Geochemical and microbiological characterization of some Azorean volcanic muds after maturation

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Abstract

Four Azorean volcanic muds were submitted to maturation (ageing process) with mineral water for 60 days under different abiotic conditions. This study intends to assess the geochemistry of these natural resources before and after maturation and to evaluate the ability of materials to be colonized by microorganisms under different mixing and light maturation procedures. Besides the geochemical and mineralogical analyses, bacterial quantification and diatom community identification and relative quantification were also provided. Principal component analysis was carried out on geochemical data. Our data reveal that muds have potentiality for thermal application and the modifications induced by maturation are affected by raw materials properties. Slightly geochemical modifications were verified probably due to the weathering of primary minerals. The bacteria and mainly the algae development on samples under light and no mixing maturation conditions were recorded rendering to muds potentialities for therapeutic uses.

Keywords: Natural resources, human health, volcanic muds, geochemistry, microbiology, phycology.

Introduction

Volcanic thermal muds have been applied "in situ" or in Spa Centres for therapeutic purposes since a long time. To be considered suitable for therapeutic application, a thermal mud (mixture of a solid phase with mineral water) should be submitted to a maturation (ageing) procedure in order to improve the quality of the raw materials and to allow the microorganisms colonization during the process. Microflora developed during the process is mainly represented by prokaryotes (mainly Cyanobacteria) and eukaryotic organisms mainly consisting of green algae and specially diatoms. The mud's anti-inflammatory action was attributed to a product of microorganisms' metabolism, the sulphoglycolipids which render mud its suitability for use, which is achieved after 50-60 days of maturation[1-5].

Maturation is a complex process affected by several factors such as water temperature, light exposure and mixing procedures that determine the final mud characteristics. There have been some indications that mud quality is also affected by granulometry, mineralogy and physico-chemistry of the raw material and geochemistry of mineral water[6-8]. Some researches evidenced that water retention, consistency, bioadhesiveness, ease of handling, pleasant sensation while applied to the skin, cooling kinetics and exchange capacity through mud/skin interface are important features for the formulation of peloids.[5, 6, 8, 9]

Therapeutic action of mud is not only due to heat sensation that leads to multiple reactions (vasodilatation, perspiration, stimulation of cardiac and respiratory frequency) but also due to anti-inflammatory action and cation exchange between mud and skin[10]. Due to the anti-inflammatory action, thermal muds have been indicated for chronic rheumatic diseases, osteoarthrosis, spondylosis myalgias and skin diseases.[4, 5, 10-12]

São Miguel Island, one of the nine islands of the Azores Archipelago (Portugal), is a particular case where some of the hyperthermal springs hold muds that have been formerly mixed with local thermal water and applied mainly at a regional Thermal Centre taking advantage of muds high temperature and high sulphur content. Recent studies carried out by our research team assessed physical and chemical properties of S. Miguel Island mud samples. Muds were considered suitable for application as cataplasms[13].

Few studies have been developed all over the world on volcanic muds concerning either maturation or therapeutic action and none is known in Portugal. The aim of this study is to characterize the maturation process of volcanic muds in what concerns the geochemistry of materials and to assess the potentiality of these muds to be colonized for microorganisms during the process. As best maturation conditions are unknown, it is also our goal to develop the maturation of muds under different abiotic conditions (light and remixing) in order to optimize the development of useful microorganisms and to obtain the best mud quality.

Material and Methods

Geological settings: The Azores Archipelago, located in the North of the Atlantic ocean, near the European western coast (1500 km west of Portugal) sits between latitudes 36°55'43" N and 39°43'23"N and longitudes 24°46'15"W and 31°16'24"W. The archipelago is composed by islands of volcanic origin that lies within a complex geodynamic setting by the proximity to the triple junction between the American, the African and the Eurasian plates. The Middle Atlantic Ridge divides the western islands (Flores and Corvo) from the other islands that are scattered along a WNW-ESE-trending strip[14, 15].

São Miguel, Terceira, Pico, Faial, Graciosa and Flores Islands exhibit hydrothermal manifestations such as mineral and thermal waters and also fumarolic fields as a result of their geological settings[14]. In São Miguel, the major island of the archipelago, the fumarolic fields occur mainly in the three active volcanic centers, especially at Fogo and Furnas volcanoes. Furnas village and Furnas Lake fumarolic fields located inside the Furnas caldera as well as Ribeira Grande fumaroles on the northern flank of the Fogo volcano are related to important tectonic features[14, 16]. Terceira Island landscape is dominated by four main strato-volcanoes. Pico Alto volcano and Santa Bárbara volcano are still active and Cinco Picos and Guilherme Moniz are extinct volcanoes with incomplete calderas[17]. Furnas do Enxofre with a central position in the island is a notable hydrothermal manifestation controlled by a fault system.

