

Enzymological characterization of the sapropelic muds in the lakes Ocna Sibiului

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Abstract. Besides purely scientific interest, the spa waters and therapeutic muds consecrated research have a remarkable practical importance. The evaluation of the water and therapeutical muds's physico-chemical, microbiological and enzymological potential constitutes a remarkable instrument with very high utility to the balneotherapy specialists.

Key Words: dehydrogenase activity, catalase activity, phosphatasic activity, sediments.

Rezumat. Pe langă interesul major științific, apele minerale și nămolurile terapeutice au facut specificul unor cercetări consacrate cu o remarcabilă importanță practică. Evaluarea potențialului enzimologic, microbiologic și fizico-chimic al apelor și nămolurilor terapeutice constituie un instrument remarcabil cu mare utilitate pentru specialiștii în balneoterapie.

Cuvinte cheie: activitatea dehidrogenazică, activitatea catalazică, activitatea fosfatazică, sedimente.

Introduction. The extreme environments are serious challenges for scientists (Gagyi-Palfy & Stoian 2008; Pricop & Negrea 2009; Petrescu-Mag & Rasiga 2009) and one of the most interesting aspects of these environments is their enzymological characterization (Carpa & Butiuc-Keul 2009). The scope of present work was the enzymological characterization of the sapropelic muds in the lakes Ocna Sibiului (Romania, European Union). Our enzymological surveys are continuing those of Bulgăreanu et al (1981, 1989), Drăgan-Bularda et al (1982, 1985), Arie et al (2006) and Poplăcean (2008).

Material and Methods

Determination of dehydrogenase activity of sediments. To determine dehydrogenase activity of the sediment, the sediment CaCO_3 is added (to neutralize the acid formed during incubation) and TTC solution (2, 3, 5 chloride - triphenyltetrazolium) which serves as transferred hydrogen acceptor by the dehydrogenase, which is reducing itself to a compound in red - phormazan. The phormazan is extracted with organic solvents (ethanol, methanol, acetone) and determined spectrophotometrically at 485 nm. The higher the phormazan concentration is, the dehydrogenase activity is higher. Current and potential dehydrogenase activity is expressed in mg phormazan/10 g sediment.

Determination of catalase activity of sediments. The sediment samples that are thermally active and inactive are added to an aqueous buffer and substrate solution (H_2O_2). After incubation, it is determined permanganometric the decomposed peroxide. Enzyme activity is expressed in mg H_2O_2 / g mud.

Determination of sediment phosphatasic activity. For determination of phosphatase activity of sediment is added toluene (to prevent proliferation of microorganisms) and the aqueous solution of enzyme substrate (disodium phenylphosphate). Reaction mixtures are incubated at 37°C, 24 hours. During incubation, the disodium phenylphosphate is breaking hydrolytically under the action of phosphomonoesterase producing phosphate and disodium

phosphate. Phenol reacts with 2, 6-dibromchinon-chlorimide (Gibbs reagent), forming a blue compound (an indophenol) which is determined photocolometric. If the indophenol concentration is higher, the phosphatase activity will be also higher.

Results and Discussion

Mud dehydrogenase activity monitoring in salt lakes from Ocna Sibiu. Dehydrogenasic activity may be considered an overall indicator of organisms biological activity, but also it was used as an ecotoxicological test to assess the effects of pollutants on soil or sediment microbiota.

Lakes, depending on mud dehydrogenasic activity, are grouped as:

- **Lakes with very low mud dehydrogenase enzymatical activity**, which shows a low microbial activity in the mud - Lake cu Insulă (Island Lake) and Lake Rândunica (Swallow's Lake). Seasonal fluctuations are relatively low, the minimum value recorded was 0.23 mg phormazan / 1 g mud (dry weight), the maximum value was 0.94 mg phormazan / 1 g mud (dry weight) and annual average dehydrogenase activity for these lakes was about 0.7 mg phormazan / 1 g mud (dry weight).

- **Lakes with moderate mud dehydrogenasic activity**, with high seasonal fluctuations - Lake Brâncoveanu (see Plate 1), Lake Pânzelor (Linen's Lake), Lake Negru (Black Lake, see Plate 1), Lake Fără Fund (Bottomless Lake), Lake cu Nămol (Mud Lake), Lake Gura Minei (Mine's Mouth Lake). Dehydrogenase activity varied between 0.11 µg phormazan / 1 g mud (dry weight) and 1,70 µg phormazan / 1 g mud (dry weight), and the average value for the monitored period was approximately 1.00 µg phormazan / 1g mud (dry weight)

- **Lakes with high dehydrogenasic activity** - Lake Ocnița (Duck or Little Saline Lake). Changes in mud dehydrogenasic enzymatical activity in the lake are between 0.61 and 1.80 mg phormazan / 1 g mud (dry weight). Lake Ocnița presents maximum annual mud dehydrogenase activity (1.29 mg phormazan / 1 g mud-dry weight), which was increased by 52% compared with that of the Lake cu Insulă and Lake Rândunica (see Fig 1).

