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Enzymological classification of salt lakes in Romania

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Abstract. Sediments of 56 salt lakes from Romania were studied enzymologically. The following 7 enzymatic and nonenzymatic catalytic activities have been measured: phosphatase, H_2O_2-splitting in nonautoclaved (catalase) and autoclaved samples, TTC reduction in nonautoclaved (dehydrogenase) and autoclaved samples, without or with glucose addition. A formula is proposed for the evaluation of the enzymatic potential of salt lake sediments or other habitats. A hierarchy of the studied lakes has been established on the basis of the enzymatic potential of their sediments expressed as enzymatic indicator.

Key words: enzymatic indicator, sediment, therapeutic mud

Introduction

In Romania there are many salt lakes, the sediments of which constitute muds with therapeutic qualities. Among them, the Techirghiol lake, situated near the Black Sea, is the biggest producer of therapeutic mud in Europe (Ţuculescu, 1965).

The aim of this paper is to evaluate the enzymatic potential of sediments in 56 salt lakes, according to the values of the enzymatic indicators of their quality. The enzymatic indicators are calculated according to a formula which takes into account the intensity of the following 7 enzymatic and nonenzymatic catalytic activities: phosphatase, H_2O_2-splitting in nonautoclaved (catalase) and autoclaved samples, TTC reduction in nonautoclaved (dehydrogenase) and autoclaved samples, without or with glucose addition.

We were encouraged in our trial by other similar attempts at classifying aquatic habitats on the basis of intensity of some enzymatic activities made by researchers in many countries.

Lenhard and co-workers (1962, 1965) were the first to utilize enzymological data for the classification of natural waters. They classified the sediments of the Apies and Vaal rivers (South Africa) on the basis of their dehydrogenase activity. Jones et al. (1972, 1979) classified 16 lakes from Cumbria

(England), based either on the phosphatase activity of water, or on dehydrogenase and phosphatase activities of water and sediments. Nechesov (1977) compared 6 lakes from the upper basin of the Angara River by using values of catalase activity in their sediments. Maksimov and Chernykh (1979) differentiated 24 zones of the Baikal Lake considering dehydrogenase activity of their sediments as a criterion of differentiation.

Many enzymological researches have been carried out on salt lake sediments from Romania in the last 20 years (Kiss et al., 1979, 1986; Rădulescu et al., 1980; Bulgăreanu et al., 1981; Drăgan-Bularda et al., 1982; Paşca et al., 1985, 1991; Crişan et al., 1988). For the present classification we use some of the analytical data already published and also unpublished data.

Kiss et al. (1986) classified 37 salt lakes from Romania based on the 7 enzymatic and nonenzymatic catalytic activities of their sediments studied in this paper. The intensity of the global enzymatic activity was expressed by means of an enzymatic indicator of sediment quality. The enzymatic indicator was obtained by totalling the relative values of the 7 activities. The relative values were calculated in percentages, for each of the 7 activities, the most intense activity in the 37 lakes studied being considered 100 per cent. This classification has the inconvenience that for establishing the position of a lake not studied before in the existing classification system, it is necessary to recalculate the enzymatic indicators for all lakes already classified.

In this paper we propose a new modality for appreciation of the enzymatic potential of the sediments, or other habitats, based on the calculation of an enzymatic indicator. The suggested formula makes possible the immediate introduction of new lakes or other habitats in the existing classification system.

In order to emphasize the advantage of comparing different habitats on the basis of some synthetic indicators, which take into account more than one parameter, we record the proposal of Beck (1984). The author calculated a microbial indicator of soil quality based on the total microbial biomass and the intensity of the activities of three hydrolases (saccharase, protease and alkaline phosphatase) and two reductases (dehydrogenase and catalase), respectively.

