

RESEARCH ARTICLE

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Clay barriers for protecting historic buildings from ground moisture intrusion

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Abstract

Background: Conservators and architects working with built heritage at risk from ground moisture intrusion face a difficult choice. Leaving the building unprotected can lead to moisture related deterioration; installing vertical and/ or horizontal barriers using standard materials requires chemical or mechanical intervention with the historic fabric. The use of compacted clay to form a barrier is commonplace in some areas of environmental engineering, and can be applied to built heritage conservation. Naturally occurring or slightly modified soils were traditionally used for protecting buildings in a number of vernacular techniques. Knowledge of what types of soils are suitable for use could provide certain regions with a low-cost, low-impact alternative for protecting historic buildings. Three commercially available products previously shown to have low hydraulic conductivity were analysed in order to identify waterproofing mechanisms; these included two specialised bentonite—sand mixtures and a Saxonian glacial till.

Results: The till relies on a high proportion of densely agglomerated fine grains to achieve a low permeability when consolidated, possibly assisted by the presence of calcite. The specialised mixtures rely on an engineered grain size distribution, with practically no silt sized grains, and a fraction of sodium bentonites capable of forming colloidal suspension when compacted. All materials showed a low risk of shrinkage when compacted at optimum moisture content.

Conclusion: Different mechanisms appear to account for the low hydraulic conductivities found in clay barrier material. The implication is that a range of soil material may be suitable for protecting built heritage from ground moisture intrusion and its associated decay mechanisms. The suitability of glacial till may present large areas of Northern Europe with locally available barrier material.

Keywords: Clay barrier, Historic buildings, Conservation, Damp-proof, Natural materials, Till, Bentonite

Background

Conservators and architects working with built heritage at risk from ground moisture intrusion face a difficult choice. Leaving the building unprotected can lead to rising damp, salt contamination and costly maintenance or irreparable damage [1, 2]. Retroactive damp-proofing generally requires chemical and mechanical intervention with the historic fabric; the irreversible nature of such techniques makes them unsuitable for contemporary heritage management [3]. Clay barriers have recently emerged as an alternative, but remain underrepresented in literature [4].

Whilst a commonplace vernacular roofing material across semi-arid climates [5], verified early evidence of clay used to protect against ground moisture is limited to a few farmsteads in Saxony [6]. These date mostly to the eighteenth and nineteenth centuries. The clay was compressed into a barrier between 20 and 30 cm thick at the foot of external masonry. There are indications that a similar technique was traditionally used to combat rising damp in the Jinggangshan region of China [7]. Whilst further as yet undiscovered vernacular examples in regions with suitable soils are probable, proliferation of such techniques is likely to have been inhibited by a general tolerance of damp cellars [3]. Evidence of lime and clay rich soils or mixtures used as flooring in important civic buildings dates back to the late Neolithic. Examples

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include *Huwwar*, found in Bronze Age Palestine [8], and *Opus Signinum*, used by the Romans specifically for waterproofing purposes [9]. By the time ground moisture intrusion was being widely and effectively combated however, the industrial production of bituminous products was well underway [10].

Use of earthen barriers persisted in hydraulic engineering. An early industrial application was the use of puddled clay to seal British canals, reservoirs and dams from the late eighteenth century [11]. This technique was gradually superseded by rolling out mats of pre-tamped clay, and in turn by the development of geotextile linings in the mid twentieth century. These encapsulate a thin layer of high swelling bentonite clay in panels or between membranes. Use of clay barriers has also become widespread in sealing landfill and waste repositories. Whilst specialised bentonite—sand mixtures or geotextiles are commonly selected, naturally occurring soils have been tested for suitability and used as alternatives [12, 13].

