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Approaches to the microbial ecology of the Dead Sea

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Abstract

The Dead Sea is a highly dynamic ecosystem in which two types of biological changes can be observed: a long-range process of adaptation and selection towards higher salt tolerance as a result of the increase of the salinity and changes in salt composition with relative increase in magnesium, and seasonal changes due to variations in temperature and salinity (winter floods). As microorganisms live in the Dead Sea at the upper limit of their salt tolerance a small decrease in salinity causes a strong increase in microbial growth and activity. This explains the heavy bloom of *Dunaliella* and halobacteria observed in summer 1980.

Introduction

The Dead Sea occupies a depression in the deepest part of the Dead Sea-Arava segment of the East African Rift Valley. Its physical, chemical and limnological properties have been described (BEYTH 1980, NEEV and EMERY 1967, NISSENBAUM 1975, STEINHORN et al. 1979). Presently its water level is about 403 meter below sea level, and the overall salinity amounts to approximately 339.6 g salts per liter (BEYTH 1980).

Until the pioneering work of ELAZARI-VOLCANI (1940) in the thirties the Dead Sea was generally considered to be devoid of living organisms. ELAZARI-VOLCANI isolated and characterized a great variety of bacteria (red halobacteria and others), unicellular green algae, cyanobacteria and protozoa. Since ELAZARI-VOLCANI's work very few biological studies have been performed in the Dead Sea: the work of KAPLAN and FRIEDMAN (1970), including the first quantitative estimates of biomass, and a study by KRITZMAN (1973). The available data till 1975 have been reviewed by NISSENBAUM (1975).

During the last twenty years the chemical and limnological properties of the lake have changed drastically: as the Dead Sea is a terminal lake, the water level depends on the balance between water inflow (Jordan river, rain water floods, springs) and evaporation. As water flow through the Jordan river is strongly diminished as a result of human activities the water balance of the Dead Sea is negative, and the lake's water level drops yearly about 1/2-1 meter. This has had far-reaching effects on the limnology of the lake: in 1976 the shallow south basin was detached from the deep northern basin, and as a result of the continuing evaporation the salt concentration in the upper layers equalled those of the lower layers, resulting in winter 1978–1979 in a complete overturn of the lake (STEINHORN et al. 1979) which had been permanently stratified for at least a hundred years (NEEV and EMERY 1967).

It may be expected that these changes in the lake's chemistry and limnology have had profound influences on biological processes: before turnover for example, the lower water mass (below 40 m depth) was anaerobic and contained H_2S (NEEV and EMERY 1967); today oxygen penetrates also the deepest parts of the water column, restricting activity of anaerobic bacteria (e.g. sulfate reduction) to the sediments only.

The recent changes in the lake's limnology renewed the interest in the biology of the Dead Sea, the microorganisms living there, their numbers, their activities and the factors limiting their development.

In this study we will present preliminary data on the biology of the Dead Sea from January-September 1980, and make an attempt to analyze the factors governing microbial distribution and activity.

Materials and methods

1. Sampling localities and procedures.

Samples were taken from the deepest part of the lake, about 8 km east from Ein Gedi, by means of Nansen sampling bottles. Additional samples were taken from the shore near Ein Gedi. All samples were processed within 12 hours after sampling.

2. *Dunaliella* counts.

In all samples investigated *Dunaliella* was the only alga observed. It was counted by filtering aliquots of water (20–50 ml) treated with 0.1 ml 0.1 n iodine through millipore filters (1.2 μm mean pore size, 25 mm diameter) and counting of cells on the filters by means of a light microscope (40 x 8 enlargement). The transparency of the filters was increased by adding a drop of immersion oil between filter and coverslip.

Chlorophyll was determined in acetone extracts of cells collected on glass fiber filters (Whatman GF/C) according to STRICKLAND and PARSONS (1968).

3. Bacterial counts.

a) Total counts. Cells from 10–20 ml water aliquots were collected by centrifugation (15 min, 25,000 x g) and resuspended in a small volume (0.1–0.2 ml) of filtered, sterile Dead Sea water. Cells were counted in a Petroff-Hauser counting chamber at a magnification of 100x8, using phase optics. Preliminary attempts to count cells on filters, using acridine orange staining and epifluorescence microscopy gave comparable results.

b) Viable counts. Highest bacterial viable counts were obtained by using a modification of the method developed by KRITZMAN (1973): 0.5 ml of tenfold dilutions of the water sample in sterile Dead Sea water were mixed with 4.5 ml of melted soft agar at 45 °C, containing 80 % (v/v) Dead Sea water and 20 % (v/v) destilled water with addition of 0.1 % peptone, 0.1 % yeast extract, 2 % potato starch and 1 % agar, pH 6.8. Subsequently the mixture was poured on a petri dish containing a layer of similar medium with 3 % agar. After solidification of the upper layer the plates were incubated at 35 °C for 2 weeks.

