ABUNDANCE AND ACTIVITY OF HETEROTROPHIC BACTERIAL COMMUNITY IN THE WATER COLUMN AND SEDIMENTS OF TEKIRGHIOL LAKE

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The aim of the paper is to evaluate the bacterial abundance and microbial dehydrogenase activity in hypersaline region of the Techirghiol Lake. Samples were taken during August–October 2006 from two horizontal transects and one vertical profile. Water temperature ranged between 20.3°C and 20.8°C while salinity showed slight fluctuation from 57.27 g L^{-1} to 70.3 g L^{-1}. Organic matter concentration was significant high during our observations and its distribution varied significantly both horizontally and vertically. Total number of culturable bacteria ranged in the surface water from 18×10^3 CFU ml^{-1} to 98×10^3 CFU ml^{-1} while vertically it had a peak of approximately 29×10^3 CFU ml^{-1} at 2.0 m depth. Abundance of bacteria estimated by direct microscopic count was 5 times higher than the number of culturable bacteria but the vertical pattern of both parameters was more or less similar. In the sediments, actual dehydrogenase activity was almost uniform ranging from 110 µg TPF g^{-1} wet mud day^{-1} to 135 µg TPF g^{-1} wet mud day^{-1}. Potential dehydrogenase activity was more patchiness depending on the organic substrata and site sampling and had values between 157 µg TPF g^{-1} wet mud day^{-1} in the presence of glycerol at St. 7 and 810 µg TPF g^{-1} wet mud day^{-1} at St. 7 when lactate was used as hydrogen donating substratum.

Key words: Bacterial abundance; Dehydrogenase activity; Tekirghiol Lake; Microbial mat.

INTRODUCTION

Hypersaline lakes defined as bodies of water with a salinity range above the salinity of seawater are characterized by shorter trophic chains and high productivity due to the absence of predators[1–3] Mass mortality of organisms allows the accumulation of large amount of dead organisms in the water column and sediments. Organic matter undergoes a slower and sometimes incomplete degradation[4–7]. Therefore, large quantities of organic matter participate by means of both living and nonliving processes in bottom regions to genesis of mud with therapeutic properties. Slower breakdown of organic matter is believed to be the particularity of microbial community inhabiting the hypersaline ecosystems. These microorganisms belonging physiologically to halophilic group of bacteria are relatively well studied[8–13]. During the last decades Tekirghiol Lake has experienced changes of its natural saline regime. In some regions its waters have progresively changed to brackish or even freshwater salinity range. It is believed that the main factor responsible for salinity changes is the freshwater input with origin in the underground water. At present, Tekirghiol Lake have three distinct regions as salinity separated by artificial dams, one hypersaline region, one with brackish waters and one region with freshwater (Fig. 1). The organism community has completely changed in region of freshwater concomitantly with absence of therapeutic mud production. Tekirghiol Lake is relatively well known in regard of its biological community and its changes of productivity and saline regime have been well studied and documented

over time[14–19]. Since the salinity seems to be the critical factor regulating the biological productivity and most important the production of therapeutic mud, it is important to assess the extent to which living organisms has changed in consequence of salinity modifications. The present paper has the aim to evaluate some aspects of microbial abundance and activity in hypersaline region of the lake as first step to a comparative study with other two regions and to address the question how the microbial community could change not only in terms of abundance but also as activity.

MATERIAL AND METHODS

Sampling site. Samples were taken from eight stations (Table 1), located on two horizontal transects (Fig. 1).

Table 1

Spatial coordonates of stations

	GPS
St. 1	44°03'33 N 28°36'46 E
St. 2	44°03'31 N 28°36'28 E
St. 3	44°03'26 N 28°36'35 E
St. 4	44°03'21 N 28°36'42 E
St. 5	44°03'12 N 28°36'58 E
St. 6	44°02'38 N 28°37'23 E
St. 7	44°02'55 N 28°38'60 E
St. 8	44°02'59 N 28°38'10 E

Vertical profile of water column consisted of six vertical points at the level of station 6. Additionally, we sampled sediments at depth ranging from 6 to 9 m at stations 6, 7 and 8. At each stations we sampled five to eight sediment cores. Water samples were taken with a Nansen bottle while mud was sampled with a Petersen grab instrument.

