use of crude jack bean extract are the majority.

119 Larsen et al.<sup>42</sup> used jack bean meal as a source of urease (with an activity of 1,700 Units/g) for  
120 plugging pervious strata in oil wells. The use of jack bean meal resulted in increasing the amount of  
121 precipitation compared to the use of high-purity commercially available urease (with an activity of  
122 150,000 Units/g). The authors of the study concluded that the presence of other proteins in crude  
123 extracts prevents the coprecipitation of the urease protein with  $CaCO_3$  by exercising a protective effect  
124 on the urease enzyme and consequently leading to greater  $CaCO_3$  precipitation. The study reported a  
125 tenfold increase in  $CaCO_3$  precipitation (from 20 to 200 grams per liter) when high-purity commercial  
126 enzyme was replaced with jack bean meal. Similar results were obtained from Tirkolaei et al.<sup>45</sup> who  
127 studied the cementation of a granular soil via EICP using crude extracts from different plants (i.e., jack  
128 bean, soybean and watermelon seeds) and commercially available enzymes. Test-tubes experiments  
129 showed that, among the plants tested, the crude jack bean provided the highest unit yield defined as  
130 urease content per initial mass of source material. Unconfined compression tests on the cemented soil  
131 demonstrated that the crude extract is also more effective at enhancing soil strength compared to  
132 commercially available highly purified urease. In line with Larsen et al.,<sup>42</sup> Tirkolaei et al.<sup>45</sup> therefore  
133 suggested that the presence of organic impurities and complementary proteins enhances the  
134 effectiveness of EICP. Park et al.<sup>36</sup> treated a sandy soil with various amounts of jack bean extract,  
135 urea, and different calcium sources (i.e., calcium chloride, calcium hydroxide and calcium nitrate).  
136 They found that, as the amount of urea increased, the unconfined compression strength of the treated  
137 soil increased up to 317 kPa. This level of measured strength was tenfold greater than that of the  
138 untreated soil and like that of the soil stabilised with 4% of high-early strength Portland cement.  
139 Furthermore, the use of calcium chloride yielded the highest strength levels compared to the other two  
140 calcium sources. Other studies<sup>43,44</sup> explored extraction of urease enzyme from soybeans and  
141 watermelon seeds with similar conclusions.

## 142 2.3 Source of $Ca^{2+}$ ions

143 Mixing concentrated solutions of  $Ca^{2+}$  and  $CO_3^{2-}$  ions leads to the precipitation of calcium carbonate  
144 in three consecutive steps: a) formation of amorphous calcium carbonate characterised by low stability

145 and high solubility, b) transformation of amorphous calcium carbonate into vaterite and c)  
146 transformation of thermodynamically unstable vaterite into stable calcite.<sup>49-51</sup> Furthermore, different  
147 sources of  $\text{Ca}^{2+}$  ions induce precipitation of calcium carbonate crystals with different shapes as  
148 revealed by electron microscopy. The rhombohedral shape, which is induced by calcium chloride  
149 ( $\text{CaCl}_2$ ), is characteristic of calcite, i.e. the most stable form of calcium carbonate.<sup>52-54</sup> Instead, calcium  
150 acetate induces a lettuce like or lamellar shape that is characteristic of vaterite, a metastable form of  
151 calcium carbonate. Finally, calcium lactate and calcium gluconate induce a more complex growth of  
152 vaterite crystals with spherical shapes.<sup>55</sup> Overall, calcium chloride appears the best option to enhance  
153 urease activity and maximise the production of a stable form of calcium carbonate.<sup>56</sup>

#### 154 **2.4 Concentration of chemical reagents (urea and $\text{Ca}^{2+}$ ions)**

155 Nemati and Voordouw<sup>33</sup> conducted one of the earliest investigations into the parameters that affect the  
156 efficiency of EICP. Their objective was to enhance fossil fuels recovery by consolidating sand in oil  
157 reservoirs and tracing contaminants in ground water systems. Nemati and Voordouw<sup>33</sup> conducted test-  
158 tubes experiments to evaluate the influence of the amount of urease enzyme and the concentration of  
159 reagents (i.e. urea, calcium chloride and skim milk powder as stabiliser) on the rate and quantity of  
160 calcium carbonate precipitation. They concluded that, for a constant concentration of reagents, an  
161 increase in the amount of urease enzyme produced a faster reaction. Conversely, a proportional  
162 increase of reagents concentration resulted in a slower reaction, though the final quantity of precipitate  
163 increased if sufficient time was allowed. Nemati and Voordouw<sup>33</sup> concluded that, as the concentration  
164 of reagents increases, longer curing times are needed for completely converting reactants into  
165 products. Other studies<sup>34,35,39,56,58,59</sup> confirmed that, for a fixed amount of urease enzyme, higher  
166 concentrations of urea and calcium chloride yield greater quantities of precipitate as curing time  
167 increases. Nevertheless, if the amount of urease enzyme and curing time are both fixed, there is an  
168 optimal concentration of the reagents leading to the highest precipitation of calcium carbonate.

169 Urease activity also plays a key role in determining the optimal amount of enzyme, i.e. the amount that  
170 maximises reaction rate while avoiding inhibition of catalysis at high reagents concentrations. Nemati  
171 and Voordouw<sup>33</sup> used purified urease with a very high activity of 26,100 Units/g, which explains why  
172 only a very little amount of enzyme was necessary (0.01-0.1 g/L) to initiate the ureolytic reactions  
173 without any inhibition of catalysis at all reagents concentration. Other studies, which employed  
174 enzymes of different nature and activity, observed instead inhibition of catalysis at large reagents  
175 concentrations with a consequent reduction of calcium carbonate precipitation.

#### 176 **2.5 pH**

177 The pH of the reaction medium is a crucial abiotic factor that influences the activity of the urease  
178 enzyme during hydrolysis of urea.<sup>60</sup> Rohy et al.<sup>61</sup> observed that an acidic reaction medium delays the  
179 EICP reaction rate, which is also consistent with the results from Cheng et al.<sup>62</sup> about delayed MICP  
180 stabilisation. Rohy et al.<sup>61</sup> investigated the compressive strength of stabilised soil samples with 100  
181 mm height and 50 mm diameter where, for each increment in molarity of the reagents (urea- $\text{CaCl}_2$ ), a  
182 greater quantity of purified urease was used. Measurements of pH showed that acidity tended to  
183 increase with the increase in molarity of the reagents with values of pH equal to 5.69 and 4.46 for 1  
184 mol/L and 3 mol/L solutions, respectively. This decrease of pH was shown to be due to the increase in  
185 urea concentration and the subsequent addition of free urease led to a slower rate of carbonate  
186 precipitation. Distinct samples were then prepared with a 3 mol/L solution and cured for 3, 7 and 14  
187 days, respectively. Samples cured for 14 days showed a compressive strength of 3 MPa, which is  
188 equal to an increase of 500% compared to the strength of samples cured for only 3 days. This increase  
189 of strength confirmed that a period of 3 days was not enough for the full stabilisation to take place  
190 because of the lower rate of carbonate precipitation at lower levels of pH.