Sampling and maturation procedures: Samples were obtained from the surface bubbling mud inside the springs. Water/mud temperature and pH were measured with a portable instrument (HI 9025 pH meter), calibrated in the field. Three volcanic muds from S. Miguel Island – Furnas Village (10), Furnas Lake (16), Ribeira Grande (19) (65-68°C and pH – 1.7 to 1.8, field data) and one from Terceira Island – Furnas do Enxofre (L) (42°C and pH - 7.5, field data) were collected in November 2008 (Figure 1) and brought to laboratory. All samples were sieved at 500 μm before the maturation procedures. Small tanks (ca 1.3 dm^3) were filled with mud and covered by a layer of S. Miguel thermal mineral water during the process (ratio 5:1). Tanks were kept uncovered during the experiment. For the maturation process a thermal mineral water from São Miguel Island was selected, this particular water was used some years ago on a local Thermal Centre.

Replicates were made from each mud sample. Three different combinations of light conditions (ambient light /artificial light /darkness) and two different agitation conditions (remixing/ no mixing) were tested in a controlled room temperature (about 18°C during the day and 15°C at night). This experiment totalized 24 different maturation conditions and took place during the 60 days of muds maturation.

Volcanic muds and maturation water characterization: For each sampling site at the beginning and at the end of the maturation process, a portion of sample was dried at constant temperature (50°C) until constant weight. Samples were disaggregated before analyzed.

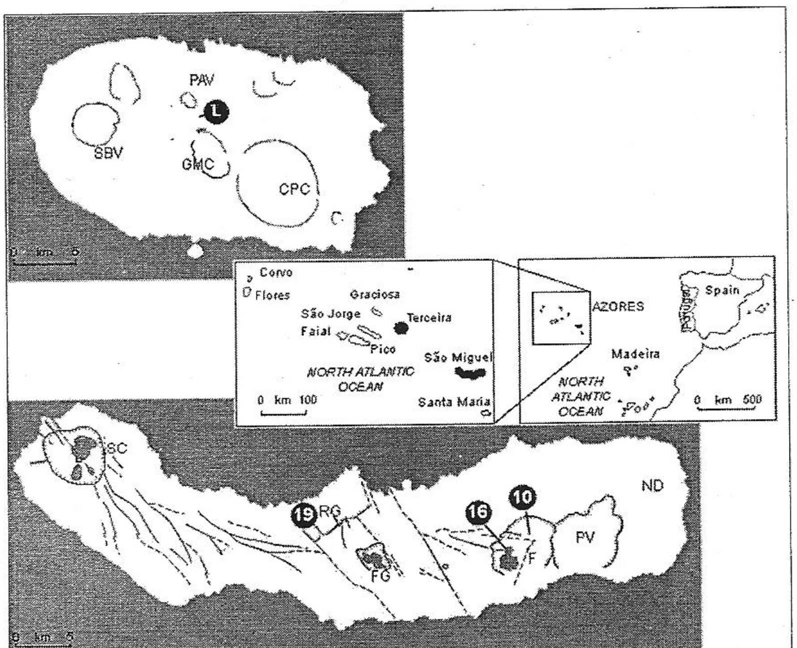

Fig. 1: Setting of the Azores Archipelago in the North Atlantic Ocean. A- São Miguel Island map and most prominent regions (SC: Sete Cidades; RG: Ribeira Grande; FG: Fogo; F: Furnas; PV: Povoação ; ND: Nordeste)[18]; B - Terceira Island map (SBV: Santa Barbara volcano; GMC: Guilherme Moniz caldera; PAV: Pico Alto volcano; CPC: Cinco Picos caldera)[17].The location of the volcanic muds studied (10, 16, 19 and L) are marked in the maps.