4. Primary production.

60 ml stoppered bottles were filled completely with the water sample tested and incubated in the laboratory in the dark or under continuous illumination (fluorescent lamps, incident light intensity 5×10^3 erg cm^{-2} s^{-1}) at 25 °C for 24 h in the presence of 38 μCi $NaH^{14}CO_3$ (0.7 μmol) (Amersham). Subsequently the water samples were

filtered through glass fiber filters (Whatmann GF/C), which were treated afterwards with acetic acid to remove excess CO_2, dried and counted in a gas-flow counter. CO_2 concentrations in the water were not measured, and the value of 2.5 mM (SASS and BEN-YAAKOV 1977) was used in the calculations.

5. Bacterial strains used in the growth experiments.

The following strains of Dead Sea halobacteria were used:
CD-1 = „*Halobacterium* from the Dead Sea", isolated by GINZBURG et al. (1970).
CD-2 = *Halobacterium volcanii* DS-2, isolated by LARSEN (MULLAKHANBHAI and LARSEN 1975).
CD-3, isolated December 1979 from surface water sampled near Massada, by enriching the water with 0.1 % peptone, 0.1 % yeast extract and 10^{-4}M EDTA, and incubating with shaking for 7 days at 35 °C followed by isolation on plates according to MULLAKHANBHAI and LARSEN (1975).
CD-4, isolated March 1980 from surface water sampled at Ein Gedi beach, by enrichment in 95 % Dead Sea water, 0.5 % tryptone and 0.5 % yeast extract, and isolation by plating.

CD-5, isolated March 1980 from surface water at the deepest part of the lake, by enrichment in 80 % Dead Sea water, 0.5 % tryptone, 0.5 % yeast extract and 1 % $NaNO_3$ in a completely filled stoppered bottle, followed by isolation on plates.

6. Growth of Dead Sea halobacteria in different magnesium concentrations.

Cells were precultured in a shaking water bath at 35 °C in 50 ml Erlenmeyer flasks containing 25 ml of the following medium : NaCl 12.5 % (20 % in the case of CD-1), $MgCl_2.6H_20$ 5 %, K_2SO_4 0.5 %, $CaCl_2.2H_2O$ 0.013 %, tryptone 0.5 %, yeast extract 0.5 %, pH 6.8. At the end of the logarithmic growth phase 0.5 ml portions of the cultures were inoculated into series of 50 ml Erlenmeyer flasks containing 25 ml of the growth medium in which the NaCl concentration was lowered to 10 % and the $MgCl_2$ concentration varied from 0–2 M. Incubation with shaking at 35 °C was continued and daily the turbidity of the cultures was measured in a Gilford 300-N spectrophotometer at 600 nm.

Results

The green alga *Dunaliella*, the presence of which was previously reported from the Dead Sea (ELAZARI-VOLCANI 1940, KAPLAN and FRIEDMAN 1970; KRITZMAN 1973), was absent from all water samples investigated from January to April 1980. In May 1980 the alga reappeared (figure 1), and showed a large bloom in August, reaching cell densities of up to 8800 cells per ml, which decreased slowly afterwards. Chlorophyll concentrations of up to 31 μg per ml were measured, more than 100 times the average value reported from measurements in the Gulf of Elat (LEVANON-SPANIER et al. 1979). *Dunaliella* was the only eucaryotic organism observed in the samples. Figure 1 additionally shows data on salinity and temperature of the surface water. An influence of the drop in salinity of the surface water caused by heavy rainfall and floods in winter as well as the increase in water temperature during the summer months on the algal development was observed.

The algal distribution is limited to the upper 5 – 10 meters of the water column (figure 2), probably as a result of light limitation (see below), though the higher salinity in the lower water layers may also be involved. The population density at the surface is generally lower than at 5 meter depth, which is probably due to photo-oxidative effects.