#### 191 **2.6 Temperature**

192 Neupane et al.<sup>63</sup> investigated the effect of temperature on both EICP reaction rate and amount of  
193 precipitate inside coarse sand samples of 5 cm diameter and 100 cm height. Their objective was to  
194 reduce the reaction rate in order to achieve an uniform distribution of precipitate over large soil areas.  
195 The sample columns were injected with two cementing solutions, containing fixed amounts of urea,  
196 calcium chloride and purified urease enzyme at mixing temperatures of 5 °C and 23.5 °C, respectively.  
197 The solution at temperature of 23.5 °C generated very large amounts of calcium carbonate near the  
198 injection point. Precipitation progressively reduced to a depth of 60 cm, beyond which no significant  
199 amount of precipitate was detected. On the contrary, the columns injected with cementing solution at  
200 temperature of 5 °C, exhibited a smaller but much more uniform precipitation of calcium carbonate.  
201 This indicates that a decrease of temperature from 23.5 °C to 5 °C reduces the reaction rate and  
202 induces smaller amounts of precipitation. These results are in agreement with those of Nemati and  
203 Voordouw,<sup>33</sup> who found that the reactants conversion rate was 100% after 120 hours at temperatures  
204 between 30°C and 50°C but it dropped to 70% after 300 hours at a temperature of 20 °C. Similarly,  
205 van Paassen<sup>64</sup> found that the rate of ureolysis doubled approximately every 8 °C as temperature  
206 increased from 5 °C to 70 °C.

207 Nevertheless, increasing temperature to very high levels may lead to a progressive denaturation of the  
208 urease enzyme. For example, Illeová et al.<sup>65</sup> demonstrated that enzyme activity was completely lost  
209 after 40 min of exposure to a temperature of 87.5 °C. Dhami et al.<sup>66</sup> found that the urease enzyme was  
210 stable at 35 °C but its activity decreased by almost 47% when temperature increased to 55 °C. Wu et  
211 al.<sup>67</sup> investigated urea hydrolysis in the absence of a calcium source and observed decreasing rates of  
212 ammonium production at temperatures above 30 °C with no ammonium production at 50 °C.

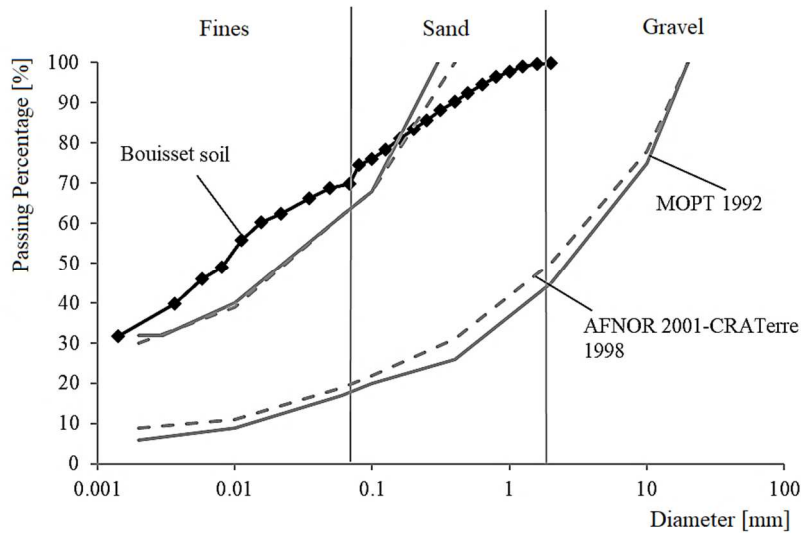
### 213 3. MATERIALS AND METHODS

214

#### 215 3.1. Bouisset soil

216 The soil used in the present work was provided by the brickwork factory Bouisset from the region of  
217 Toulouse (France). It is one of the five soils, supplied by different brickwork factories in France,  
218 which were tested in the project TERCRUSO.<sup>68</sup> The project TERCRUSO<sup>68</sup> showed that the Bouisset  
219 soil possesses similar hygro-mechanical characteristics, but better water durability properties,  
220 compared to the other four soils, which is the reason why it was selected in the present study.

221 Fig. 1 shows the grain size distribution of the Bouisset soil, which was determined as the average of  
222 three independent measurements by means of wet sieving and sedimentation tests in compliance with  
223 the norms XP P94-041<sup>69</sup> and NF P 94-057.<sup>70</sup> Fig. 1 also presents the lower and upper limits of the  
224 recommended grading ranges for the manufacture of compressed earth bricks according to AFNOR<sup>71</sup>;  
225 CRATerre-EAG<sup>72</sup> and MOPT.<sup>73</sup>



226

227 Fig. 1: Grain size distribution of the Bouisset soil in relation to existing recommendations for the manufacture of  
 228 compressed earth bricks by AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> and MOPT.<sup>73</sup>

229 Inspection of Fig. 1 indicates that the Bouisset soil exhibits a well graded grain size distribution that is  
 230 slightly finer than the limit prescribed by current recommendations for compressed earth bricks. The  
 231 specific gravity of the solid particles,  $G_s$  is reported in Table 1 and was measured as the average of  
 232 four pycnometer tests performed according to the norm NF P 94-054.<sup>74</sup> The plasticity properties of the  
 233 fine fraction (i.e. the fraction smaller than 0.400 mm) were measured according to the norm NF P94-  
 234 051.<sup>75</sup> In particular, the Atterberg limits, i.e. the liquid limit  $w_L$ , plastic limit  $w_P$  and plasticity index  $I_P$ ,  
 235 were all determined as the average of four independent measurements and are given in Table 1.

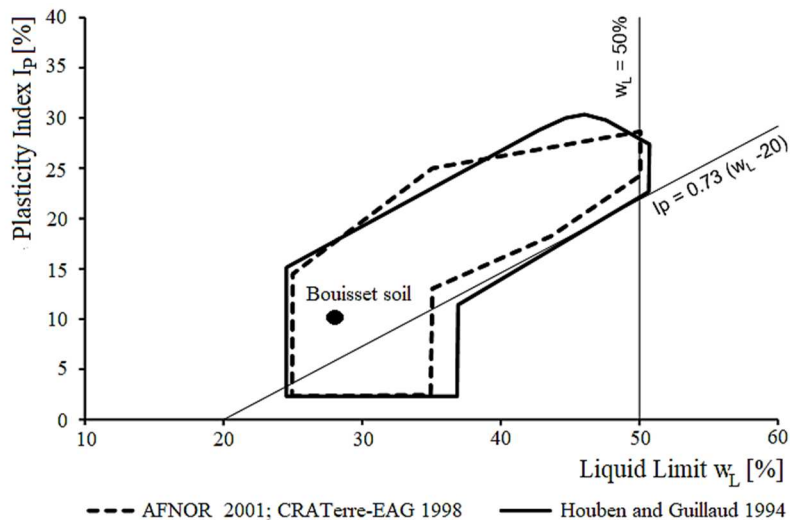
236 Fig. 2 shows the plasticity characteristics of the Bouisset soil with reference to the Casagrande chart,  
 237 which indicates that the material can be classified as a low plasticity clay. Note also that the Bouisset  
 238 soil falls inside the recommended plasticity region for the manufacture of compressed bricks according  
 239 to AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> and Houben and Guillaud.<sup>76</sup>

240 Previous studies of the Bouisset soil<sup>68</sup> have indicated a predominantly kaolinitic clay fraction, which  
 241 indicates a small tendency to swell/shrink upon wetting/drying and, therefore, makes this soil suitable  
 242 for earth construction. Another measure of the soil sensitivity to water is the clay activity,  $A$ , which is  
 243 defined as the ratio between the plasticity index and the percentage of clay fraction (i.e. the percentage  
 244 of the soil that is smaller than 0.002 mm). For the Bouisset soil, the activity has been established to be  
 245 0.30, which classifies this material as low active.<sup>76</sup>

246

Table 1: Main properties of Bouisset soil (after Cuccurullo et al.<sup>78</sup>).

