Physico-chemical and Microbiological Monitoring of Solotvino Salt Lakes.

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Solotvino Resort located on the basis of salt mines is one of the oldest resorts in Transcarpathia. In 1986 in the resort the therapeutic effect of salt mines has been used and the underground branch of Allergic Hospital was founded [1-3]. Since the XIX century the method of hydrotherapy- salt lakes water application has been widely used, especially in the rehabilitation of patients with psoriasis [4-5]. These lakes are formed by karst and situated on the surface of the dome of Solotvyno solid salt.

Healing properties of salt lakes are due to physico-chemical and bacterial composition. So important is the study of physical and chemical properties of the water for the development of medical technologies and treatments of monitoring observations of the properties in terms of environmental issues.

Temperature studies were carried out using the craft, directly on the lake with chromel-aluminium thermocouples and resistive temperature sensors with an accuracy of 0.5 C.

With specially made waterselector water samples were taken from different depths of 0.5m discreetness of different parts of the lake. Chemical analysis of water was conducted in the laboratory. Total mineralization was determined by conductometric method, and content of certain ingredients by potentiometric method using ion-selective sensors was made. The studies were conducted from August 1997 up to the present with intervals of one month.

Table 1 shows the results of chemical analysis of water taken from different depths of Solotvyno Lake in March 2001. The obtained data show that the total mineralization and therefore the density of water is not uniform in depth. If the salinity of the surface layer of the lake is 15.53 g/dm3, the deepest point of the lake at a depth of seven meters by 153.64 g/dm3, i.e with increasing depth. From the table one can see that the water is chlorided and in addition to salt it contains ions of calcium and magnesium. Moreover, the distribution of each element on the depth of the lake is an apportionment of the total concentration.

TABLE 1.
Chemical analysis of water. Solotvino, sampling 23.03.2001

№	Places of sampling	Density g/cm^3	Cations g/dm^3			Anions g/dm^3			M, g/dm^3
			K^++Na^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	
1	0 м	1,011	5.8178	0.0352	0,1644	9.0752	0.2882	0.1287	15.52
2	1 м	1.064	35.7196	0.0826	0.5173	55.3020	1.1535	0.1220	92.90
3	2 м	1.062	34.2260	0.0875	0.5614	53.1750	1.0359	0.1281	89.21
4	3 м	1.066	36.4435	0.0875	0.5534	56.3606	1.3206	0.1342	94.91
5	4 м	1.066	36.4487	0.0875	0.5534	56.3655	1.3315	0.1345	94.92
6	5 м	1.080	45.1379	0.0899	0.5654	69.6593	1.5023	0.1403	117.10
7	6 м	1.105	59.5948	0.1045	0.6857	92.1700	1.5459	0.1403	154.24
8	7 м	1.104	59.3142	0.1094	0.7215	91.8135	1.5465	0.1403	153.65

A similar distribution of chemical elements in the deep water of the lake is observed in samples taken since 1997. Fig. 1 shows the dependence of the density of salt water lake on the depth of the deepest point of the lake Kunigunda in different months in 2001. In this graph the considerable interest is that there is no linear increase of the total concentration throughout the depth, and at the same time is available plateau which is characteristic for almost all months in the calendar year. The difference between them is only the width of the plateau.

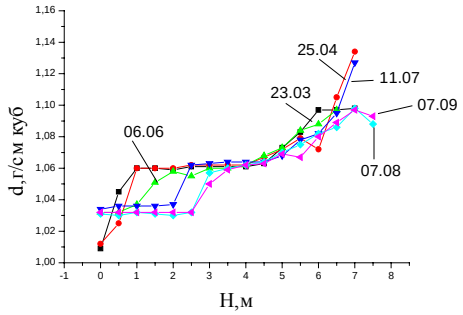

Fig.1 Dependance of the density of water from the depth in the salt lake Kunikhunda in 2001