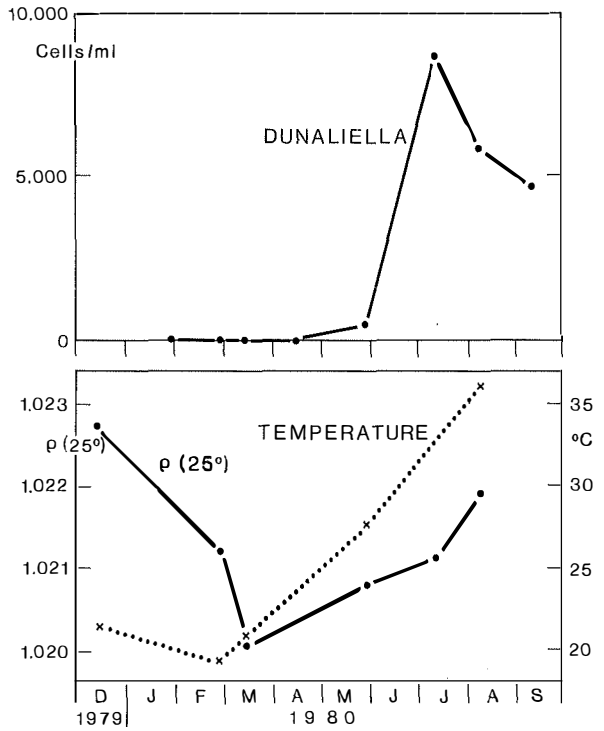

Figure 1

Dunaliella counts in surface water, sampled at Ein Gedi beach. Data on water temperature and salinity (expressed as specific gravity of the water at 25 °C) were obtained from Dr. D. Anati, Dr. M. Stiller and our own measurements.

The measurement of primary production in the Dead Sea by means of the light incorporation of $^{14}CO_2$ in the algae presents special problems: because of the high calcium concentration the Dead Sea is saturated with respect to CO_2 (SASS and BEN-YAAKOV 1977), and cristals of $CaCO_3$ (as aragonite or calcite) can often be found in the water (NEEV and EMERY 1967). The addition of labelled carbonates may thus lead to their precipitation, lowering their biologically available concentration. Yet we used the method, assuming that the small quantity of bicarbonate added would not cause precipitation during the experiment. Our measurements were carried out in the laboratory, and we are aware of the possibility that the values thus measured may differ significantly from those in situ. Primary production generally paralleled algal counts; the maximal value measured was 6.9 mg C m^{-3} h^{-1} (7 August 1980, depth 5 m), much higher than, for example, values reported from the Gulf of Elat (LEVANON-SPANIER et al. 1979). However, expressing primary production per quantity of chlorophyll (the assimilation number) shows that photosynthesis in the Dead Sea is highly inefficient: while in the Dead Sea the assimilation number calculated was 0.15–0.22 mg C h^{-1} mg^{-1} chlorophyll, from the Gulf of Elat a maximal value of 5.3 was reported, with an average of 1–2 (LEVANON-SPANIER et al. 1979).

Bacteria in the water samples were counted by direct microscopic observation in a counting chamber after concentrating the cells by centrifugation. However, the

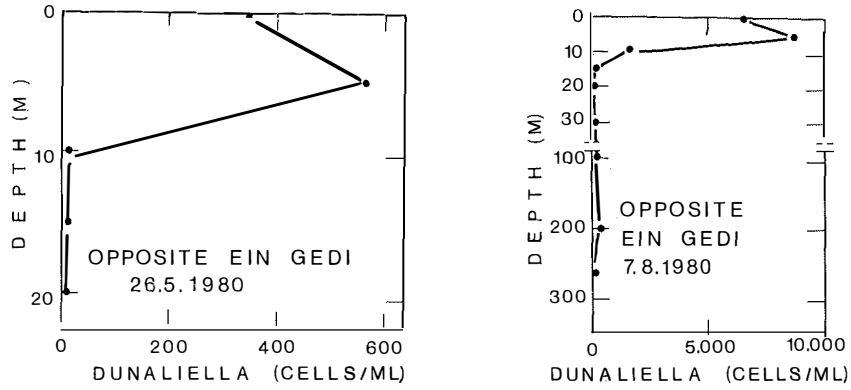

Figure 2

Dunaliella counts at different depths on 26.5.1980 and 7.8.1980 in water sampled at the deepest part of the lake.

