Archaeological medicinal earths as antibacterial agents: the case of the Basel Lemnian sphragides

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Abstract: This paper presents the scientific investigation of three Lemnian sphragides (terra sigillata, stamped earth), a famed medicinal clay in antiquity, dated to the sixteenth–seventeenth centuries, and presently in the Museum for the History of Pharmacy, University of Basel. The three specimens are compared with clays from the purported locality of its extraction, at Kotsinas, NE Lemnos, Greece. The study suggests a local origin for the Basel samples; it also demonstrates, for the first time, that the three Lemnian sphragides have a significant antibacterial effect against Staphylococcus aureus, a common Gram-positive pathogen, but have no such effect against Pseudomonas aeruginosa, a Gram-negative microorganism. Clay samples from the purported locality of extraction showed no antibacterial effect against S. aureus. Subsequent analysis with ultra-performance liquid-chromatography mass spectrometry (UPLC-MS) revealed the presence of organic constituents in one sphragis which were absent from a sample of modern clay. A fungal secondary metabolite is proposed here as the active ingredient but other factors may also play a role. The ongoing investigation into the bioactivity of some medicinal clays might aid in the re-evaluation of Belon’s statement included at the start of this paper, namely, that the Lemnian earth worked only because people in the past wished it to work.

During the past ten years there has been a renewed interest in the study of Lemnian earth, a medicinal clay extracted from the island of Lemnos in the NE Aegean (Fig. 1). It was celebrated in antiquity as an antidote to poison (Theophrastus, On Stones 52; Dioscorides De Materia Medica V.13; Pliny Nat. Hist. XXXV.14; XXVIII.24; XXIX.33; Galen On Simple Drugs IX, II). During the Ottoman occupation of the island (fifteenth–early twentieth century) it was used against various ailments and as a preventive against ‘the plague’ (Hasluck 1909–1910; Sealy 1919; Hasluck & Hasluck 1929; Tourptsoglou-Stephanidou 1986, p. 562). It was concluded that the medicinal clay bore no pharmacological properties other than those attributed to it by those who believed in its efficacy. The earths of the Aegean and their potential antibacterial properties are currently the subject of investigation by our team and include not just Lemnos but also other islands in the Aegean (Photos-Jones et al. 2015, 2016).

The Museum for the History of Pharmacy of the University of Basel, Basel, Switzerland, established in 1925, has in its collection 36 such sphragides, part of a larger collection consisting of c. 420 similar artefacts, making it one of the most extensive collections of terra sigillata on display in the world.
shape the sphragides are spheroidal, triangular or square; they range in size between 1 and 8 cm in diameter. In colour they vary over a range of white, beige, dark brown, light red, dark red violet or yellow.

Of these medicinal earths the majority of more than 150 specimens originate from Silesia, the region between Poland, Germany and the Czech Republic and known in the texts as terra Silesia. The remainder derive from Laubach in Germany, Bohemia (today in the Czech Republic) and also from Malta and Cyprus (Häfliger 1931). Most of the Lemnian sphragides in the Basel collection are of unknown provenance. What is known, however, is that half of the Lemnos specimens came into the possession of the museum via the pharmaceutical institute of the Swiss Federal Institute of Technology in Zurich (ETHZ) in the 1930s. The sphragides formed part of the pharmacognostic collection started by Professor Eduard Schar between 1873 and 1892 and continued by Professor Carl Hartwich until 1917 (Sticher-Levi & Sticher 1995). The rest came from the private collection of Dr Joseph Anton Haefliger, a Basel apothecary, who bequeathed his collection to the university in 1925 and which formed the basis for the Museum for the History of Pharmacy. However, a detailed history of the collection is yet to be written.

The Lemnian sphragides examined here (Fig. 2a–c) display a script on their surface which can be attributed to the sixteenth century (Richard Todd, pers. comm. 2015). The grey sphragis (no. 01424) reads tin-makhtum (sealed clay) in a style of script that is very similar to Belon’s illustration of Lemnian sphragides in circulation at the time of his visit to Lemnos in the middle of the sixteenth century; it is reproduced from his book in Figure 2d. We can conclude that the grey sphragis is probably dated to the same period. The other two sphragides have a somewhat different style of script. Although less clear, they also probably read tin-makhtum (Richard Todd, pers. comm. 2015).