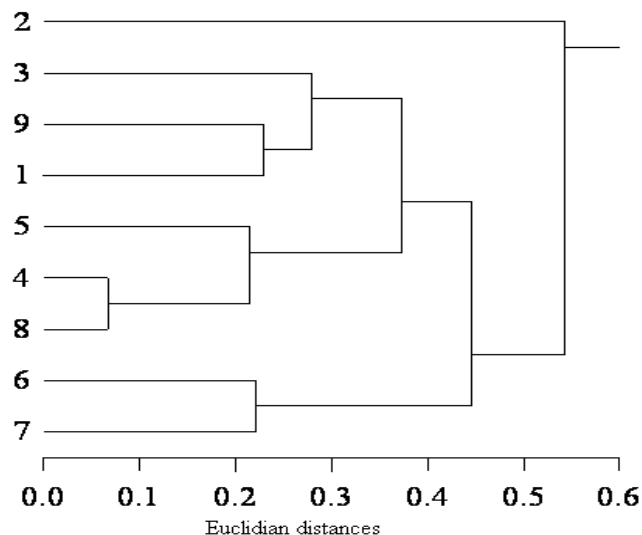


Figure 1. Cluster tree made on the values of dehydrogenase activity of lakes' mud (grouping based on average values of dehydrogenase activity on the Euclidean distance and method for group nearest neighbor - a simple amalgamation, 1 - Bottomless Lake, 2 - Duck (Little Saline) Lake, 3 - Mud Lake, 4 - Swallow's Lake, 5 - Brâncoveanu Lake 6 - Linen's Lake, 7 - Black Lake, 8 - Island Lake, 9 - Mine's Mouth Lake)

Mud phosphatase activity monitoring in salt lakes from Ocna Sibiu. Grouping lakes according to mud phosphatase activity is so:

- **Lakes with mud low phosphatase potential** - Lake cu Insulă, Lake Rândunica and Lake Fără Fund - here phosphatase activity varied between 63.22 μg phenol / g mud and 95.21 μg phenol / g mud, the average being 77.61 μg phenol / g mud.

- **Lakes with moderate mud phosphatase activity** - Lake Gura Minei, Lake Pânzelor, Lake cu Nămol, Lake Negru, Lake Brâncoveanu - the minimum phosphatase being 73.5 μg phenol / g mud, and maximum value being 155.53 μg phenol / g mud. Annual average phosphatase activity of the mud to these lakes is around 100 μg phenol / g mud.

- **Lakes with intense phosphatase activity** - Lake Ocnița - average annual phosphatase was 154.43 μg phenol / g mud and seasonal fluctuations falls between 117.5 μg phenol / g mud and 192.24 μg phenol / g mud (see Fig 2).

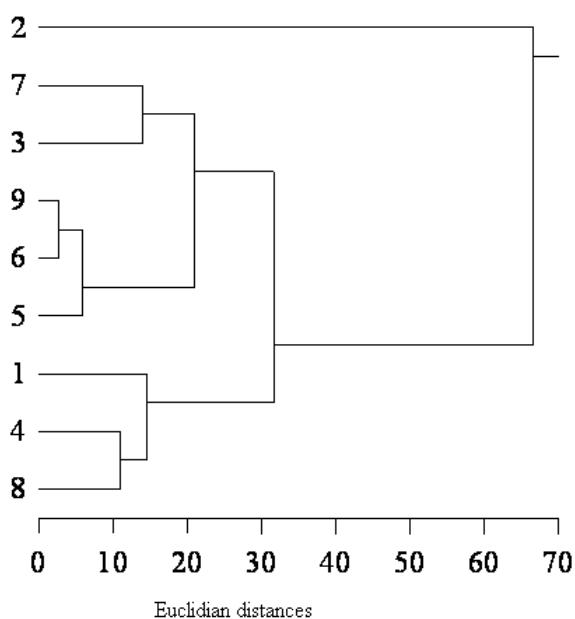


Figure 2. Cluster tree made on the values phosphatase activity of lakes' mud (grouping based on average values of phosphatase activity on the Euclidean distance and method for group nearest neighbor - a simple amalgamation, 1 - Bottomless lake, 2 - Duck (Little Saline) Lake, 3 - Mud Lake, 4 - swallow's Lake, 5 - Brâncoveanu Lake 6 - Linen's Lake, 7 - Black Lake, 8 - Island Lake, 9 - Mine's Mouth Lake)

Mud catalase activity monitoring in salt lakes from Ocna Sibiu. Depending on the activity of catalase enzyme, the lakes are grouped as:

- **Lakes with low mud catalase activity** - Lake cu Insulă, Lake Rândunica, Lake, Lake fără fund, Lake Brâncoveanu, Lake Gura Minei. Seasonal variation and difference between the enzyme potential of these lakes was between 0.15 and 1.1 $\text{mg H}_2\text{O}_2$ / g dry mud, and the annual average recorded value was up to 0.5 $\text{mg H}_2\text{O}_2$ / g dry mud.