Materials and methods

The sediments of 56 salt lakes were studied enzymologically, in different periods, as shown in Table 1. The samples were centrifuged 30 min at 4,000 rotations/min. After the removal of the supernatant, the dry matter and the seven enzymatic and nonenzymatic catalytic activities specified below were measured in the sediments. For measurement of the nonenzymatic catalytic H_2O_2-splitting and nonenzymatic TTC reduction, a part of the sediment

Table 1. Hierarchy of the salt lakes according to values of the enzymatic indicator of their sediments.

Position	Lake	Locality	Eastern longitude (E) northern latitude (N)	Period of analyses	Total number of sediment samples	Enzymatic indicator
0	1	2	3	4	5	6
1	Roşu	Ocna Şugatag	23°57′ E 47°47′ N	1981–1982	7	0.655
2	Horea	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	9	0.649
3	Baia Roşie	Slănic Prahova	24°56′ E 45°14′ N	1977–1978	4	0.609
4	Aluniş	Sovata	25°4′ E 46°35′ N	1979	9	0.608
5	Băile	Cojocna	23°51′ E 46°45′ N	1978–1979	20	0.584
6	Privighetorii	Turda	23°46′ E 46°35′ N	1986	3	0.577
7	Nr. 3	Cojocna	23°51′ E 46°45′ N	1978–1979	4	0.572
8	Crişan	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	7	0.569
9–10	Cloşca	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	10	0.556
9–10	Baia Baciului	Slănic Prahova	24°56′ E 45°14′ N	1977–1984	14	0.556
11	Bătrânilor	Turda	23°46′ E 46°35′ N	1987	14	0.504
12	Csiky	Turda	23°46′ E 46°35′ N	1986	9	0.489
13	Pânzelor	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	5	0.473
14	Mierlei	Sovata	25°4′ E 46°35′ N	1977–1978	12	0.464
15	Ştrand	Turda	23°46′ E 46°35′ N	1987	10	0.463
16	Techirghiol	Techirghiol	28°37′ E 44°3′ N	1984–1988	29	0.440
17	Roşu	Sovata	25°4′ E 46°35′ N	1977–1979	12	0.431
18	Fundata	Fundata	27°10′ E 44°37′ N	1987	3	0.430
19	Durgău	Turda	23°46′ E 46°35′ N	1986	9	0.428
20	Tarzan	Turda	23°46′ E 46°35′ N	1986	9	0.425
21	Negru	Sovata	25°4′ E 46°35′ N	1990–1994	43	0.421
22	Bătrân	Ocna Şugatag	23°57′ E 47°47′ N	1981–1982	8	0.420
23	Sărat 1	Brăila	27°55′ E 45°14′ N	1982–1983	5	0.417

Table 1. Continued.

Position	Lake	Locality	Eastern longitude (E) northern latitude (N)	Period of analyses	Total number of sediment samples	Enzymatic indicator
0	1	2	3	4	5	6
24–25	Ursu	Sovata	25°4′ E 46°35′ N	1990–1994	106	0.409
24–25	Verde	Sovata	25°4′ E 46°35′ N	1979	9	0.409
26	Sinoe	Sinoe	28°52′ E 44°37′ N	1983	9	0.394
27	Balta cu Nămol	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	6	0.364
28	Fără	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	2	0.359
29	Costineşti	Costineşti	28°38′ E 43°57′ N	1989	2	0.357
30	Tineretului	Turda	23°46′ E 46°35′ N	1987	7	0.354
31	Amara	Amara	27°17′ E 47°37′ N	1987	6	0.352
32	Ocniţa	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	9	0.351
33	Nuntaşi	Nuntaşi	28°43′ E 44°32′ N	1979–1980	25	0.345
34	Balta Albă	Balta Albă	27°20′ E 45°17′ N	1987	6	0.332
35	Nou Format	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	5	0.324
36	Găvrilă	Ocna Şugatag	23°57′ E 47°47′ N	1981–1982	15	0.315
37	Câineni	Câineni	27°18′ E 45°14′ N	1986	8	0.305
38	Movila Miresei	Movila Miresei	27°38′ E 45°13′ N	1987	15	0.303
39	Poporului	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	9	0.294
40	Baia Neagră	Slănic Prahova	24°56′ E 45°14′ N	1977–1984	7	0.276
41	Minei Mari	Ocna Dejului	23°48′ E 47°7′ N	1982–1983	12	0.269
42	Istria	Istria	28°43′ E 44°34′ N	1985	4	0.249
43	Sărătura II	Murighiol	29°9′ E 45°2′ N	1985	8	0.248
44	Sărătura I	Murighiol	29°9′ E 45°2′ N	1985	10	0.231
45	Cabdic	Ocna Dejului	23°48′ E 47°7′ N	1982–1983	11	0.212
46	Sfântu Ion	Ocna Sibiului	24°4′ E 45°53′ N	1978	2	0.211

