Disponibilidade de elementos traço para absorção cutânea em tratamentos com a lama negra de Peruíbe

Availability of trace elements for skin absorption in treatments using Peruíbe black mud

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RESUMO

Introdução: No Brasil, o uso de recursos naturais passou a fazer parte do Sistema Único de Saúde (SUS) com a implantação da "Política Nacional de Práticas Integrativas e Complementares" e a Lama Negra, localizada na cidade de Peruíbe, Estado de São Paulo, tem sido amplamente utilizado para tratamentos terapêuticos, por exemplo psoríase, dermatite periférica, neuropatia, acne e seborreia, mialgia, artrite e processos reumáticos não articulares.

Objetivo: O objetivo deste estudo foi verificar a mobilidade dos elementos da Lama Negra de Peruíbe, e avaliar quais (benéficos ou tóxicos) estão disponíveis para transferência ao paciente durante o tratamento terapêutico tópico. Para tanto, foram empregados dois métodos de extração: procedimento de extração sequencial e extração única com emprego de suor artificial.

Métodos: Dois métodos de extração foram empregados para medir os elementos extraídos: espectroscopia de emissão atômica com plasma indutivamente acoplado e espectrometria de absorção atômica com forno de grafite.

Resultados e discussão: Os resultados mostraram que os elementos Ca, Cd, Mg, Mn e Na são altamente extraídos na fração trocável e resultados semelhantes foram observados na extração com suor artificial, entretanto, isso pode não ser um problema durante o tratamento terapêutico.

Conclusão: Todos os outros elementos investigados foram extraídos em baixas concentrações, indicando que os efeitos adversos à saúde devem ser desprezíveis, embora até o momento haja pouca ou nenhuma evidência de absorção pela pele.

Palavras-chave: Extração sequencial. Suor artificial. BCR-701. Lama Negra de Peruíbe.

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ABSTRACT

Introduction: In Brazil, the use of natural resources became part of the Unified Health System (SUS, in Portuguese) with the implementation of the "National Policy for Integrative and Complementary Practices" and the Black Mud, found in Peruíbe city, São Paulo State, has been extensively used for therapeutic treatments e.g. psoriasis, peripheral dermatitis neuropathy, acne and seborrhea, myalgia, arthritis, and non-articular rheumatic processes.

Objective: The aim of this study was to verify the PBM's chemical elements mobility, and evaluate which ones (beneficial or toxic) are available for transfer to the patient during topical therapeutic treatment. To get this goal, two extraction methods were employed: sequential extraction procedure and a single extraction by employing artificial sweat.

Methods: Two extraction methods were employed to measure the extracted elements: Inductively coupled plasma atomic emission spectroscopy and Graphite furnace atomic absorption spectrometry.

Results and discussion: The results showed that the elements Ca, Cd, Mg, Mn and Na are highly extracted in the exchangeable fraction and similar results were noted in extraction with artificial sweat, however, it may not cause adverse problems during the therapeutic treatment.

Conclusion: All the other elements investigated were extracted in low concentrations indicating that adverse health effects should be negligible even though until this moment there is little or no evidence for skin absorption.

Key words: Sequential extraction. Artificial sweat. BCR-701. Peruíbe black mud.

INTRODUCTION

Clay minerals are substances found throughout the earth's surface, as they are the main component of soils and pelitic sedimentary rocks. Their use for therapeutic purposes is an ancient practice, particularly with regard to smectite, kaolinite, and palygorskite.[1–3] For a long time, clay minerals have been used in wound healing to relieve skin irritation with anti-inflammatory purposes and to treat gastrointestinal disorders. Currently, due to their frequency of occurrence and particular properties, clays minerals have been used as fundamental components in several medicinal products in the pharmaceutical.[3] This is highlighted in its use as both an active ingredient and as excipient, due to its high adsorption capacity, specific surface, easy handling and also because it promotes drug disintegration, influencing the processes of medicine release when orally administered. Furthermore, clay minerals are used as topical cosmetics, for their feature of adsorbing substances such as fats and toxins.[4] In this context, therapeutic treatments using clays have acquired increasing economic importance. In Brazil, the use of natural resources became part of the Unified Health System (SUS, in Portuguese) with the implementation of the National Policy for Integrative and Complementary Practices (PNPIC)[5] which establishes the inclusion of thermal treatment, in addition to other integrative practices, as a therapeutic option in the public health system.