Several commercial producers of clay barrier material now also market specialised mixtures for building works. These optimised bentonite-sand mixtures are designed to be compacted against external foundations to form vertical or horizontal damp-proof courses without the need for any further fixing [4]. Beyond these, a number of recent conservation projects in Saxony and Brandenburg have utilised local till as barriers. Besides being reversible, naturally occurring clays have the additional benefit of providing nearby areas with a local source of material. This can further reduce the embodied energy of the technique in comparison to the use of specialised mixtures, which rely on geographically limited bentonites and may involve long distance transportation of finished products. A comparison of material used in conservation projects in Saxony to the specialised products can identify and distinguish properties for achieving low hydraulic conductivity, in turn facilitating the location of other suitable naturally occurring clays.

This paper presents an analysis of three commercially available products that have been repeatedly used as clay barriers in building engineering. Two are industrially produced bentonite—sand mixtures and one is an unmodified Saxonian glacial till. The objective is to examine the mechanisms by which they achieve a low hydraulic conductivity. The aim is to facilitate the selection and modification of naturally occurring, local soils for use in retroactive damp-proofing of built heritage.

Theory of waterproofing with clay

Clay is a very fine-grained soil material, typically particles less than 2 μm in diameter [14]. The clay fraction is made up mainly of clay minerals, laminates of electrochemically bound phyllosilicates. Due to a negative

charge across their sheet structures, certain clay minerals have the ability to adsorb and exchange cations such as sodium or calcium. These can in turn bind large amounts of water within the interlaminate zone and thus cause the mineral structure to approximately double in volume, as a polymolecular film forms and progressively forces the laminates apart. This is known as crystalline swelling. The most expansive clay mineral is montmorillonite, the main component of bentonite. Sodium montmorillonite can also undergo a secondary, more gradual yet far greater form of swelling. This is known as osmotic swelling [15]. It occurs when diffuse clouds of positively charged ions overlapping the clay minerals cause the mineral structures to entirely delaminate and peptise to form a colloidal suspension [16]. Compaction of sodium bentonite will increase the overlap of diffuse clouds and the effect of osmotic swelling (Fig. 1). Drying out leads to shrinkage, which can form cracks within the soil as a whole. If the clay fraction makes up a significant percentage of the total grain-size distribution in a soil, then that soil is referred to as a clay. Loam is a soil with a medium clay fraction. A clay or loam may contain numerous types of clay minerals as well as other grain factions such as silt and sand, and other mineral components or additives.

These characteristics can result in various properties that will contribute towards low hydraulic conductivity when a soil is compacted:

- A favourable grain distribution may result in a densely consolidated matrix, with progressively finer particles filling pore space between coarser aggregate [17–19]
- The presence of swelling clays can bind water during hydration. Besides retarding flow-rate, this will constrict pore space and further reduce permeability as the clay minerals expand or peptise into open porespace. In order for this mechanism to take full effect, outward volumetric expansion (heave) of the clay mass must be restrained [19, 20].
- A certain optimum moisture content will be necessary in order to achieve Proctor compaction (maximum density of consolidated grains) and therefore the lowest possible porosity.
- The proportion of clay to silt and sand should not allow for shrinkage to form cracks in the compacted mass during dehydration, as these would significantly increase permeability during any subsequent hydration [20].
- The presence of certain mineral components can reduce permeability. This can be due to the effect of various natural components or artificial additives, such as natural or slaked lime, cement or fly-ash, and

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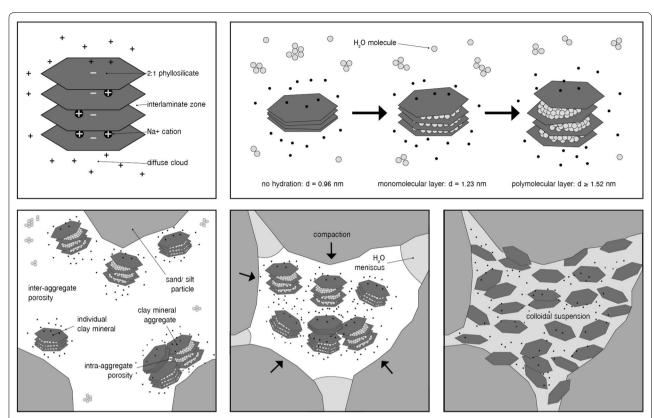


