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SPELEOTHERAPY EFFECTS ON WISTAR RATS REFLECTED BY PULMONARY AND DERMAL FIBROBLASTS CULTURES

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Abstract: Speleotherapy – a special kind of climatotherapy, uses the certain conditions of caves and salt mines to cure several diseases, especially respiratory and skin diseases. Atmospheric dust could cause allergic reactions or asthmatic attacks. The cave air is very low on dust. This fact reduces any kind of irritation. In this way, the symptoms of the diseases are reduced or eliminated completely, while the patient is in the cave. But that does not explain how it should have a longer lasting effect. Curing asthma involves spending 2-3 hours a day underground in subterranean caves or salt mines over a 1-2 month period. An old study describes a speleotherapy course, which was 4 hours a day for 6-8 weeks, with 100 COPD (Chronic Obstructive Pulmonary Disease) and asthma patients and reported improvement that lasted 6 months to 7 years (Skulimowski, 1965).

Our objectives were to explore the effects of speleotherapy on cellular morphology and physiology of pulmonary and dermal fibroblasts obtained from tissues of Wistar rats, in normal and Ovalbumin challenged, “asthmatic” conditions. 60 Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Ten animals of each lot were send to Cacica, Turda and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment. Pulmonary and dermal fibroblasts cultures were prepared from Wistar rat lung and respectively dermal tissue.

Trying to identify the biological mechanisms of speleotherapy, our experimental design was made for cell morphology, physiology and biochemical evaluation of cells in cultures obtained from animals that were treated by speleotherapy. The complex picture of results was analysed and explained through biological mechanisms comparing to the control cell cultures obtained from healthy, untreated Wistar rats. In this article we describe the supposed biological mechanisms that explain the protective effects of speleotherapy.

Conclusion: Speleotherapy induces changes on the morphology and protein expression of pulmonary and dermal fibroblasts in vitro, and these changes - by comparing with ovalbumin sensitised animals, supports the beneficial effects of speleotherapy.

1. Introduction

Asthma is a disorder characterized by chronic inflammation of the airways, airways hyper-responsiveness, and changes in airway architecture, termed remodeling. The cells responsible for maintenance of lung structure are the parenchymal cells of the lung, including epithelial cells, mesenchymal cells, and endothelial cells. Recent studies have suggested that the function of epithelial cells, smooth muscle cells, and fibroblasts cultured from lungs of individuals with asthma differs from the function of cells similarly cultured from individuals without asthma. These functional differences, particularly as they relate to repair and remodeling, could contribute to airway structural alterations (Sugiura et al., 2007). Bronchial asthma affects up to 10% of the developed countries population, its prevalence increasing in all world.

Therapy with bronchodilators, corticosteroids, leukotriene inhibitors, mastoid cells stabilizers and recent with IgE receptor antagonists have been shown an improvement of asthma symptoms.

To solve the existing problems in allergy, pulmonology and medical recovery field and for use of natural therapeutic factors in patient treatment with different pathologies, international scientific community appealed to specialists, medical, ecological and social programs.

The new scientific and practical directions in therapy of the most severe allergic diseases - bronchial asthma - use underground medium of salt mines and caves. This therapy method was named speleotherapy from greece „spelaion”- cave, gap and „therapy”- treatment. Today the speleotherapy is recognized as therapy in underground of salt mines and caves with natural theraoeutic factors for many diseases (Iu.Simionca and al.,2005, 2008).

Primary cell cultures can readily be obtained from human and animal skin using the explant method or trypsineisation. Full thickness skin, also called the integument, is a composite of three tissues (epidermis, dermis and subcutaneous tissue), none of which constitutes a homogenous entity. Epidermis normally is composed of keratinocytes, which represent the largest population numerically, and lesser numbers of melanocytes, Langerhans’cells, and occasional cells of the lympho-reticular system, which are, however, transient members of the community.

Although the bulk of the dermis is noncellular (collagen and ground substance), within this compartment is also a variety of cell types, including fibroblasts, histocytes, mast cells, macrophages, lymphocytes and Schwann cells, endothelial cells of blood vessels and lymphatics, striated muscle cells of erector pili muscles, and smooth muscle of blood vessels.
The subcutaneous tissue includes most of the dermal cell types and fat cells as well (Flaxman, 1974).

The current study was designed to investigate the influence of salt mine medium from Cacica, Turda and Dej Salt Mines upon the cell morphology and electrophoretic expression of pulmonary and dermal fibroblasts in vitro obtained from Wistar rats tissues, in normal and Ovalbumin challenged “asthmatic” conditions.

Fibroblasts were cultured from lung and dermal parenchyma of control, ovalbumin-sensitized, and speleotherapy treated rats after ovalbumin-sensitization. Fibroblasts shape in culture can vary in accordance with the substrate, which on they is growing, and the space they have for movement.

Using pulmonary and dermal fibroblasts cultures to verify the therapeutic properties of saline mines medium, described as speleotherapy, represents an innovative and scientific new way to establish the medical methodology of preventing, treating and recovery of patients with various pulmonary problems.

Speleotherapy presents a great scientific interest and is a future direction in health and environmental area.

2. Geography and geology

One of the perspective salt mines used in medical and balnoclimatic tourism purpose from Romania is Turda Salt Mine.

TURDA SALT MINE is one of the historical monuments of Romania, from Cluj and a touristic attraction at national and international level especially for Bai Sarate Turda, Durgau salted lakes and the ruins of Potaissa roman castrum where was stationed the Vth Macedonica Legion 2000 years ago.

The exploitation of salt from Turda in current microdepression of Baile Sarate has a special interest during the roman occupation in Dacia. The first documentary of mine attestation dating from XII century when avid rocks, minerals and fossils collector - Joanne Fridvaldscky says- „is so famous that has no equal in all eastern”.

With Saraturile Turzii was declared natural reserve with national interest and became a historical museum of salt mining.

Turda Salt Mine joined to touristic circuit in 1992 (Ov. Mera si al., 2010) and benefit from EU funding under PHARE CES Programme 2005 through „Improving the attractiveness of the tourist potential of the balneray resort Lacurile Sârate-Zona Durgâu-Valera Sărata and Turda Salt Mine” project; modernization works of Turda Salt Mine has start in 2008 and have lasted two years.

Turda Salt Mine has legally all prerequisites, for therapeutic use: mines with furnished rooms, tailored for both tourists and sick persons, including disabled persons, mines rooms are large space, isolated rooms; no exploitation activities; in Terezia Mine there are a salin lake adapted for recreation.

Official opening of modernized Turda Salt Mine took place on 22 January 2010.

SALT MINE CACICA - it is situated in the locality with the same name, in the N-E part of the Romania, at 42 km W from Suceava Town and the 17 km N from Gura Humorului. The air strongly ozonized, the purity and beauty of nature, make from this place an attractive destination in any season, both for rest, pleasure and the treatment of respiratory disorders.

The entrance into the salt mine is made on fir tree stairs that are over 200 years old, mineralized by the salty water that penetrated the wood. The work by chisel gab and sledge hammer of the miners that ones worked here left real works of art, that bear the seal of the talent access stairs cut in the salt massif; vaulted ceilings or huge galleries. The real measure of the craftsmanship of those who dug the salt with the hammer is given by the small church built in salt at a depth of 27 metres and the dance hall located at a depth of 37 metres.

This underground Catholic chapel sanctified in 1800 has been gathering all the inhabitants, for the last two centuries, on the feast of St. Varvara protector saint of the miners.

OCNA DEJ SALT MINE is located in Romania, in the iddle of the Transylvanian Basin 3 km from the city of Dej and 60 km from Cluj-Napoca. Importance of salt in the development of human civilization and the exceptional quality of the salt deposit made the salt to be exploited since antiquity in Ocna Dej.