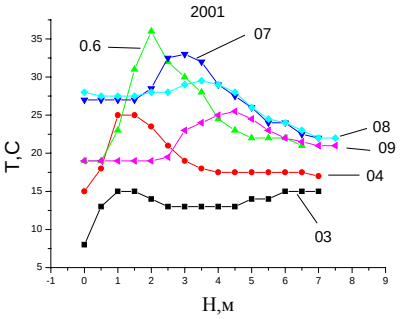

Fig.2 The distribution of water temperature on the depth of the salt lake

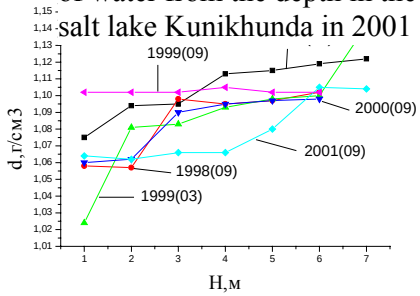

Fig. 3 The distribution of the density of water on the depth of the lake in different years

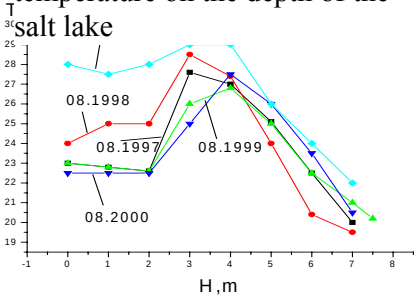

Fig.4 The distribution of water temperature on the depth of the salt lake in different years

In order to determine the nature of this anomaly, temperature distribution measurement are performed directly in the lake depth. These results clearly indicate the presence of a temperature gradient in the depth of the lake which is characterized by a water layer with high temperature, Figure 2. As can be seen from the graphs that layer of water lies below the surface depends on the season shifts in depth. Thus, during periods when the ambient temperature is low, warm layer of water rises closer to the surface, and in periods of high temperature - goes deeper. In winter, an anomalous temperature variation in lake water generally can almost disappear (Fig. 3). The presence of a layer of water with anomalous with respect to freshwater lakes, the temperature distribution with depth is observed across the salt lake area and depth of occurrence of the same in one and the same time. This fact indicates the absence of thermal springs within the lake, which could cause an abnormal course of the temperature dependences.

Periodic monitoring, sampling and chemical analysis of samples during the years 1997-2001 showed that the water level in the lake and the distribution of the total salinity is not constant. In Fig. 3 it is shown the distribution of the density of the water depth of the lake in different years. As can be seen from the graphs the total mineralization decreased substantially. At the same time, the distribution of density with depth has not changed. In Fig. 4 it is shown the temperature distribution of water in the lake at the above period, from which it is seen that the course of the temperature anomalies in lake hardly changes despite a significant decrease in the salinity of water.

This fact also does not speak in favor of the physical and chemical nature of the anomaly. The only exception is the sample taken in September 1999, an analysis which showed that water throughout the depth of the lake is homogeneous and its total concentration was ~ 150 g/dm3. This is obviously due to the fact that during this period there was an accident in Solotvyno mines and almost saturated solution of salt partially fell into the lake.

Table 2.

Chemical analysis of water. Solotvino, sampling 10.11.2003

№	place of sampling	Cations g/dm^3			Anions g/dm^3			Cations g/dm^3
		K^++Na^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3-	
1	0 м	28,8438	0,0608	0,5093	44,9222	0,7520	0,1220	75,21
2	2м	63,4439	0,1361	1,0426	98,6928	1,7484	0,1220	165,19
3	7м	66,4856	0,1361	1,0506	103,4573	1,6579	0,1220	175,91

In summer 2003 the lake began to drain with the water pumped from the mine #9. Table 2 shows the results of chemical analysis of water from different depths (the sample was taken 10.11.2003). As you can see the total mineralization of water began to rise.

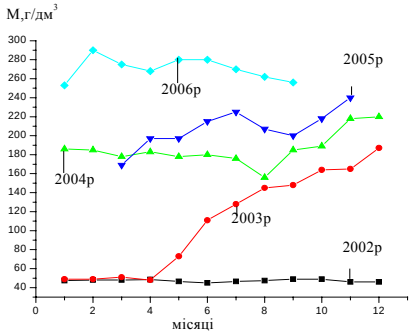

Fig.5 The distribution of water density on the depth of the lake in different years.