The locality of the extraction of the raw material for the Lemnian sphragis (Fig. 3) near Kotsinas, NE Lemnos has been well documented in the sources mentioned above, as has the ritual of extraction and its subsequent processing. About 40 travellers’ accounts written between the late fifteenth century and early twentieth century have been translated and edited by the historian Tourptsoglou-Stephadidou (1986). Information about the Lemnian earth formed an integral part of any description of the island during that period, together with its flora,
Fig. 2. (a) Lemnian sphragis (red). MA no. 01422/44. (b) Lemnian sphragis (grey). MA no. 01424. (c) Lemnian sphragis (white). MA no. 01432. (d) Belon’s illustration of the stamps on the Lemnian sphragides at the time of his visit (Belon 1553, p. 55).
fauna or customs of its inhabitants. Most of the sources agree that the raw material was extracted/dug out of a pit once a year during the course of a single day (6 August) and was subsequently ‘washed’. There were different grades of Lemnian earth, and only the finest variety was stamped. Apart from the pit, there have also been suggestions that the water of nearby natural spring(s) may have been involved in the process of enrichment of the medicinal variety. For example, Belon (1553) referred to two springs, Albacario (c. 1680s) to three springs and finally Covel (c. 1670s) to one spring (Tourptsoglou-Stephanidou 1986, p. 162). There are today three natural springs in the locality known as Phthelidia, Strongyle and Kokala (Roumelioti 2013).

The exact nature of the involvement of the springs is not clear; however, there are some tantalizing suggestions. For example, Joos van Ghistele, a Dutchman who visited Lemnos in 1485, wrote that (Terra Sigillata) is produced in Lemnos in a pool which dries up every summer and is full of water in winter. When this pool begins to dry up, a thick scum, variegated in colour, forms on its surface. This is skimmed off and laid on clean planks as required, according to the method in use locally. When dry, it is made up into round pellets or flat cakes.

It has been pointed out that the Dutch/Flemish word for scum is equivalent to ‘a scum on the surface of beer or wine caused by fungus’ (Hasluck & Hasluck 1929, p. 674). Another account suggests that ‘this earth [was collected] from the mud of a spring’ (Carlier de Pinon in the late 1500s; see Tourptsoglou-Stephanidou 1986, p. 111). A third account pointed out that the presence of a water source kept the pit moist all year round. It follows that the pit may have acted as a settling tank throughout the year (Jacopo Soranzo in 1580s; see Tourptsoglou-Stephanidou 1986, p. 119). Apart from van Ghistele, and in reference to the ‘fothiness’ associated with the pit, another writer reported that ‘the sacred earth “jumps” and “overflows”’ (Sibthorp in 1810s; see Tourptsoglou-Stephanidou 1986, p. 451).

The coastal hamlet of Kotsinas, which is first mentioned in Byzantine records, became well known for its potting tradition (Tourptsoglou-Stephanidou 1986, p. 50). Indeed, the remains of several workshops/kilns in various stages of abandonment could still be found in 2007. Ethnographer Psaropoulou (1986, p. 235), who interviewed the local potters in the 1970s, mentions that some of their forebears ‘made cups from the earth taken from a locality known as Kokalas’. The name Kokala(s) must have been associated with the third spring mentioned above.

The aim of this paper is to examine the Basel Lemnian sphragides not simply from their mineralogical and chemical make-up but also from a...
microbiological perspective, namely from the perspective of their potential antibacterial properties. There is no way of establishing for certain that the Basel Lemnian sphragides did indeed originate from Lemnos. Stamped Lemnian earth was well sought after; fakes were extensively circulating throughout central Europe and the bazaars of the Ottoman Empire (MacGregor 2013). However, although it is important to attempt to embed the Basel sphragides within the landscape of their origin (i.e. Kotsinas), it is well acknowledged that provenance studies require large datasets deriving from extensive sampling and analysis, which are currently unavailable. The small dataset presented here can therefore only be considered indicative rather than conclusive.

A total of eight samples have been examined, including the three Lemnian sphragides (Fig. 2a–c) and five samples of sedimentary clays retrieved from various depths in Kotsinas and within an area of weathered volcanic tuffs (Fig. 3) (E. Zagana, pers. comm. 2013; Roumelioti 2013). There is no way of knowing what the boundaries of the area of clay extraction were over a period of 500 years or more. It is very likely that many pits would have been opened over a considerable area and, once emptied in the course of each year, it is unlikely that they would have been reworked as their contents would have been depleted. This implies that, although we assume today that only the area within these volcanic tuffs may have been worked, other areas may also have been sampled and used for the preparation of the medicinal earths. Furthermore, one or more pits may have been involved.