The first statement concerning the Ocna Dej salt exploitation dating from Roman times can be observed today in the form of excavation remains clogged. Roman has operated the mine until XII–XIII centuries when they consider the current perimeter begins Ocna Dej salt mining plant.

Today, Ocna Dej salt mine is part of National Salt Company SA and its main activity is extraction, preparation and marketing of gemstones salt.

The Ocna Dej salt mine is characterised by: temperature: 12.4–14.5 °C, pressure: 1,018–1,020 hPa, humidity: 65–71%, the presence of saline aerosols, lighting artificial and own ventilation system. A higher concentration of NaCl is ensured by continuous operation of the mine.

These environmental conditions provided by the Ocna Dej salt mine led researchers to undertake studies on evaluating the possibility of using this mine, not only for salt extraction, but also for the development of the radon therapy and speleotherapy in Romania ( Calin M.R.. and Calin M.A., 2010)
3. Methods

Materials: Phosphate Buffer Solution (PBS): NaCl 0,13M + KCl 2,6mM + NaHPO₄ x12 H₂O 8mM + KH₂PO₄ 1,4mM; HAM-F12 culture medium (Sigma); penicillin 100 U/ml streptomycin 100µg/ml; neomycin 50µg/ml; fetal bovine serum (Sigma).

Rat Wistar Model of Allergic Asthma

Wistar rats of 75-100g weights were sensitized to Ovalbumin by i.m. injections.

Primary pulmonary fibroblasts culture

After anaesthesia with chloroform, rats were killed. The thorax was opened and then the lungs were removed en bloc in a laminar flow hood using sterile technique and put into ice-cold sterile Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + NaHPO₄ x12 H₂O 8mM + KH₂PO₄ 1,4mM). 1mm tissue pieces were suspended in 0,125% trypsin and 0,001% DNase and repeatedly stirred for 6 minutes and centrifuged at 1000g. The pellet was resuspended in HAM-F12 medium with 4500mg/l glucose, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µg/ml neomycin and 10% fetal bovine serum (Sigma). The cell viability was tested with the techniques described by Laemmli (1979). The cultures have been washed with PBS, curretted from the culture plate and lyzed in buffer containing 0,5M Tris-HCl, pH 6,8 + 0,05% BPB + 10% glycerol + SDS 10%.

4. Results

4.1. Speleotherapy results on dermal fibroblasts

Control skin cells culture of 7 days has a heterogenic aspect with a high pre-confluence level. The cell division is to a high level and the cell morphology shows a typical microscopic view, described in the specific literature (fig.3). There are two types of cells: epithelial and fibroblastic.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control skin cell culture, being observed a sensible number reducing of dermal fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 7 days of culturing, the pre-confluence level is much lower than in the control case.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. By phase contrast microscopy, it is possible to observe a rising of the cells number.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized and treated by speleotherapy in Dej Salt Mine shows also an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. It is observed the rising of the cell population density and that of cell viability.

Skin cells cultures were homogenized with Laemmli buffer pH 6,8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure. Samples of 10µl were loaded into wells in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20; 14.2 and 6.5 KDa.

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands within the gel (Towbin et al., 1979).

Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis, allowed us to compare the profiles of the total proteins expression.
TABLE 1: SDS polyacrylamide gel electrophoresis of the dermal cells cultures

Table 1: SDS polyacrylamide gel electrophoresis of the dermal cells cultures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>2nd day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>3rd day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>4th day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>5th day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>6th day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
<th>7th day skin cells culture from Ovalbumin-assisted rat exposed to the olive medium of the Oil Salm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>CONTROL (---)</td>
<td>OVALBUMIN (---)</td>
<td>CONTROL (---)</td>
<td>OVALBUMIN (---)</td>
<td>CONTROL (---)</td>
<td>OVALBUMIN (---)</td>
<td>CONTROL (---)</td>
</tr>
</tbody>
</table>

Fig. 3: Experimental results for dermal fibroblasts cultures

TABLE 2: Protein expression analysis of the skin cells cultures

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Peak width (kDa)</th>
<th>CONTROL (ng/10µl)</th>
<th>OVALBUMIN (ng/10µl)</th>
<th>CACICA (ng/10µl)</th>
<th>DEJ (ng/10µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250 - 260</td>
<td>3.0</td>
<td>5.0</td>
<td>2.6</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>220 - 230</td>
<td>3.1</td>
<td>2.4</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>200 - 210</td>
<td>4.0</td>
<td>0.8</td>
<td>2.0</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>180 - 190</td>
<td>2.1</td>
<td>0.8</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>5</td>
<td>160 - 170</td>
<td>1.5</td>
<td>2.3</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>140 - 150</td>
<td>2.4</td>
<td>1.5</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>120 - 130</td>
<td>1.7</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>100 - 110</td>
<td>4.0</td>
<td>2.1</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>9</td>
<td>80 - 90</td>
<td>2.5</td>
<td>1.6</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>10</td>
<td>60 - 70</td>
<td>2.3</td>
<td>1.2</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>11</td>
<td>40 - 50</td>
<td>1.1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>12</td>
<td>20 - 30</td>
<td>1.0</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>13</td>
<td>0 - 10</td>
<td>0.9</td>
<td>1.5</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>14</td>
<td>10 - 20</td>
<td>1.1</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>15</td>
<td>0 - 10</td>
<td>1.0</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>16</td>
<td>0 - 10</td>
<td>0.9</td>
<td>1.5</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Total amount of protein in 10 µl of sample</td>
<td>134.1</td>
<td>79.45</td>
<td>101.19</td>
<td>65.71</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 10: TOTAL amount of proteins in 10 µl of sample

Fig. 3: Experimental results for dermal fibroblasts cultures
4.2. Speleotherapy results on pulmonary fibroblasts

Control pulmonary fibroblasts culture of 9 days has a homogenic aspect with a high pre-confluence level. The cell division is at a high level and the cell morphology shows a typical microscopic view, described in the specific literature.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control pulmonary fibroblasts culture, being observed a sensible number reducing of pulmonary fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 9 days of culturing, the pre-confluence level is much lower than in the control case.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged asthmatic rats. By phase contrast microscopy, it is possible to observe a rising of the cells number.

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Pulmonary fibroblasts were homogenized with Laemmli buffer pH 6.8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into wells in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20; 14,2 and 6,5 KDa

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands in the gel (Towbin et al., 1979). Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis, allowed us to compare the profiles of the total proteins expression.
5. Discussion

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts and epithelial cells obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats. Compared with control culture, skin cells cultures from Ovalbumin-sensitized rats and Ovalbumin-sensitized treated in Cacica and Dej Salt Mines rats demonstrated the positive role of the saline medium for the sensitized rats.

The current study focused on skin cells, which are believed to play a major role in the organism – environment interaction. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to lesion repair in dermatological problems. Cells cultured from the skin of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura et al., 2007).

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats, a model of asthma that results in airway hyperresponsiveness and chronic airway remodeling, as other authors had presented.

Compared with control fibroblasts, fibroblasts obtained from lung parenchyma of the “asthmatic” rats and Ovalbumin-sensitized rats treated in Cacica and Dej Salt Mines demonstrated the positive role of the saline medium for the “asthmatic” rats.

The current study focused on fibroblasts, which are believed to be cells that play a major role in the maintenance and remodeling of interstitial connective tissue. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to airway remodeling in asthma. Fibroblasts cultured from the lungs of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura et al., 2007).

6. Conclusions

- Phase contrast microscopy analyses of primary skin cells cultures reveals an cellular regeneration after animal exposure to saline medium in Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitised rats.
- The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of Ovalbumin-sensitized animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of skin cells in cultures;
- Wistar rats sensitised with Ovalbumin have a low number fibroblasts in skin cells cultures, with a more sensitive morphopathologic level.
- Phase contrast microscopy analyses of primary fibroblasts cultures reveals an cellular regeneration after animal exposure to saline medium in Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitised rats.
- The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of Ovalbumin-sensitized animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of pulmonary fibroblasts in cultures;
- Wistar rats sensitized with Ovalbumin have a low number pulmonary fibroblasts output cultures, with a more sensitive morphopathologic level.