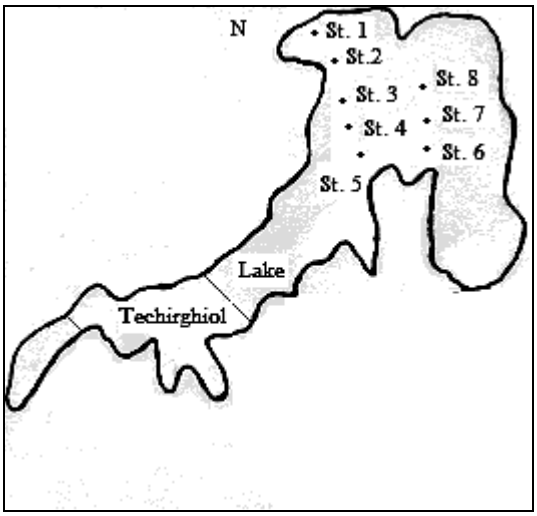

Fig. 1. Distribution of stations.

Bacterial abundance was recorded both by direct microscopic count and cultivation technique. For direct counts cells were harvested from water samples by centrifugation at 10 000 rpm/min, 5 min. Subsequently, a small aliquote of concentrated sample (0.1 ml) was placed on a clean sterile microscopic slide, covered with coverslip and cells were counted in phase contrast at 1000x magnification with a Novex microscope equiped with a high resolution digital camera (Euromex). For each sample it has been counted ten microscopic fields, the average count was expressed as number cells/ml by using an appropriate conversion factor.

Total heterotrophic culturable bacteria. The total number of culturable bacteria was counted on Nutrient Agar: bactopeptone Difco, 5.0 g; beef extract Difco, 5.0 g; agar-agar, 15.0 g; NaCl 55.0, 1000 ml; pH=7.6–7.8.

Dehydrogenase activity was assayed as described by Cassida[20] with slight modifications. Sediment samples were taken from three different stations (St. 6, St. 7 and St. 8) with a Petersen grab instrument at depths ranging from 6 to 8 m. A sterile glass tube was used to subsample in laboratory a central core of mud which was subsequently weighted and placed in sterile test tubes. Potential dehydrogenase activity was assayed by using glucose, sodium lactate and glycerol as hydrogen-donating substrata. Each gram of mud received 1 mL of 0.5 M substratum, 1 ml phosphate buffer (pH=7.4) and 1 ml of 2,3,5 – triphenytetrazolium chloride (TTC) (3% w/vol). Actual dehydrogenase activity has been determined by adding to one gram of mud 1 ml TTC (3% w/vol), 1 mL of sterile water and 1 ml of phosphate buffer (7.4). Mud blank received 2 ml of water and 1 ml of phosphate buffer (7.4) per gram. All experimental variantes were prepared in triplicate. Samples were shaken and incubated at 28^{o}C for 24 h. Subsequently, triphenylformazan (TPF) was extracted by using a mixture of 96^{o} ethyl alkohol and acetic acid (20:1). Extracted solutions were read at 485 nm by using a Spektronic 20D spectrophotometer. The values obtained were compared against a standard curve and they are recorded as µg/gram wet mud. Statistical analysis was done with Statistica 5.5.A software package (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISCUSSIONS

Physicochemical parameters

Horizontal distribution of inorganic nutrients has proved significant fluctuation. Salinity exhibited moderate variability ranging from 61.46 g L^{-1} to 68.8 g L^{-1} while organic matter concentration varied strongly on small distance between 25.5 g L^{-1} and 109.1 g L^{-1} (Fig. 2a). Nitrates and phosphates progresively decreased from St. 1 to St. 8 (Fig. 2b, c). We did not find a significant correlation between these parameters suggesting that the water dynamics would be the main responsible factor of horizontal distribution of nutrients. On vertical profile, salinity and organic matter demonstrated a tendency to decrease from surface to bottom. Decreasing was sharply between 1.0 and 3.5 m after that these parameters were more or less constant with depth.

No significant correlations were found between depth on the one hand and organic matter and salinity on the other hand but vertical distribution of salinity and organic matter correlated strongly positive each other (r=0.93).