**Materials and methods**

As mentioned earlier, geological samples were taken at depth as clearly suggested by the texts. Depth from ground surface is designated on the sample number; for example, LE6-1.60 signifies a sample taken at a depth of 1.6 m. All samples are buff coloured like the grey Lemnian sphragis (Fig. 2b), with the exception of LE8-surf which was red and was collected from the surface. Samples were analysed with quantitative X-ray powder diffraction (PXRD) to establish mineral structure and are interpreted using the Diffrac Plus software package from Bruker and the Powder Diffraction File (PDF). The quantitative analysis was performed by the Rietveld method, using the TOPAS software from BRUKER. The samples were ground to a grain size of less than 60 μm. For the PXRD analysis two samples were prepared, one oriented and one unoriented. Because of the overlapping of montmorillonite with chlorite on the 15 Å peak, the samples were also treated with glycerol. As a result of the presence of chlorite the 15 Å peak is split into two peaks: one for montmorillonite with c. 17 Å, because of swelling; and a second with 14 Å which belongs to chlorite. The results are presented in Table 1.

**PXRD**

The mineralogical analysis was carried out via PXRD on a Bruker D8 Advance Diffractometer using Ni-filtered Cu Kα radiation (35 kV, 35 mA) with a Lynx Eye strip silicon detector. Data were collected for 2θ values in the range of 3–70° with a step size of 0.02° and a count time of 1 second per step. The diffractograms were analysed and interpreted using the Diffrac Plus software package from Bruker and the Powder Diffraction File (PDF). The quantitative analysis was performed by the Rietveld method, using the TOPAS software from BRUKER. The samples were ground to a grain size of less than 60 μm. For the PXRD analysis two samples were prepared, one oriented and one unoriented. Because of the overlapping of montmorillonite with chlorite on the 15 Å peak, the samples were also treated with glycerol. As a result of the presence of chlorite the 15 Å peak is split into two peaks: one for montmorillonite with c. 17 Å, because of swelling; and a second with 14 Å which belongs to chlorite. The results are presented in Table 1.

**ICP-MS**

The samples were analysed with ICP-MS (7500CX coupled with Autosampler Series 3000, both by Agilent Technologies). About 200 mg of each sample were digested with 8 ml aqua regia in a microwave digestion device (Multiwave 3000, Anton Paar), following the EPA3051 method. The method is not suitable for determination of Si due to partial dissolution of the silicates and of S due to analytical constraints. The analysis of the major elements is therefore not included. The precision of the analyses was tested using suitable standards. The results of the trace element analyses are presented in Table 2.

**Microbiological testing**

Eight samples, five sedimentary clays and three sphragides, were prepared for microbiological testing by grinding into fine powders and subsequent sterilization by dry heat at 200°C for 2 hours. Two fully susceptible bacterial strains, one Gram-positive organism (S. aureus ATCC 25923) and one Gram-negative organism (P. aeruginosa ATCC 27853), were chosen. A 0.5 McFarland dilution (a standard bacterial dilution utilizing optical density to create a standard inoculum) of each bacterial strain was made using sterile saline and a densitometer. A total of 100 μl of this suspension was added to a sterile micro tube containing 0.02 g of the sample. Positive and negative controls were included, consisting of: (a) bacterial suspension only (with no mineral samples added); and (b) sample only (100 μl of sterile saline added instead of bacterial suspension). Each combination was set up in
Table 1. Results of quantitative PXRD analysis for the three sphragides and a set of five sediments

| Sample     | Dolomite | Illite | Kaolinite | Quartz | Albite | Montmorillonite | Hematite | Chlorite | Gypsum | Alunite | Cristobalite | Trymite | Calcite |
|------------|----------|--------|-----------|--------|--------|-----------------|----------|----------|--------|---------|--------------|---------|---------|
| LE1 (WHITE)| 65.2     | 9.9    | 17.3      | 7.6    | –      | –               | –        | –        | –      | –       | –             | –       | –       |
| LE2 (GREY) | –        | 18.1   | –         | 6.9    | 9      | 66              | –        | –        | –      | –       | –             | –       | –       |
| LE3 (RED)  | –        | 41     | 37.4      | 17.7   | –      | –               | 3.8      | –        | –      | –       | –             | –       | –       |
| LE1-3.2    | –        | 14.9   | 2         | 33.2   | 15.1   | 18.1            | –        | 5.7      | 1      | –       | –             | –       | 9.9     |
| LE2-2.7    | 12.2     | 1      | 23        | 6.9    | 30.5   | –               | 10.4     | –        | –      | –       | –             | –       | 15.8    |
| LE5-3.3    | 13.3     | 1      | 21        | 12.7   | 35.1   | –               | –        | 8.9      | –      | –       | –             | –       | 8.1     |
| LE6-1.6    | 22       | 1.3    | 23.8      | 10.1   | 16.9   | –               | 9.9      | –        | –      | 22.5    | 4.5           | 1.9     | –       |
| LE8-surf   | –        | –      | 69.3      | –      | –      | –               | 1.8      | –        | –      | –       | –             | –       | –       |