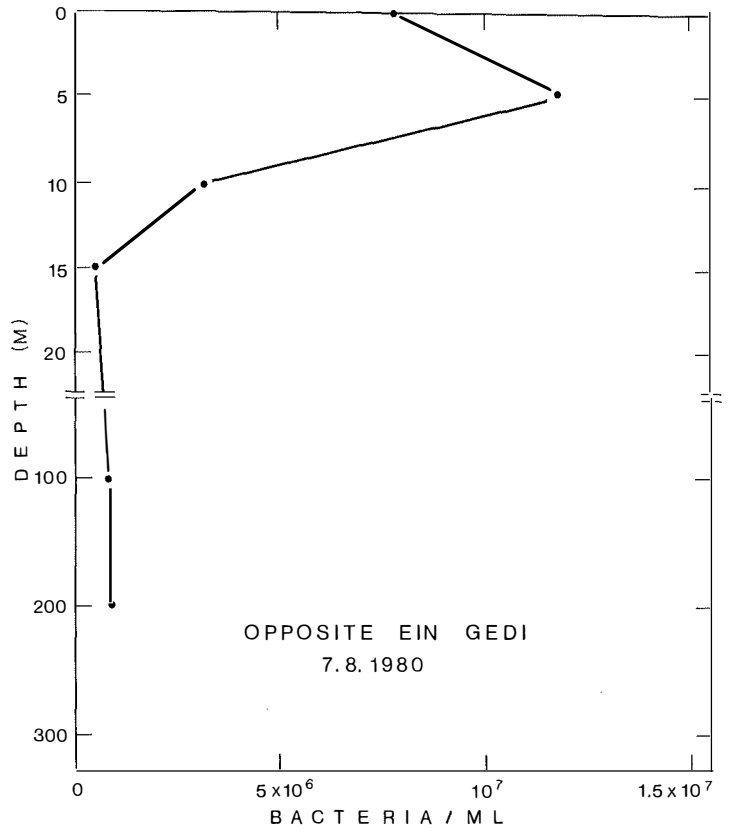

Figure 3

Bacterial counts (made by direct microscopical observation) in water samples taken from different depths at the deepest part of the lake, 7.8.1980.

pleomorphic nature of red halobacteria like *H. volcanii* (MULLAKHANBHAI and LARSEN 1975) which are the dominant types in the Dead Sea (KAPLAN and FRIEDMAN 1970) makes identification of particles as bacteria difficult, and counts obtained may thus be too high. Bacterial densities counted varied between $2x10^4$ and $1.2x10^7$ cells per ml. In summer a large bacterial bloom was observed, visible as a red coloration of the water. Figure 3 shows the bacterial densities measured in the water column. The numbers obtained are similar to those measured by means of epifluorescence microscopy with acridine orange staining. A great similarity between the depth distribution can be observed of algae (Figure 2) and bacteria (Figure 3).

Counts of viable cells on plates were without exception very low (up to 1500 cells per ml): The plating method appeared to be highly selective, most of the colonies found to develop were rod-shaped red halobacteria; the pleomorphic red halobacteria which were found to be the dominant microorganisms by direct microscopical observation did not appear. However, the method of viable counting used gave the highest counts of the many media and incubation procedures tested.

A number of pleomorphic red halobacteria have been isolated in the past from the Dead Sea (GINZBURG et al. 1970, MULLAKHANBHAI and LARSEN 1975) and additional strains were isolated by us (see Materials and Methods). Morphologically all strains are alike, and corresponding with the description given for *H. volcanii* (MULLAKHANBHAI and LARSEN 1975), all are red and lack gas vacuoles. However, the different isolates investigated varied in a number of properties like their plasma content (A. OREN and R.D. SIMON, unpublished results) and in their ability to metabolize different sugars. Growth experiments (Table 1) show that, though all strains are very magnesium-tolerant compared with e.g. *H. salinarium* (as to be expected for organisms living in the Dead Sea), rather large differences in magnesium tolerance were found between them. The upper limit of magnesium concentrations tolerated is in the range of the concentrations reported from the Dead Sea: upper water mass in 1959–1960 1.48 M, average concentration in 1977 1.81 M (BEYTH 1980).

Table 1

Magnesium tolerance of Dead Sea halobacteria in the presence of 10 % NaCl at 35 °C. For comparison a *H. salinarium* strain lacking gas vacuoles (obtained from Prof. R. D. Simon) was included in the studies.

Strain	Date of isolation	Highest Mg^{2+} concentration enabling growth
H. salinarium		0.9 M
CD-1	around 1969	1.15 M
CD-5	March 1980	1.45 M
CD-3	December 1979	1.65 M
CD-2	around 1970	1.9 M
CD-4	March 1980	1.95 M