Fig. 1 Sodium montmorillonite activity. *Top left* clay mineral with layer charges; *top right* crystalline swelling; *bottom* osmotic swelling after compaction. Diagram not to scale (adapted from [14, 15])

the result of various mechanisms, such as cementation of pore space, hydraulic binding or chemical reaction with the clay fraction [21–25].

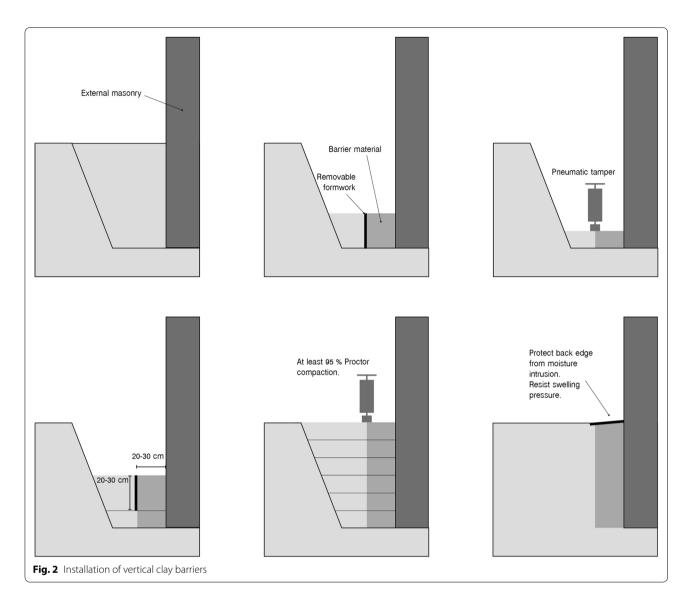
Installation of suitable materials involves pouring layers into a formwork constructed in an excavation pit (Fig. 2). Layers are successively compressed with a pneumatic tamper to form a vertical barrier. The moisture content should enable a Proctor compaction rate of at least 95%; the density should be monitored at regular intervals. Care must be taken to prevent water penetration occurring between the barrier and the masonry. Settling, shear forces and dimensional expansion due to clay mineral activity must be resisted. High moisture loads may require a drainage at the foot of the barrier [4]. Horizontal barriers running underneath foundations have been installed; in existing buildings this will clearly necessitate excavation of the existing floor.

Methods

Two specialised clay mixtures and a naturally occurring soil were selected for analysis (Fig. 3). The natural soil (hereafter referred to as till) is excavated from a terminal moraine in southern Saxony; the specialised mixtures (hereafter referred to as mixture A and mixture B) are manufactured in a factory environment by two separate producers.

Each of the products has been subject to previous testing and used to form damp-proof courses. Mixtures A and B have been employed in a large number of buildings, including conservation projects. The till was selected to be representative of material used in Saxony and Brandenburg, areas with a history of using clay barriers to protect buildings [6]. The products have previously been tested for composition, hydraulic conductivity and Proctor compaction rate (Table 1); they have each been classified as practically impermeable (tests on mixture A were conducted by MTM Baustoffe, Munster, Germany in 2014; tests on mixture B were conducted by Erdbaulaboratorium Essen, Essen, Germany in 2004; tests on the till were conducted by GEOS Ingenieurgesellschaft, Freiburg, Germany in 2016). The mixtures are known to contain bentonite. The till is known to contain approximately 5% CaCO₃ (Calcite) and 1% CaMg(CO₃)₂ (Dolomite). The purpose of this study is not to test the suitability of these materials for use as clay barriers or the applicability of the technique as a whole. The purpose of this study is to determine the specific mechanisms by

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which these products achieve low hydraulic conductivity and to highlight any differences therein.