Acknowledgments

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References


The origin of high silicon content in potentially medicinal groundwater of Gran Canaria (Canary Islands, Spain). Modelling of chemical water-rock interactions

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Abstract
Groundwater of Gran Canaria (Canary Island, Spain) have been appreciated and used as an element of health tourism since the 19th Century. This activity was abandoned in the second half of 20th Century when springs disappeared due to groundwater drawdown. The chemistry of groundwater from 19 intakes in volcanic rocks of the north part of Gran Canaria was studied by applying geochemical modelling for quantifying processes responsible for high Si concentrations. Studied groundwater has temperature of 16.3°C–25.5°C, pH of 4.40–7.40, and usually HCO₃-(Cl)-Mg-Ca-Na hydrochemical types. At near-neutral pH, fresh groundwater usually has 0.1-0.3 mM of Si. In studied groundwater Si concentrations are 0.42 to 1.82 mM, and show positive correlation with ionic strength and temperature. Volcanic bedrocks consist of, generally, easily reactive silicate minerals. Weathering is not supported by low rainfall; however, it shall be intensified by high influx of salts from marine aerosols and lithogenic carbon dioxide into groundwater. Geochemical modelling has found water-mineral reactions which reflect properly diversity of bedrock mineralogy. Based on those chemical reactions, contributions of particular silicate minerals to the pool of silicon dissolved in groundwater were calculated. Understanding the processes responsible for water chemistry might help in proper management and protection of groundwater. 

The Si-rich waters might be found in numerous places of Gran Canaria in all volcanic rocks. Silicic acid is the only form of silicon which is biologically available, and is regarded as a component which provides balneotherapeutic benefits. Many studies have showed beneficial and essential aspects of silicon in humans. Studied groundwater from Gran Canaria has an unexploited balneotherapeutic potential, and due to very high Si contents they seem to be ideal for testing the health benefits of such waters to humans. Hydrogeochemical methods, including geochemical modelling, provide effective tools for protection of such medicinal waters.

1. Introduction and aims
Silicon, the second most abundant of the elements constituting Earth’s crust, occurs in groundwater at concentrations which are no match numerically for many other elements which are less abundant in the crust. In comparing to rocks, natural waters comparatively are impoverished into silicon. Fresh groundwater usually contains about 0.1 mM Si. The higher Si concentrations, usually up to about 2 mM, are found in thermal and/or of extreme-pH groundwater. Silicon content in drinking water is not regulated by standards. At present, this element is regarded as a beneficial, and even an essential for human health (e.g. Exley, 1998, 2009a, 2009b). However, the health benefits of silicon in medicine, also in balneology, were appreciated already in 19th Century (Guibert, 1865; Weber, 1907; Wohlmann, 1914).

Silicon is mentioned as a component which gives therapeutic properties for medicinal waters. In some countries the element is taken into account in balneological regulations, e.g. the threshold value for siliceous curative water was established on 50 mg/L (≈0.64 mM) and on 70 mg/L (≈0.9 mM) of H₂SiO₃ in Hungary and Poland, respectively.

Groundwater of Gran Canaria (Canary Island, Spain) have been appreciated and used as an element of health tourism since the 19th Century, in localities like Azuaje, Berrazales, Teror and Telde. This activity was abandoned in the second half of 20th Century (e.g. Jiménez et al., 2012; Rodríguez et al., 2012), when springs disappeared due to the generalized groundwater levels drawdown in the island. At present, chemically valuable groundwater from several localities of Gran Canaria from exploited wells are used for
production of bottled water only. Volcanic history and geological setting of Gran Canaria are predestining their groundwater for being enriched into some compounds, like carbon dioxide, silica or fluorine. The presented study has focused on the composition of groundwater in northern part of Gran Canaria at places where increased Si content has been documented. Studied groundwater has high contents of dissolved silicon, often higher than in many balneologically-used medicinal waters of European spas.

Geochemical modelling has been applied with the aim of elucidating and quantifying the main chemical reactions responsible for high silicon concentration in groundwater of northern Gran Canaria. The balneological potential of studied groundwater is underrated. Understanding the processes responsible for water chemistry might help in proper management and protection of groundwater.

2. Materials and methods
Studied groundwater occurs in volcanic rocks divided to three main volcanic suites: Miocene Phonolites, Pliocene Roque Nublo Group including mafic lavas and breccia type ignimbrites deposits with juveniles of phonolitic composition (Mangas et al., 1993), and Quaternary Post Roque Nublo basalts and basanites. Groundwater from 19 large-diameter (2.5 and 3 m) wells was sampled and analysed (Fig. 1). In water samples physicochemical parameters (pH, T, SEC) were determined in the field and dissolved constituents were determined in the labs. A multivariate statistical cluster analysis technique was applied to groundwater data. The data were subjected to Q-mode hierarchical and to k-means cluster analyses by using STATISTICA program (StatSoft, 2005) for classifying the groundwater samples into hydrochemical groups, and for selecting representative water wells for geochemical modelling. The Ward's linkage method was applied, and Manhattan distance was chosen as a measure of similarity.

The main geochemical reactions responsible for groundwater chemistry have been defined and quantified by applying geochemical inverse mass balance modelling by using PHREEQC program (Parkhurst and Appelo, 1999) with llnl thermodynamic database. For geochemical modelling four water wells (nos. 3, 9, 17, 18 – Fig. 1) were selected, representing almost homogeneous type of bedrocks. Wells 3 and 9 show groundwater which flows mainly through Miocene trachyte-phonolites; well 18 – water through rocks of Roque Nublo group, and well 17 – through Post Roque Nublo rocks.

Outlines of conceptual geochemical model are presented on Figure 2. At first step, the pure water was equilibrated with respect to atmospheric CO2 and evaporated for imitating clean rain water chemistry (input water in the model). Evapotranspiration coefficients and mean air temperatures at recharge zone of each well were applied (Cruz et al., 2012). Next, inverse mass balance modelling was done between input water (recharging water), defined in such a way, and groundwater from particular wells (output water). Three main kinds of source phases which might release substances into solution have been included in the conceptual model: marine aerosols, minerals of volcanic bedrocks, and CO2 of volcanic origin (Fig. 2). Marine aerosols were assumed to be of mean composition Na0.779Mg0.0875Ca0.0145K0.017Cl0.906(SO4)0.047 based on the mean sea water chemistry. Minerals included in models cover primary minerals (mainly silicates) and secondary clay minerals. There have been selected minerals which were documented by petrologic studies and present chemistry the closest to genuine minerals.

3. Geological setting and hydrogeology
The study area, located in the northern part of Gran Canaria (Canary Island, Spain) (Fig. 1), is shaped by a series of radial gullies formed in the centre of island (at max. 1949 m a.s.l.), which run into the sea. The climate of Gran Canaria is subtropical. Average annual rainfall is 375 mm/y, varying between 820 mm/y in the highlands and 115 mm/y on the coast. The air temperature is moderate. Average annual temperature varies from 12°C in the highlands to 22°C at the coast, with an average temperature of 18°C.

The geology of the study area shows volcanic and sedimentary materials (Fig. 1). On the surface, basaltic, basanitic and trachybasaltic
lava flows of the Roque Nublo and Post-Roque Nublo suites, and sediments of Las Palmas detritic Formation mainly occur. Miocene trachytic and phonolitic lavas outcrop usually in the coastal area and at the bottom of ravines, where alluvial deposits are also observed (Aulinas et al., 2010; Perez-Torrado et al., 1995). Volcanic rocks mostly consist of diopside, olivine and labradorite (mainly in basaltic, basanitic and trachybasaltic lavas), andandesine, kaersutite, anorthoclase and hauyne in Miocene trachyte-phonolite rocks (Perez-Torrado, 1992; Aulinas et al., 2010). Sediments, weathering cover, and fillings of fractures also include calcite, dolomite, gypsum and clays (illite, and smectites, usually montmorillonites), and zeolites (analcime, chabazite, phillipsite).