Table I

Geochemical results of muds before and after maturation process. Data is rounded according to detection limits

Sample		Furnas Village (10)							Furnas Lake (16)							Ribeira Grande (19)							Furnas do Enxofre (L)							Reference samples			
Label		10i	10A	10B	10C	10D	10E	10F	16i	16A	16B	16C	16D	16E	16F	19i	19A	19B	19C	19D	19E	19F	Li	LA	LB	LC	LD	LE	LF	Benet.[a]	Calda[b]	Capp.[c]	Comm.[d]
Condition		WM	AL +M	AL +W	ArL +M	ArL +W	D +M	D +W	WM	AL +M	AL +W	ArL +M	ArL +W	D +M	D +W	WM	AL +M	AL +W	ArL +M	ArL +W	D +M	D +W	WM	AL +M	AL +W	ArL +M	ArL +W	D +M	D +W				
Major chemical elements (%)	SiO_2	85.8	77.2	77.0	68.7	68.7	70.3	69.9	47.1	48.4	50.6	49.5	48.5	49.7	49.8	52.0	52.4	52.4	52.5	50.9	50.2	54.2	40.7	32.2	32.3	40.7	40.7	39.7	39.6	58.9	52.0	44.1	55.4
	Al_2O_3	4.7	8.7	8.7	14.2	14.0	12.3	12.4	20.3	20.4	19.8	20.0	19.9	20.0	19.8	19.3	19.8	19.8	21.5	19.2	20.8	20.4	29.9	30.2	30.0	29.8	29.8	31.0	30.9	18.0	13.8	11.9	19.8
	Fe_2O_3	0.9	1.6	1.6	2.6	2.6	2.6	2.5	7.6	6.7	5.8	6.2	6.4	6.1	6.1	1.6	1.7	1.7	1.9	1.6	1.8	1.7	12.0	12.7	12.6	12.1	12.0	11.3	11.3	4.4	5.8	5.3	7.3
	MnO	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
	MgO	0.0	0.1	0.1	0.5	0.4	0.3	0.3	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	2.4	2.7	2.8	3.0
	CaO	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	9.4	13.9	0.9
	Na_2O	0.7	1.4	1.4	1.1	1.1	1.2	1.3	3.3	3.2	3.5	3.3	3.4	3.4	3.4	1.9	1.8	1.8	1.8	1.6	1.6	1.6	1.2	0.9	1.1	1.2	1.3	0.9	0.9	1.0	0.8	0.7	2.0
	K_2O	0.8	1.9	1.9	2.2	2.2	2.4	2.4	4.7	4.6	4.7	4.6	4.6	4.7	4.7	3.1	2.8	2.8	2.8	2.5	2.6	2.6	1.2	1.8	1.9	1.1	1.2	1.1	1.1	1.7	2.2	2.0	1.2
	TiO_2	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.9	1.9	1.8	1.8	1.8	1.8	1.8	1.6	1.6	1.6	1.6	1.5	1.5	1.6	1.4	2.0	1.9	1.4	1.4	1.3	1.3	0.5	0.7	0.6	0.9
	P_2O_5	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.4	0.4	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1
	S	0.2	0.3	0.4	0.4	0.5	0.5	0.6	5.0	4.9	4.3	4.7	4.9	4.4	4.4	5.2	4.4	4.6	2.9	5.6	5.7	3.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	n.p.	0.2	n.p.	n.p.
Trace elements (ppm)	Cu	5.4	3.3	3.5	2.6	2.5	1.7	1.6	10.8	11.0	10.8	9.9	9.9	9.9	10.4	35.2	29.0	28.1	47.6	28.7	27.1	44.3	2.4	4.1	4.0	3.9	3.9	3.1	2.5	n.p.	27.0	24.0	n.p.
	Pb	2.4	4.3	4.3	7.7	7.9	8.1	8.5	9.6	10.2	7.8	8.8	9.5	8.9	8.1	18.2	16.3	15.8	20.2	14.2	14.5	20.4	12.5	51.7	52.9	26.4	26.1	17.2	16.4	n.p.	14.0	8.5	n.p.
	Zn	1.0	4.0	4.0	7.0	7.0	5.0	6.0	17.0	18.0	19.0	17.0	20.0	17.0	19.0	5.0	7.0	9.0	6.0	9.0	7.0	6.0	52.0	40.0	42.0	56.0	55.0	49.0	50.0	n.p.	109.0	67.0	n.p.
	Ni	1.0	0.8	0.8	0.8	1.2	0.9	1.0	7.0	8.1	7.9	7.2	7.9	7.0	7.1	2.4	5.2	6.3	5.2	7.5	5.2	7.2	0.6	1.2	1.4	1.6	1.4	1.1	1.0	n.p.	58.0	67.0	n.p.
	Mn	16.0	34.0	36.0	90.0	102.0	74.0	82.0	45.0	51.0	49.0	47.0	52.0	46.0	50.0	5.0	8.0	11.0	8.0	12.0	9.0	9.0	370.0	316.0	326.0	429.0	408.0	369.0	364.0	n.p.	n.p.	n.p.	n.p.
	As	6.1	26.0	27.2	71.3	73.0	59.3	62.0	3.6	4.2	3.9	3.4	3.9	3.4	3.4	1.1	1.4	1.6	1.8	2.1	1.8	2.1	1.8	2.4	2.1	2.6	2.6	2.2	1.9	n.p.	4.0	3.4	n.p.
	Th	1.1	2.9	3.0	7.9	8.0	6.0	6.0	6.7	7.1	7.1	7.0	7.3	7.3	7.1	14.6	16.7	17.2	20.8	16.9	17.3	20.3	19.1	20.9	21.4	19.8	20.1	19.5	19.8	n.p.	n.p.	n.p.	n.p.
	Cd	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.1	0.2	0.3	0.2	0.1	0.1	0.3	0.2	0.2	0.2	n.p.	0.5	0.1	n.p.
	Sb	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.6	0.5	0.5	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	n.p.	0.0	<0.05	n.p.
	Ba	125.0	48.0	51.0	22.0	20.0	21.0	25.0	44.0	54.0	47.0	49.0	48.0	49.0	48.0	12.0	14.0	14.0	15.0	14.0	14.0	16.0	30.0	23.0	21.0	19.0	19.0	19.0	19.0	n.p.	n.p.	n.p.	n.p.
	Hg	0.1	0.2	0.2	0.2	0.3	0.2	0.2	1.3	1.2	1.0	1.1	1.2	1.1	1.1	1.8	1.6	1.6	1.4	1.6	1.7	1.4	0.8	0.9	1.0	0.8	0.8	0.8	0.8	n.p.	0.1	<0.02	n.p.