<b>Grain size distribution</b>	
Gravel content (> 2 mm, %)	0
Sand content ( $\leq 2$ mm, %)	31
Silt content ( $\leq 63$ $\mu\text{m}$ , %)	35
Clay content ( $\leq 2$ $\mu\text{m}$ , %)	34
<b>Specific gravity, <math>G_s</math></b>	2.65
<b>Atterberg limits</b>	
Plastic limit, $w_P$ (%)	18.7
Liquid limit, $w_L$ (%)	29.0
Plasticity index, $I_P$ (%)	10.3
<b>Clay activity, <math>A</math></b>	0.30



247

248 Fig. 2: Plasticity properties of the Bouisset soil in relation to existing recommendations for the manufacture of  
 249 compressed earth bricks by Houben and Guillaud<sup>76</sup> and AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> (after Bruno<sup>9</sup>).

### 250 3.2. Plant-derived urease enzyme

251 The present work has explored a new cost-effective EICP stabilisation method that is based on the use  
 252 of crude urease enzyme extracted from soybeans. Soybeans have been either centrifuged into a juice or  
 253 crushed into a fine powder to provide two alternative sources of the urease enzyme, which have been  
 254 tested to assess their effectiveness.

#### 255 3.2.1. Soybeans liquid extract

256 The procedure for obtaining the liquid urease extract consisted in soaking dry soybeans into distilled  
 257 water (in a ratio of 1g:10 ml) for 24 hours before centrifuging both beans and water in a 700-Watt  
 258 blender for 1 minute (Fig. 3). This resulted in the extraction of a semi-transparent grey-yellow juice,  
 259 which was collected in a glass while the crushed beans were retained in the blender. To increase  
 260 enzyme concentration, the crushed beans were recovered from the blender and centrifuged two more  
 261 times to extract additional liquid that was mixed with the previously collected juice.



262

263 Fig. 3: Procedure for obtaining soybeans liquid extract: soybeans are soaked in water (a), soaked soybeans are  
 264 centrifuged in a blender (b), soybeans juice is collected (c).

#### 265 3.2.2. Fine soybeans powder

266 An alternative procedure was also followed to obtain the urease enzyme in the form of fine soybeans  
 267 powder. Soybeans were crushed by using an electrical grinder and passed through a sieve to collect the  
 268 finest particles with dimensions smaller than 0.400 mm (Fig. 4). This cut-off size was chosen to boost  
 269 the fine fraction of the earth mix and to avoid that material imperfections, caused by the presence of  
 270 large inclusions, may increase the vulnerability of the stabilised earth to water erosion.



Fig. 4: Sieving of the fine fraction (smaller than 0.400 mm) of crushed soybeans.

### 3.3. Chemical reagents

The chemical reagents used in this work are urea and calcium chloride supplied by Labbox (Fig. 5), whose main properties are synthetically described in Table 2. Urea is a colourless and odourless organic material, which serves an important role in the metabolism of nitrogen compound and is the main nitrogen containing substance of the urine of mammals. It is also highly soluble in water and practically non-toxic. The hydrolysis of urea exhibits a high carbonate conversion rate compared to other calcium carbonate precipitation processes.<sup>14,79</sup> Calcium chloride is instead a salt that provides an excellent source of calcium ions because of its high hygroscopicity and solubility in water. The water solubility of calcium chloride at room temperature is up to 100 times greater than that of other calcium salts such as calcium hydroxide or calcium nitrate, which makes calcium chloride a very effective reagent to generate high concentrations of calcium ions. This is an important aspect to consider during EICP stabilisation because larger concentrations of calcium ions correspond to greater calcium carbonate precipitation.<sup>36</sup>



Fig. 5: Urea ( $\text{CO}(\text{NH}_2)_2$ ) powder and calcium chloride ( $\text{CaCl}_2$ ) flakes.

Table 2: Properties of the urea ( $\text{CO}(\text{NH}_2)_2$ ) and calcium chloride ( $\text{CaCl}_2$ ) used in the present work.

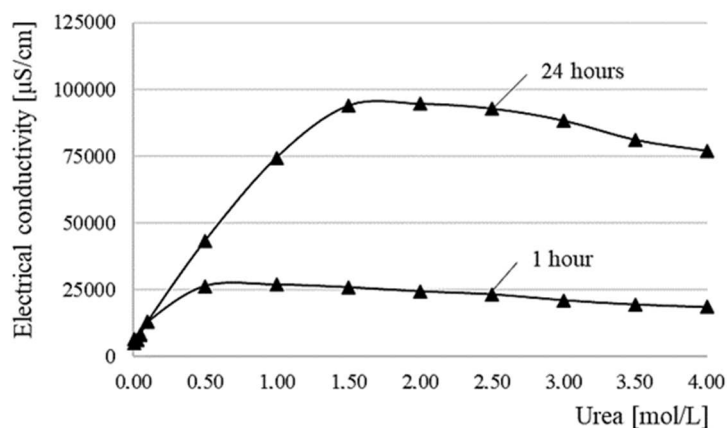
	Chemical formula	Labbox reference	MW (g/mol)	Appearance	Assay (%)
Urea	$\text{CO}(\text{NH}_2)_2$	UREA-00P-1K0	60.06	White crystals, powder	>99.6 %
Calcium chloride	$\text{CaCl}_2$	CACH-A0P-1K0	110.98	White pearls	>94 %

### 3.4. Preliminary test-tubes experiments

#### 3.4.1. Liquid soybeans extract

This section briefly recalls past research by the authors of this study<sup>80</sup> which is propaedeutic to the developments presented in this paper. This research investigated the influence of reagents concentration on the efficiency of the EICP reactions inside test-tubes containing liquid soybeans

296 extract. Twelve test-tubes with a capacity of 40 ml were filled with fresh liquid soybeans extract  
 297 produced as described in Section 3.2.1. The extract in each test-tube was mixed with twelve different  
 298 equimolar concentrations of urea and calcium chloride, namely 0.010 mol/L, 0.025 mol/L, 0.050  
 299 mol/L, 0.10 mol/L, 0.50 mol/L, 1.00 mol/L, 1.50 mol/L, 2.00 mol/L, 2.50 mol/L, 3.00 mol/L, 3.50  
 300 mol/L and 4.00 mol/L. The urea was added to the fresh liquid extract shortly after centrifugation of the  
 301 soybeans, while the calcium chloride was added after 24 hours. Electrical conductivity was measured  
 302 at 1 and 24 hours after the addition of urea. Fig. 6 compares the values of electrical conductivity  
 303 measured at these two times inside the test-tubes containing different concentrations of urea. The peak  
 304 value of electrical conductivity changes from about 27000  $\mu\text{S}/\text{cm}$  for an urea concentration of 0.50  
 305 mol/L after 1 hour to about 100000  $\mu\text{S}/\text{cm}$  for an urea concentration of 2.00 mol/L after 24 hours.  
 306 After 24 hours, equimolar concentrations of calcium chloride were introduced in each test-tube, which  
 307 induced the rapid precipitation of calcium carbonate at the bottom of the tubes as visually observed.  
 308 An equilibrium condition was reached after about 72 hours from the addition calcium chloride, with  
 309 no more material being deposited at the bottom of the tubes (Fig. 7). The material inside each test-tube  
 310 was then filtered and weighted, which showed that the amount of precipitate was largest for a  
 311 concentration of reagents between 1.50 mol/L and 2.50 mol/L. This concentration level is also  
 312 consistent with the electrical conductivity values measured in Fig. 6 and with the visual observation of  
 313 precipitate inside test-tubes in Fig. 7.



314  
 315 Fig. 6: Measurements of electrical conductivity 1 and 24 hours after adding urea to the liquid soybeans extract  
 316 (urea concentration varying from 0.010 mol/L to 4 mol/L) (after Cuccurullo et al.<sup>80</sup>).