Grain size distribution was determined according to DIN 18123 in order to evaluate various aggregate content and classify soil type [26]. Samples of approximately 250 g were oven dried before being sieved through progressively decreasing mesh sizes down to 0.063 mm. The aggregate caught in each mesh was weighed. Remaining aggregate with a diameter smaller than 0.063 mm was then poured into a hydrometer and the specific gravity of the water–soil mix was recorded at intervals as smaller particles progressively settled to the bottom [27]. The results were plotted on a grain size distribution curve (Fig. 4).

In order to assess hygroscopicity, water mass adsorptivity was determined according to DIN 18132 [28]. Aggregate larger than 0.4 mm was removed and oven-dried

samples of approximately 0.5 g were placed in an Enslin–Neff apparatus (Fig. 5). The amount of water drawn out of the horizontally aligned pipette and through the glass filter was recorded after 60 min. The experiment was repeated until consecutive results with less than a 10% margin of difference were obtained.

Using values for Proctor density and optimum moisture content determined in previous tests, it is possible to calculate bulk density, porosity and degree of saturation for the compacted masses according to the following formulae:

$$\rho_{\rm d} = \rho_{\rm Pr}/(1 + w_{\rm Pr}) \tag{1}$$

$$n = (1 - \rho_d/\rho_s) * 100 \tag{2}$$

$$S = (\rho_d/(\rho_w * w_{Pr}))/(n/100)$$
(3)

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Fig. 3 Tested materials. Top dry grains; bottom moist mass. a Mixture A, b Mixture B, c Saxonian till

whereby ρ_d is the bulk density, ρ_{Pr} is the Proctor density, ρ_s is the measured particle density and ρ_w is the density of water in g/cm³; w_{Pr} is the optimum moisture content as a percentage of mass; n is the total porosity as a percentage of total volume of the consolidated mass, and S is the degree of saturation as a percentage of total porosity. Particle density was determined according to DIN 18124 [29]. Oven dried samples of approximately 20 g were placed in a pycnometer and mixed with 0.1 l of water; the weight of the resulting displaced liquid is used to determine the initial particle density. Tests were repeated three times and average values used.

Shrinkage limits were determined according to DIN 18122-2 [30]. The shrinkage limit is defined as the point at which further loss of moisture will not result in further reduction of volume. Aggregate larger than 0.4 mm was removed. Wet samples were compacted into steel rings with a diameter of 76 mm and a height of 20 mm and oven dried. This enabled a calculation of the shrinkage limit according to the formulae

$$w_s = (V_d/m_d - 1/\rho_s) * \rho_w$$
 (4)

whereby w_s is the moisture content at shrinkage limit as a percentage of total mass; V_d the volume of the dried sample in cm³; m_d the mass of the dried sample in g; ρ_s the particle density of the sample and ρ_w the density of water in g/cm³.

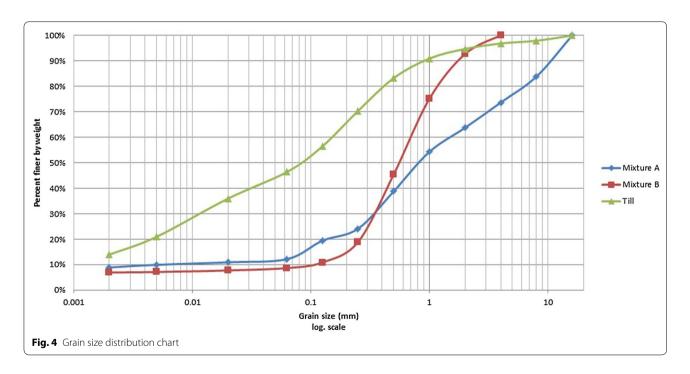
In order to determine the presence of sodium bentonites and associated osmotic swelling, swelling deformation was examined in an Oedometer (Fig. 5) [15]. Samples were compacted into steel rings with a diameter of 76 mm and a height of 20 mm at optimum moisture content. Due to the small size of available steel rings any aggregate ≥10 mm was removed from the samples. Samples were subject to a load of 0.5 kN/m² and placed in distilled water. To determine deformation, one dimensional heave was measured at increasing intervals until no or little change was evident across two subsequent intervals. The results were plotted on a graph as percentage change of the entire volume over time in order to assess the dominant swelling mechanism.