The therapeutic use of clay minerals is called mud therapy or pelotherapy, employed as part of thermal treatments, in which the clays are employed in mud form, composed by a mixture of organic and inorganic materials with sea, salt lake or mineral–medicinal water. This mixture is usually called peloid. For the peloid obtainment, the clay minerals remain in contact with water in time periods varying from a few months up to 1-2 years depending on the spa-tradition, and this process is called maturation.[2,6,7] During the period of maturation the peloid acquires its characteristic greasiness, in part due to the growth of organic constituents.[8]

The peloids can be applied to different parts of the body or on the whole body by means of masks

and poultices, or even by bathing the body partially or totally, for therapeutic or cosmetic purposes. In most cases, the peloid is applied in temperatures varying from 40 to 45 °C.[7]

The mechanism of therapeutic action of the peloids is still unclear. Some researchers believe that heat is the main therapeutic agent.[7–9] In general, balneology and thermotherapy cause thermal stress in a short time, which is manifested by increasing noradrenaline, adrenaline, cortisol, growth hormone and β-endorphin secretion.[10,11]

This physical stress can exert an analgesic effect based on different mechanisms: the anti-inflammatory effect of cortisol and catecholamines positively influences the metabolism of prostaglandins. Thus, it is believed that apart from the mechanism of action of the various alternative therapy forms used in spas, the relaxation experience, reduction of emotional tension and elevation of the stress mood, decrease in muscle tone and possibly the pain or the pain perception that those patients have to come to suffer from.[12,13] Alternatively, various researchers have also pointed out the importance of the mud chemical constitution in the therapeutic effects due to the possible element mobility during the treatment in case of topical use.[14,15] Whether beneficial or toxic for the organism, ions and other compounds may pass from the peloid to the skin and enter the bloodstream.[16,17]

In Brazil the Peruíbe Black Mud (PBM), found in Peruíbe city, São Paulo State, has been extensively used for therapeutic treatments. In this city, the mud treatment is done in a therapeutic clinic called "Complexo Termal de Lama Negra " also called "Lamário" and this practice is nowadays sponsored by the Brazilian Public Health System. In the "Lamário", before being matured, the collected mud is sieved, in a 2 mm aperture sieve, to separate any coarse material, such as stones, leaves and pieces of branches. The maturation is then performed, keeping the mud in contact with sea water, taken 2 km from the coast, at least for two months in a maturation pond. During this period, the sea water is periodically changed. No agitation is employed in the maturation process. PBM is commonly used for the treatment of psoriasis, peripheral derma-

titis neuropathy, acne and seborrhea, myalgia, arthritis, and non-articular rheumatic processes, applied to different body parts or all over it, for therapeutic or cosmetic purposes and in most cases applied at approximately 40° C. The PBM therapeutic activity and anti-inflammatory efficacy has already been tested, and described by Britschka et al[18] (2011).

The objective of this paper was to verify the PBM's chemical elements mobility, concluding which ones (beneficial or toxic) are available for transfer to the patient during topical therapeutic treatment. To get this goal, two extraction methods were employed: the BCR-701 sequential extraction procedure (SEP)[19] and a single extraction by employing artificial sweat.

The BCR-701 SEP was chosen because it is considered that this technique can provide detailed information on the mode of occurrence, availability, mobilization and transport of metals in clay mineral matrices with satisfactory precision and accuracy.[19–21] The optimized BCR-701 SEP is performed in three steps resulting in the following four fractions: exchangeable, reducible, oxidizable and residual.

The extraction with artificial sweat, at the same length of time and temperature as the mud is applied, can provide a more realistic scenario for the element transference from mud to body, since the sweat is the only interface of contact between the skin and the mud during its topical application.[8]

MATERIALS AND METHODS

Sampling description

Two samples of matured black mud were collected in the "Complexo Termal de Lama Negra" in Peruíbe city, São Paulo State, Brazil, from the trough used to mature and store it. Before any analysis, samples were dried in a ventilated oven at 60 °C till constant weight, transferred to a mortar previously decontaminated with nitric acid, HNO_3, then crushed to a size particle of 200 mesh and homogenized.

A physicochemical, geochemical, elemental and radiological characterization by this peloid has been done by Silva et al[22] (2015) and the results obtained by

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these authors are summarized in table 1. This study showed that the mud deposit is quite homogeneous presenting a small variation between the 10 sampled points along its length. Therefore, these results indicate that the two samples analyzed in this study can be considered representative of the mud deposit.