Mineral composition normalized to %.
triplicate. The micro tubes were then incubated overnight in an upright position on top of a rotating mixer to help prevent sedimentation. To help estimate bacterial counts, ten-fold serial dilutions were made using sterile saline and either: (a) the bacterial suspensions prior to overnight incubation; (b) the bacterial suspensions following overnight incubation; or (c) the bacterial suspensions + samples following overnight incubation. A total of 10 ml of each dilution was plated onto blood agar in duplicate and incubated overnight at 37°C. Following overnight incubation of these plates, bacterial counts were quantified by counting the number of colony-forming units on each plate. Results are displayed as graphs in Figures 5 and 6.

**UPLC-PDA-MS**

Only one sample of Lemnian sphragis (LE2 (GREY) (bioactive)) and only one of the sedimentary clays (LE5-3.3 (non-bioactive)) were analysed; 20 mg of each sample was extracted in 1 ml 50% aqueous methanol. Each sample was sonicated using a Soniprep 150 (MSE, UK) for 5 min in 1 min intervals (100 W, 17 kHz, maximum amplitude). The samples were subsequently centrifuged at 13 000 g for 10 min and supernatants were transferred to HPLC vials.

The combined system of chromatography with mass spectrometry was a Waters Acquity Ultra-performance LC coupled to a photodiode array and a Xevo quadrupole time-of-flight mass spectrometer (UPLC-PDA-MS). Liquid chromatography separates the various components within a compound as a function of retention time as they travel through a column. Identification of each component requires mass spectrometry, and in this study direct infusion-electron spray ionization was the method used to introduce the components into the mass spectrometer.

![Fig. 4. X-ray diffractogram of sample LE2 (GREY) sphragis, after Rietveld refinement.](image-url)
Samples were separated on a BEH C18 column (100 × 2.1 mm; 1.7 μm particle size) which was maintained at 40°C. The mobile phase was a mixture of (a) Milli-Q Water plus 0.1% formic acid and (b) acetonitrile plus 0.1% formic acid. Separation was achieved using a gradient increasing from 20% (b) to 70% (b) over 10 min, followed by a 100% (b) wash step and re-equilibration. Data were acquired in positive ion electrospray scanning from m/z 50 to 2000 (mass-to-charge ratio) with a scan time of 2 s and inter-scan delay of 0.1 s. Low-voltage scans were acquired at 6 V and high-voltage using a ramp over 25–40 V, providing parent ion and characteristic fragment data, respectively. Instrument control, data acquisition (centroid) and processing were achieved using MassLynx v4.1. The results for direct infusion (positive and negative ionization) for both the sphragis and the local clay

Fig. 5. (a) Effect of the three Lennian sphragides on bacterial count of *S. aureus* (left column/blue) and *P. aeruginosa* (right column/red). All three sphragides showed an antibacterial effect against *S. aureus* but not against *P. aeruginosa*. The white and grey sphragides appear to exert a more significant effect than the red. (b) Effect of the Kotsinas sediments on bacterial count of *S. aureus*. None of the five sediments showed an antibacterial effect against this pathogen.
are shown in Figure 7 and the reverse-phase chromatograms for the same samples are shown in Figure 8.

Results

Lemnos is dominated by young Cenozoic (Upper Eocene–Lower Oligocene) sedimentary rocks which consist of both marine and terrestrial sediments (Fig. 2) (Photos-Jones & Hall 2011, p. 51). Such sedimentary rocks underlie the topographically gentle areas in the north-central, northeastern and southern parts of the island. Younger Lower Miocene volcanic rocks, both lavas and pyroclastics, crop out in a general east–west zone through the central area of the island. Extensive outcrops of mafic to felsic intrusive rocks of calc-alkaline affinities cross-cut the sediments in the extreme southern and northwestern parts of the island, but from their distribution on the geological map these do not appear to relate to well-defined magmatic centres.