Table 1. Continued.

Position	Lake	Locality	Eastern longitude (E) northern latitude (N)	Period of analyses	Total number of sediment samples	Enzymatic indicator
0	1	2	3	4	5	6
47	Ocna de Apă	Coştiui	24°2′ E 47°56′ N	1981–1982	8	0.192
48	Plopu	Murighiol	29°9′ E 45°2′ N	1985	10	0.179
49	Baia Verde 3	Slănic Prahova	24°56′ E 45°14′ N	1980–1981	17	0.174
50–51	Sărătura III	Murighiol	29°9′ E 45°2′ N	1985	2	0.169
50–51	Rotund	Turda	23°46′ E 46°35′ N	1987	6	0.169
52	Auster	Ocna Sibiului	24°4′ E 45°53′ N	1978–1979	2	0.166
53	Marinel	Turda	23°46′ E 46°35′ N	1987	3	0.163
54	Ocnei	Turda	23°46′ E 46°35′ N	1987	8	0.154
55	Grota Miresei	Slănic Prahova	24°56′ E 45°14′ N	1977–1978	2	0.143
56	Baia Verde 2	Slănic Prahova	24°56′ E 45°14′ N	1980–1981	10	0.136

samples was autoclaved at 120 °C for 1 h in three consecutive days. The enzymatic and nonenzymatic catalytic activities were measured only in sediments because the preliminary tests did not register their presence in the supernatant after centrifugation.

Phosphatase activity was measured according to the Krámer and Erdei (1959) method, at the natural pH of sediments, in reaction mixtures without buffer. The reaction mixtures consisted of 2.5 g sediment plus 2 ml toluene (antiseptic) plus 10 ml of 0.5 per cent disodium phenylphosphate solution. The incubation was carried out at 37 °C for 24 h. Phosphatase activity is expressed in mg phenol/2.5 g sediment dry matter.

Catalase activity in active samples and nonenzymatic H_2O_2-splitting in autoclaved samples were measured with a technique based on Kappen's (1913) method. The reaction mixtures consisted of 1.5 g sediments plus 10 ml distilled water plus 2 ml of 3 per cent H_2O_2 solution. The incubation was carried out at room temperature for 1 h. Catalase activity and nonenzymatic H_2O_2-splitting are expressed in mg decomposed H_2O_2/1.5 g sediment dry matter.

TTC (2,3,5-triphenyltetrazolium chloride) reduction in nonautoclaved samples without glucose addition (actual dehydrogenase activity), or with glucose addition (potential dehydrogenase activity), and that in autoclaved samples without or with glucose addition (nonenzymatic TTC reduction) were assayed according to the method of Casida et al. (1964). The reaction mixtures consisted of 0.5 g sediment, 0.5 ml of 3 per cent TTC solution, 1 ml distilled water or 1 ml of 3 per cent glucose solution. The incubation was carried out at 37 °C for 24 h. TTC reduction activities are expressed in mg triphenylformazan/0.5 g sediment dry matter.

The control reaction mixtures consisted of sediment without substrate solutions, and substrate solutions without sediment, respectively.