Sequential extractions procedure (SEP)

The BCR-701 procedure is summarized in the following and full details are reported in Sutherland[19] (2010).

Step 1 (water/acid soluble and exchangeable fraction/carbonate included): To 1 g of black mud, 40 mL 0.11M acetic acid, CH_3COOH, was added followed by an end-over-end shaking (30 ± 10 rpm) for 16 hours at room temperature. The solid residue was separated from the extract by centrifugation at 3500 rpm for 20 minutes, the residue was then decanted and the supernatant separated for analysis. The residue was washed with 20 mL ultrapure water, shaken for 15 minutes and centrifuged with the supernatant discarded, taking care to not discard any solid residue.

Step 2 (reducible fraction): 40 ml of 0.1 M hydroxylammonium chloride, $NH_2OH.HCl$, adjusted to pH of around 2 by adding HNO_3, was added to the step 1 residue, followed by an end-over-end shaking (30 ± 10 rpm) for 16 hours at room temperature. The separation of the extract, collection of the supernatant and rinsing of residues were the same as described in Step 1.

Step 3 (oxidisable): Next, the residue of the second step was added carefully, dropwise, 10 mL oxygen peroxide H_2O_2, covered and digested for 1 hour at room temperature with occasional manual shaking, heated to 85 ºC for 1 hour in a water bath and reduced the volume to 3 mL (uncovered); a further 10 mL H_2O_2 was added and again heated to 85 ºC for 1 hour; 50 mL 1M ammonium acetate, CH_3COONH_4, (pH 2) was added and shaken for 16 hours at room temperature. The separation of the extract, collection of the supernatant and rinsing of residues were the same as described in Step 1.

The residue from Step 3 was dissolved by using aqua *regia digestion* (a mixture of 12M HCl and 16M HNO_3 in a 3:1 ratio) and concentrated HF in Teflon beakers, then heated on a hot plate until complete dissolution.

All the extracted solutions were stored in polyethylene falcon tubes. The concentration of aluminium (Al), antimony (Sb), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), silver (Ag), sodium (Na), titanium (Ti) and zinc (Zn) was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) and the concentration of cadmium (Cd) and Lead (Pb) was determined by Graphite Furnace Atomic Absorption Spectroscopy (GF AAS).

The reproducibility of the extraction procedure for elements determined in this study was evaluated by the standard deviation of five replicates for each sample.

Artificial sweat

The artificial sweat was prepared following European norm EN 1811:1998+A1: 2008. The solution contained 0.5wt.% sodium chloride, 0.1 wt.% lactic acid, 0.1 wt.% urea and a 1 vol.% ammonia solution.[8] This analysis was made similar to the sequential extraction: 10 ml of artificial sweat solution was added to 1 g of black mud followed by an end-over-end shaking (30 ± 10 rpm) for 16 hours at room temperature. The solid residue was separated from the extract by centrifugation at 3500 rpm for 20 minutes, the supernatant was then analyzed. Aiming to simulate the mud contact time with the patient during treatment, the stirring time was also tested, changing the stirring time from 16 hours to 20 minutes.

Inductively coupled plasma atomic emission spectroscopy

For inductively coupled plasma atomic emission spectroscopy, a Spectro ARCOS ICP optical emission spectrometer (Spectro Analytical Instruments Co, Kleve, Germany), equipped with an axially viewed plasma was used. Sample introduction system was composed by a cross flow nebulizer and a Scott double pass spray chamber and instrumental parameters selected to optimize each elemental determination.

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Graphite Furnace Atomic Absorption Spectroscopy measurements were performed by using a Perkin Elmer Analyst 800 graphite furnace atomic absorption spectrometer (Perkin Elmer, Vernon Hills, Illinois, USA), calibrated with diluting certified standard solution (SPEX Certiprep Inc., USA) of the elements of interest. Matrix modifiers of $NH_4H_2PO_4$ 0.5% (m/v) and $Mg(NO_3)_2$ 0.03% (m/v) were used. Analyses were carried out in duplicate with differences between the measurements of up to 10%.

RESULTS

Table 2 shows the results of the concentration and coefficient of variation (CV) of each fraction.