A significant concentration of intrusive rocks of calc-alkali mafic to felsic composition occurs in sediments in the extreme southern part of the island. However, the notable development of andesitic to felsic lavas in the western part of the island attests to the presence of a volcanic centre in the vicinity of the study area. In the area of the extraction of the Lemnian earth there are light-yellow to yellow slightly altered pyroclastic volcanic rocks of dacitic-andesite composition. Within light-coloured surface material, rare relicts of fresh volcanic rock and isolated feldspar phenocrysts are observed. The Kotsinas area consists largely of Miocene tuffs which are bordered to the west by Holocene alluvium and to the east and south by shallow-marine calcareous mudstones and sandstones (Fig. 3).

The results of the quantitative PXRD analyses (Table 1) suggest that the three sphragides are not mineralogically identical: the white sphragis consists largely of dolomite and kaolinite, with some illite and quartz; the grey sphragis (Fig. 4) consists of montmorillonite, illite, albite and quartz; and the red sphragis consists mainly of illite, kaolinite and quartz. The fifth sample of sedimentary clay, surface sample LE8, consists primarily of kaolinite, alunite and hematite. The main difference between clay sediments and sphragides is the presence of calcite and chlorite, which are both absent from the sphragides. If calcite was originally present it could have been removed by some sort of treatment, for example dissolution in some organic acid. However, the removal of chlorite by beneficiation is less likely. Dolomite in LE1 (WHITE) is absent from any of the sediments analysed here, while LE2 (GREY) is particularly rich in montmorillonite, suggesting a volcanic tuff environment; on the other hand LE8-surf, with kaolinite and alunite, suggests an acidic alteration in a volcanic environment.

Table 2 presents the ICP-MS results for trace elements in an attempt to highlight potential

Fig. 6. Top row: the agar plates show the differences in colonial appearance when S. aureus was mixed with a Lemnian sphragides sample: (a) white, (b) red and (c) grey Lemnian sphragides. Bottom row: normal colonies of S. aureus which grew from our positive control. Both the number and size of colonies has diminished. Such an effect was not seen with P. aeruginosa. The appearance of S. aureus colonies returned to normal when they were subjected to a fresh agar plate.
Fig. 7. Positive ionization spectrum of (a) LE2 (GREY), the bioactive sphragis and (e) LE5-3.3 sedimentary clay. Comparison of the two spectra shows that the clay displays far fewer peaks than the sphragis. Negative ionization spectrum of (b) LE2 (GREY) bioactive sphragis and (d) LE5-3.3 sedimentary clay. Comparison of the two spectra shows that there is an overlap between clay and sphragis for some peaks (e.g. 325.2110). However, other peaks (e.g. 731.1160) are present only in the sphragis.
differences in metallic element content which might have adverse effects on antimicrobial properties. Pb content is relatively elevated in the sphragides compared to some of the sediments, but not all (i.e. LE6-1.60). Cu content is considerably higher in LE1 (WHITE) while As content is elevated in LE3 (RED). Ti, Co, Cr, Ni and Mn are overall relatively lower in concentration in the sphragides compared to the sedimentary clays.

After chemical and mineralogical analysis, the three sphragides were subsequently tested microbiologically against S. aureus and P. aeruginosa; the results are shown in Figure 5a. A logarithmic scale on the y-axis of the graph displays the bacterial counts for both S. aureus and P. aeruginosa, expressed as the mean number of colony-forming units per 10 ml. The graphs illustrate the changes in bacterial counts, both after an overnight (18 h) incubation and with or without addition of mineral samples. Error bars demonstrate the standard error of the mean (SEM), calculated on Microsoft Excel by first working out the standard deviation of sample results and then dividing this number by the square root of the number of samples. Significance (p value) is relative to the positive control (bacterial suspension only, at 18 h), and was determined on Microsoft Excel using a two-tailed t-test. Only p values ≤ 0.05 were deemed significant.

All three varieties of sphragides were associated with a statistically significant reduction in bacterial counts of S. aureus, but not P. aeruginosa (Fig. 5a). The most significant antibacterial effect was elicited by the white and grey Lemnian sphragides. Bacterial counts of S. aureus were reduced by 3 log (p = 0.0006 and 0 = 0.009, respectively). Although the red Lemnian sphragis reduced bacterial counts of S. aureus by less than 1 log, it reached statistical significance (p = 0.009); it also affected the morphology of the bacterial colonies (Fig. 6).