Discussion

This study demonstrates that the Dead Sea is a very dynamic biotope, showing slow biological changes as a result of the changing limnology of the lake during the last decades, as well as changes as a reaction to seasonal salinity and temperature variations. In the last twenty years the salinity of the lake has increased significantly and the relative concentrations of the different cations have changed. Thus the overall

magnesium concentration in 1977, just before the overturn of the lake, was 22% higher than its concentration in the upper water mass in 1959–1960 (BEYTH 1979; STEINHORN et al. 1979). The microbial population in the Dead Sea had to adapt to the new conditions, especially as the overturn caused the disappearance of the slightly less saline upper water mass, in which organisms with a somewhat lower salt tolerance could find a refuge. The fact that the Dead Sea is still "alive" must be due to the ability of the microbial communities to adapt to the increasing salinities and the changing chemical composition of the water. We have shown that representative strains of pleomorphic halobacteria isolated in the Dead Sea differ in their plasmid content and biochemical properties and show differences in their tolerance to magnesium. It is tempting to speculate that the process of drying out of the lake and the resulting increase in total salinity and especially in magnesium content caused a succession of species with increasing salt tolerance, resulting in development of different types one after the other. This hypothesis is, however, difficult to prove because of the small number of strains isolated in the past that have survived. However, the process of drying out of the lake is expected to continue during the years to come, and it may be expected that the process of selection and evolution will continue, together with the increase in overall salinity of the lake and the changes in salt composition.

The finding that the microorganisms live in the Dead Sea at the upper limit of their salt tolerance (Table 1) explains why small changes in salinity have a profound influence on the growth rate (Figure 1). Additionally, temperature changes may be very important, especially as the salt tolerance of halophilic bacteria increases with temperature (MULLAKHANBHAI and LARSEN 1975). This explains the enormous seasonal fluctuations in microbial activities: the winter rains and floods lower the salinity of the uppermost water layer, and its heating up in summer leads to a massive bloom of red halobacteria, visible as a red coloration of the lake, and of *Dunaliella*.

It is possible that in addition to salinity and temperature other parameters are involved that limit microbial activity. For example, organic substrates available in the Sea may be an important factor regulating heterotrophic activity. Measurements performed by NISSENBAUM et al. (1972) about ten years ago showed that the sediment contains high concentrations of amino acids (60–790 mg per kilogram dry sediment) and fatty acids (4–14 mg per kilogram dry sediment). Also the concentrations of organic carbon and organic nitrogen have been reported to be high in the water column: 4–8 mg per liter and 0.3–3 mg per liter, respectively (NEEV and EMERY 1967).

It is still not clear whether phosphate can be a limiting factor in the Dead Sea: its solubility is low as limited by the high calcium and magnesium concentrations, and the phosphate concentration amounts to about 30–40 μg P per liter (M. STILLER, personal communication). It is not clear, however, to what extent the phosphate is freely available to microorganisms, or bound as complexes.

Heavy metal ions like Zn^{2+}, Pb^{2+} and Cu^{2+} were found in the Dead Sea in very high concentrations (NISSENBAUM 1977), and it can well be imagined that these inhibit microbial growth; dilution of the water will lower these concentrations and enable a higher growth rate. KRITZMAN 1 (1973) found indications that the heavy metal ions indeed inhibit microbial activity in the Dead Sea since the addition of chelating agents such as citrate and EDTA results in increased growth rates.

For photosynthesis and primary production light limitation can be an important factor: as the Dead Sea is very turbid the possibility for photosynthesis is limited to the upper 5–10 meters only. However, in the upper meters light intensity seems to be too high, and we generally found higher algal counts in 5 m depth than at the surface (figure 1).

The conditions under which microorganisms live and grow in the Dead Sea are thus more or less clear, but the cause of their death is much less understood. Predating organisms feeding on algae or bacteria are absent in the Dead Sea. ELAZARI-VOLCANI (1943, 1944) indeed isolated a number of protozoa from the lake and also KRITZMAN (1973) observed amoebae, but their importance in controlling microbial population size is not proven in this case, and all isolated strains have been lost. We did not observe any protozoa in the many samples investigated. One or more of the following processes may be responsible for decreases in microbial populations:

1. Microbes may serve as centers around which salts cristallize in the Dead Sea, such as NaCl, $CaCO_3$, $CaSO_4$, and during whitening of the lake (mass precipitation of $CaCO_3$ as aragonite cristals large populations may be removed by precipitation (NEEV and EMERY 1967).

2. Insufficient maintenance energy caused by extreme limitation of organic substrates may result in cell death. It is equally possible that increase in salt concentration beyond the tolerated limits not only inhibits growth but also causes irreversible damage.

3. Halophilic bacteriophages may control the size of bacterial populations; a number of phages attacking halobacteria are known, but no attempts have as yet been made to prove the existence of phages against Dead Sea halobacteria, nor have attempts to isolate such phages from the Dead Sea been carried out.

It may be concluded that the Dead Sea is a very dynamic ecosystem. The properties of the organisms living there and the factors controlling the microbial activity are as yet only very incompletely known. Major efforts will be made in the coming years to broaden our understanding of the biological processes taking place, before the inflow of Mediterranean water with the completion of the planned connection between the Mediterranean and the Dead Sea will again significantly change this unique ecosystem.

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