It is possible to notice that in the exchangeable fraction (F1) the element cadmium presented the highest CV, with 58.1%, followed by Pb with 23.2%. In this fraction, Zn presented the lowest CV (0.9%).

Such as the exchangeable fraction, the resultant residue after 3 steps of extraction was the one that presented the lowest number of elements with CV greater than 20%: Cd (47.7%) and Pb (28.8%). In this fraction, the elements that presented the lowest CV were Al (2.3%), and with very close CVs, Ba, Ca, K and Ti presented CVs of 2.4%, 2.8%, 3.3% and 3.3%, respectively.

In the reduced fraction (F2) Cu presented the highest variation (62.7%), followed by Na (51.6%), Zn (49.1%), Cd (39.3%), Cr, (26.2%), K (29%) and Fe (23.2%). The other elements presented CV between 3.2 and 17.2%.

The oxidizable fraction (F3), as well as the reducible fraction showed a higher number of elements with high CV, varying from 22.6 to 53.7%, whereas the elements had variations between 0.28% (Ba) and 15.9 % (Cu).

The chemical composition of PBM from the three steps of sequential extraction and the residue dissolution are shown in figures 1 to 4 and artificial sweat results are shown in Table 3. Graphs are in logarithmic scale due to the difference in order of magnitude of the element concentrations.

Sequential extraction showed that in the exchangeable fraction the elements Ca, Cd, Mg, Mn and Na are extracted in high proportion (Figs. 1 and 3).

With the results obtained in the extraction with artificial sweat, it is possible to notice that there was no significant difference in the concentrations of the elements according to the time of agitation and only Ca, Cd, Fe, K, Mg and Na were above the detection limit.

The elements that were extracted in the first step from sequential extraction (exchangeable fraction) are ones that are ready to be absorbed via patient skin during therapeutic treatment.

DISCUSSION

In general, the CV obtained in all fractions was less than 20% and this value is in agreement with values obtained by other authors for the same type of extraction.[23–25]

It was observed that the elements Ni and Zn were preferentially bound to the residual fraction although they were also found in considerable amounts in the exchangeable fraction. The elements Al, Ba, Cr, Cu, Fe, K and Li, were bounded preferentially in the residual fraction while Pb was found in the reducible and residual fractions, with insignificant amounts in exchangeable and oxidizable ones. The Ag concentration was below the detection limit in all sample fractions.

Although Cd is mostly present in the exchangeable fraction, it is possible to notice its presence considerably in the residual fraction.

Calcium is an essential element whose tolerable daily intake limit is 2.500 mg/day. In the samples it is present in the exchangeable fraction in considerably higher proportions, varying from 1203 to 1776 µg g^{-1}. For extraction with artificial sweat, the values varied from 174 to 406 µg g^{-1}. Although this element is one of the most abundant in the human body with its importance for bone composition[26] there are no reports concerning its skin absorption. Calcium is also involved in several metabolic processes such as in the metabolism of nitric oxide (NO), that in turn, is involved in inflammatory processes.[27] It has also been shown that calcium crystals are found in synovial fluid of osteoarthritis (OA) patients and that they are associated with cartilage lesion and the severity of the OA forms.[28] The naturally occurring

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calcium crystals may also contribute to cartilage degradation and synovitis in OA.

Magnesium is one of the most important micronutrients for human health and is strongly associated with immune responses. It is known that magnesium may have an inflammatory pathogenesis and may have an anti-inflammatory property.[29,30] Among the factors potentially useful for preventing knee OA is magnesium intake, because it is a micronutrient for humans[30] but data directly relating to magnesium absorption by skin is lacking. The concentration of magnesium was found in the exchangeable fraction varying from 2430 to 2948 µg g^{-1}. It was also possible to note that there is a small proportion in the reducible fraction of this element (594 µg g^{-1}). In artificial sweat the results varied from 342 to 779 µg g^{-1}. The maximum daily intake of magnesium that is unlikely to pose an adult health risk is 350 mg/day.

Manganese is an essential mineral for humans. It is necessary for macronutrients metabolism, tissue and bone formation and reproductive processes.[31] In PBM, manganese present in the exchangeable fraction is around 105 µg g^{-1}. Studies reporting systemic effects in animals after dermal exposure to organic manganese compounds, indicate that skin absorption has occurred.[31] Relatively high proportions of this element were also observed in the reducible fraction (28.2 µg g^{-1}) probably associated with the high mobility of reduced Mn forms. In the artificial sweat the concentration of this element was 1.16 µg g^{-1}. The recommended daily dose of manganese for ingestion by adults is 1.6 to 2.3 mg/day.

