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RED WATER IN THE LAKE »MALO JEZERO« (ISLAND OF MLJET)

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RED WATER IN THE LAKE „MALO JEZERO" (ISLAND OF MLJET)

by

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Malo jezero (the Small Lake) is situated in a submerged bay of upper-chalky limestone on the north-western side of the Adriatic island of Mljet. — According to V u l e t i ć (1953) its surface comprises 241.320 m², its volume 3.348.964 m³ and its greatest depth attains 29 m. The lake is joined by means of a canal (2.5 m. wide and 0.20 m. deep) with Veliko jezero (the Great Lake) and the latter by means of a canal (4.5 m. wide and 0.60 m deep) with the bay of Solina, and beyond it with the open sea. — The scanty ventilation of deep waters in the »Malo jezero« accounts for the formation of H_2S below a depth of 19—20 m. as it was found by M. B u l j a n (1952). — It represents the first finding of sulphur-hydrogen in the Mediterranean sea water. — This boundary of waters contaminated with H_2S is not a constant one but varies during the year from a depth of 20 to 24,5 m. and may come to a full ventilation, that is a disappearance of H_2S. Owing to the springs of fresh water and the rain water which flow from the surrounding ground into the lake, the salinity of the lake water is lower (29,18 to 37,86 ‰) than the one in the open sea. The bottom of the lake in the zone fouled with H_2S is in general composed of clay and argil, and otherwise sandy or sand-argillous (V u l e t i ć 1953).

In the compass of the important works which have been undertaken in the region of the island of Mljet, bacteriological examinations of the water have been taken seven times in the year 1953 and each time from depth of 0, 5, 10, 15, 20 and 25 m. respectively of 0, 10, 20, and 25 m. In the month of May 1953 some water of a red-pinkish colour was drawn for the first time from a depth of 20 m. and the same phenomenom was observed also in the months of June and August in the same year. In 1953 tests were taken also in the months of January, March, September and November 1953 but there was no red water to be found. The bacteriological analysis of the red water ascertained that it was due to a rich population of red bacterial strain. We passed to examine the characteristics of this bacterial strain and under which circumstances it develops to its maximum, provoking the phenomenon of the red water.

All the hydrological data of »Malo jezero« quoted in this paper have been handed over for use by Dr. Miljenko B u l j a n (and they derive from

the paper he is preparing on the »Hydrochemical researches of the lakes on the island of Mljet») and we thank him both for his obligingness and his useful remarks about the paper. We likewise thank the abs. chem. techn. Joseph Špan for the perfomed chemical analyses of the water during the experiments. The laboratory technicians Ojdana Marović and Petar Bilić gave a full and devoted assistance in the execution and control of the exeriments and also to them we tender our heartiest thanks.

BACTERIAL STRAINS AND THEIR PIGMENT

Already in cleaning the strains by means of passages on the agar medium, we ascertained that, as a matter of fact, in this case it was the question of two red rodlike strains differing between them in the size of the cells as well as the intensity of the red stain of the colony. — We gave to one strain the laboratory mark »O« and to the other »P«. The determination of the characteristics of both the strains which we are going to treat in their particulars elsewhere, showed that it was a question of rods with rounded ends, coming singly and some slightly bent. Their size varies from 0,5 to 1 $\mu \times 2{,}1$ till 4,27 μ, they are mobile with a polar flagella and are gram-negative. In their cells they have no sulphuric drops. They grow also under anaerobic conditions, but do not create pigment, while during their growth under aerobic conditions they create a red pigment. For their growth they need organic substances, they melt the gelatine, from carbohydrates they create some acid but no gas. The most favourable pH is 7.8 and the most suitable temperature is 23—24° C.

It appeared furthermore that it was actually a question of two strains which differ from each other since the strain »O« has fewer cells than the strain »P« because strain »O« grows slower on the agar stroke and broth, its colonies on the agar-medium have at he adge a white-yellowish concentric ring, whereas the colonies of the strain »P« have none. — The difference consists also in the pigment, since the one of the strain »O« is of a light-red colour with the young colonies and later it darkens a little, while the pigment of the strain »P« is of a fiery red colour with the young colonies and dark red-violet in the older cultures.