The island aquifer is conceptualised as a stratified and heterogeneous water-body, with groundwater flows from recharge area (at the summits in the central part of island) towards the coast. There are hydraulic connections between waters occurring in different rocks, which actually form one common hydrogeological system in the island (SPA-15, 1975; Custodio and Cabrera, 2008). In the study area, groundwater comes predominantly from Roque Nublo group rocks and underlying Miocene trachytic and phonolitic lavas. Groundwater exceptionally flows towards the deep gullies (“barrancos”) and generally discharges to the coast located at the north (Fig. 1). An important amount of groundwater is also discharged artificially due to withdrawals from wells and galleries. Agriculture, which is mainly practised on the coastal areas, is supplied from both surface and groundwater resources, and locally causes an important irrigation return flows (Cabrera and Custodio, 2004; Custodio and Cabrera, 2008), which promote increase of soil and groundwater salinity.

4. Silicon in studied groundwater

Studied groundwater are low-mineralized with specific electric conductivity (SEC) ranging between 280 and 2760 µS/cm, and mostly weakly acid, with pH varying from 4.40 to 7.40 (Tab. 1). Bicarbonate waters of HCO₃-Mg-Ca-Na hydrochemical types prevail (Fig. 3). Main parameters of groundwater chemistry were summarized in Table 1. Silicon in fresh groundwater of near-neutral pH, also those which occur in volcanic rocks, usually shows concentrations of 0.1-0.3 mM (Dobrzyński, 2006, 2007). In studied groundwater Si concentrations are clearly higher, and vary from 0.42 to 1.82 mM (mean 1.28 mM). Dissolved silicon presents correlation with ionic strength of solution (Fig. 4), and noticeable chemical diversity. Groups of wells distinguished on Figure 4 apparently refer to hydrogeological and hydrogeochemical conditions. It might be assumed that revealed diversity mainly is being triggered through: (1) reactivity of volcanic bedrocks, and (2) local hydrogeological and anthropogenic factors. Groundwater sub-populations indentified on Figure 4 are caused by above mentioned factors. Groundwater of group I (wells nos. 1, 5, 6, 11, 13, 17, 19) contacts with rocks of Post Roque Nublo and Roque Nublo groups. Waters of wells nos. 2, 3, 8, 9, 10, 12, 14, 15, 16 occur mainly in Phonolites and Roque Nublo rocks, and might be divided into two groups (Fig. 4): “IIa” and “IIb”. Waters of IIb-group have lower mineralization, and occur at higher altitudes than waters of IIa-group. Water from well 18 shows an intermediate chemistry mainly formed due to contact with Roque Nublo rocks. Groundwater from wells 4 and 7 (group III) demonstrate mineralization higher than other waters, what results from irrigation activity and/or sea water intrusion. A pattern of groundwater subpopulations very similar to that showed on Figure 4 has been also recognized by means of both hierarchical (Fig. 5) and k-means (Tab. 2) cluster analysis. In most groundwater, silicon occurs as (ortho)silicic acid (H₄SiO₄), and it’s concentration is chiefly governed by temperature and pH. Also in studied groundwater, the higher temperature, the higher Si concentration (Fig. 6A). The Si-temperature pattern is only “disturbed” by salinity-affected waters (wells 4 and 7), which show relatively high temperatures.

In studied groundwater, with pH between 4.40 and 7.40, silicic acid remains undisassociated. Increase of Si concentrations at this pH range depends on factors other than dissociation, like temperature, bedrock
reactivity, water chemistry (aggressiveness), water residence time. Waters of wells 2, 10, 16 (IIb group on Figure 4), which occur at the highest altitudes, and represent shorter water residence times, have the lowest Si concentrations (11.8-23.1 mg/L Si) (Fig. 6B, Tab. 1). Salinity-affected waters (wells 4 and 7) and waters from wells 6, 12 and 15 have, at pH of 6.0-6.7, slightly higher Si concentrations (27.7-34.6 mg/L Si) than waters of the IIb group. Remaining waters have lower pH (4.40-6.20), what probably results from still un-neutralised pool of CO₂, and contain higher Si concentrations (35.3-51.0 mg/L Si). Concentrations of dissolved silicon in studied groundwater are high and close to the solubility limit of silicic acid (1.942 mM), at 25°C (Fig. 4).

At chemical equilibrium in the groundwater-rock system, Si concentrations are controlled by solubility of minerals. However, in aquifers of so-called active zone, like in the case of studied fissured volcanic aquifers, non- or meta-stable equilibrium usually occur, and Si concentration meets a quasi steady-state conditions. Generally, the chemical composition of groundwater, including silicon concentration, in particular points of the geochemical system depends on steady-state conditions between three main groups of processes: (1) releasing substances into a solution from decaying/transforming minerals, (2) immobilizing substances in a newly formed secondary solids (colloids, minerals), and (3) removing solutes together with ground and surface waters running off. About 90% of the continental crust is built of silicate minerals, which create a vast reservoir of source mineral phases releasing Si into natural waters. However, resistance to weathering and usually low solubility of silicate minerals significantly limit Si concentration in waters.

The influx of lithogenic (volcanic) CO₂ into groundwater of Gran Canaria increases their aggressiveness with respect to bedrocks. Results show that CO₂ influx, at first, destabilises the chemical equilibrium, and responds for rapid increase of both dissolved inorganic carbon (DIC) and Si concentrations, until finally the partial chemical equilibrium with some minerals is probably reached and Si content quasi-stabilized at level of about 1.4 mM (Fig. 6C). Achieving the chemical equilibrium with secondary, neo-formed minerals, mainly clay minerals, favours stabilization of silicon concentrations.

5. Geochemical modelling

Inverse geochemical modelling provided data on reactions which are responsible for the water chemistry in Post Roque Nublo, Roque Nublo, and Phonolites aquifer-rocks. Representative groundwater selected for geochemical modelling (wells – nos. 3, 9, 17, 18) contact with most homogeneous bedrock and present chemical composition least changed by the human activity. In lithological profiles of wells 3 and 9 phonolite rocks dominate. In the profile of well 17 Post Roque Nublo rocks are only present, while in profile of well 18 Roque Nublo rocks prevail.

Several models have been found for groundwater from each particular wells. Three models have been found for water from well no. 3, six models for well no. 9, and four models both for waters from wells nos. 17 and 18. Models recognized phases which deliver substances into a solution (source phases) and phases which immobilize solutes from water (so-called sink phases), and quantified amount of each (dissolved or precipitated) phases by giving a mole transfer (in mol/kg H₂O).

Because the models which have been found for groundwater from each aquifer-rock types presented considerable resemblances, their results are expressed in a synthetic way (Tab. 3). Phases which have been included in every model play an essential role in the forming of groundwater chemistry. Reactions with mineral phases which occurred optionally in models might be displaced by reactions with some other minerals while water-rock interactions in the natural systems.

In all aquifer-rock types, important effects of both lithogenic CO₂ and marine aerosols were confirmed. The mean influx of CO₂ and marine aerosols ranges from 15.5 to 50.8 mmol/L, and from 0.52 to 3.39 mmol/L, respectively (Tab. 3). The dissolved CO₂ presents the highest mole transfers amongst phases included in models.