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Table 1 Key properties of tested materials in comparison to products suitable for use as landfill barriers

	Mixture A	Mixture B	Saxonian till	Bentonite-sand mix [42]	Volcanic soil, Chile [12]	Ankara clay [13]
Clay fraction (d < 0.002 mm)	9%	7%	14%	10%	3–5%	51.8-64.2%
Coarse fraction (d > 0.06 mm)	88%	91%	54%	90%	43-53%	10.5-19.8%
Calculated conductivity (m/s)	1E ⁻⁵	1.5E ⁻⁴	8.5E ⁻⁹			
Measured conductivity (m/s) ^a	8.5E ⁻¹¹	5E ⁻¹⁰	8.5E ⁻¹¹	10E ⁻⁹	5.2-6.5E ⁻⁹	0.9-3E ⁻¹¹
Water adsorption (% of initial mass)	148%	226%	43%			
Particle density (g/cm ³)	2.62	2.60	2.60	2.58	2.45-2.66	(2.6)
Proctor compaction ^a (g/cm ³)	2.09	1.76	2.0	1.7	1.12–1.15	1.28-1.50
Opt. moisture ^a (% of total mass)	8.5%	17%	9%	17%	41-42%	16-26%
Bulk density (g/cm ³)	1.92	1.5	1.84	1.45	0.79-0.82	1.02-1.29
Porosity (% of total volume)	27%	42%	29%	45%	69%	60%
Degree of saturation (% of hydrated porosity)	85%	21%	69%	20%	3%	7%
Opt. moisture (% of total mass; d < 0.04 mm)	24%	47%	11%			
97% dry moisture (% of total mass; d < 0.04 mm)	20%	27%				
Shrinkage limit (% of total mass; d < 0.04 mm)	25%	37%	15%			

^a Values taken from past tests



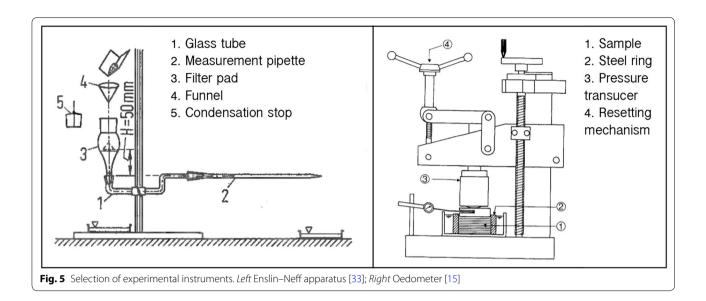
Results and discussion

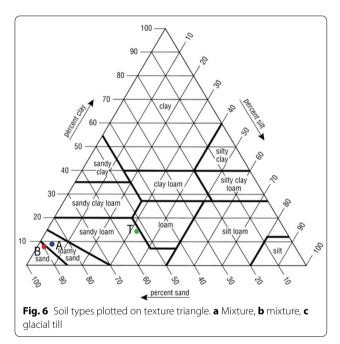
Results are summarised in Table 1. This table also includes two recently assessed natural soils suitable for use in landfill engineering and a typical bentonite—sand mixture for comparison.

Grain size distribution

The grain size distribution curves identify significant differences in the composition of the till and the two specialised mixtures (Fig. 4). Technically, none of the soils are true clay; the mixtures are sands or loamy

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sands, whilst the till is a sandy loam or loam (Fig. 6) [28].