It obviously appears to be a question of subjects of the family *Athiorhodaceae* (Molish) and precisely *Rhodopseudomonas*. In our further exposition we shall maintain the laboratory marks for the strains, that is: strain »O« and strain »P«.

The strains »O« and »P« originate a red pigment only if they grow under aërobic conditions and if in the medium there are enough organic substances, lactic acid and calcium carbonate. — If such conditions are complied with, the red pigment appears after 96 hours in the development of the culture and, as we have already stated, there is some difference between the strains »O« and the strains »P«. During their

growth under anaërobic conditions the cultures and colonies of the strains »O« and »P« have the colour of ivory. — The strains »O« and »P« do not stain the agar medium and the broth culture filtered through the Berkefeld filter gives a colourless broth.

The cells of the strains »O« and »P« dried in the air and treated with different dissolvents showed that the pigment of both the strains melts in chloroform, carbon dissulphide, ether, acetone, ethylene and methyl alcohol and does not melt in distilled water. The pigment extracted by means of chloroform or methyl alcohol, gives with sulphuric acid a violet hue. Concentrated nitric acid decolorizes the filter paper soaked with pigment, while in a concentrated hydrohloric acid the filter paper remains unchanged and is stained red. If dipped in concentrated sulphuric acid the filter paper soaked with red pigment turns to a violet hue.

Therefore the pigments of the strains »O« and »P« are carotenoid and it will be necessary to examine them chromatographically and separately to determine to which group of carotinoides they belong and which are their specific properties.

HYDROGRAPHICAL CONDITIONS

At the time when the red water appeared in »Malo jezero« at a depth of 20 m. in the months of May June and August 1953, the following hydrographic conditions were prevailing:

The temperature during those months from the surface to the bottom was risen (at 20 m. on average 16.28° C) while in the months of January and March as well as in September and November it was remarkably lower (at 20 m. on average 14.78°). — The salinity in the months when the red water appeared was at a depth of 20 and 25 m. higher (on average 37.90 ‰) than at the beginning of the year when it was on average 37.07 ‰ and a little lower than towards the end of the year when it was of 37.18 ‰. — Only in September the salinity rose against the three previous months when the red water appeared.

At the time of the red water there was oxygen, only in May while in the other two months, that is in June and August at a depth of 25 m. and 20 m. there was enough H_2S (about 2.93 mgr/lit. at 25 m. and about 1.16 mgr/lit. at 20 m. of depth). At the beginning and toward the close of the year at a depth of 20 m. there was about 2.32 cm^3 of oxygen pro litre.

The quantity of total phosphate was in the layer of the surface until 20 m. of depth at the time of »red water« about 6.8 mgr. per ton and in the layer at 20—25 m. it was of 18.8 mgr. per ton, so that the maximum of total phosphates was at a depth of 20 m. The quantity of total phosphates at the beginning and at the close of the year, namely when there was no »red water« was in the layer of the surface until a depth of 20 m. about

9.4 mgr. per ton, and in the layer 20—25 m. there was about 13.6 mgr. per ton, the maximum in those months was at a depth of 25 m. It is particularly striking that in May and June, when the »red water« appeared the quantity of the total phosphates was by far greater (23.0—26.0 mgr/ton) than in the course of the whole year when it varied from 4.8 to 15.9 mgr/ton.

At the time of the red water till a depth of 20 m. there was in the layer free iron for about 2.1 mgr/ton and in the layer at 20—25 m. there was 46.7 mgr. per ton. The maximum of iron was found at 20 m. (70 mgr. per ton) and it differed remarkably from the quantity of iron at 25 m. (28 mgr. per ton). In January and March as well as in September and November in the layer at 20 m. there was free iron about 4.6, and in the layer 20—25 m. about 19.0 mgr. per ton. — The maximum of free iron too was at that time found at 25 m. depth, excepting in the month of September, when the maximum was at 20 m. of depth.

The concentration of H-ion varied at the time of the red water at a depth of 20 m. from 7.25 to 7.82 so that it was the same as when there was no red water, and when at such a depth pH was from 7.30 to 7.68. Otherwise the concentration of H-ion during the year varied from 8.18 to 7.25 and the maxima were always in the layers of the surface, with a lessening towards the deeper water.