Apart from CO₂ and marine aerosols, primary and secondary minerals have been considered in found models. Reactions with those...
minerals depend on bedrock mineralogy. In all aquifer rocks the main source mineral phases are various feldspars and diopside (pyroxene). In Roque Nublo and Phonolites also feldspathoids (haüyne, nepheline) and biotites play the role, while in Post Roque Nublo - olivines. The secondary silicates considered in all models are montmorillonites of various chemical compositions. Mole transfers for silicate minerals are usually of 0.01 to 1 mmol/L (Tab. 3). Modelling results were used for the quantitative description of the contribution of particular minerals to amount of dissolved silicon. Contributions are expressed in the percentage of total Si released into solutions (Tab. 4). In all aquifer rocks, acid plagioclase end-member, albite (avg. 30-49 %) and diopside (avg. 19-42 %) contribute the most of silicon. The anorthite contribution varies between 1.5% and 10.3%. Potassium feldspars play the role only in Post Roque Rock bedrock, contributing about 13% of Si. Apart from feldspars and diopside, in Phonolite and Roque Nublo bedrocks feldspathoids (avg. 13.7-15.3 %) and biotites (11.2-18.8 %) play secondary role, while in Post Roque Rock olivines contribute 5.5% of dissolved Si on average. Contributions from kaersutite and titanite are negligible.

6. Conclusions

In the north of Gran Canaria, Si-rich groundwater might be found in rocks of all volcanic complexes. Obtained data shall be useful in hydrogeochemical forecasting of the groundwater quality. Understanding the processes which form chemistry of groundwater is important from both pure and applied view-points, because it helps us to protect quantity and quality of groundwater resources, like valuable medicinal waters. The performed geochemical modelling provides information about the total effect of water-rock interactions, irrespectively of the residence time of water in bedrocks. The longer time of water-rock contact usually supports increase of solutes concentration. Obtaining quantitative information about the intensity of particular chemical reactions in individual parts of the studied area would require further studies, e.g. on environmental tracers in groundwater.

Young volcanic bedrocks in studied area consist of, generally, easily reactive minerals. Weathering is not supported by low rainfall, however, it shall be intensified by dissolving of salts from marine aerosols and lithogenic carbon dioxide in groundwater. Diversity of bedrock mineralogy is well reflected by found water-mineral reactions. The common features of all volcanic aquifer-bedrocks are decaying of feldspars and pyroxenes, and formation of smectites. Silicic acid is the only form of silicon which is biologically available. In some countries, silicon (silicic acid) is regarded as a beneficial component which provides balneotherapeutic benefits. Many studies have showed beneficial and essential aspects of silicon in humans, e.g. because it limits bioavailability of toxic aluminium, and detoxifies organisms. Silicic acid at concentration greater than 0.5 mM in drinking waters has been suggested that show some protection against Alzheimer's disease (Exley et al., 2006). Bioavailable silicon is depleted from the most of products in our diet; also waters we drink usually have very low Si concentration of about 0.1 mM. Naturally Si-rich (fresh and medicinal) groundwater are used only locally. Unfortunately, therapeutic potential of Si-rich waters is still underrated (Dobrzyński and Exley, 2010). The potential Si-rich therapeutic water might be found in numerous places of the northern part of Gran Canaria island. While planning to use these waters it is necessary to consider hydrogeological and environment pollution issues. An earlier identification and an assessment of hydrogeological and hydrogeochemical conditions are required. Hydrogeological and hydrogeochemical methods, including geochemical modelling, provide effective tools for protection of medicinal waters.

Studied groundwater from the north part of Gran Canaria have very high Si contents, higher than in many balneologically-used medicinal waters of Europe, and an unexploited balneotherapeutic potential. The studied waters have silicic acid concentration much higher than above mentioned 0.5 mM level, and they seem to be ideal for testing the health benefits of such waters to humans.
Acknowledgements
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Figure 1. Localization, geologic map (after Balcells et al., 1990, modified) and groundwater head contours map (Benavides and Galindo, 2011) for 2008 of study area in the northern part of Gran Canaria. Explanations: 1-19 - numbers of sampled water wells.

Figure 2. Scheme of conceptual geochemical model for water-rock interactions in Gran Canaria island.

Figure 3. Stiff diagrams map reflecting groundwater chemistry of sampled wells.

Figure 4. Silicon concentration versus ionic strength of groundwater. Explanations: 1-19 - numbers of sampled wells. Groups of wells: I - groundwater chemistry dominated by interactions with Post Roque Nublo and Roque Nublo volcanic rocks; IIa, IIb - groundwater chemistry dominated by interactions with Phonolites and Roque Nublo volcanic rocks; III - groundwater chemistry affected by irrigation and/or sea salinity. Green line – minimum threshold value of Si content (0.5 mM) suggested as a beneficial for human health (after Exley et al., 2006); red line – solubility limit of silicic acid (1.94 mM), at 25°C; dark blue lines – ranges of silicon content typical for fresh groundwater. Groundwater used in inverse geochemical modelling (wells nos. 3, 9, 17, and 18) are marked as red filled circles.

Figure 5. Hierarchical Q-mode cluster dendrograms of studied groundwater based on: 5A - major solutes chemistry data; 5B - major and minor chemistry data, water table data, and contribution of bedrocks in alimentation zone. 1-19 - numbers of sampled wells. Dashed lines added for highlighting groups of clustered water members.

Figure 6. Silicon concentration versus temperature (6A), pH (6B), and dissolved inorganic carbon (6C) in groundwater. 1-19 - numbers of sampled wells.
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<th>Cl</th>
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<td>Cueva de Matos</td>
<td>20.0</td>
<td>1200</td>
<td>6.14</td>
<td>48.100</td>
<td>7</td>
<td>781.0</td>
<td>52</td>
<td>32</td>
<td>1.2</td>
<td>0.27</td>
<td>94.62</td>
<td>52.91</td>
<td>62.57</td>
<td>13.41</td>
</tr>
<tr>
<td>9</td>
<td>1254-TP</td>
<td>Cuesta La Arena</td>
<td>22.5</td>
<td>1156</td>
<td>5.62</td>
<td>38.760&lt;1</td>
<td>659.0</td>
<td>66</td>
<td>18</td>
<td>11</td>
<td>0.24</td>
<td>48.11</td>
<td>53.31</td>
<td>91.07</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1277-TP</td>
<td>El Laurel</td>
<td>17.7</td>
<td>700</td>
<td>5.80</td>
<td>21.379&lt;1</td>
<td>231.9</td>
<td>52</td>
<td>55</td>
<td>105</td>
<td>0.34</td>
<td>40.39</td>
<td>28.82</td>
<td>41.87</td>
<td>11.35</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1485-TP</td>
<td>Las Pellas</td>
<td>20.0</td>
<td>490</td>
<td>5.30</td>
<td>36.827</td>
<td>35</td>
<td>292.9</td>
<td>35</td>
<td>6.3</td>
<td>9.4</td>
<td>0.2</td>
<td>27.45</td>
<td>23.3</td>
<td>37.76</td>
<td>5.53</td>
</tr>
<tr>
<td>12</td>
<td>1993-TP</td>
<td>Los Lomitos</td>
<td>20.0</td>
<td>965</td>
<td>6.52</td>
<td>34.627&lt;1</td>
<td>305.1</td>
<td>97</td>
<td>87</td>
<td>35</td>
<td>0.36</td>
<td>50.92</td>
<td>40.91</td>
<td>73.69</td>
<td>7.83</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2178-TP</td>
<td>El Rapador</td>
<td>21.5</td>
<td>617</td>
<td>6.15</td>
<td>51.013</td>
<td>22</td>
<td>353.9</td>
<td>29</td>
<td>7</td>
<td>0.24</td>
<td>48.18</td>
<td>29.98</td>
<td>37.28</td>
<td>8.36</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2250-TP</td>
<td>San Antón</td>
<td>21.0</td>
<td>1145</td>
<td>6.08</td>
<td>46.380</td>
<td>4</td>
<td>671.2</td>
<td>59</td>
<td>39</td>
<td>3.1</td>
<td>0.43</td>
<td>91.1</td>
<td>47.15</td>
<td>60.23</td>
<td>11.13</td>
</tr>
<tr>
<td>15</td>
<td>2558-TP</td>
<td>Hoya del Pedregal</td>
<td>20.0</td>
<td>832</td>
<td>6.01</td>
<td>32.937</td>
<td>3</td>
<td>549.2</td>
<td>39</td>
<td>24</td>
<td>0.35</td>
<td>66.68</td>
<td>40.21</td>
<td>45</td>
<td>8.69</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2730-TP</td>
<td>Finca Los Palos</td>
<td>19.3</td>
<td>678</td>
<td>7.00</td>
<td>23.153&lt;1</td>
<td>402.7</td>
<td>39</td>
<td>45</td>
<td>17</td>
<td>0.84</td>
<td>34.88</td>
<td>32.68</td>
<td>47.51</td>
<td>9.95</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>3309-TP</td>
<td>Cazadores</td>
<td>21.0</td>
<td>469</td>
<td>5.77</td>
<td>40.819&lt;1</td>
<td>317.3</td>
<td>20</td>
<td>2.5</td>
<td>0.5</td>
<td>0.27</td>
<td>36.16</td>
<td>21.97</td>
<td>27.95</td>
<td>6.33</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>3968-TP</td>
<td>Cañado del Lance</td>
<td>20.0</td>
<td>869</td>
<td>6.21</td>
<td>43.936</td>
<td>4</td>
<td>549.2</td>
<td>47</td>
<td>31</td>
<td>3.9</td>
<td>0.23</td>
<td>62.37</td>
<td>37.39</td>
<td>52.66</td>
<td>9.07</td>
</tr>
<tr>
<td>19</td>
<td>6138-TP</td>
<td>Finca La Palma</td>
<td>19.9</td>
<td>303</td>
<td>4.40</td>
<td>35.983</td>
<td>31</td>
<td>109.8</td>
<td>55</td>
<td>14</td>
<td>17</td>
<td>0.8</td>
<td>9.32</td>
<td>8.16</td>
<td>37.52</td>
<td>4.96</td>
</tr>
</tbody>
</table>