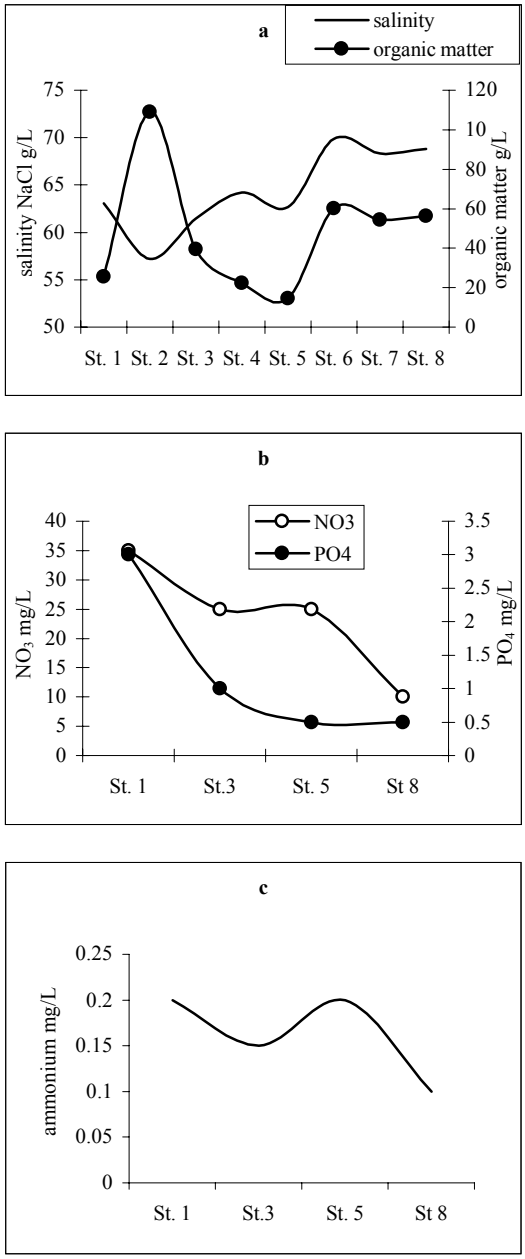

Fig. 2. Horizontal distribution of salinity, organic and inorganic nutrients: a – salinity and organic matter; b – phosphates and nitrates; c – ammonium.

Salinity and organic matter pattern seemed a little unsual at first sight, but probably higher salinity from surface water layer was compensated by increased volume due to the higher temperature in comparison with bottom water. This layer has been exposed to strong sunlight and to intense evaporation during the day. A little more dense than the water below, this layer accumulated organic matter by preventing the settling of particles from to bottom layer. Inorganic nutrients had an opposing trend with depth. Nitrates decreased with depth suggesting that their production was more intense in the surface water with high organic load.

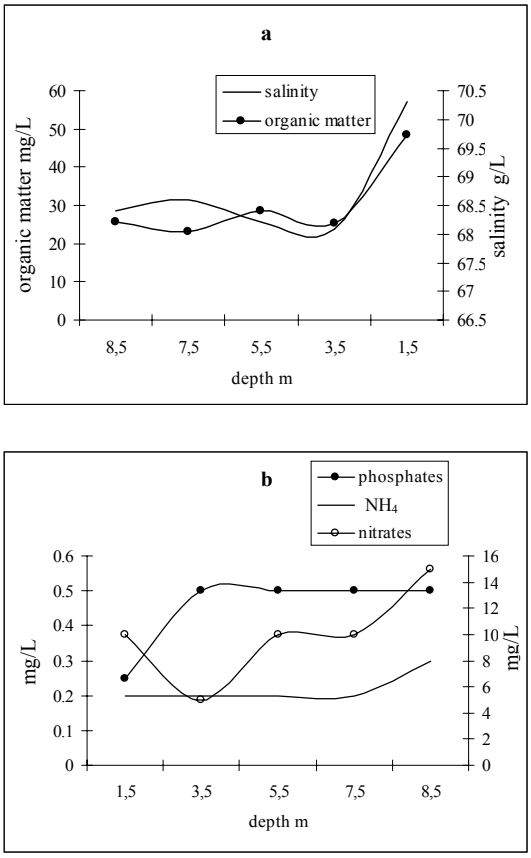

Fig. 3. Vertical profile of salinity, organic and inorganic nutrients: a – organic matter and salinity; b – nitrates, phosphates and NH_4^+.

As opposed, phosphates had the tendency to increase with depth indicating an increased uptake in the surface layer by bacteria and phytoplankton as a result of their intense metabolic activity.

Bacterial abundance

Abundance of culturable bacteria showed significant fluctuations as horizontal distribution, ranging from 18×10^3 cell mL^{-1} to 98×10^3 cell mL^{-1} (Fig. 4). Presence of living and nonliving particulate matter, and water movements itself support a very dynamic environment and the abundance of bacteria cannot often correlate apparently with most physicochemical parameters.

We recorded indeed horizontal variations of inorganic nutrients, salinity and organic matter, but without a clear connection with bacterial abundance. Most probable, in the absence of a sharp physical gradient the horizontal distribution of bacteria and nutrients was mainly regulated by small scale water dynamics.