According to the above mentioned hydrographic data we can establish that the red water appeared at a depth of 20 m. at a period of the year when the temperature is slightly risen, the salinity somewhat augmented, the layer of contaminated water with H_2S risen to a depth of 20 m. with a logical disappearance or lessening of the quantity of oxygen and with the highest appearance of total phosphates and iron at such a depth.

If we compare the hydrographic conditions of »Malo jezero« in the year 1953 with those in the year 1952 and 1951 we are impressed by the fact that in the year 1953 the quantity of free phosphates was by far greater. In the months of May, June and August 1953 there was on average in the layer from the bottom up to a depth of 20 m. 18.8 mgr. of free phosphates per ton, while at the very same depth during the same months in 1951 there was on average about 4.2 mgr. per ton and in 1952 about 2.8 mgr. per ton. — The maxima of phosphates were in 1951 and 1952 always at a depth of 25 m. while in 1953 they were at 20 m., namely on the upper boundary of the sulphur-hydrogenic zone, precisely in the months when the »red water« appeared. — In 1953 when the »red water« appeared, the temperature at a depth of 20 m. was also higer of about 1° C. than the one in 1952 and the salinity was higher too in 1953 for on average 1.5 ‰. — At any rate we can conclude that these exceptional hydrographic conditions in 1953 were one of the elements which conditioned the stronger development of red bacteriological strains, which caused the appearance of the »red water« during the summer months of 1953.

EXPERIMENTS

I: — The strains »O and P« have been inoculated on ten different selective media, to ascertain on which of them they grow the best and generate pigment. It has been ascertained that both the strains grow the best on the nutrient medium for heterotrophic reductors of sulphate (media M 10):

Sea water 75%, distilled water 25%

K_2HPO_4	0.02 %
$MgSO_4 . 7 H_2O$	0.02 %
$/NH_4/_2SO_4$	0.10 %
$FeSO_4 . /NH_4/_2SO_4$	0.01 %
Peptone	0.25 %
Yeast extract	0.25 %
Na_2SO_3	0.01 %
Ca-lactat	0.30 %
Ascorbic acid	0.01%

pH — 7.6

By a gradual elimination of the single ingredients from the above mentioned medium, it was possible to ascertain that for the most favourable growth of the strains »O and P« peptone or yeast extract and Ca-lactata are absolutely necessary. This was also proved by a second experiment, in which it was shown that on the aforesaid medium which had not the above three, respectively at least two of the said ingredients, Ca-lastata and peptone or yeast extract, the strains did not grow at all.

It was necessary to determine what quantity of peptone, respectively of yeast extaxt is required for the most favourable growth of the strains. In the experiment in which the quantity of peptone varied and the other ingredients were fixed (Ca-lactata 0.3%), the strains grew the best when the quantity of peptone was 0.50—1.0%: but with these quantities they did not generate pigment. With the quantity of peptone 0.20—0.30% the strains grew also fairly well and generated pigment. If in the medium varied also the yeast extract, then, the strains grew the best with 0.20% peptone and 0.20% yeast extract. During the further course of the experiment we varied the quantity of Ca-lactata and then most favourable growth was with 0.40% peptone, respectively 0.20% peptone and 0.20% yeast extract and 0.40—0.50% Ca-lactata.

When we addel to the medium various quantities K_2HPO_4 the strains scarcely grew od did not grow at all. The same thing happened if we added various quantities of $Fe_3/PO_4/_2$. — However when we added together also calcium and iron phosphate, 0.1% of each, then the growth was good, but not the most favourable. We therefore conclude that phosphates in this form do not stimulate the growth of the strains O and P.

On media in which besides peptone were added organic phosphates Lecithin or natrium — glycerophosphate, the growth of the strains was as follows: Both strains grew the best and generated pigment (particu-

larly the strain »P«) on a medium with 0.2% lecithin and on a medium with 0.15% Na-glycero-phosphates. It appeared therefore that organic phosphates stimulate the growth of the strains »O« and »P«.

Consequently the most suitable nutrient medium for the most favourable growth of the strains O and P should be composed of:

0.20 % peptone
0.20 % Yeast extract
0.50 % Ca-lactata
0.20 % lecithin
75 % sea water
25 % destilled water
ph—7.6

On this medium has been performed the determination of the strains O and P.