1 – SEC - specific electric conductivity
Table 2. Clusters and their members in Q-mode, k-means clustering analysis of studied groundwater.

<table>
<thead>
<tr>
<th>Aggregations</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wells</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>14</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>
Table 3. The mean mole transfer (in mol/kg of H₂O) of phases responsible for formation of groundwater chemistry in volcanic bedrocks (based on results of inverse mass balance geochemical modelling).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Formula</th>
<th>Post Roque Nublo</th>
<th>Roque Nublo</th>
<th>Phonolites</th>
<th>Well 9 (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Well 17 (4)</td>
<td>Well 18 (4)</td>
<td>Well 3 (3)</td>
<td>Well 9 (6)</td>
</tr>
<tr>
<td>Lithogenic CO₂(g)</td>
<td></td>
<td>2.108E-02</td>
<td>1.553E-02</td>
<td>4.374E-02</td>
<td>5.081E-02</td>
</tr>
<tr>
<td>Marine aerosols²</td>
<td>Na₀.⁷⁷₉Mg₀.₀₈₇Ca₀.₀₁₄K₀.₀₁⁷Cl₀.₉₀₆(S</td>
<td>5.171E-04</td>
<td>1.465E-03</td>
<td>3.386E-03</td>
<td>2.057E-03</td>
</tr>
<tr>
<td>Potassium Feldspar</td>
<td>KAlSi₃O₈</td>
<td>2.502E-04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albite</td>
<td>NaAlSi₃O₈</td>
<td>9.105E-04</td>
<td>5.783E-04</td>
<td>8.729E-04</td>
<td>1.670E-03</td>
</tr>
<tr>
<td>Anorthite</td>
<td>CaAl₂Si₂O₈</td>
<td>2.891E-04</td>
<td>7.465E-05</td>
<td>5.452E-05</td>
<td>4.687E-04</td>
</tr>
<tr>
<td>Diopside</td>
<td>CaMgSi₂O₆</td>
<td>6.261E-04</td>
<td>1.181E-03</td>
<td>1.079E-03</td>
<td>8.809E-04</td>
</tr>
<tr>
<td>Kaersutite</td>
<td>NaCa₂Mg₄Ti₅Si₆Al₂O₂₃(OH)</td>
<td>2.927E-07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hauyne</td>
<td>Na₂CaSi₃Al₂O₁₂SO₄</td>
<td>2.542E-04</td>
<td>2.890E-04</td>
<td>9.094E-05</td>
<td></td>
</tr>
<tr>
<td>Nepheline</td>
<td>NaAlSiO₄</td>
<td>1.483E-04</td>
<td>1.053E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlogopite</td>
<td>KMg₃AlSiO₁₀(OH)₂</td>
<td>2.073E-04</td>
<td>2.951E-04</td>
<td>6.265E-04</td>
<td></td>
</tr>
<tr>
<td>Aninite</td>
<td>KFe₃AlSiO₁₀(OH)₂</td>
<td>2.987E-08</td>
<td>2.987E-08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forsterite</td>
<td>Mg₂SiO₄</td>
<td>3.066E-04</td>
<td>4.480E-08</td>
<td>4.479E-08</td>
<td></td>
</tr>
<tr>
<td>Fayalite</td>
<td>Fe₂SiO₃</td>
<td>4.479E-08</td>
<td>4.480E-08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titanite</td>
<td>CaTiSiO₅</td>
<td>2.508E-07</td>
<td>2.927E-07</td>
<td>2.509E-07</td>
<td>2.300E-07</td>
</tr>
<tr>
<td>Fluorapatite</td>
<td>Ca₅(PO₄)₃F</td>
<td>2.455E-06</td>
<td>4.535E-06</td>
<td>1.756E-06</td>
<td>2.844E-06</td>
</tr>
<tr>
<td>Fluorite</td>
<td>CaF₂</td>
<td>5.882E-06</td>
<td>3.791E-06</td>
<td>5.972E-06</td>
<td>4.901E-06</td>
</tr>
<tr>
<td>Montmorillonite-Ca³</td>
<td>Ca₀.₁₆₃Mg₀.₃₃₃Al₁.₆₇₃Si₅.₃₄O₁₀(OH)</td>
<td>-9.304E-04</td>
<td></td>
<td>-9.723E-04</td>
<td></td>
</tr>
<tr>
<td>Montmorillonite-Mg³</td>
<td>Mg₀.₄₉₅Al₁.₆₇₃Si₅.₃₄O₁₀(OH)</td>
<td>-8.84E-04</td>
<td>-8.72₄E-04</td>
<td>-1.04₂E-03</td>
<td>-7.29₁E-04</td>
</tr>
<tr>
<td>Montmorillonite-Na³</td>
<td>Na₀.₃₂₃Mg₀.₃₃₃Al₁.₆₇₃Si₅.₃₄O₁₀(OH)</td>
<td>-1.17₅E-03</td>
<td>-1.₁₆₁E-03</td>
<td>-1.₃₈₄E-03</td>
<td>-₉.₇₀₀E-04</td>
</tr>
<tr>
<td>Montmorillonite-K³</td>
<td>K₀.₃₃₃Mg₀.₃₃₃Al₁.₆₇₃Si₅.₃₄O₁₀(OH)</td>
<td>-1.₁₇₅E-03</td>
<td></td>
<td></td>
<td>-1.₂₄₄E-03</td>
</tr>
</tbody>
</table>