Reference samples: [a]Thermal mud used in the spa of Benetutti (Northern Sardinia)[6], [b]Thermal mud used in the spa of Calda (Southern Italy)[9], [c]Thermal mud used in the spa of Cappetta (Southern Italy)[9], [d]Commercial peloid (Laviosa Chimica Mineraria, 1996)[6]. Wmat: without maturation; AL: ambient light; ArL: artificial light; D: dark; M: mixing; W: without mixing; n.p.: not presented. Table 1 only presents the trace elements with content above the detection limit.

Mud grain size distribution was assessed using wet sieving and X-ray grain size analyzer (Sedigraph 5100). Qualitative and semi-quantitative mineralogical analyses were carried out by X- ray diffraction (XRD) using a XRD-Philips X'Pert Pro-MPD[19-21]. Major chemical elements were assessed by X-ray fluorescence (XRF) using a XRF-Panalytical Axios PW4400/40. Samples were submitted to a multi-element analysis in an Accredited Lab in Canada (ACME Anal., ISO 9002) for minor chemical elements determination.

A 0.5 g split was leached in hot (95°C) aqua regia (HCl-HNO_3-H_2O) for one hour. The solutions were analyzed by Inductively Coupled Plasma-Emission Spectrometry for 27 chemical elements detection (Ag, As, Au, B, Ba, Bi, Cd, Co, Cr, Cu, Ga, Hg, La, Mn, Mo, Ni, Pb, Sb, Sc, Se, Sr, Th, Tl, U, V, W, Zn). The geochemical features of three samples in use on Italian spas centers: Benetutti, Calda and Cappetta muds and one commercial mud were also considered in order to evaluate the suitability of the Azorean volcanic muds for thermal centre application. Chemical analysis of mineral water was performed by ICP-Mass Spectrometry for determination of 72 chemical elements such as Ag, Al, As, Au, B, Ba, Bi, Ca, Co, Cr, Cu, Fe, Ga, Hg, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Sr, Th, Ti, U, V and Zn.

Principal component analysis (PCA) was carried out on geochemical data from maturated and initial volcanic muds.

Microbiology

Bacteria: The separation of microorganisms from clay and fine sand is very difficult. The main reason is their adhesion to particles. The analysis of bacteria was preceded by one-step extraction of the mud (5 g of mud from the sediment uppermost layer) by mechanical shaking for 30 min under controlled environment with 50 ml of 0.2% $Na_4P_2O_7$ to release the microorganisms followed by sedimentation for about 5 min[22].

The number of colonies of heterotrophic soil bacteria and fungi were determined on universal plate count media. Plate counts were performed at the beginning after 1 month and at the end of maturation period. Samples (1 ml) were inoculated by pour-plate method in Yeast Extract Agar. Bacteria colony-forming units (CFU) were counted after 48 h and 72 h of incubation at 37°C and 22°C respectively. For all samples, appropriate dilutions (using NaCl 0.9%) of extracts after sedimentation were made before plating.