The five Kotsinas sedimentary clays were subsequently tested against S. aureus but not against P. aeruginosa. As can be seen in Figure 5b none of them reduced significantly the bacterial counts of S. aureus. Sample LE8-sulf was associated with a slight reduction in the bacterial count of S. aureus, but this was a reduction of less than 1 log and did not reach statistical significance (p = 0.27). The bacterial colonies of S. aureus which grew following exposure to the Lemnian sphragides were noticeably smaller and paler than the positive controls (Fig. 6), suggesting stunting of bacterial growth. The appearance of S. aureus colonies returned to normal when they were subjected to a fresh agar plate, suggesting that the damage was reversible.

Having assessed the antibacterial activity of the three Lemnian sphragides, it is now essential...
to direct attention to the molecules that might be responsible for it. Direct infusion with positive ionization of the extract from Lemnian *sphragis* gave a spectrum with an intense ion at $m/z$ 579.2228, whereas no prominent ions were detected in the extract from LE5-3.3 (Fig. 7). Matching the main peak to a natural compound database (Laatsch 2014) revealed that it has a close similarity to a family of compounds of fungal origin known as bioxan-thracenes. These are a group of fungal metabolites which have antimalarial and cytotoxic properties (Laatsch 2014).

Further to the above, the extracts were separated by reversed-phase chromatography using a method routinely used for analysis of natural metabolites from cyanobacteria. The ion chromatograms revealed that the Lemnian *sphragis* contained many peaks with mass between 300 and 750 amu in addition to that at $m/z$ 579 and also 705 which were not present in the extracts (Fig. 8).

**Discussion and conclusions**

On the basis of mineralogy and geochemistry it is clear that the three Basel Lemnian *sphragides* could not have originated from any of the sedimentary clays discussed here. However, there is a strong probability that the three *sphragides* did originate from the greater Kotsinas area, the ‘original’ boundaries of which, if they ever existed, cannot be known; neither can we be confident of the depth from which clay for *sphragides* was retrieved. We examine each *sphragis* individually.

Regarding the dolomite-rich *sphragis* (LE1 (WHITE)), samples with similar composition have been obtained from the grey-blue clays of the Romanou–Kontopouli area which lies to the east of the Kotsinas area (Fig. 3). The area includes the now-deserted hamlet of Aghios Ypatios where Covel (Tourtspoglou-Stephanidou 1986, p. 160) tells us that ‘washing’ of the earth did take place. Analyses of such clays have revealed the following composition: dolomite 19.25%; illite 27.22%; kaolinite 19.80%; albite 9.36%; and quartz 24.39% (I. Marantos, pers. comm. 2015). It is possible that similar deposits occur at Kotsinas at depth. In the Kotsinas area there is a geological fault where Miocene tuffs occur at Kotsinas at depth. In the Kotsinas area (Fig. 3). The area includes the now-deserted hamlet of Aghios Ypatios where Covel (Tourtspoglou-Stephanidou 1986, p. 160) tells us that ‘washing’ of the earth did take place. Analyses of such clays have revealed the following composition: dolomite 19.25%; illite 27.22%; kaolinite 19.80%; albite 9.36%; and quartz 24.39% (I. Marantos, pers. comm. 2015). It is possible that similar deposits occur at Kotsinas at depth. In the Kotsinas area there is a geological fault where Miocene tuffs occur at Kotsinas at depth. In the Kotsinas area there is a geological fault where Miocene tuffs occur at Kotsinas at depth. In the Kotsinas area there is a geological fault where Miocene tuffs occur at Kotsinas at depth. In the Kotsinas area there is a geological fault where Miocene tuffs occur at Kotsinas at depth.
There is also the possibility of a brominated metabolite, but its identity is not presently known.

Having established the presence of a possible secondary metabolite, it is important to be able to exclude the possibility that fungal growth responsible for bioactivity may have been acquired as a result of long-term storage. Instead, it is important to be able to demonstrate that the molecules responsible for the bioactivity of the three Basel Lemnian sphragides were acquired as a result of mineral beneficiation at the time of its extraction and packaging. Although the possibility of a placebo effect (as implied by Belon’s statement at the start of the paper) cannot be fully discarded, it is still possible that the clay-based Lemnian sphragis may have not been ‘made precious’ simply by means of a ceremony but rather by means of a carefully determined plan of combined beneficiation and appropriate choice of raw materials.

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