Transfers of phases which occur in each of the found models, i.e. essential phases, are bolded; unbolded values refer to an optional phases which occur only in particular model(s). 1 – number of found models; 2 – composition of marine aerosols based on the mean sea water chemistry; 3 – chemical formulas of montmorillonites used in the models are given accordingly to "llnl" thermodynamical database.
Table 4. The weathering contribution (in %) of silicate source mineral phases to the pool of silicon dissolved in studied groundwater. Mean values are given in brackets.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Post Roque Nublo</th>
<th>Roque Nublo</th>
<th>Phonolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well 17</td>
<td>Well 18</td>
<td>Well 3</td>
</tr>
<tr>
<td>Potassium Feldspar</td>
<td>7.469 – 26.370 (12.981)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaersutite</td>
<td>0.028 – 0.035 (0.032)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annite</td>
<td>0.001 – 0.002 (0.001)</td>
<td></td>
<td>0.001 – 0.001 (0.001)</td>
</tr>
<tr>
<td>Forsterite</td>
<td>4.148 – 6.763 (5.666)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fayalite</td>
<td>0.001 – 0.001 (0.001)</td>
<td>0.001 – 0.001 (0.001)</td>
<td></td>
</tr>
<tr>
<td>Titanite</td>
<td>0.004 – 0.005 (0.005)</td>
<td>0.005 – 0.006 (0.005)</td>
<td>0.004 – 0.004 (0.004)</td>
</tr>
</tbody>
</table>

Contributions of an essential phases are bolded; unbolded – an optional phases. *-optional phase included only in the one found model.
Figure 1. Localization, geologic map (after Balcells et al., 1990, modified) and groundwater head contours map (Benavides and Galindo, 2011) for 2008 of study area in the northern part of Gran Canaria. Explanations: 1-19 - numbers of sampled water wells.
Figure 2. Scheme of conceptual geochemical model for water-rock interactions in Gran Canaria island.

Figure 3. Stiff diagrams map reflecting groundwater chemistry of sampled wells.
Figure 4. Silicon concentration versus ionic strength of groundwater. Explanations: 1-19 - numbers of sampled wells. Groups of wells: I - groundwater chemistry dominated by interactions with Post Roque Nublo and Roque Nublo volcanic rocks; IIa, IIb - groundwater chemistry dominated by interactions with Phonolites and Roque Nublo volcanic rocks; III - groundwater chemistry affected by irrigation and/or sea salinity. Green line – minimum threshold value of Si content (0.5 mM) suggested as beneficial for human health (after Exley et al., 2006); red line – solubility limit of silicic acid (1.94 mM), at 25°C; dark blue lines – ranges of silicon content typical for fresh groundwater. Groundwater used in inverse geochemical modelling (wells nos. 3, 9, 17, and 18) are marked as red filled circles.
Figure 5. Hierarchical Q-mode cluster dendrograms of studied groundwater based on: 5A - major solutes chemistry data; 5B - major and minor chemistry data, water table data, and contribution of bedrocks in alimentation zone. 1-19 - numbers of sampled wells. Dashed lines added for highlighting groups of clustered water members.
Figure 6. Silicon concentration versus temperature (6A), pH (6B), and dissolved inorganic carbon (6C) in groundwater. 1-19 - numbers of sampled wells.
Investigation of pH variation of blood during peloidotherapy

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3. Monica Surdu – MD, resident physician, Constanța Emergency County Hospital.

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**Background.** The main aim of the study is to evaluate the variation of acid-base balance of human body during peloidotherapy. The secondary objective is balneal safety for the patient suffering of joint degenerative diseases and post-traumatic sequelles of the limbs, undergoing mud therapy. This paper work presents only the results obtained on pH variations. **Materials and methods.** The study plot was composed by forty three patients undergoing mud therapy for rehabilitation in Techirghiol Balneal and Rehabilitation Sanatorium. Daily therapeutic intervention consists in: thermoneutral bath (alternatively mud bath and salt bath), kinetotherapy, massage and electrotherapy. Proofs prelevations (capillary blood) were performed in four moments of the cure: at the bigining of the cure, immediately after first balneal application, at twenty four hours after first application and at the end of the cure of ten balneal application. Blood determinations were made using CCX Nova Biomedical 6 analyser for critical care. Results obtained were interpreted in correlation with the results of VIASAN 317/2004 study upon variations of biochemical composition of mud after therapeutic use. In order to use results of VIASAN study we asked and we received aproval from the owner and incumbent of the study, Techirghiol Balneal and Rehabilitation Sanatorium. All patients get to know about the rhythm of blood prelevation and possibility of results publication and they signed the informed consent. Statistic analysis were made with the soft SPSS 12.0 upon witch Constanța Ovidius University holds the licence of using. **Rezults.** Variations of blood pH in the four moments of cure were within physiological limits. They were statistically insignificant with one exception: immediatly after first salt bath application pH increased statistic significant but within physiological limits (p=0.02<0.05). **Conclusions.** During peloidotherapy the acidity of blood measured through pH, variates within the frame of physiological limits. This variation might be corelated with the variation of mud bath solution`s pH after application and possibly with skin`s pH variation. Balneal application of mud and salt water of the lake is a safe procedure for the patients. In order to establish the possible correlation between three medium implicated in the physiological answer to peloidotherapy, respectively: mud, skin and blood are needed more studies.

Key words: blood pH, acid-base balance, peloidotherapy, thermoneutrality.

**Introduction**

The normal biological functions of the body imply the keeping of some dynamic parameters in balance such as: body temperature, water distribution, ions quantity and distribution and pH. The last one, define the body's internal environment acidity and represents the decimal logarithm of the hydrogen ions with the sign changed. The activity of enzymes, membranare channels through which is made the transport of molecules and ions, etc have an optimally function at a specific temperature and a certain pH. Keeping almost constant the blood pH is achieved through the intervention of buffer systems: the inorganic carbonates, phosphates, hemoglobin, some organic acids. Adjusting the components of these systems is carried out quickly (some minutes) by respiratory mechanism whereby carbon dioxide is removed and by the renal mechanism, witch is slower (some hours ) whereby the bicarbonate is removed. [(1),(2),(3)]

One of the first studies on acid-base balance in healthy person belongs to american authors N. W. Shock and A. B. Hastings and was
published in the March 1934 issue of the journal „The Journal of Biological Chemistry” (4).

Currently, most studies on the acid-base balance and fluid-electrolyte are related to post-traumatic, respiratory, renal and/or metabolic disorders. Although significant progress has been made in the study of acid-base balance, in the literature that I had access and I studied it, I didn’t found studies to evaluate changes of this important balance parameters for the human body when applied in the external cure of mineral waters and/or mud, although balneotherapy is "disturbing" homeostasis parameters, at least, by:

- Increase skin temperature (due to thermopexic qualities of the mud and salt water used for bathing);
- Changing electrical charges of skin (due to biophysical properties and biochemical composition of the therapeutic environment that acts as an ion exchangers);
- Change the pH skin (due to the different pH bath);

In this context I consider that I must study physiological and/or pathophysiological possible changes of the acid-base balance, as a step in the reliance on scientific basis of peloidotherapy (evidence based balneology), as a holistic and integrative medicine. This paper presents the variation of one parameter (pH of the blood) between the twenty-eight parameters determined with the analyzer CCX Nova Biomedical 6.

**Materials and methods**

Study of acid-base balance variation under the peloidotherapy impact was conducted in autumn season when the climate impact is reduced. I chose the thermoneutral application with minimum disturbance for the body functions. For the Techirghiol sapropelic mud the thermoneutral value is 38°C and 37°C for the saline water.

In accordance with the methodology approved by National Institute of Rehabilitation, Physical Medicine and Balneology, therapeutic application consists in alternative use of mud and salt water of the lake. Specific working methodology of Rehabilitation Sanatorium Techirghiol is balneotherapy (peloid bath alternative with salt bath), physical therapy, electrotherapy and masotherapy, prescribed and applied in accordance with the therapeutic targets required by each patient suffering. Daily therapeutic program of patients was achieved in the working diagram of the sanatorium. Thus some patients began therapy with saline bath and other with peloid bath. Due to the alternation of the applications, at the end of the cure all patients accumulate the same number of baths: five salt bath and five mud bath.