An approximation of hydraulic conductivity through the compacted grains of a particular grain size distribution can be calculated using formulae supplied by Beyer and Kaubisch [17, 31] (Table 1).

$$K = C * d_{10^2}$$
 (5)

whereby K is the hydraulic conductivity in m/s, C is a uniformity coefficient and d_{10} is the diameter of grains in the 10th percentile in mm, can be applied to soils where $0.06 < d_{10} < 0.6$ mm. It was applied to mixture A and the till.

$$K = 10^{0.0005P^{2} - 0.12P - 3.59}$$
 (6)

whereby K is the hydraulic conductivity in m/s and P is the percentage of fine grained material (d < 0.06 mm), can be applied to soils where 60% > P > 10%. It was applied to mixture B.

The results of these calculated hydraulic conductivities classify the two mixtures as permeable $(10^{-4} \text{ m/s} > \text{K} > 10^{-6} \text{ m/s})$ and the till as weakly permeable $(10^{-6} \text{ m/s} > \text{K} > 10^{-8} \text{ m/s})$; the previously measured conductivities all result in a classification of very weakly permeable (K < 10^{-8} m/s) [32]. This indicates that whilst the hydraulic conductivity of the till is almost representative of the porosity within its consolidated grain matrix, the mixtures are predominantly reliant on a further property to achieve low conductivity.

Hygroscopicity

The water mass adsorptivity test enables a classification of the finer aggregate, in order to give an indication of the presence of clay minerals and their likely plasticity [28, 33]. The very high adsorptivity of the specialised mixtures falls in the region of \geq 130%, confirming the presence of highly plastic clays or bentonites. The water mass adsorptivity of the natural clay falls into the region of 40 to 60%, indicative of moderately plastic silts and clays.

Porosity

Measured particle densities are similar to those of other clay barrier materials and fall within the expected range of 2.6–2.7 g/cm³ for sandy soil types [34]. The total porosities of Proctor compacted mixture A and the till are noticeably lower than similar barrier materials, including that of compacted mixture B, in which almost

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half the volume is made up of pore space. This is reflected in the degree of saturation: despite the higher moisture content, only 20% of the pore space in mixture B will be saturated following Proctor compaction, whereas 70% of the till and 85% of mixture A will be saturated.

Studies have shown clay structures to exhibit dual-porosity [35, 36]: an intra-aggregate region at the nanometer scale, which contributes little to the effective porosity in which conductivity is possible [19, 37], and an interaggregate region at the micron scale, which decreases with increasing dry density and saturation [20, 38]. The relatively high Proctor densities and saturation of mixture A and the till suggest they are dominated by intraaggregate porosity. If sodium montmorillonite is present compaction can induce osmotic swelling and result in a colloidal suspension, further reducing effective porosity [16] (Fig. 1). A possible explanation for the relatively low bulk density of mixture B could be an optimised pore space closure in order to limit outward expansion and swelling pressure of the compacted mass.

Stability

The shrinkage limit enables an assessment of shrinkage risk. To do this it is necessary to calculate the moisture content of the examined aggregate according to the formula

$$w_f = (w_{PR} - w_c * d_c)/d_f$$
 (7)

whereby w_f is the moisture content of the examined fine aggregate, w_{PR} the optimum moisture content of the total aggregate and w_c the moisture content of the removed coarse aggregate as a percentage of mass; d_c the diameter of the smallest coarse aggregate, and d_f the diameter of the largest fine aggregate in mm. Values of 1% were set for w_c [30].

The optimum moisture content of the till and mixture A are at or below the shrinkage limit. This indicates only minimal volumetric change can occur with any further loss of moisture. Whilst mixture B lies slightly above the shrinkage limit at optimum moisture content, reducing to 97% dry Proctor compaction brings it well below the limit. This suggests the mixtures should be compacted slightly to the dry side of optimum moisture to avoid any risk of shrinkage; it should be noted that mixture B is supplied with a moisture content that enables this.