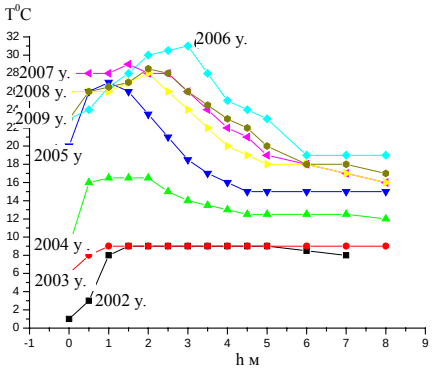

Fig.6 The distribution of water temperature on the depth of the lake in different years.

In Fig. 5 it is shown the total mineralization of water in a depth of 2m during 2002-2006. Total mineralization increased from 2003 to 2006. In 2006 it began to stabilize and remains at that level today. For 2003- 2005 the temperature gradient is decreased and only since 2006 it began to grow. Fig. 6 shows that the layer with anomalous temperature rose closer to the surface of the lake.

In water samples taken in the lake in the laboratory for special culture medium was carried out to highlight the seeding bacteria. The results are shown in Table 3.

Strain was isolated halophilic ***Halobacterium solinarum***, containing bacteriorhodopsin (BR). Halophilic bacteria, mostly sticks with a diameter of 0.5 microns and a length of 3.10 (Fig. 7). At the opposite ends of the cell there are flagella with the help of which it moves. Bacterial membrane consists of protein-lipid bilayer. For this membrane fraction, purple membrane was isolated consisting of fragments of shell membrane, which contains hundreds of thousands of protein molecules, in biochemical properties similar to visual rhodopsin molecule and, therefore, was later named bacterial rhodopsin.

Table 3.

The amount of bacteria and temperature on months.

h, м	march		april		june		july		august		septembe r		october		november	
	bacter ia /мl	t	bacte ria /мl	t	bacte ria /мl	t	bacter ia /мl	t	bacteria /мl	t	bact eria /м	t	bacter ia /м	t	кфо/ мл	t
0	2,5 x 10^3	8	10^3	15	2 x 10^3	19	3 x 10^5	27	5 x 10^3	28	10^5	19	10^4	17	2,5 x 10^3	9,5
0,5	**3,4 x 10^4**	13	1,2 x 10^2	18	1,6 x 10^4	19	**5 x 10^5**	27	**9 x 10^5**	27, 5	**1,7 x 10^6**	19	**5 x 10^4**	23 ,5	2,1 x 10^3	12
1	2 x 10^3	**15**	2,5 x 10^3	25	1,1 x 10^4	23	10^3	27	8,1 x 10^5	27, 5	**8 x 10^5**	19	**4,2 x 10^4**	**25**	10^2	13
1,5	**5 x 10^3**	**15**	**4,6 x 10^3**	**25**	1,5 x 10^4	31	10^3	27	1,9 x 10^5	27, 5	5,5 x 10^5	19	3,4 x 10^4	25	10^2	14
2	4 x 10^3	14	4,3 x 10^3	23,5	2 ,2 x 10^4	36	**5,5 x 10^5**	**28 ,5**	2,8 x 10^4	28	2 x 10^5	19	**2,3 x 10^4**	**24 ,5**	4 x 10^2	14
2,5	4,5 x 10^3	13	2,25 x 10^3	21	**2,5 x 10^4**	**32**	**2 x 10^5**	**32 ,5**	**4,2 x 10^5**	**28**	4 x 10^4	19 ,5	2,1x 10^3	23	2 x 10^2	14
3	3 x 10^3	13	5 x 10^2	19	2,3 x 10^4	30	5 x 10^4	33	**2,6 x 10^5**	**29**	2 x 10^3	23	1,6 x 10^3	23	1,2 x 10^3	15
3,5	3 x 10^3	13	3 x 10^2	18	2 x 10^4	28	7,4 x 10^3	32	5 x 10^4	**29, 5**	1,6 x 10^3	24	1,2 x 10^3	23	1,5 x 10^2	19
4	5 x 10^3	13	6,5 x 10^2	17,5	2 x 10^4	24	5,3 x 10^3	29	3,6 x 10^3	29	1,2 x 10^3	25	1,1 x 10^3	23	**1,3 x 10^4**	**23**
4,5	2,6 x 10^2	13	3 x 10^2	17,5	1,3 x 10^4	23	4 x 10^3	27 ,5	2 x 10^3	28	**10^3**	**25 ,5**	10^3	23	2,1 x 10^3	23
5	3 x 10^2	14	2,8 x 10^2	17,5	7 x 10^3	22	3,3 x 10^3	26	1,5 x 10^3	26	10^3	24 ,5	10^3	24	9,4 x 10^3	22
5,5	3 x 10^3	14	2,3 x 10^2	17,5	5 x 10^3	22	2,3 x 10^3	24	10^3	24, 5	6 x 10^2	23	8,1 x 10^2	23 ,5	1,7 x 10^3	22
6	3 x 10^2	**15**	2,5 x 10^2	17,5	3 x 10^3	22	3,1 x 10^3	24	10^3	24	3 x 10^2	22	3 x 10^2	23	9,3 x 10^2	22
6,5	4 x 10^2	**15**	2 x 10^2	17,5	2 x 10^3	21	4 x 10^3	22 ,5	10^3	23	2 x 10^2	22 ,5	10^2	22 ,5	9,2 x 10^2	22
7	5 x 10^2	**15**	10^2	17				22	10^2	22	1,5 x 10^2	21 ,5	10^2	21 ,5	1,7 x 10^2	21,5
7,5									10^2	22	10^2	21	10^2	20	2 x 10^2	20,5