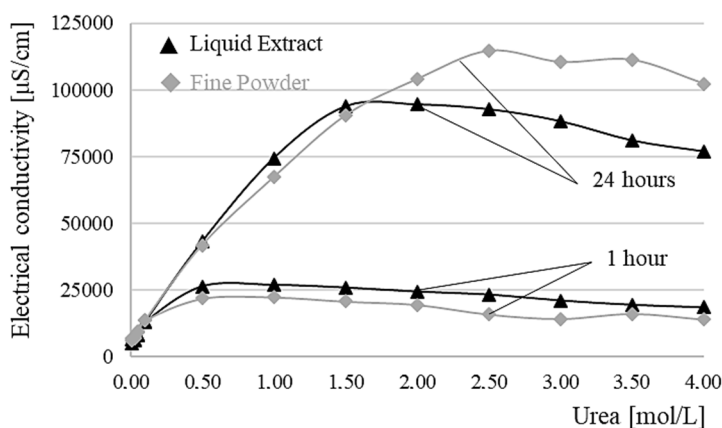
317  
 318 Fig. 7: Visual inspection of test-tubes 72 hours after adding calcium chloride (equimolar urea and calcium  
 319 chloride concentrations varying from 0.010 mol/L to 4 mol/L).

320 **3.4.2. Fine soybeans powder**

321 The previous test-tube investigation was repeated in this work on the fine soybeans powder instead of  
 322 the liquid soybeans extract. As before, the objective was to find the optimum equimolar concentrations  
 323 of urea and calcium chloride that maximise precipitation of calcium carbonate. Twelve test-tubes were  
 324 filled with 40 ml of distilled water which was mixed with 4 g of the fine soybeans powder produced as  
 325 described in Section 3.2.2. This amount of soybeans powder was chosen for consistency with the  
 326 liquid extract, which was produced by centrifuging dry soybeans and distilled water in the proportion  
 327 of 1g:10 ml (see Section 3.2.1).

328 Similar to the previous tests, urea was added to each test-tube in twelve different concentrations of  
 329 0.010 mol/L, 0.025 mol/L, 0.050 mol/L, 0.10 mol/L, 0.50 mol/L, 1.00 mol/L, 1.50 mol/L, 2.00 mol/L,  
 330 2.50 mol/L, 3.00 mol/L, 3.50 mol/L and 4.00 mol/L. Fig. 8 shows the measurements of electrical  
 331 conductivity 1 and 24 hours after the addition of urea to the test-tubes. As before, the electrical  
 332 conductivity appears to increase significantly over time with a maximum value of about 125000  
 333  $\mu\text{S}/\text{cm}$  for a concentration of 2.50 mol/L after 24 hours. Fig. 8 also includes the previous  
 334 measurements of electric conductivity on the liquid soybeans extract and indicates that the fine powder  
 335 leads to higher levels of electrical conductivity for higher concentrations of urea after 24 hours while a  
 336 negligible difference between the two cases is observed after 1 hour.

337



338

339 Fig. 8: Comparison of electrical conductivity measurements 1 and 24 hours after adding urea to test-tubes (urea  
 340 concentration varying from 0.010 mol/L to 4 mol/L).

341 The subsequent addition of equimolar concentrations of calcium chloride to the test-tubes led to the  
 342 rapid formation of a sticky yellow semi-liquid paste (Fig. 9). Unlike the case of the liquid soybeans  
 343 extract, the relatively high consistency of this paste did not allow collection of the precipitated calcium  
 344 carbonate by means of filtration (see Section 3.4.1.).



345

346 Fig. 9: Formation of a semi-liquid paste after addition of calcium chloride to test-tubes containing distilled water,  
 347 soybeans fine powder and urea (urea/calcium chloride concentration of 2.50 mol/L).

## 348 4. EXPERIMENTAL PROGRAM ON STABILISED EARTH

### 349 4.1. Samples manufacture

350 Three different stabilisation methods were selected for the subsequent campaign of laboratory tests on  
 351 earth samples. For two of these methods, the source of urease enzyme was provided by either the  
 352 liquid soybeans extract or the suspension of fine soybeans powder in distilled water. In the third  
 353 method, the previous two sources of urease enzyme were combined into a suspension of fine soybeans  
 354 powder in liquid soybeans extract. In all cases, the cementing solution was completed by adding urea  
 355 and calcium chloride in equimolar concentrations of 2.50 mol/L. This concentration was chosen based  
 356 on the previous test-tube experiments (see section 3.4), which suggested a maximum carbonate  
 357 precipitation for this amount of reagents.

358 The three different stabilisation methods are summarised in Table 3 using the following codes:

- 359 - stabilisation using liquid soybeans extract is referred as SC;
- 360 - stabilisation using fine soybeans powder suspended in distilled water is referred as SP;
- 361 - stabilisation using fine soybeans powder suspended in liquid soybeans extract is referred as SCP.

367 Table 3: Composition of cementing solution for SC, SP and SCP stabilisation methods.

Stabilisation method ID	Liquid soybeans extract	Fine soybeans powder	Distilled water	Reagents (2.50 mol/L urea/calcium chloride)
SC	×	-	-	×
SP	-	×	×	×
SCP	×	×	-	×

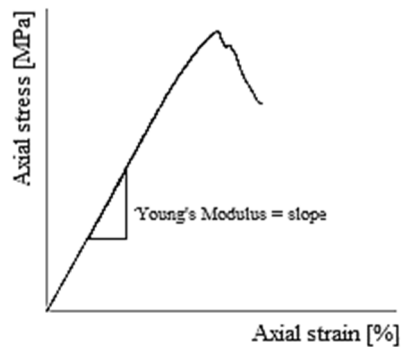
368 For the SP and SCP stabilisation methods, the amount of soybeans powder was equal to 1/12 of the  
 369 dry sample mass. This relatively little amount of soybeans powder was chosen after some preliminary  
 370 tests to limit the growth of mould on the sample surface during prolonged exposure to water. Finally,  
 371 as a term of comparison, a set of control (unstabilised) samples made of dry earth and distilled water  
 372 were prepared according to the same manufacturing procedure, as described below.

373 Cylindrical earth samples with a diameter of 50 mm and a height of 100 mm were manufactured as  
 374 follows: (i) the dry earth was passed through a 2 mm sieve to remove the largest particles; (ii) the  
 375 sieved material was carefully mixed with an amount of cementing solution equal to the Proctor  
 376 optimum water content of the Bouisset soil, that is 12.4%; (iii) the moist soil was statically compacted  
 377 in 10 layers inside a cylindrical mould to attain the Proctor optimum dry density of the Bouisset soil,  
 378 that is 1.97 g/cm<sup>3</sup> (note that the earth was compacted immediately after mixing it with the cementing  
 379 solution to prevent the premature precipitation of calcium carbonate crystals); (iv) the surface of each  
 380 compacted earth layer was lightly scarified before applying the subsequent layer; (v) the final samples  
 381 were equalised under controlled humidity (40 ± 5 %) and temperature (20 ± 5 °C) until the mass  
 382 changed less than 0.1 % over at least one week.

#### 384 4.2. Mechanical properties

385 After equalisation, the cylindrical samples were subjected to unconfined compression tests with a  
 386 constant axial displacement rate of 0.001 mm/s. Three samples were tested for each stabilisation  
 387 method to confirm the repeatability of measurements. The peak compressive strength and Young's

388 modulus were calculated as the average of these three tests. The Young's modulus was simply  
389 measured as the slope of the tangent to the stress-strain curve over the initial stress range where the  
390 material response is reasonably linear (Fig. 10).