Total coliforms were counted from samples of 0 and 60 maturation day to evaluate the microbial quality of the mud. CFU were obtained after filtration of 1 ml of decimal dilutions of sample through cellulose acetate filters (0.45 μm) and incubation on Chromocult® Coliform Agar media for 24 h at 37°C.

Algae: The edges of some fumaroles in São Miguel, under a thin pellicle of water, show green, brown and golden brown patches indicating the presence of photosynthetic organisms. Samples from the edges of the fumaroles (sampling area) were scraped or collected by syringe suction and kept alive for laboratory mud inoculation. A second sub-sample was immediately preserved with formalin (10% final concentration) for posterior study.

Temperature and pH measurements at each sampling site were carried out using a HI 9025 pH meter. Samples from the experimental tanks were collected on sediment uppermost layer at the beginning and at the end of the maturation process for analyses.

The study of diatoms requires the oxidation of samples to clean frustules for posterior identification which is based on valve morphology. Samples were cleaned using HNO_3 (65%) and potassium dichromate ($K_2Cr_2O_7$) at room temperature for 24 h, followed by multiple centrifugations (1500 rpm) for acid removal. Cleaned diatoms were mounted in Naphrax® before observation under light microscope (Leitz Biomed 20 EB) at 1000 x under oil immersion for diatom identification and semi-quantification. A total of about 400 valves were counted randomly from each sample except in those where diatoms were rare. Taxonomy was based on floras commonly used for Freshwater diatom studies [23-30].

Results and Discussion

pH and temperature in the water/mud maturation: During the maturation process, water pH decreases from 3 to 1.5 for samples 16 and 19; sample 10 preserves the pH value around 2 as well as sample L with a pH close to 4. Mud temperature decreases from 22°C to 18°C in all experimental tanks.

Geochemistry, mineralogy and grain-size distribution: Maturation water is bicarbonated-sodium type with higher content on Na (386 mg/L), Cl (156 mg/L), Si (150 mg/L), K (40 mg/L), Ca (16 mg/L), S (15 mg/L), B (9 mg/L) and Mg (8 mg/L); the remaining elements contents are below the 0.5 mg/L. Grain size analyses revealed that the raw materials have high content on fraction under 63 μm (sample 10 ≈ 77%; sample 16 ≈ 98%; sample 19 ≈ 94%; sample L ≈ 87%). Sample L has the highest content on fraction under 2 μm (60%) while sample 16 shows the lowest one (13%).

XRD analyses reveal that mineralogical composition of the muds is dominated by quartz, alunite, K-feldspars and plagioclase although present with different proportions on samples. Besides this common mineralogical composition, other phases are also present: magnetite-maghemite (samples 10 and L), pyrite (samples 16 and 19), hematite and opal (sample L) and sulphur (sample 19). Kaolinite is the main clay mineral present on all samples, being more abundant in L sample while sample

10 reveals the lower content on kaolinite and higher content on primary minerals such as quartz.

Geochemical composition of the muds (Table 1) shows similar features when compared to reference samples, particularly sample 16. Azorean volcanic muds show lower content on CaO and MgO but they are enriched in S and P_2O_5. Concerning trace elements muds reveal lower content on Cu, Zn and Ni. Despite few exceptions, the amount of toxic metals such as Cd, Hg, Pb and Sb in the muds studied are of the same order of magnitude as in comparative muds. Attention must be paid to the amount of As, whose presence should be avoided in particular on sample 10 (especially on the maturated ones).

The high content of Na, Si, K, Ca, S and Mg in the mineral water may explain the slightly enrichment of some of the maturated muds on these elements. The content on S may be an important feature for the Azorean muds because this element can be absorbed through skin and it has been reported to produce an analgesic effect[31].