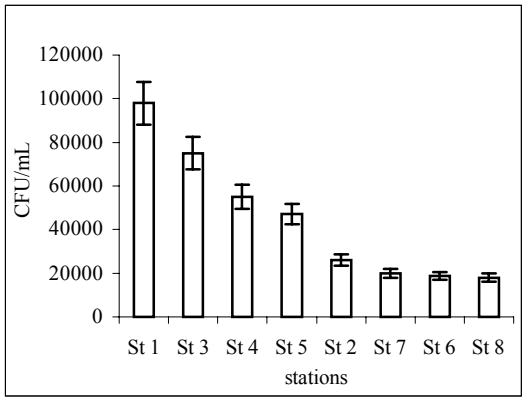

Fig. 4. Horizontal profile of abundance of culturable bacteria.

We have recorded simultaneously the vertical abundance of bacteria by both direct microscopic count and cultivation methods on a vertical profile from 0.5 to 9.0 m depth.

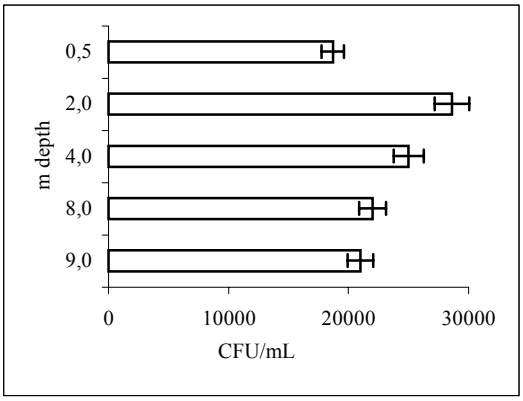

Fig. 5. Abundance of culturable bacteria in the water column.

Culturable bacteria increased in number slightly from 0.5 to 2.0 m after that their abundance lowered slowly with depth (Fig. 5). In general, abundance as revealed by direct microscopic count had more or less similar trend with vertical profile of culturable bacteria (Fig. 6). Lower numbers in superficial layer were followed by two peaks at 2,0 and 6.0 m (about $1{,}7\times10^6$ cell mL^{-1}) that decreased afterwards to about 0.79×10^6 cell mL^{-1} in bottom water (Fig. 6). Vertical profile of direct count and culturable bacteria correlate weak positively each other (r=0.87). It should be noted the obvious discrepancy between direct count measurements and cultivations determinations, first showing a value of 5× higher than the plate count abundance. Culturability of bacteria in aquatic environments has been extensively disscussed[21].

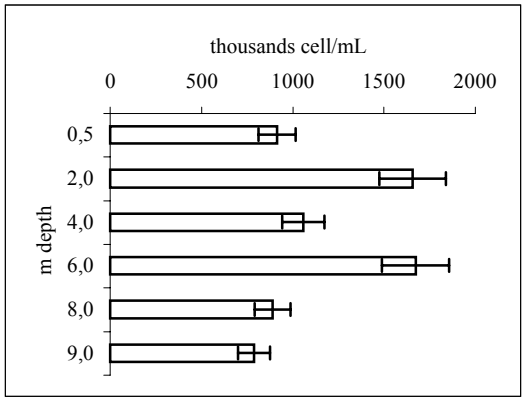

Fig. 6. Abundance of total count in the water column.

It has been reported that direct microscopic count can reveal a number of bacteria of 10 to 100× higher than cultivation techniques[22]. This wide range of percent of culturable bacteria demonstrate that their ability to grow on rich nutrient laboratory media might vary for different habitats[23,24]. We have observed a differential ability of bacteria from Tekirghiol to grow on nutrient agar. One group of bacteria was fast growing giving countable colonies only after 24–48 h while a second group was slow growing with visible colonies after 6–7 days of incubation.

Dehydrogenase activity

Actual activity in mud samples proved a reduced variability at the three stations, ranging from 110 µg TPF g^{-1} day^{-1} to 135 µg TPF g^{-1} day^{-1} (Fig. 7). This pattern suggested that *in situ* organic loading and available hydrogen-donating substrata were quantitatively less or more similar in space. Enrichment of sample mud with organic substrata enhanced dehydrogenase activity and it has been found a heterogenous response that varied significantly not only with chemical structure of donors but also as level of spatial intensity of dehydrogenase activity. High activity of dehydrogenase was recorded in the presence of glucose, ranging from 394 TPF g^{-1} day^{-1} (St. 6) to 680 TPF g^{-1} day^{-1} (St. 8) while glycerol supported less intense activity (Fig. 7). Lactate significantly increased dehydrogenase activity at St. 7 (520 TPF

g^{-1} day^{-1}) exceeding the level recorded for glucose (Fig. 7). Since potential activity is 3 to 5 times higher than actual activity, it can be assumed that *in situ* available organic substrata could quantitatively limit the microbial activity in sediments.