Sodium is necessary to transmit nerve impulses and stimulate the muscular action, being the most abundant cation in the extracellular fluid of the human body.[32] The sodium concentration in the exchangeable fraction is around 25.182 µg g^{-1}. The tolerable daily sodium intake is 2.3 g/day.

According to the results, it is possible to note that Na was extracted in larger amounts than the other elements, independent of shaking time (20 minutes or 16 hours). The sodium concentration varied from 27022 to 36360 µg g^{-1}. Comparing this result with some muds used in Spanish spas[8], the results agree,

with values varying from 650 to 28400 µg g^{-1}. Low values were also observed for the elements Ca (174 to 406 µg g^{-1}) and K (618 to 761 µg g^{-1}) when compared to clays from Spanish spas, whose concentrations vary from 300 to 8430 µg g^{-1} for Ca, from 50 to 2390 µg g^{-1} for K. However, the Mg concentration is considerably higher, varying from 343 to 779 µg g^{-1} (this study) and 3.5 to 7.2 µg g^{-1} (Spanish spas).

Zinc compounds are used in the drug industry as ingredients of common products like sun blocks, diaper rash ointments, deodorants, athlete's foot preparations, acne and poison ivy preparations and anti-dandruff shampoos. The mechanism of zinc absorption through skin has been investigated and the degree of zinc absorption through the skin is not known, but findings in rats indicate that it can be readily absorbed although in very small amounts.[33] In the exchangeable fraction 12 µg g^{-1} of zinc was extracted. It is also possible to note the presence of this element in the reducible fraction varying from 6.6 to 8.7 µg g^{-1} and 22 to 28 µg g^{-1} in the residual fraction. In artificial sweat, Zn concentration was below the detection limit. The inhalation of zinc is regulated in many countries at 5–10 mg/m^3 consistent with guidelines of the International Labor Organization (ILO) to prevent respiratory illness.[34] The maximum daily intake limit for zinc, which would probably not pose a health risk in an adult, is 8 to 13 mg per day and the daily tolerable limit is 40 mg.[32] These values are three orders of magnitude higher than those found in the exchangeable fraction.

Considering the more toxic elements, cadmium has no known biological function in humans. This element is widely present in the environment and can cause various health problems in the exposed population. Cadmium has been classified as carcinogenic to humans by the IARC (International Agency for Research on Cancer).[35] This element causes a spectrum of toxic effects to a number of organ systems including kidneys and lungs. Persistence in the environment makes it a problem for hazardous waste cleanup. Cadmium is naturally present in trace amounts in most environments including soil and water. Despite all this, the absorption of Cd compounds through the

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skin is negligible.[36] Cadmium is found mainly in the exchangeable fraction (0,019 and 0,031 µg g^{-1}) and in artificial sweat, the results varying from 0.0033 to 0.007 µg g^{-1}. In the residual fraction, the concentrations varied from 0.005 and 0.019 µg g^{-1}. All these concentrations are much lower than the provisional tolerable weekly intake limit for this element, which is 25 µg kg^{-1} body weight.

The finding of toxic effects following dermal exposure suggests that chromium is absorbed through the skin, although information on the absorbed percent is limited.[33,37] Penetration of Cr through skin has been studied in animals as well as in humans, previously analyzing biopsies from skin or evaluating the "disappearance measurement" of isotopes of Cr in salt formulations. Chromium concentration was only found in a significant amount in the residual fraction varying from 19 µg g^{-1} to 25 µg g^{-1}; the exchangeable fraction showed a concentration of around 1.4 µg g^{-1}. In artificial sweat Cr concentration is below the detection limit. These results show that the absorption of this element through the skin is very unlikely. Following the RDA (Recommended Dietary Allowance) studies, the limit of ingestion of Cr is 20-35 µg per day.[32]

Lead is considered a toxic metal and its exposure causes various health problems. Lead salts are used in medicine as an antiseptic and astringent, always taking care due to its high toxicity. In PBM, Pb was preferentially bounded to the reducible fraction with 9.3 µg g^{-1} and 10.1 µg g^{-1}. Also it was possible to note a considerable concentration of this element in the residual fraction (6.6 µg g^{-1} and 5 µg g^{-1}). In artificial sweat Pb concentration is below the detection limit. The skin absorption of lead is considered negligible and it can be concluded that the low levels of these elements found in the samples, may not pose any harm to the patients in the black mud treatment.[38] The provisional tolerable weekly intake limit for this element is 25 µg kg^{-1} body weight.