For the phosphatase and the TTC reduction activities, the incubation was carried out at 37 °C in order to ensure optimal conditions for the reactions, a practice frequently used in research on aquatic enzymology or soil enzymology, the domain from which we took the methods. For H_2O_2-splitting activities, the incubation was carried out at room temperature because in one hour at this temperature the entire quantity of H_2O_2 could be decomposed in the active samples.

The enzymatic indicator is calculated according to the following formula:

$$EI = \frac{1}{n} \cdot \sum_{i=1}^{n} \frac{A_r(i)}{A_{\max}(i)}$$

where EI = enzymatic indicator; n = number of activities; $A_r(i)$ = real individual value of the activity, the measured intensity of the activities after the incubation period; $A_{\max}(i)$ = maximal theoretical individual value of the activity according to the substrate quantity added to the reaction mixtures.

From the reaction mixture compositions, we have calculated the maximal theoretical individual values of the activities. These values are:

- 21.56 mg phenol (from 50 mg disodium phenylphosphate in initial reaction mixture) for the phosphatase activity;
- 60 mg H_2O_2 (the quantity added to initial reaction mixture) for the catalase activity and nonenzymatic H_2O_2-splitting;
- 13.45 mg triphenylformazan (from 15 mg TTC in initial reaction mixture) for the four TTC reduction activities.

Results

For our research, we chose enzymes from the oxidoreductase class (catalase and dehydrogenase), owing to their importance in the respiratory processes

in sediments, and phosphatase because the enzymatic hydrolysis of phosphomonoesters is actually the only way to release the biologically utilizable phosphorus (PO_4^{3-}) (Francko and Heath, 1979). At the same time, sediments are the main phosphorus reservoir in aquatic pools (Serruya et al., 1974). Likewise, the enzymological classifications of different aquatic habitats made by the researchers cited in the introduction to this paper were based on one or two from these three enzymes.

The nonenzymatic catalytic H_2O_2-splitting and TTC reduction were measured because these activities frequently had relatively high values, perhaps due to the presence in sediments of highly reduced substances (for example sulphides) and of humic acids, which can act as electron donor-acceptors and can reduce tetrazolium salts, respectively (Pamatmat, 1975; Schindler et al., 1976; Vosjan, 1982). We also tested the capacity of sediments to hydrolyse nonenzymatically sodium phenylphosphate, but since this activity could not be detected, it was not taken into account.

The enzymological analyses were carried out in different places at each salt lake studied, in different seasons, sometimes for several years. On the basis of the absolute individual values obtained in each analysis, only a mean value for each of the 7 activities has been calculated. This value has been used for the calculation of the enzymatic indicator. On the basis of the calculated value of enzymatic indicator of sediment quality, a hierarchy for the 56 salt lakes studied has been established. This classification is presented in Table 1. We mention that from the former system of classification (Kiss et al., 1986), the analytical data on the lakes studied before 1984 were assumed and the enzymatic indicators were recalculated according to the formula proposed in this paper. Also the lakes Techirghiol, Ursu and Negru, present in the former system, were re-estimated according to subsequent research data.

Of course, there were variations in the intensity of activities related to the year and the season when the sediments were sampled. The initial research followed the seasonal evolution of the activities as well. But, as already mentioned, the aim of this paper is limited to an overall evaluation of the enzymatic potential of studied sediments. That is why, for calculation of the enzymatic indicator of sediment quality in each lake, we used only the mean values of each activity, which moderated the seasonal differences. Otherwise, the simultaneous processing of so many samples would be impossible. Also due to the restrictions required by the aim of this paper, which did not intend to be exhaustive, we did not consider enzyme kinetics, or the correlation of each activity with different physico-chemical parameters of sediments, especially because we had not sufficient data for all the lakes studied enzymologically.

Since the lakes from Ocna Sibiului, Turda, Ocna Şugatag, Sovata, Ocna Dejului, Slănic Prahova, Cojocna and Murighiol have areas less than 1 km^2,

and they are grouped in relatively small areas, in Table 1 we give the same geographical coordinates for all the lakes situated in the same locality.