In the study group were included forty-three adult patients: twenty-eight women and fifteen men, twenty-four began therapy with peloid bath and nineteen with saline bath. The average age of patients in the study group was $52.27 \pm 13.04$ years (table 1).

**Table I average age of patients by type of the first applications and sex**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>The average age group</td>
<td>$52.27 \pm 13.04$</td>
</tr>
<tr>
<td>The average age subgroup inaugural mud bath</td>
<td>$54.27 \pm 12.27$</td>
</tr>
<tr>
<td>The average age subgroup inaugural saline bath</td>
<td>$51.11 \pm 13.40$</td>
</tr>
<tr>
<td>The average women’s age</td>
<td>$51.86 \pm 11.38$</td>
</tr>
<tr>
<td>The average men’s age</td>
<td>$58.40 \pm 11.11$</td>
</tr>
</tbody>
</table>

**Criteria for inclusion** in the study group were:

- patients suffering from degenerative rheumatic diseases and with posttraumatic sequelae of limb which had the correct indication of balnear treatment, apparently healthy based on clinical examination and commonly, generally laboratory tests.
Table II PH variation during peloide cure

<table>
<thead>
<tr>
<th></th>
<th>before the cure (in)</th>
<th>immediately after the first application (im)</th>
<th>at 24 hours after the first application (24)</th>
<th>at the end of the cure (fin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>average group ±DS</td>
<td>7.40 ± 0.03</td>
<td>7.42 ± 0.04</td>
<td>7.40 ± 0.03</td>
<td>7.41 ± 0.03</td>
</tr>
<tr>
<td>average subgroup BN ±DS</td>
<td>7.41 ± 0.04</td>
<td>7.43 ± 0.04</td>
<td>7.41 ± 0.03</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>average subgroup BS ±DS</td>
<td>7.39 ± 0.03</td>
<td>7.41 ± 0.03</td>
<td>7.39 ± 0.03</td>
<td>7.41 ± 0.03</td>
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<th>t-test p&lt;0.05</th>
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<tr>
<td>BN/BS</td>
<td>0.26</td>
</tr>
<tr>
<td>BN in/BN im, 24, fin</td>
<td>0.09</td>
</tr>
<tr>
<td>BS in/BS im, 24, fin</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Exclusion criteria:
- any of the situations described as contraindications for balnear cure;
- presence or inflammatory phenomena joint appearance, decompensation of cardiac and / or blood pressure during treatment;
- skin lesions that contraindicate the balnear applications and adjuvant procedures;
- respiratory, endocrine, neurological sufferings, spinal static disorder that can change blood gas homeostasis;
- cortisone medication or any other type of medication known to interfere with the determined parameters (diuretics, supplements containing minerals or carbohydrates in composition).

Patients were asked if they want to participate in the study and were informed about the collection of biological samples, that the results will be published in various scientific publications and they received assurances of confidentiality of personal data. All patients signed the informed consent.

Biological sampling was carried out in the morning before breakfast and before starting the treatment, in four moments of cure:
- before the cure (noted „in‟);
- immediately after the first application and we called it the inaugural / initiate application (noted „im‟)
- at twenty-four hours after the first application, but before the second one (noted „24‟);
- at the end of 10 applications cure (noted „fin‟).

Blood samples did not require any further processing, the determination being done on capillary whole blood, with the analyzer „CCX 6 Nova Biomedical” offered by the "Center for Scientific Research in Stress" of the "Faculty of Pharmacy" from "Ovidius University of Constanta". Analyzer was installed in the laboratory of Techirghiol Balneal and Rehabilitation Sanatorium respecting technical conditions required in the technical documentation.

Results
In accordance with the requirements of evidence-based medicine after tabled the results I calculated the average and standard deviation and then I applied multiple comparison tests (t-test) to assess the statistical significance of the results. (table 2).

Table II PH variation during peloide cure
In mathematical processing of data obtained for the parameter pH I found a normal distribution and uniform values for both types of application, for all moments in time and for all analyzed groups. Test comparison between groups showed variation in the physiological limits and insignificant statisticall for blood pH during cure, except pH measured immediately after the first saline application, which had a statistically significant increase, but the physiological limits.
It should be emphasized homogeneity distribution and very small standard deviation value, which shows the gentleness application and fine, precise control mechanisms. Acid-base homeostasis is maintaining in the first line of action by the buffer systems of plasma, followed by respiratory mechanism, completed and corrected by the renal mechanism. Slightly increased pH value obtained after the first application (figure 1) suggests the involvement of the respiratory mechanism that prompt removes carbon dioxide by hyperventilation. Comparing the reactivity of the body expressed in blood pH parameter with the method used in the group therapy (figure 2), we can summarized that although the two therapeutic applications used (saline bath and mud bath) have different biophysical properties: the temperature of thermal neutrality, hydrostatic pressure, osmotic pressure, the body reacts similarly:

- slight increase of pH after the first application skin, possible caused by the change of the temperature;
- return to values close to the initial ones during next hours by entering in action the corrective mechanisms.

During application the pH of plasma and the maintenance mechanisms are "threatened" by increasing temperature of the internal environment and the increasing lung ventilation. So:

- skin temperature may increase by 1 - 2°C while the temperature of the bath is kept constant. In the working protocol of Rehabilitation Sanatorium Techirghiol maintain the average temperature bath almost constant is done by adding a quantity of heated water to ½ length of proceedings;
- increasing temperature of the central area (core) is only 0.1 - 0.2°C. In these conditions warm-blooded and pH are not threatened, and thermoregulatory function and acid-base homeostasis are maintained easily by the body;
- respiratory function is required at minimum because the relaxant effect (muscle and general) of the application produces decrease respiratory rate and increased breath amplitude with minimum consequences: both the saline bath and the peloide one.

The body responds to this request with the appropriate functional adjustments that do not exceed physiological limits. Intensity of the response depends on the inaugural application: peloide application is more demanding than the saline one.

Interface of the biological system body - peloid environment is represented by the skin.
One of the most important functions of the skin is the physical-chemical barrier performed by the corneum stratum (metabolical inactive) and hydro-lipid film ("biochemical sponge"), that regulate skin homeostasis and by the consequence the body's internal environment. Skin coating acts as a osmotic membrane.

Lipid film formation and maintenance of skin pH is influenced by climatic factors (wind, sun, which can have different values compared with the residence town of the patient) or chemical factors (substances acting like detergents, solvents, soaps, which can be found in mud composition), that disrupts homeostasis and can reduce skin acid mantle of skin formation. (5)

Studies about the effect of mud therapy on skin pH and seborrheic secretory status showed normalization of skin pH after fourteen mud applications (6).

Analyzing the interaction between skin and bath environment showed a lower pH value of peloide solution at the end application, statistically insignificant. (7).

Comparative changes in peloide environmental of the pH measured before and after therapeutic treatment and variations of blood pH during peloidotherapy it can be assumed biological interaction between skin and mud:

• blood pH increased slightly;
• mud solution pH decreased.

Interface of two media, the skin, reacted by normalization of pH.

Conclusions.

Systematized these results we can say:
1. during peloidotherapy pH variations in the internal environment is in physiological limits, connected with changes in environmental pH bath and pH of the skin;
2. peloide application does not disrupt the acid-base balance (investigated by the parameter pH) above physiological limits, so it's a safe application.

The results obtained allow us to suggest that further studies will be required concomitant evaluation of the interaction between environment and body bath by researching environmental parameters involved: the mud, blood, etc.

Bibliography:
7. PNCDI, VIASAN proiect 317/2004,