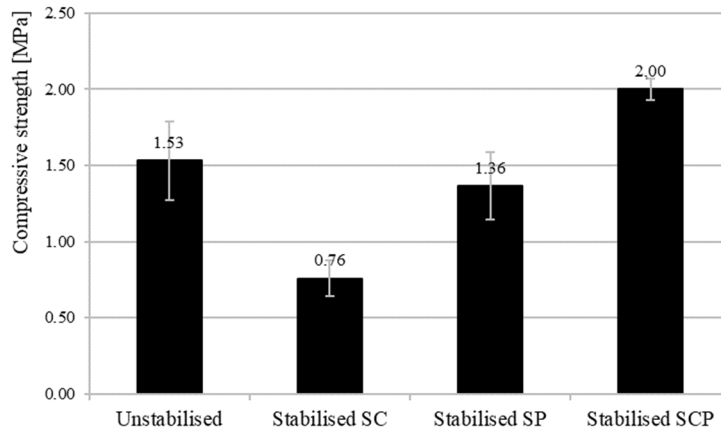


391

392 Fig. 10: Stress-strain curve from unconfined compression tests and measurement of the Young's modulus as the  
393 slope of the initial tangent to the stress-strain curve.

394 Fig. 11 shows the average peak compressive strength of all stabilised and unstabilised samples  
395 together with the corresponding standard deviation bars. Inspection of Fig. 11 indicates that  
396 compressive strength varies depending on the chosen stabilisation method with the lowest,  
397 intermediate and highest levels recorded for the SC, SP and SCP stabilised samples, respectively.  
398 Interestingly, only SCP stabilisation, which is a combination of the SC and SP methods, generates a  
399 level of strength which is higher than that of the unstabilised samples. Instead, the use of the liquid  
400 soybeans extract as a source of urease enzyme (SC stabilisation) markedly undermines the mechanical  
401 properties of the material resulting in a compressive strength that is twofold lower than that of the  
402 unstabilised samples. This result is in apparent contradictions with the observations made in test-tube  
403 experiments (Cuccurullo et al.<sup>80</sup>), where precipitation of carbonate crystals was observed in a solution  
404 of liquid soybeans extract, urea and calcium chloride. An explanation might be given by the presence  
405 of proteins, other than the urease enzyme, which may affect the mechanical performance of the earth  
406 material. This is an important aspect that should be investigated in detail during future research. The  
407 visual examination of SP and SCP stabilised samples also highlighted the formation of a crystallised  
408 white covering on the external material surface. This observation may provide further evidence of the  
409 greater efficiency of the fine soybeans powder as a source of urease enzyme, either alone or in  
410 combination with the liquid soybeans extract.

411 Fig. 12 shows the average measurements of Young's modulus of all stabilised and unstabilised  
412 samples together with the corresponding standard deviation bars. Like compressive strength, the  
413 Young's modulus varies depending on the chosen stabilisation method with the highest, intermediate  
414 and lowest levels of stiffness recorded for the SCP, SP and SC stabilised samples, respectively. In this  
415 case, all three stabilisation methods generate lower values of Young's modulus compared to the  
416 unstabilised material.

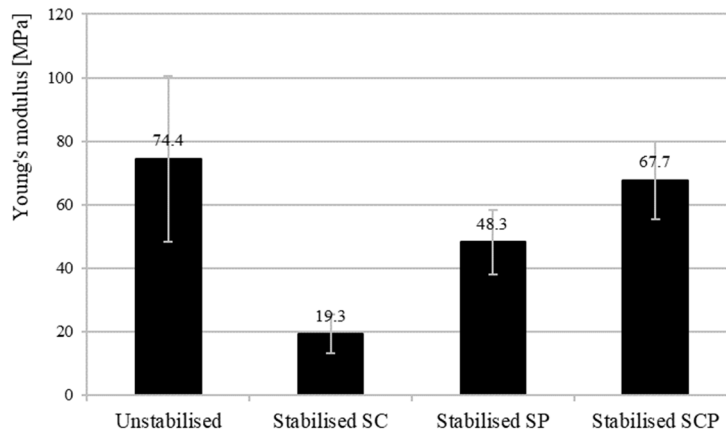


417

418

419

Fig. 11: Compressive strength measured from unconfined compression tests on unstabilised, SC stabilised, SP stabilised and SCP stabilised samples.



420

421

422

Fig. 12: Young's modulus measured from unconfined compression tests on unstabilised, SC stabilised, SP stabilised and SCP stabilised samples.

### 423 4.3. Hygroscopic properties

424 The hygroscopic behaviour of the stabilised earth was investigated via the measurement of the  
 425 moisture buffering value (MBV). Samples were exposed to step cycles of relative humidity, between  
 426 53% and 75%, under a constant temperature of 23 °C inside a climatic chamber (CLIMATS Type  
 427 EX2221HA). Each of these two humidity levels was maintained for twelve hours while the sample  
 428 mass was recorded every two hours as prescribed by the norm ISO 24353<sup>81</sup> for the characterisation of  
 429 the hygro-thermal behaviour of building materials exposed to cyclic variations of humidity over a  
 430 daily time. Each sample was placed in the upright position inside an aluminium foil pan so that only  
 431 the top and lateral surfaces were directly exposed to the atmosphere inside the climatic chamber. The  
 432 total area of the exposed surface was, therefore, higher than the minimum value of 0.010 m<sup>2</sup> required  
 433 by the norm ISO 24353.<sup>81</sup>

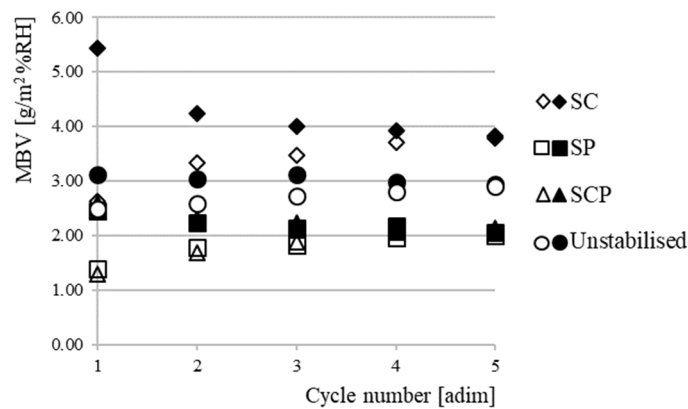
434 Before the start of the tests, all samples were equalised at a temperature of 23 °C and a relative  
 435 humidity of 53%. Equalisation was assumed to be complete when the mass of the sample changed less  
 436 than 0.1% over at least one week, which took generally a period of two weeks. After equalisation, the  
 437 samples were exposed to the cyclic changes of relative humidity, as previously described. Distinct  
 438 MBVs were calculated during the moisture uptake and moisture release stages of each cycle according  
 439 to the following equation:

440

$$MBV = \Delta m S \Delta \%RH \quad (6)$$

441 where  $\Delta m$  is the absolute value of the sample mass variation (in grams) at end of each relative  
 442 humidity stage,  $S$  is the exposed surface (in square meters) and  $\Delta \%RH$  is the imposed change of  
 443 relative humidity (in percentage). The values of  $\Delta m$  measured at the end of a moisture uptake stage  
 444 (i.e. at the end of the exposure to the high humidity level) provide the “MBV uptake” while the values  
 445 of  $\Delta m$  measured at the end of a moisture release stage (i.e. at the end of the exposure to the low  
 446 humidity level) provide the “MBV release”. To take into account the change of sample dimensions  
 447 caused by swelling, the exposed surface was calculated from the average of three height measurements  
 448 and three diameter measurements taken at the beginning of the test (i.e. at  $T = 23 \text{ }^\circ\text{C}$  and  $RH = 53\%$ )  
 449 and at the end of each moisture uptake stage (i.e. at  $T = 23 \text{ }^\circ\text{C}$  and  $RH = 75\%$ ). This assures that small  
 450 variations of sample dimensions, owed to swelling upon wetting at high humidity, are duly considered.  
 451 To confirm the repeatability of measurements, three replicate samples were tested, with the relevant  
 452 MBVs calculated as the average of these three measurements.