As one can see from Table 1, there is a wide variance in the intensities of the seven enzymatic and nonenzymatic catalytic activities as expressed by means of the enzymatic indicator value (0.136–0.655). The enzymatic indicator can theoretically have values between 0 (when no activity exists in the samples studied) and 1 (when all the real individual values are equal to the maximal theoretical individual values of all activities). Because of the recalculation of the intensities of the activities relative to dry matter, one could possibly record even real individual values higher than the maximal theoretical individual ones. This could enable us to obtain an enzymatic indicator even higher than 1. We do not exclude this theoretical possibility. In fact, it is very improbable because the proposed formula assumes an equal weight for each activity, yielding thus a dynamic equilibrium between the studied activities. By way of confirmation, as shown in Table 1, the maximal value recorded in the case of the lake with the highest enzymatic potential (Roşu from Ocna Şugatag) is only 0.655.

For statistical interpretation of our results, we applied *t*-test paired two sample for means (Microsoft–Excel program). According to the significance of differences between the enzymatic indicators of sediment quality in the 56 salt lakes studied, we can distinguish 4 categories of enzymological quality of sediments into which the salt lakes studied are grouped. Between the lakes from each group there are not statistically significant differences ($P > 0.05$). Group I includes the first 17 lakes, with the highest values of the enzymatic indicator (position 1 – Roşu from Ocna Şugatag, EI = 0.655; position 17 – Roşu from Sovata, EI = 0.431). The next 16 lakes belong to the second value group (position 18 – Fundata, EI = 0.430; position 33 – Nuntaşi, EI = 0.345). The group III includes the next 11 lakes (position 34 – Balta Albă, EI = 0.332; position 44 – Sărătura I, EI = 0.231). The lakes with the weakest enzymatic quality of their sediments are included in group IV (position 45 – Cabdic, EI = 0.212; position 56 – Baia Verde 2, EI = 0.136).

The medical exploitation of salt lake sediments has a long tradition in Romania. In the last hundred years a large network of sanatoria has been developed where therapeutic mud has been successfully used. Owing to the good results obtained in the treatment of different diseases (rheumatical, gynecological, dermatological), this network of sanatoria is still developing. The existence of a positive correlation between the enzymatic potential and the therapeutic value of the mud is empirically inferred for the time being. The superior therapeutic value of sediments in lakes like Techirghiol (EI = 0.440), or Ursu (EI = 0.409), as compared to that of sediments from other lakes also exploited medically, like Balta Albă (EI = 0.332), or Movila

Miresei (EI = 0.303) is recognized. Because of the therapeutic quality of the muds in the first two lakes, and to their important deposits, especially in Techirghiol lake, close by these lakes have developed big therapeutic bases where patients from different European countries have been treated with good results. Even the development of the medical sanatoria is an argument for the good therapeutic quality of these salt lake sediments.

This paper aims also to be a starting point for the scientific discussion of the problem of correlation between the enzymatic potential and the therapeutic quality of sediments. This approach needs the collaboration of enzymologists and therapists.

For the reasons enunciated above, we think that the evaluation of enzymatic potential of therapeutic muds has not only a theoretical importance, but a practical one, too. The enzymatic potential, expressed as an enzymatic indicator gives primary information on mud quality. It can be a useful guide for therapeutic specialists. At present, many sediments with a high enzymatic indicator are used little or not at all medically. Based on the data offered by our system, the lakes with such sediments can be studied with the purpose of developing and exploiting their therapeutic potential. In this category we mention the lakes from Turda city (Privighetorii, Bătrânilor, Csiky and Ştrand, situated in the first value group), which have the advantage of being grouped in the vicinity of a considerable urban population. At present, they are not used balneologically, though they have an enzymatic potential higher than others intensely exploited for balneotherapy (Balta Albă, Movila Miresei), which belongs to the third value group.

The principles and methods used for enzymological classification of lakes can also be applied in other environmental studies, e.g. for enzymological classification of soils, peats or other aquatic habitats.

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