CONCLUSIONS

Samples of Peruíbe black mud were analyzed using the sequential BCR and simple extraction with arti-ficial sweat procedures. In general, the coefficient of variation between the results of all fractions was less than 20% and this value is in agreement with those obtained by other authors for the same type of extraction. The sequential extraction procedure showed that the elements Ca, Cd, Mg, Mn and Na are preferentially bounded to the exchangeable fraction. The same elements were extracted in the simple extraction with artificial sweat together with small amount of Fe. In reducible fraction, only Pb was extracted in significant mount while Cr and Fe were extracted preferentially in the oxidisable one. The results showed that the elements Cr, Cu, Ni and Zn are mainly bounded in the residual fraction, presenting low availability, although Ni and Zn was found in small mounts in all fractions.

The elements extracted, in the exchangeable fraction of the sequential extraction and in the extraction with artificial sweat, may be prone to be exchanged via patient's skin, however, there is little or no evidence of this absorption, even for the toxic elements, indicating that adverse health effects should be negligible.

Considering the highest concentrations observed for Cd, Cr, Mg, Mn, Na, Pb and Zn, regardless the extraction method (BCR or artificial sweat), all results are lower them the limits established for daily intake, used as comparison parameter since there are no limits for these elements in mud topical products.

It is important to emphasize that the success of treatment of Peruíbe black mud for topical use also depends on important steps such as application time, skin surface preparation, integrity of the barrier function, resistance to permeation of trace elements by the stratum corneum and other layers of the epidermis.

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TABLE AND FIGURE CAPTIONS

Table 1 - Physicochemical, geochemical, elemental, and radiological results of Peruíbe black mud obtained by Silva et al[22] 2015.

Mineral composition: **Quartz, mica, feldspar, halite, kaulinite**
pH: 6,9

	MIN	MAX		MIN	MAX
Silt + clay (%)	31	53	Moisture (%)	34	73
Sand (%)	44	66	Organic matter (%)	5.2	20
CEC (meq/100g)	26	29.7	LOI (%)	0.8	3
Elemental composition (%)					
SiO_2	49	59	Cl	1.7	4
Al_2O_3	12	16	MgO	2.1	6.5
SO_3	3.7	6.7	TiO_2	0.3	0.8
Na_2O	2	3.3	CaO	0.09	0.6
Fe_2O_3	1.6	6.1	P_2O_5	0.11	0.18
K_2O	1.2	2.5	MnO	0.02	0.08
Elemental composition ($\mu g\ g^{-1}$)					
As	7.1	12.2	Rb	41	89
Ba	345	476	Sb	0.49	1.1
Br	88.1	172.1	Sc	8.44	13.1
Cd	7	490	Se	0.13	2

Continuação da coluna anterior.

	MIN	MAX		MIN	MAX
Elemental composition ($\mu g\ g^{-1}$)					
Ce	49	82	Sm	3.6	6.2
Co	7.5	12.7	Ta	0.54	0.9
Cr	47	83	Tb	0.43	0.8
Cs	3.6	6.9	Th	7.3	11.2
Eu	0.89	1.51	U	2.3	4.5
Hf	3.2	6.5	V	33	102
La	22.9	38.2	Yb	1.3	2.1
Lu	0.22	0.34	Zn	67	129
Nd	22	51	Zr	132	467
Pb	12.37	30.8			
Radiologicalcomposition (Bq kg^{-1})					
^{226}Ra	12.4	23.3	^{210}Pb	0.6	1.2
^{228}Ra	29.4	44.2	^{40}K	367	542

Table 2 - Concentration, in $\mu g\ g^{-1}$ (mean ± standard deviation, n=5), and coefficient of variation (CV), in %, of the elements extracted through the sequential extraction.