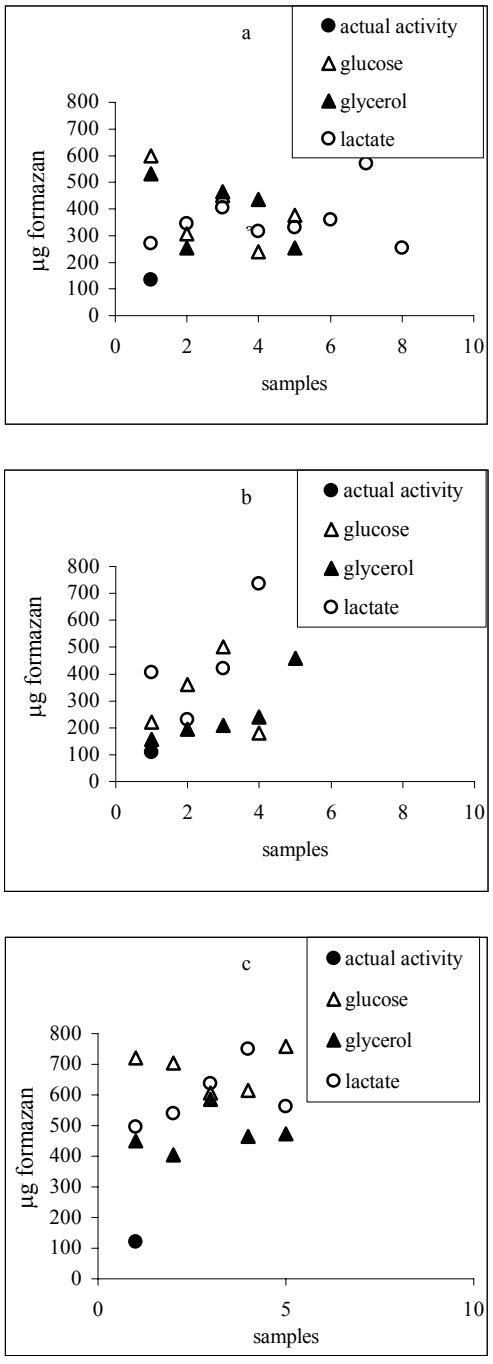

Fig. 7. Actual and potential dehydrogenase activity (µg TPF g^{-1} mud day^{-1}) in sediment samples collected from St. 6 (a), St. 7(b) and St. 8 (c).

On average, potential dehydrogenase activity significantly increased in mud samples collected at St. 8 in comparison with activity recorded at two another stations (Fig. 7). This fact in connection with a more uniform actual activity suggested that the three stations might be similar in respect of their organic loading. At the same time, the differential need for different hydrogen donors indicated a diverse microbial community as composition and activity. In general, dehydrogenase assay reflects the intensity of *in situ* microbial metabolism although there are reports showing that some microorganisms existing in a natural sample lack the ability to reduce *in vitro* TTC[25]. A series of environmental factors may affect dehydrogenase activity[26–28] and there is no a clear relationship until present between TTC reduction and abundance of total microbial community[29–31]. Supplementary data are needed to estimate accurately the microbial activity in Techirghiol Lake by using dehydrogenase as a main assay in the ecologically oriented studies. In this sense, there are necessary further researches regarding the particularities of dehydrogenases activity associated with different conditions in sediments such as aerobiosis/anaerobiosis, its relation with specific kind of organic matter (high molecular weight/low molecular weight compounds), influence of salinity and its interference with content of organic matter.

Microbial mat composition

We have also made some preliminary observations regarding the diversity of microbial community existing in shallow sediments rich in organic matter and exposed to sunlight. In hypersaline environments mat-like acumulation of microorganisms forms complex ecosystems[32–36] at milimeter scale, including a wide variety of photosynthetic, chemoautotrophic and chemoorganotrophic organisms. Within the mat microorganisms are highly ordered in space and connected each other by their metabolic activities[37,38]. Microbial mats are commonly found at the sediment/water interface in more or less extended areas of sublittoral zone of the Tekirghiol Lake. Microscopic investigation revealed that microbial mat covering the upper region of sediments consisted mainly of three groups of organisms: cyanobacteria, purple sulfur bacteria and sulfur nonphotosynthetic bacteria. During our investigation the microbial mat was predominantly pink-redish in colour due to the massive growth of purple phototrophic bacteria. The most abundant community belonged of *Chromatium sp*. (Fig. 8 a,b,c).