453 Fig. 13 shows the variation of both MBV uptake and MBV release over the consecutive humidity  
 454 cycles for all stabilised and unstabilised samples (solid markers indicate the MBV uptake while hollow  
 455 markers indicate the MBV release). As expected, the MBVs of the uptake and release stages tend to  
 456 converge towards the same steady state value as the number of cycles increases. In general, five cycles  
 457 were sufficient to attain steady state conditions for all materials.



458

459 Fig. 13: MBVs measured during the uptake and release stages of subsequent humidity cycles on unstabilised, SC  
 460 stabilised, SP stabilised and SCP stabilised samples. Solid markers indicate the MBV uptake while hollow  
 461 markers indicate the MBV release.

462 The final MBVs of the different materials were calculated as the averages of the uptake and release  
 463 values of the last three stable cycles (Table 4). A stable cycle is defined as a humidity cycle where the  
 464 uptake and release values are approximately equal, thus indicating the achievement of steady state  
 465 conditions. Inspection of Table 4 indicates that the unstabilised earth exhibits a moisture buffering  
 466 capacity that is greater than that of the SP and SCP stabilised earth, which incorporates the fine  
 467 soybeans powder as a source of urease enzyme. An explanation of this result is proposed later when  
 468 the results of water immersion tests are presented. Yet, even for the SP and SCP stabilised earth, the  
 469 MBV value remains larger than 2, which indicates good hygroscopic properties.

470 Interestingly, the highest moisture buffering capacity is recorded for the SC stabilised earth, which  
 471 incorporates the liquid soybeans extract as a source of urease enzyme. In this case, a very high MBV  
 472 close to 4 is attained, which is even greater than that of the unstabilised earth. A possible explanation  
 473 of this result might be found in the modification of the pore size distribution of the earth due to the  
 474 precipitation of calcium carbonate inside the small voids that have been penetrated by the liquid  
 475 soybeans extract. This may increase the fraction of nanopores compared to the unstabilised earth, thus  
 476 increasing the material capacity of absorbing/releasing vapour under changing ambient humidity.

477 Therefore, SC stabilisation might not be very effective in increasing the stiffness and strength of the  
478 earth but offers a way to improve the material hygroscopic properties.

479 Table 4: MBVs of unstabilised, SC stabilised, SP stabilised and SCP stabilised samples.

Sample ID	MBV [g/m <sup>2</sup> %RH]
Unstabilised soil	2.91
SC	3.79
SP	2.03
SCP	2.07

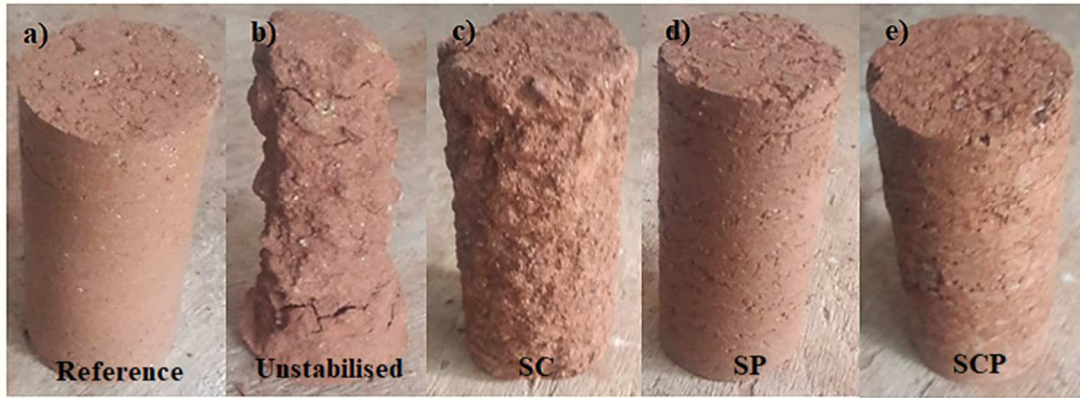
480

#### 481 4.4. Durability properties

482 Durability against water erosion was assessed by means of immersion tests according to the norm DIN  
483 18945.<sup>82</sup> After equalisation to the laboratory atmosphere (corresponding to a temperature of  $20 \pm 5$  °C  
484 and a relative humidity of  $40 \pm 5\%$ ), the samples were weighed to record their initial mass  $m_i$  and then  
485 immersed in water for ten minutes. They were subsequently removed from water and equalised again  
486 to the laboratory atmosphere to attain the same moisture content as before immersion. After  
487 equalisation, the final mass  $m_f$  was recorded and introduced, together with the initial mass  $m_i$ , in the  
488 following equation to calculate the percentage mass loss  $\% \Delta m$  :

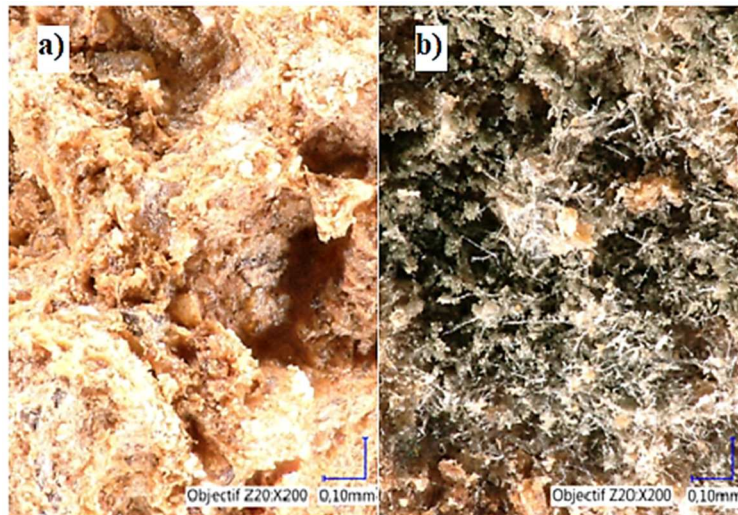
$$489 \quad \% \Delta m = \frac{m_i - m_f}{m_i} \times 100 \quad (7)$$

490 Each test was performed in three replicates to confirm the repeatability of the experimental procedure.  
491 The mass loss was about 42% for the unstabilised samples but reduced to 13% for the SC stabilised  
492 samples. An even better result was obtained by SP and SCP stabilisation, when only 1% of the initial  
493 mass was lost. Fig. 14 compares the pictures of the different samples before and after immersion  
494 confirming that a significant improvement of durability is achieved by means of enzymatic  
495 stabilisation, especially when the fine soybeans powder is used as a source of urease enzyme.  
496 Interestingly, the introduction of soybeans powder as a source of urease enzyme improves the erosion  
497 resistance of the material to levels that are acceptable for routine building applications. The lower  
498 mass loss experienced by the stabilised samples may be explained by the formation of calcium  
499 carbonate bonds between earth particles and by the partial occlusion of voids with a consequent  
500 reduction of permeability to water. Fig. 15 compares the images captured by a 4K digital KEYENCE  
501 microscope on the surface of SC and SP stabilised samples. The growth of organic ramifications on  
502 the surface of SP stabilised earth may be responsible for the occlusion of voids, which explains the  
503 hydrophobic behaviour and relatively low moisture buffering capacity of this material (see Section  
504 4.3).



505

506 Fig. 14: Reference sample before immersion (a); unstabilised sample after immersion exhibiting a mass loss of  
 507 42% (b); SC stabilised sample after immersion exhibiting a mass loss of 13% (c); SP stabilised sample after  
 508 immersion exhibiting a mass loss of 1% (d); SCP stabilised sample after immersion exhibiting a mass loss of 1%  
 509 (e).