II: — The strains O and P require then for their most favourable growth enough organic substances and Ca-lactata. In order to ascertain whether the strains O and P require for their growth only lactic acid, we put some in the medium. If in the medium there are organic substances and 0.10% of lactic acid, the strains grow perfectly well, but generate scanty pigment (strain »O«) or no pigment at all (strain »P«) whereas with 0.20% of lactic acid there is no growth at all. When we added to the medium instead of lactic acid some glycerine, the growth with 0.4% of glycerine was good but once again the generation of pigment was weakened (strain »O«) or was completely wanting (strain »P«). If to the medium we added about 0.2% $CaCO_3$ — precipitate, the strains grew well but did not generate any pigment. On the other hand when we added about 0.10% of lactic acid and 0.2% $CaCO_3$ — precipitate, the cultures of the strains O and P developed very well and generated pigment, i. e. the medium itself and the film and the sediment where coloured in red. It should be mentioned that the pigment of the strain P was of a more intensive red hue than the one of the strain. O. The strains O and P therefore require for their most favorable growth both lactic acid and calcium carbonate.

III: — In order to ascertain the development of the strains O and P under the aerobic and anaerobic conditions, respectively in water with and without H_2S an experiment was performed with broth-medium of the aforesaid compund in erlenmeyer flasks of 1 lit. Each flask contained 1 lit. of broth and they were all kept in the dark at a temperature of 23—25° C.

The flasks A and B were inoculated with the strains O and P and they were used as a control to the experiment. The strains grew well and reddened the broth already after three days and at the close of the experiments i. e. after fifty days the cultures were very well developed with a great deal of red sediments and a film on the surface. In the flasks A and B at the begining of the experiment there was pH 8.20, respectively 8.0 and at the end of it 8.0 respectively 8.08: therefore it did not change much.

The flasks C and D were inoculated with the strains O and P and then the culture was covered with a layer of paraffine oil 3 cm. thick. After a fortnight the cultures were badly developed and had generated no pigment at all. — After sixteen days the paraffine oil was removed from the cultures and eight days later the culture of the strain O (flask C) was well developed, but there was no red pigment, whereas the culture of the strain P (flask D) was also well developed, but generated also red pigment. — At the beginning of the experiment pH was 8.3 in both the flasks and at the close of the experiment 7.17 respectively 7.13 in the flask D; consequently pH had remarkably lessened. During the experiment no H_2S appeared in the flasks.

The flasks E and F were inoculated with the strains O and P and then into the broth was let for fifteen minutes gas H_2S. After three days in both the flasks the cultures were well developed, but did not generate red pigment. — After twelve days the culture of the strain O (flask E) was very well developed and coloured in pink and the strain P (flask F) was also well developed and of an intensive red hue. — At the beginning of the experiment pH broth was 8.3 respectively 8.0 and at the close, namely after a fortnight, it was 8.9 respectively 8.01. Consequently in this experiment pH did not change much.

The flasks G and H were filled each with 1 lit. of broth inoculated with the strains O and P and then in the bouillon we let for fifteen minutes H_2S and finally the cultures were covered with a layer 3 cm. thick of sterilized paraffine oil. After three and after fifteen days the cultures were ill developed in both the flasks. After a fortnight the paraffine oil was taken away from the surface of the broth and three days later the culture of the strain O developed very well but produced a poor pink pigment, while the culture of the strain P developed also very well and gave a much more intensive red sediment and a red film. At the beginning of the experiment there was pH 8.0 respectively 8.3 and at the end 7.73 respectively 7.61. — In this case too, pH lessened considerably.

It appears from these experiments that the strains O and P develop well only under aërobic conditions if they have at their disposal organic phosphates (flasks A and B, C and D) but that they attain their fullest development only if the culture at its starting stage of development has at its disposal H_2S for a longer or shorter time. In this case the cultures develop slower in comparison with the controlling one, but after the disappearance of H_2S, i. e. after the ventilation of the medium, the development of the culture is rapidly strenghtened and with regard to its copiousness and intensity of pigment it surpasses the controlling culture. — It has been ascertained during the experiments that pH lowered the most in the cultures which had developed under anaërobic conditions (0.43 till 1.17 from the starting pH).