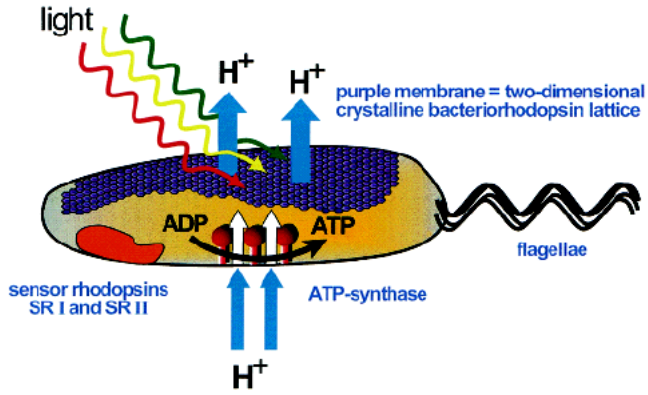

Figure 7. Schematic representation of *Halobakterium solinarum.*

Fotoenergetic Bacteriorhodopsin acts as a transducer which accumulates energy quanta in a form directly accessible to living cells. The energy distribution of solar radiation is maximum in the yellow-green region of the visible spectrum. The absorption spectrum of bacteriorhodopsin shows that the maximum absorption of light energy for halophilic located in the same area. But after illumination bacteria yellow-green portion of the visible spectrum absorption band of bacteriorhodopsin energy is transformed so that the absorption maximum shifts to long-wavelength ultraviolet area of the spectrum (UVA-spectrum).

Bacteriorhodopsin under light makes the transfer of photons from outside the cell. Formation of Bacteriorhodopsin (absorption maximum 560 nm), 412 intermediate (absorption maximum 412 nm) is associated with a proton from the chromophore and subsequent discharge it outside the cell [5,6]. Obviously halobakteria in this active state may well improve performance, and the effect of ultraviolet radiation on human skin, leading to increased photosensitivity of skin. As one of the therapeutic factors in therapy is that the the lake has healing effects of solar radiation, the "correction" of halobakteria that remain on the skin after bathing, the perception of its ultraviolet spectrum is perhaps one of the main therapeutic factors among a number of factors of balneotherapeutic lake.