510

511 Fig. 15: Images captured by a 4K digital KEYENCE microscope on the surface of SC stabilised samples (a) and  
 512 SP stabilised samples (b). Note the organic ramifications on the surface of the SP stabilised sample.

513 **4.5. X-Ray Diffraction (XRD) analysis**

514 Five grams of the unstabilised, SC stabilised and SP stabilised material were ground into a fine  
 515 powder and air dried to the laboratory environment before performing a X-Ray Diffraction (XRD)  
 516 analysis. Results from these XRD tests revealed the presence of kaolinite, microcline intermediate,  
 517 muscovite, quartz and calcium carbonate in the form of calcite (Figs. 16, 17 and 18). Calcite was  
 518 identified in all samples, both unstabilised and stabilised. An approximate XRD quantitative analysis  
 519 was performed based on the construction of diffraction peak mathematic function model by means of  
 520 polynomial whole pattern fitting to the spectral lines using computer software (Rietveld refinement).  
 521 This indicated an amount of calcite of about 2% for the unstabilised samples compared to about 8%  
 522 for the SP stabilised samples.

523  
524

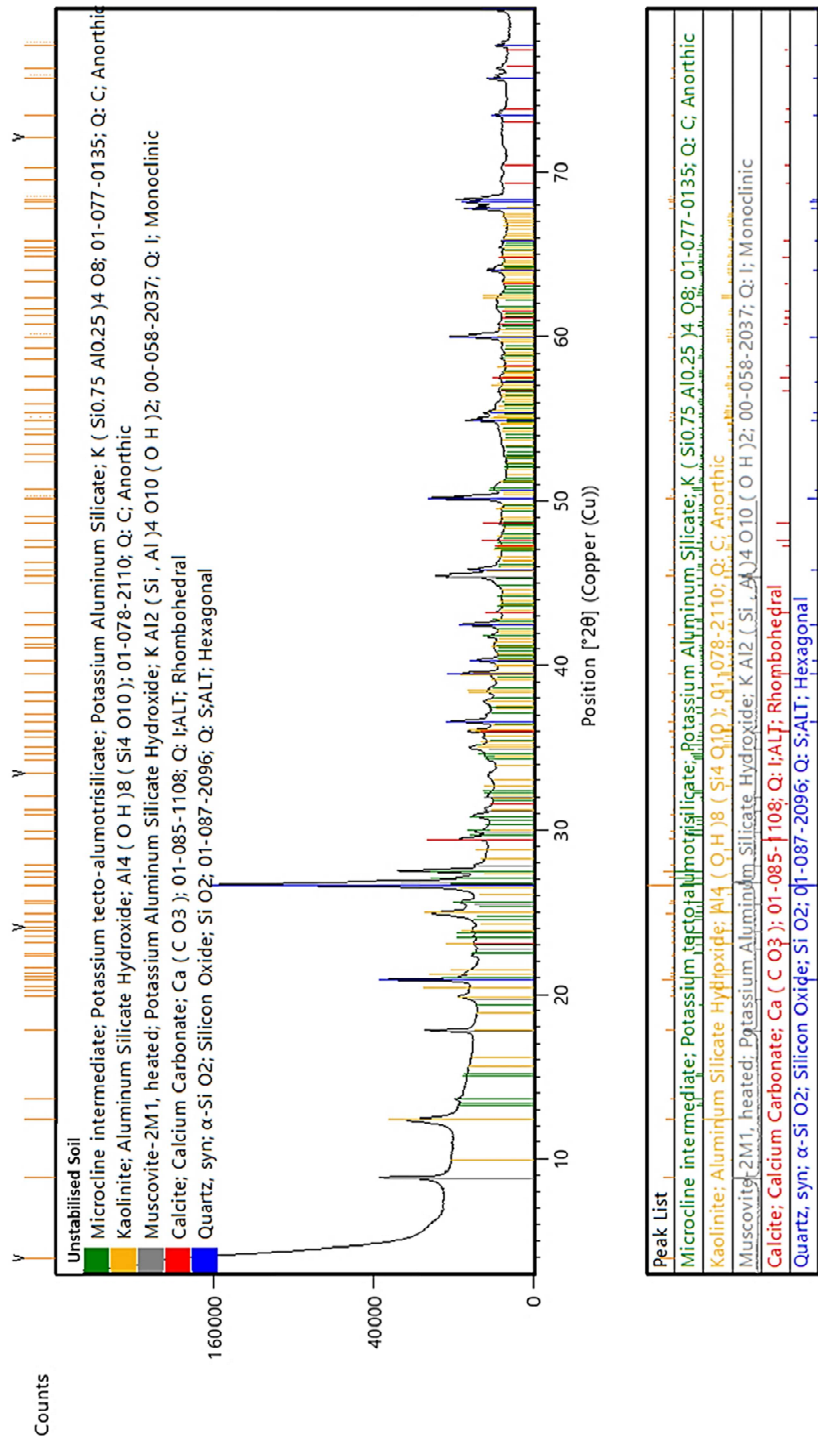


Fig. 16: XRD analysis on unstabilised sample.