After maturation, geochemical analyses (Table 1) reveal, for sample 10, a decrease on Ba and SiO_2 content and higher content in Al_2O_3, Fe_2O_3, MgO, CaO, Na_2O, K_2O, P_2O_5, As and Mn. Actually, a decrease in quartz and alunite $[KAl_3(SO_4)_2(OH)_6]$ was recorded in opposition to an increase in feldspars and kaolinite. Regarding the maturation of the sample 16, an increase in SiO_2, Ba and Mn and a decrease in Fe_2O_3 was recorded for all maturated muds. Sample 16 maturated muds only presented a slightly decrease in pyrite $[FeS_2]$ content and a discrete increase on kaolinite. Sample 19 maturation processes revealed only slightly chemical alterations comparatively to the initial sample. It seems that for this sample, the maturation process did not promote mineralogical or chemical alterations. Pb content increased in all maturated L samples. Slightly alterations on SiO_2, Al_2O_3, K_2O, and TiO_2 content were noted in muds maturated with ambient light (LA and LB samples). Matured samples revealed a decrease on plagioclase feldspars and a slightly increase on kaolinite.

PCA analysis: PCA data matrix included the 4 initial muds and the 24 mud samples submitted to different maturation conditions (A, B, C, D, E and F) and 18 active variables: SiO_2, Al_2O_3, Fe_2O_3, MgO, CaO, Na_2O, K_2O, P_2O_5 (major elements), S, Cu, Pb, Zn, Mn, As, Th and Ba (trace elements). The explained variance % and the cumulative variance of PCA axes are shown in table 2.

The first three axes explained 85.76% of the total inertia. The three axes retention was based on an empirical criterion of eigenvalues >1[32]: axis 1 explained Al_2O_3, Fe_2O_3, Pb, Zn, Mn and Th; axis 2 explained SiO_2 and As in opposition to CaO, Na_2O, K_2O, TiO_2, P_2O_5 and S; and axis 3 explained MgO and Ba in opposition to Cu.

Table 2
Correlations between variables and PCA axes, eigenvalues, percentage of explained variance and percentage of cumulative variance for initial and maturated muds

	Axis 1	Axis 2	Axis 3
SiO_2	0.83	-0.53	0.05
Al_2O_3	-0.93	0.28	-0.06
Fe_2O_3	-0.84	0.11	-0.52
MgO	0.39	-0.13	-0.59
CaO	0.58	0.70	-0.38
Na_2O	0.36	0.86	-0.28
K_2O	0.46	0.85	-0.19
TiO_2	-0.14	0.90	-0.16
P_2O_5	-0.40	0.87	-0.11
S	0.35	0.83	0.32
Cu	0.10	0.41	0.87
Pb	-0.80	0.11	0.12
Zn	-0.89	-0.03	-0.38
Mn	-0.87	-0.30	-0.36
As	0.45	-0.60	-0.27
Th	-0.86	0.06	0.47
Ba	0.48	0.00	-0.40
Eigenvalues	6.77	5.22	2.51
% Explained variance	39.85	30.72	14.74
% Cumulative variance	39.85	70.57	85.31

In axis 1, Al-Fe-Pb-Zn-Th association reflected the mineral signature of sample L [magnetite (Fe_2O_4)-maghemite (Fe_2O_3), hematite (Fe_2O_3) and kaolinite $(Al_2Si_2O_5(OH)_4)$ high content] and high content on Pb, Zn and Th. Axis 2 discriminates two groups of variables: Si-As and Ca-Na-K-Ti-P-S, matching to sample 16 and 10 mineralogical composition respectively. Sample 10 is characterized by a higher content on quartz (SiO_2) and As and sample 16 by a higher content on K-feldspars $(KAlSi_3O_8)$ and plagioclase $[(Na,Ca)(Si,Al)_4O_8]$ as well as Ti, P and S. Axis 3 discriminates the sample 19 features: low content in Mg and Ba in opposition to the Cu higher values.

The variable projection on PCA's first factorial plane established groups of chemical elements according to their affinity (Figure 2). PCA analyses exposed discrete changes of the maturated muds composition comparatively to the initial ones and assemblages the maturated samples according mainly to the light conditions, especially for L and 10 samples. The mixture procedure seems to have less repercussion on the geochemical features. Moreover, the period of maturation can influence significantly the alterations outcome so maturation for longer periods should be tested for these volcanic muds.