		F1		F2		F3		F4*	
		Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
Al	MAT (A)	266 ± 11	4.3	1158 ± 123	10.8	899 ± 62	6.9	35400 ± 2834	7.9
	MAT (B)	200 ± 17	8.9	1410 ± 243	17.2	1610 ± 72	4.5	43980 ± 998	2.3
Ba	MAT (A)	-	-	6.4 ± 0.4	6.9	7.58 ± 0.02	0.28	169 ± 14	8.2
	MAT (B)	-	-	7 ± 1	16.4	13.1 ± 0.1	1.2	223 ± 5	2.4
Ca	MAT (A)	1176 ± 34	1.9	382 ± 32	8.4	41 ± 14	34.5	787 ± 60	7.7
	MAT (B)	1203 ± 25	2.1	350 ± 12	3.5	-	-	870 ± 24	2.8
Cd	MAT (A)	0.031 ± 0.002	6.8	0.007 ± 0.003	39.3	-	-	0.019 ± 0.004	21.6
	MAT (B)	0.02 ± 0.01	58.1	0.0129 ± 0.008	6.4	0.008 ± 0.003	37.7	0.006 ± 0.003	47.7
Cr	MAT (A)	1.4 ± 0.1	10.1	3.2 ± 0.2	7.0	10.5 ± 0.8	7.8	20 ± 1	6.27
	MAT (B)	1.3 ± 0.1	11.3	3.0 ± 0.8	26.2	10 ± 5	53.7	25 ± 1	4.85
Cu	MAT (A)	-	-	1.3		2.4 ± 0.4	15.5	7.4 ± 0.3	4.1
	MAT (B)	-	-	2 ± 1	62.7	3.5 ± 0.6	15.9	7.4 ± 0.5	6.8
Fe	MAT (A)	530 ± 70	13.3	3220 ± 282	8.8	4288 ± 324	7.6	10554 ± 727	6.9
	MAT (B)	435 ± 60	13.9	2657 ± 615	23.2	6818 ± 371	5.4	13940 ± 1815	13.0
K	MAT (A)	849 ± 35	4.1	853 ± 74	14.0	177 ± 10	5.5	9166 ± 868	9.5
	MAT (B)	857 ± 19	2.3	416 ± 120	29.0	190 ± 22	11.8	11950 ± 398	3.3
Li	MAT (A)	2.06 ± 0.09	4.7	1.6 ± 0.2	13.8	3 ± 1	32.9	24 ± 2	8.3
	MAT (B)	2.2 ± 0.11	5.3	1.79 ± 0.06	3.5	4.57 ± 0.06	1.4	29 ± 1	4.9
Mg	MAT (A)	2948 ± 93	3.1	573 ± 75	13.0	320 ± 34	10.6	2128 ± 151	7.1
	MAT (B)	2430 ± 66	2.7	594 ± 55	9.2	718 ± 48	6.7	2641 ± 98	3.7
Mn	MAT (A)	104 ± 2	2.3	24 ± 1	5.2	30 ± 2	5.2	59 ± 4	6.8
	MAT (B)	102 ± 3	2.6	28 ± 3	9.2	49 ± 2	4.6	79 ± 3	4.3
Na	MAT (A)	25182 ± 674	2.7	251 ± 23	9.3	496 ± 11	2.3	2615 ± 285	10.9
	MAT (B)	13430 ± 76	0.6	262 ± 135	51.6	1132 ± 227	20.0	3288 ± 132	4.0
Ni	MAT (A)	2.4 ± 0.2	8.9	3.0 ± 0.2	8.2	2.8 ± 0.3	9.2	9.5 ± 0.5	5.4
	MAT (B)	3.5 ± 0.3	10.0	3.2 ± 0.5	14.2	2.6 ± 0.9	36.2	11.4 ± 0.7	6.2
Pb	MAT (A)	0.9 ± 0.2	23.2	10.3 ± 0.6	5.6	0.2 ± 0.1	49.0	5 ± 1	28.8
	MAT (B)	0.28 ± 0.02	5.8	9.3 ± 0.3	3.2	0.5 ± 0.2	31.7	6.6 ± 0.4	5.5
Sb	MAT (A)	-	-	-	-	-	-	0.9 ± 0.1	11.0
	MAT (B)	-	-	-	-	-	-	1.2 ± 0.9	7.7
Ti	MAT (A)	-	-	-	-	27 ± 3	11.1	1826 ± 143	7.8
	MAT (B)	-	-	-	-	42 ± 12	29.0	2112 ± 69	3.3
Zn	MAT (A)	10.6 ± 0.3	2.6	6.7 ± 0.4	6.2	6 ± 1	22.6	22 ± 2	7.7
	MAT (B)	12.1 ± 0.1	0.9	8 ± 4	49.1	4.8 ± 0.7	15.5	28 ± 2	9.0

*Residual fraction.