The presence of calcium carbonate is likely to further reduce hydraulic conductivity in the till. With sufficient humidity, cation exchange can replace metallic ions in swelling clays with the calcium ions of calcareous minerals [21, 23]. This forms agglomerations of fine particles which enable a more densely compacted, stabilised grain matrix. The stabilising effect of lime additives in clayey soils has been investigated with respect to calcium oxide,

calcium hydroxide and calcium carbonate, however it is not entirely understood to what degree cation exchange and to what degree other stabilisation processes are responsible. A stabilising effect of calcite found in nature is the cementation of calcareous loess following gradual moisture redistribution in deposits [22]. The cementation generally associated with quicklime (CaO) has been shown to reduce permeability of clayey soils at certain threshold quantities [24]. Cement and fly-ash additives have also been shown to reduce hydraulic conductivity in certain clays [25].

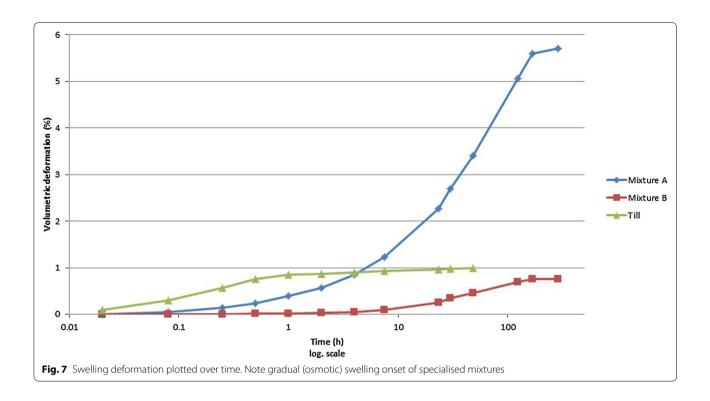
Swelling

The objective of this experiment was to determine the predominant swelling mechanism by plotting deformation over time. Measured deformation itself is to be viewed with caution given the limited sample sizes used.

Swelling deformation measured over time produces a very different curvature in the till compared to the mixtures (Fig. 7); the total heave of the two mixtures is noticeably different in itself. The till swelled by approximately 0.8% within the first 0.5 h, and leveled out at 1% after 50 h. Mixture B reached a similar overall deformation of 0.75%, however only after nearly 300 h of gradual swelling. Mixture A also swelled gradually, however achieved a total deformation of almost 6% after 300 h. The rapid heave of the till is likely to be a result of its overall higher clay content, indicating the final stages of crystalline swelling as the sample fully saturates. The steady heave of the two mixtures is highly indicative of the gradual onset of osmotic swelling [15], indicating a significant proportion of sodium-montmorillonite. The lower overall deformation of mixture B is likely to be a result of its higher porosity, which allows more room within the matrix for the delaminating clay minerals to expand into.

That the specialised mixtures rely on osmotic swelling to achieve low hydraulic conductivity is significant for the future design of sand-bentonite mixtures suitable for protecting historic buildings. Mishra et al. found sodium-bentonite content to be a controlling factor in the hydraulic conductivity of sand-bentonite mixtures [39]. Calcium bentonite is also widely used in landfill and repository engineering, but undergoes far less swelling and its primary role may be in filtering leachates via ion exchange [40]. The implication is that the limited natural occurrence of sodium bentonites will restrict use in sustainable conservation strategies to certain regions [11, 14]. Furthermore, groundwater chemistry has been shown to have an effect on the long-term stability of bentonite [41], and a gradual exchange of sodium to calcium has been observed in bentonite panels [4]. Highly saline ground moisture loads may preclude the use of sandbentonite mixtures.

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That any deformation was measured in the till is significant in that it confirms the presence of swelling clays, which are capable of cation exchange [14]. This can result in the formation of agglomerations with calcite minerals that could potentially contribute to the low measured hydraulic conductivity.