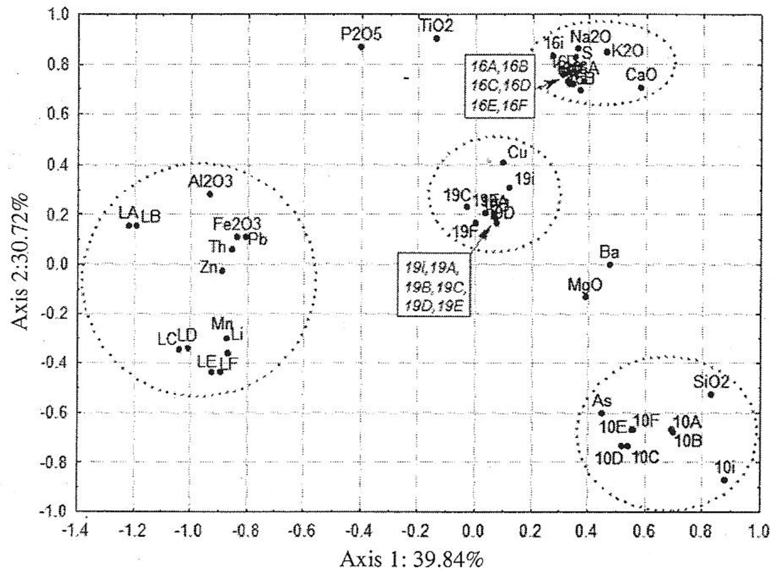

Fig. 2: Principal component analysis of Azorean volcanic muds: projection of variables and samples on the first factorial plane (axis 1/axis 2).

Microbiological characterization: Results of microbial counts (Table III) allow the arrangement of the four samples into two different groups in close correlation to their behaviour during the maturation process. Group one (comprising samples 10, 16 and 19) revealed similar evolution of the bacterial counts while the second group (including only sample L) behaved differently. While the first group revealed a decrease of total coliforms during the entire process, sample L showed an increase along the maturation period. Concerning the viable microorganisms, sample L revealed higher content of microorganisms growing at 37°C and 22°C when compared to the first group. Our findings suggest that samples 10, 16 and 19 may have some antimicrobial activity against pathogenic bacteria.

Microbial communities developed at 37°C and 22°C in sample L probably have a different composition from the one that colonized the other samples because they grew at different maturation conditions. It is admissible to consider that pH, taxonomic composition of the initial inoculate and smaller particles size features played a major role in the development of viable microorganisms and specifically, those of the coliform group.

Group one demonstrated high content of microorganisms growing at 37°C especially under light and without mixing. A similar behaviour was exhibited for viable microorganisms living at 22°C, but in this case a decrease was generally observed in samples collected half-way through the maturation period (30 day) and at the end of the process (60 day). Samples L submitted to mixture procedures, displayed a high amount of viable microorganisms.

The current work recorded slightly geochemical alterations after maturation process, especially for sample 10 which contains higher content on primary minerals (such as quartz). These alterations can be related with primary minerals weathering caused by an extremely low pH and microorganisms combined action. Sample 10 maturated muds revealed very low pH ($\approx$2) and a high content on microbes living at 22°C when compared to samples 16 and 19. Although sample L revealed the highest content on microorganisms, the raw material has already high content on secondary minerals (kaolinite) and exhibited the highest pH values that can influence the alterations rates induced by maturation.

Many microorganisms have long been known to play a significant role in mineral-weathering processes for their nutritional constituents; silicate minerals for example, often contain the limiting nutrients P and Fe as well as trace elements necessary for microbial growth. The microbial community establishment and development is mainly dependent on geochemistry, temperature and pH features[33, 34]. Dissolution minerals rates are also enhanced by pH values away from neutral. For example, silicate and aluminum-silicate dissolution increases with pH in the acidic range[35, 36]. These facts are consistent with the major alterations recorded on sample 10. Minor alterations detected on sample 16 maturated muds concerning Fe content may be related with pyrite oxidation caused by microorganisms. This hypothesis is consistent with other studies that attribute to a few chemolithotrophic species living in extreme environments the pyrite dissolution, over a restricted range of environmental conditions (pH 2-3, 20-30°C)[37].

Table 3
Results of bacterial analyses (no. CFU/0.1g mud)