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Table 3 - Concentration, in, µg g⁻¹ (mean ± standard deviation, n=5), of the elements extracted through the artificial sweat solution after 20 minutes and 16 hours of agitation.

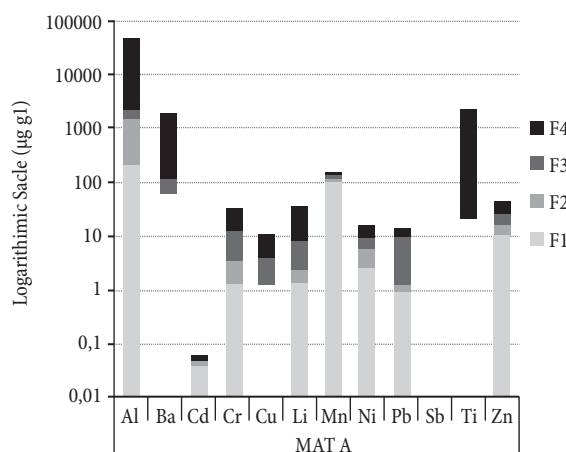|	20 minutes		16 hours	
	MAT A	MAT B	MAT A	MAT B
Ca	406 ± 51	174 ± 11	352 ± 27	257 ± 32
Cd	0.007 ± 0.001	0.004 ± 0.002	0.007 ± 0.001	0.0033 ± 0.0002
Fe	-	-	-	10 ± 2
K	681 ± 19	710 ± 25	761 ± 14	759 ± 24
Mg	779 ± 134	343 ± 24	572 ± 49	530 ± 127
Mn	-	-	-	1,16 ± 0,01
Na	36360 ± 981	27022 ± 1605	35900 ± 1225	28650 ± 1343

Figure 1 - Concentration (%) of Ca, Fe, K, Mg, and Na in the fractions obtained by sequential extraction in MAT A.
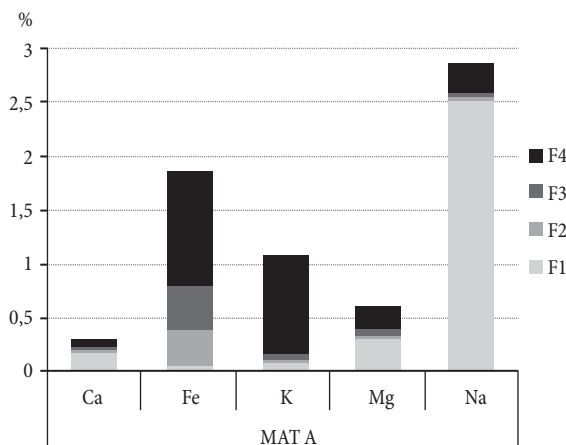
Figure 3 - Concentration (%) of Ca, Fe, K, Mg and Na in the fractions obtained by sequential extraction in MAT B.
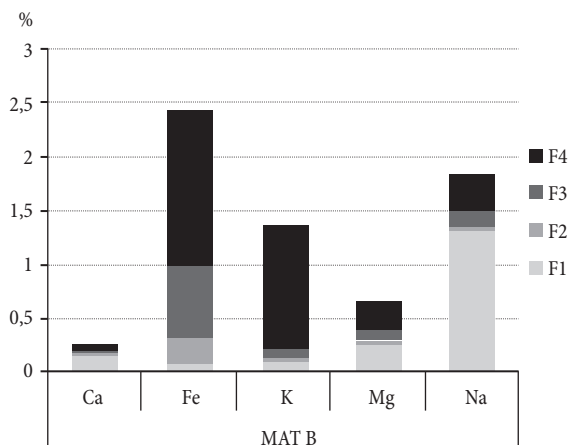
Figure 2 - Concentration (µg g⁻¹), in logarithmic scale, of Al, Ba, Cd, Cu, Cr, Li, Mn, Ni, Pb, Sb, Ti and Zn in the fractions obtained by sequential extraction in MAT A.

Figure 4 - Concentration (µg g-1), in logarithmic scale, of Al, Ba, Cd, Cu, Cr, Li, Mn, Ni, Pb, Sb, Ti and Zn in the fractions obtained by sequential extraction in MAT B.