Outlook

Comparing the products with some of the many soil types and mixtures used in landfill and repository engineering can help direct future research into potential barrier material. The specialised mixtures fall within the broad range of sand-bentonite mixtures used in landfill and repository engineering [39, 42], with the proviso of containing predominantly sodium-montmorillonite. Whilst effective in retarding moisture flow, the highly localised provenance of sodium-bentonite will limit its use in sustainable conservation strategies to certain areas [11, 14]. Where it is available, sodium-bentonite powder should be mixed with sand or gravely sand; high silt content is likely to increase the risk of shrinkage. Calcareous additives should be avoided to prevent cation exchange.

The suitability of the glacial till suggests large areas of Northern Europe may have a locally available source of suitable clay deposited at end moraines [14]. Calcite content and an even grain size distribution are likely to be key factors; a small fraction of swelling clays will be beneficial.

Mixing sand with calcareous loess may result in a material with similar characteristics. The till is quite different to some other naturally occurring clay barrier materials with similarly low measured hydraulic conductivities [12, 13]. The volcanic Chilean soil and the clastic Turkish clay examined in these studies display marked differences themselves, although the presence swelling clays and calcareous minerals was noted in both. This implies that other soil types will be suitable for protecting historic buildings. Modification of unsuitable soil types with lime or even industrial waste material presents a further option [24, 25], although any potential material should be tested in laboratory conditions before use as barrier.

Conclusion

The objective of this research was to identify the key waterproofing mechanisms of clay barriers used to protect buildings from ground moisture intrusion by examining three commercially available products. The aim was to facilitate the selection and modification of naturally occurring clays that may be locally available to heritage sites. Each of the tested materials exhibited different properties in order to achieve low hydraulic conductivity:

 Mixture A has an even grain size distribution and forms a dense matrix. A small fraction of sodium bentonites further reduces conductivity by peptising when the mixture is compacted.

- Mixture B also has a small fraction of sodium bentonites; these appear to be optimised to close an otherwise porous, sandy matrix following compaction, resulting in minimal heave and swelling pressure.
- The glacial till has a high proportion of fine aggregate, giving its consolidated matrix a low effective porosity. This includes a relatively large clay fraction, with some swelling clay minerals present. The calcium carbonate content may further reduce conductivity, by cementing pore space through dissolution, or increasing the density and stability of the matrix due to cation exchange.
- A resistance to shrinkage is notable in all products, in that their optimum or supplied moisture content is at or below the shrinkage limit. This will reduce the risk of shrinkage during dehydration.

Based on the limited number of materials examined here, some broad recommendations can be made. Calcareous materials and/or swelling clays can contribute positively to low hydraulic conductivity; till and loess deposits may provide useful resources. Silty, moderately plastic clays or loams with a risk of shrinkage could be stabilised with sand and cementing additives such as lime or fly-ash. Conversely, sand and loamy sand could be conditioned with sodium bentonite powder; in this case calcareous material should be avoided. Moisture content during installation should enable Proctor compaction and be at or beneath the shrinkage limit to avoid fracture. Further analysis of proven barriers should be used to establish a laboratory protocol, in order to evaluate the suitability of local clays before further modification and testing.

The broad implication of this research is that a range of soil material may be suitable for protecting built heritage from ground moisture intrusion and its associated decay mechanisms. The use of engineered bentonite—sand mixtures will increasingly provide an alternative as the industry grows; however the prospect of locally available, low cost barrier material should encourage further investigation of less specialised resources. Even in cases where local soils and additives cannot achieve the low conductivities of the products examined here, reversible mitigation may be preferred to the irreversible intervention of most damp-proofing techniques.

Authors' contributions

MM participated in the design of the study, carried out the experiments and drafted the manuscript. RL coordinated the study, and participated in its design. CZ conceived of the study, and participated in its design. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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