- **Lakes with moderate mud catalase activity** - Lake cu Nămol and Lake Pânzelor. The average mud catalase potential was 0.87 $\text{mg H}_2\text{O}_2$ / g dry mud, ranging from 0.23 $\text{mg H}_2\text{O}_2$ / g dry mud at 2.0 $\text{mg H}_2\text{O}_2$ / g dry mud.

- **Lakes with intense mud catalase activity** - Lake Negru and Lake Ocnița. Minimum activity of catalase was 0.31 $\text{mg H}_2\text{O}_2$ / g dry mud in Lake Negru in January and a maximum of 2.30 $\text{mg H}_2\text{O}_2$ / g dry mud was determined to Lake Ocnița in the month of October. Annual average being 1.25 $\text{mg H}_2\text{O}_2$ / g dry mud (see Fig 3).

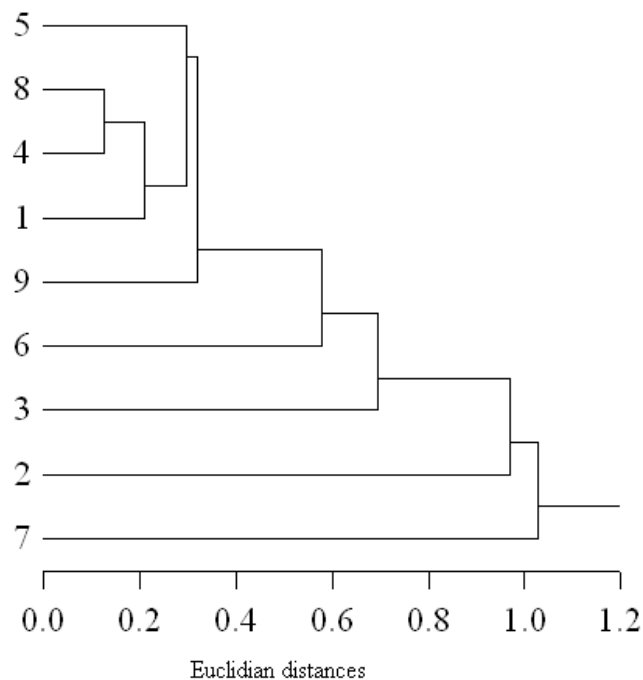


Figure 3. Cluster tree made on lakes' mud catalase activity values (grouping based on average values of catalase activity on the Euclidean distance and method for group nearest neighbor - a simple amalgamation, 1 - bottomless lake, 2 - Duck (Little Saline) Lake, 3 - Mud Lake, 4 - swallow's Lake, 5 - Brâncoveanu Lake 6 - Linen's Lake, 7 - Black Lake, 8 - Island Lake, 9 - Mine's Mouth Lake)

Conclusions. In terms of mud enzymatical potential expressed by dehydrogenase activity, phosphatase and catalase activity we found that:

- Lake Ocnița –the three enzymatic mud activities have intensified in the monitorized period. The mud presents so intense microbial activity, high oxidation-reduction potential and high organic loading.
- Lake Negru has increased catalase activity and moderate dehydrogenase and phosphatase activity, which shows an intense oxidation-reduction potential of mud, which is not polluted.
- Lake Pânzilor and Lake cu Nămol presents a moderate intensity of all the activities investigated.
- Lake Brâncoveanu and Lake Gura Minei record moderate dehydrogenase and phosphatase activity, and reduced catalase potential.
- Lake Fără Fund presents reduced phosphatase and catalase potential and moderate dehydrogenase activity.
- Lake Rândunica and Lake cu Insulă presents the minimum intensity of enzymatic activities characteristic of sapropelic mud, indicating its poor therapeutic efficiency.

Phosphatase activity is reduced in March and July and increased in January and October, consistent with accumulation of organomineral biocomponents. Dehydrogenase activity in contrast, presents high weight in March and July and low in winter and autumn. Oxidation-reduction potential of the mud, reflected the catalase activity is low during winter and spring and increased in summer and autumn.



Brâncoveanu Lake



Black Lake

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