The community was located in the deeper anaerobic region of the mat. *Chromatium* organisms are phototrophic sulfur bacteria and they are commonly described in hypersaline waters [39,40]. They inhabit the anaerobic region of the mat and can grow at weak intensity of light of only 1% of total direct sunlight due to their specific assimilatory pigments[39]. Cells are highly motile and can move vertically to horizons optimal as light intensity and hydrogen sulfide concentration[41]. H_2S is supplied from underlying black layer rich in sulfate reducing bacteria and it is oxidized by *Chromatium* organisms to another sulfur intermediates. Their abundance and position within the mat reflect a balance between light intensity and hydrogen sulfide concentration[39]. The upper region of the mat is colonized by cyanobacteria that need sunlight and provide the community with oxygen and organic substrate. In Tekirghiol Lake at the interface sediment/water we found a relatively abundant community of filamentous cyanobacteria belonging to *Phormidium* (Fig. 9 a, b) and *Oscillatoria* genera (Fig. 9 c). They support the growth of a rich aerobic heterotrophic bacteria community living within the top oxic region. The position of cyanobacteria may be variable within the mat since they can move vertically depending the light intensity[42]. At higher intensity of light they usually reach a deeper horizon to avoid UV damage[43]. At the boundary where oxygen and H_2S overlap abundant populations of *Beggiatoa* (Fig. 10 a, b, c) formed a third layer. *Beggiatoa* requires simultaneously H_2S and O_2 for metabolism and their position within mat is regulated by the redox conditions. During the day these organisms may be found close to the black region rich in sulfide while at night they can reach the surface layer of the mat. Well developed mats may be found often in aquatic oligotrophic habitats[44] since they are very productive ecosystems due to the efficient nutrient cycling. A careful analysis of conditions in which microbial mats develop in Tekirghiol Lake may reveal some distinct particularities of primary source of organic carbon. Here the microbial mats flourish on the massive accumulation of dead bodies of brine shrimps and macrophites. Enhanced degradation of organic matter generates anaerobiosis and high amounts of hydrogen sulfide which in turn stimulates a rich sulfur bacterial community. At the same time anaerobiosis limits the growth of aerobic heterotrophic bacteria only in the upper region of the mat where they can find oxygen enough. Thus we suppose that microbial mat might have a critical role in genesis of therapeutic mud on the sediments of Tekirghiol Lake. Microbial mat functioning prevents the complete degradation of organic matter by maintaining the anaerobiosis and favours the accumulation of organic substrata that gives therapeutic properties to the mud. The high salinity is the key factor for optimal activity of microbial mats from Tekirghiol Lake. It favour not only the high productivity of the lake but also particular decomposition processes. It has been observed in other aquatic habitats that salinity changes as result of fresh water input might alter and eliminate the microbial mats[45,46] changes clearly observed in brakish and freshwater regions of the Tekirghiol Lake. In the absence of these microbial systems therapeutic mud cannot accumulate. Therefore, among other topic of research, investigation of microbial mats biochemistry and activity from Tekirghiol Lake might contribute to a better understanding of therapeutic mud production.

CONCLUSIONS

During our observations the bacterial abundance as revealed by direct microscopic count and cultivation techniques was relatively high in the water column. This rich bacterial community was supported by high organic load consisting mainly of brine shrimp and macrophites bodies. Dehydrogenase activity suggested an intense microbial metabolism in the sediments. The high discrepancy between actual and potential dehydrogenase could indicate that microbial activity would be limited by the availability of suitable organic substrata. Since the organic load was significant high during our observations the *in situ* limited dehydrogenase activity could be explained most probably by competition of microorganisms for low molecular weight organic substrata in sediments. Probably, macromolecules were the major part of organic matter that should be first converted to simple compounds before feeding the metabolism of most bacterial groups in the sediments. Microbial mats covering the sediments consisted mainly of sulfur purple bacteria, cyanobacteria and sulfur non-photosynthetic bacteria. Their activity is essential for accumulation of important quantities of intermediate products of anaerobic metabolism and play a critical role in therapeutic mud genesis.

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