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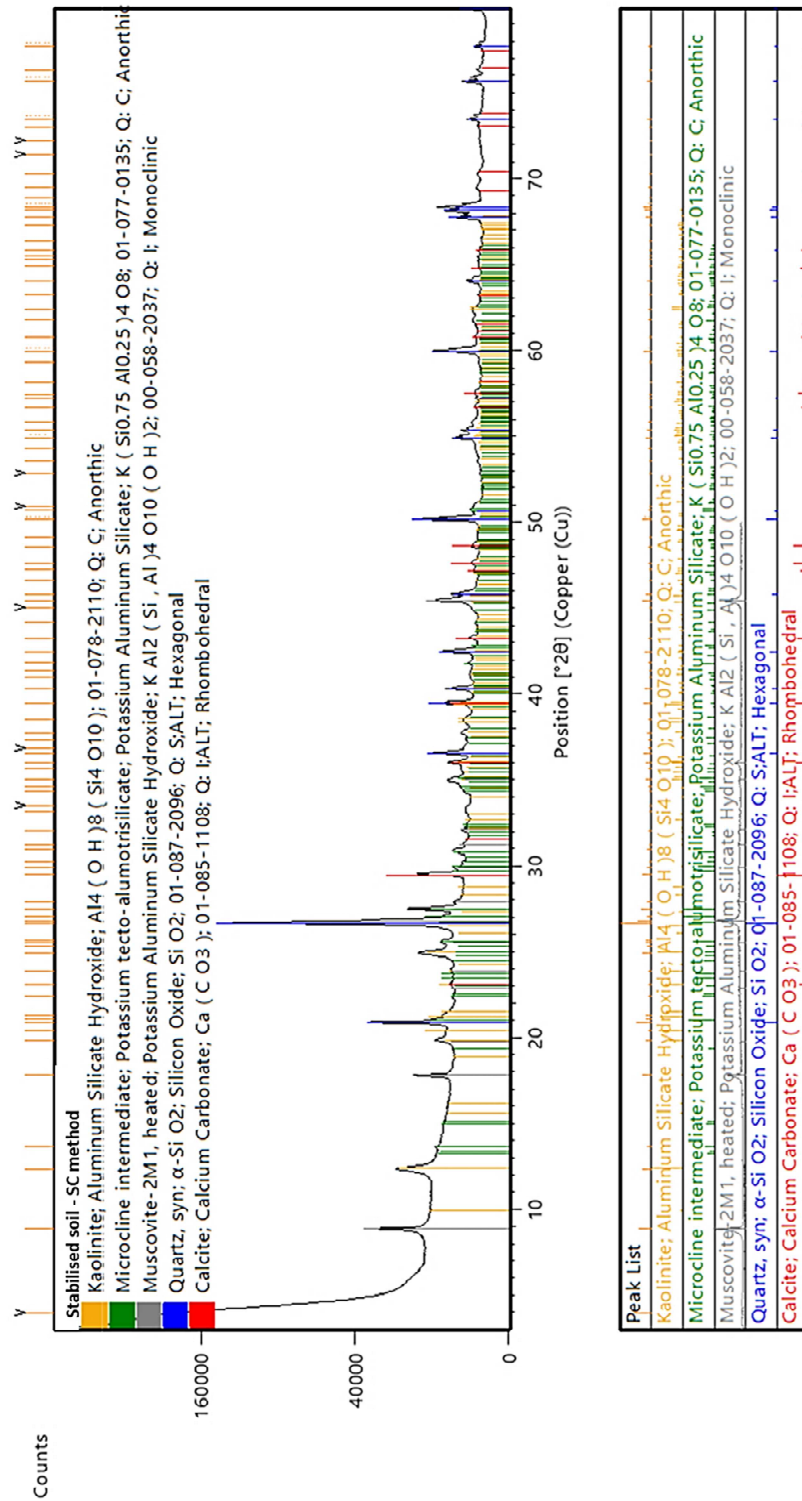
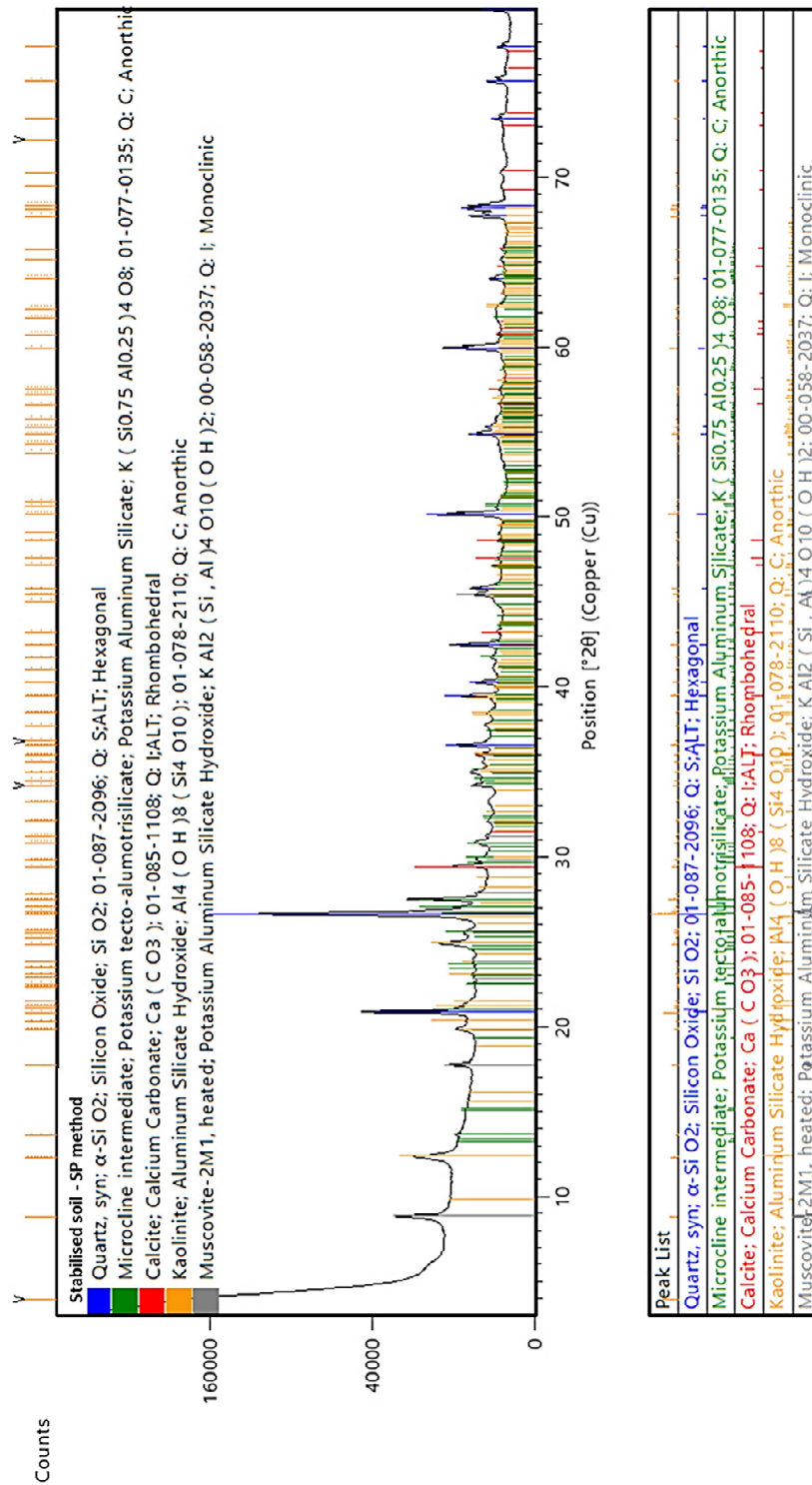


Fig. 17: XRD analysis on SC stabilised sample.



527  
528

Fig. 18: XRD analysis on SP stabilised sample.

## 529 5. CONCLUSIONS

530 This paper has presented an experimental study of enzyme induced carbonate precipitation (EICP) as a  
 531 suitable technique for the stabilisation of soils and, more specifically, for improving the hygro-  
 532 mechanical and durability properties of compacted earth in building applications. The main outcomes  
 533 of this work are summarised as follows:

- 534 - Earth materials have been stabilised by exploiting the urease enzyme that is present in  
535 soybeans. Both fine soybeans powder and liquid soybeans extract provide a good source of the  
536 urease enzyme as a catalyst of the hydrolysis of urea, which leads to the production of  
537 carbonate ions and the precipitation of calcium carbonate as a binding phase.
- 538 - Results of unconfined compression tests on stabilised earth samples indicate that material  
539 strength changes depending on the chosen stabilisation method with the highest levels of  
540 strength obtained when the fine soybeans powder and liquid soybeans extract are used  
541 together as a source of the urease enzyme. Interestingly, if the fine soybeans powder and  
542 liquid soybeans extract are used alone, strength levels drop to values that are lower than those  
543 of the unstabilised material.
- 544 - Unstabilised earth has an excellent capacity to buffer moisture and hence exhibits a high  
545 hygro-thermal inertia. This capacity is not significantly changed by the stabilisation methods  
546 tested in the present work. The moisture buffering capacity of the material reduces slightly  
547 when the fine soybeans powder is employed while it increases when the liquid soybeans  
548 extract is used.
- 549 - The water durability of both unstabilised and stabilised earth has been assessed by immersion  
550 tests according to the norm DIN 18945<sup>82</sup>. During water immersion, the unstabilised samples  
551 lost about 42% of their initial mass while the samples stabilised with a combination of liquid  
552 soybeans extract and fine soybeans powder exhibited a mass loss of only 1%. This negligible  
553 mass loss makes this stabilised material adequate for exposure to natural weathering according  
554 to the norm DIN 18945<sup>82</sup>. Digital microscope images have showed the presence of organic  
555 ramifications on the surface of the earth stabilised with the fine soybeans powder. This may  
556 explain the hydrophobic behaviour and smaller moisture buffering capacity of this material  
557 compared to the unstabilised one.
- 558 - Results from X-Ray Diffraction (XRD) analyses of both stabilised and unstabilised earth has  
559 revealed the presence of minerals like kaolinite, microcline intermediate, muscovite, quartz  
560 and calcium carbonate in the form of calcite. An approximate quantitative interpretation of  
561 these results detected an amount of calcite of about 2% in the unstabilised samples, which  
562 increased to about 8% in the samples stabilised with the fine soybeans powder.

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