Maturation condition	Sample label	Total coliforms		Viable microorganisms (37°C)			Viable microorganisms (22°C)		
		a	c	a	b	c	a	b	c
AL + M	10A	-	-	25	440	175	3	650	-
AL + WM	10B	-	-	25	6100	6100	3	5300	550
ArL + M	10C	-	-	25	-	-	3	3500	1750
ArL + WM	10D	-	-	25	9650	9650	3	15300	11550
D + M	10E	-	-	25	-	-	3	9900	5775
D + WM	10F	-	-	25	260	260	3	uncount.	5475
AL + M	16A	75	-	25	-	75	43	1550	1000
AL + WM	16B	75	-	25	100	75	43	500	50
ArL + M	16C	75	-	25	-	-	43	9800	-
ArL + WM	16D	75	-	25	135	135	43	3250	1075
D + M	16E	75	-	25	-	50	43	290	975
D + WM	16F	75	-	25	65	65	43	245	-
AL + M	19A	75	-	-	-	-	93	4100	-
AL + WM	19B	75	-	-	490	490	93	15950	1050
ArL + M	19C	75	-	-	-	-	93	3150	50
ArL + WM	19D	75	-	-	295	295	93	3800	-
D + M	19E	75	13	-	-	50	93	5900	325
D + WM	19F	75	-	-	-	-	93	380	375
AL + M	LA	5	1513	88	193600	188000	6	219200	277000
AL + WM	LB	5	1750	88	65200	42500	6	25075	32467
ArL + M	LC	5	1575	88	213400	527000	6	22785	226500
ArL + WM	LD	5	1000	88	103600	87850	6	19600	4800
D + M	LE	5	813	88	278200	630000	6	145000	370000
D + WM	LF	5	113	88	264000	464500	6	9600	343000

AL: ambient light; ArL: artificial light; D: dark; M: mixing; WM: without mixing; a: maturation sampling day 0; b: maturation sampling day 30; c: maturation sampling day 60; -: absence; uncount.: uncountable.

Concerning the microalgae characterization, the community was able to develop in samples that were submitted to maturation under light and without mixing conditions. With the exception of maturated samples 19D and LB, the genus *Nitzschia* was the dominant taxon on all maturated muds, contributing more than 60% of the diatom content (Table IV). Our results reveal that a diatom community is able to adapt and to develop in Azorean volcanic muds under maturation processes which can be a considerable finding for their therapeutic effectiveness because previous studies reported mainly the diatoms, as responsible for muds anti-inflammatory action[1, 2, 4, 5, 38, 39]. The genus *Nitzschia* was the best adapted to these specific micro-environments. Consistent with our results, other studies found a similar diatom community developing at extreme environments, mainly with acidic ones[40-43].

Conclusion

A few investigations on maturation procedures, especially using an argillaceous phase have been carried out in order to establish the conditions and to study mainly physical, chemical and mineralogical effects of maturation[2,44]. However, no studies have been done so far with volcanic muds, as in the present work. This report brought together results from biological, mineralogical and geochemical approaches and must be considered as a first-step towards the optimization of the maturation process for the application of these natural resources in benefit of human well being.

Table 4
Diatom community and relative abundance on inoculates and maturated mud samples

	VOLCANIC MUD SAMPLES										
DIATOMS	**10 inoc.**	**10D**	**16 inoc.**	**16B**	**16D**	**19 inoc.**	**19B**	**19D**	**L inoc.**	**LB**	**LD**
Nitzschia sp. Hassal	◊◊	♦♦♦	♦♦♦	♦♦♦	♦♦♦	♦	♦♦	◊◊	◊◊	◊◊	♦♦
Pinnularia sp. Ehrenberg	♦♦	-	-	◊	◊	♦	♦	♦♦	◊	◊	◊
Achnanthes sp. Bory	◊	◊	-	◊	-	◊	◊	-	◊	-	-
Aulacoseira sp. Thwaites	◊	◊	-	◊	◊	-	-	-	-	-	-
Eunotia sp. Ehrenberg	-	◊	-	◊	-	◊	◊	◊	♦	◊◊	◊◊
Navicula sp. Bory	◊	◊	-	◊	-	◊◊	◊	◊◊	♦	♦♦	♦
Fragilaria sp. Lyngbye	◊	◊	-	-	-	◊	◊	◊	-	-	-
Frustulia sp. Rabenhorst	-	-	-	-	-	-	-	-	◊◊	-	-
Gomphonema sp. Ehrenberg	◊◊	◊	-	-	-	-	◊	-	-	-	-

inoc.: inoculate; ◊ <5%;◊◊ 5-20%; ♦20-50%;♦♦ 50-85%; ♦♦♦ >85%

The colonization of these muds during maturation by diatoms and bacteria render to muds potential application for therapeutic use, however further evaluation and improvement is required. Interesting considerations can be made from the current work data: (1) maturation is a very complex process affected by several features; (2) the pH and microorganisms content seem to play an important role on geochemical maturation modifications; (3) bacteria and microalgae are able to colonize Azorean volcanic muds which can endow the materials with therapeutic action; (4) light procedures with no mixing are the selected conditions to the forward maturation tests.; (5) sample 16 submitted to maturation with light and no mixing seems to be the most indicated for further maturation tests because the geochemical composition is closer to the reference sample, shows an absence of pathogenic bacteria and enables the development of microflora.

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