In bacteriorhodopsin, halofilic cells synthesize proteins, nucleic acids, carotenoids, lipids, glycerol, kardiolinin, glycolipids, vitamins A, E, C, beta carotene, B1, B2, B3, B6, D, PP. Almost all halobakteria with yellow, orange or purple color. This is due to a high content of carotenoids in their tissues that protect the bacteria from harmful UV radiation.

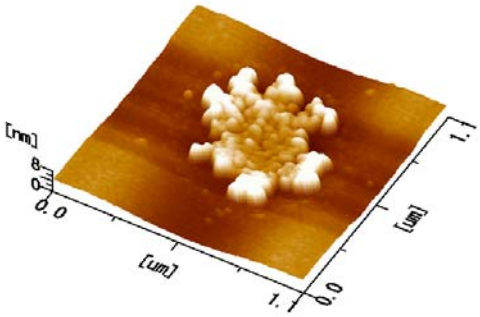

Figure 8. Detail of single purple membrane.

Today, the biotechnology laboratory of Uzhgorod National University reproduced method of growing halophilic bacteria in artificial conditions. Extreme halofilics for growth and development requires special medium with a high salt content of Na, K, Mg and organic additives. The cultivation of biomass is produced within 7 days. Climate cabinet CC designed for fast growing bacteria in artificial controlled conditions. Cultivation system consisting of six plane-parallel ditch (10 liters of medium in a cuvette) lamp lighting

LH-40, power supply and clean air bubbler, temperature controllers. Photo of one of the ditch in working condition is shown in Figure 9. Performance of the system is more than 300 grams of biomass per month.

To enable long-term transportation and storage without loss of medical properties the device is designed and setup for freeze-drying of biomass halophilic bacteria in sterile distilled water and 10% aqueous solution of sodium chloride. Cool-dehumidified supply used for suspension of bacterial lysates for subsequent applications and organization of artificial treatment ponds.

Based on SRO "Rehabilitation" conducted research on the use of lysates of cells as a biological photosensitizer. The standard UV light irradiated two symmetrical parts almost untanned healthy skin without visible pathological areas, one of which is superimposed applications of 1%, 2.5%, 5%, 10%, 15% solution of lyophilized culture of halophilic bacteria [strain 353 - L] prepared in Laboratory of Biotechnology Uzhgorod National University. Another area of skin was considered the controlled one and was intact. For standard exposure time was taken 2 min. After 24 hours a slight redness (erythema) of the skin were found at the site, which was applied 5% solution of culture halophilic bacteria and at the site of 10% and 15%, respectively, erythema was greater. For untanned skin redness without any application it was required UV irradiation for 3-4 min. Application of halophilic bacteria reduces erythema dose increases photosensitivity of the skin without apparent adverse effects. A 5% solution of halophilic bacteria lysate was most appropriate.

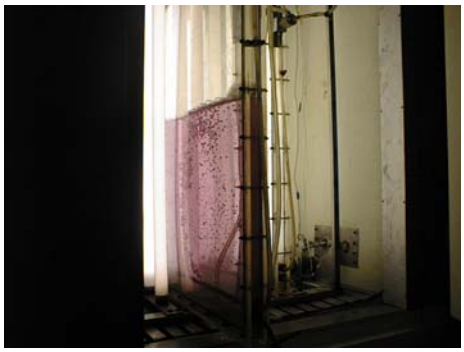

Figure 9. Cuvette cultivator during cultivation of halophilic bakteria.

On the basis of SRO "Rehabilitation" (Uzhgorod) artificial treatment ponds for treatment of psoriasis were developed [7]. The complex procedures are brine baths, mud baths and artificial ultraviolet irradiation of two biodoses. According to the research it can be concluded that in addition to the above procedures to treat psoriasis the applications of halophilic 5% lysate were added, followed by UV irradiation and thus to approximate the treatment of psoriasis in the bath complex of SRO "Rehabilitation" to the natural one conducted in the village of Solotvino during treatment at Lake Kunihunda.

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