IV: — In the further experiment we wished to push the conditions of development of the strains O and P. the nearest to the natural conditions of the sea in which the red water appears. We took three flasks of 8 lit.

and in each we poured seven lit. of sea water (sterilized in the Koh-pot for three days) and added some pine needles (*Pinus* sp.). — We inoculated the flasks I ind II with strains O and P and the flask III with some mud from the sea bottom of »Malo jezero« on the island of Mljet and with the strains O and P. — In all the three flasks we put then on the surface of the water a layer of sterilized engine-oil about 3 cm. thick so that the fermentation should happen under anaërobic conditions. The flasks were kept in the dark at a temprature of 22—25° C. The first eleven days we followed the variation of the conc. H-ion, and the experiment lasted ninety days in all. In the flasks I and II pH varied from the starting 8.2 towards a swift lowering and already after 48 h. it amounted to 6.93, respectively to 7.06 and after eleven days pH was 6.21, respectively 6.58. — In the flasks I and II appeared no H_2S, not even after ninety days. In the flask III pH after 48 h. fell to 6.72 and after seven days to 6.49 and after eleven days it rose again to 7.01. — In the flask III after seven days appeared H_2S in a rather big quantity and after ninety days there was 183.24 mgr H_2S lit. The water was already troubled after 48 hours in all the three flasks and particularly much so in the flask III, but no red pigment appeared in any of the flasks.

After seven days for a control of the growth of the strains O and P the samples of water in the flasks I, II and III were inoculated on the broth in the test tubes and kept under aërobic conditions. It was ascertained that in the flasks I, II and III the strains O and P were growing. After ninety days a big growth of free phosphates was ascertained in the bottles. In the water of the flasks I and II there were 708, respectively 773 mgr. of free phosphates per m^3. and in the water of the flask III there were 1056 mgr/m^3 while free iron was not detected in any of the bottles in a bigger quantity than it is to be found in normal sea water. It has also been ascertained (by means of Uffermans reagent) that in the water of the bottles I, II and III there was some lactic acid.

As a further control of the growth of the strains O and P after ninety days 150 ccm. of water were extracted under sterilized conditions frome ach of the bottles I, II and III and poured into sterilized small bottles and then contaminated under aërobic conditions. After 48—96 hours in the water from the bottles I and II appeared a slight red-pinkish pigment and a microscopic research showed that there were O and P strains. — In the water from the bottle III the red pigment did not appear at once but only after 5—6 days, that is when the water contained no longer H_2S and it was of a fiery red hue. — At that time in the water from the bottles I and II there was pH 7.60 respectively 7.63 and in the water from the bottle III there was pH 7.99.

In these experiments it was determined too that the strains O and P develop very poorly under anaërobic conditions regardless of the fact whether H_2S is present or not. Besides it has benn ascertained that the strains regenerate from the organic substances fairly big quantities of phosphates and let free the lactic acid. It has been furthermore shown that the culture of the strains O and P which developed under anaërobic

conditions when coming into aërobic conditions develops much better in a surroundings in which there was previously H_2S, but that its development is slower.

It was possible to ascertain again that in aërobic cultures of strains O and P there is a considerable lowering of pH.

Discussion

The flowing of »red water« in the various seas, gulfs and salt lakes, has already been noted by a lot of authors. — Some of them found (Joubin 1918, Heldt 1932, Allen 1938) that the red water is caused by various organisms such as Copepodes (*Calanus, Diaptomus*) Phyllopode (*Artemia*) *Dinoflagellate* (*Dunaliella, Gonyaulax*) and Diatomeae, and some authors sustain that the red water is caused by bacteria. So Warming (1875) Düggeli (1924) Gietzen (1931) Heldt H. J. (1952) Hadjikakidis (1952) and others have ascertained that the red water appears when the conditions for the most favourable development of some species of bacteria (*Chromatium* sp. *Thiopedia* sp. and some other chromosynthetic kinds of bacteria) are fulfilled. In almost the cases found, it was a question of localities where the sea or fresh water were relatively shallow, in which sulphur-hydrogene appears either constantly or periodically.

The phenomenom of the red water in the »Malo jezero«, on the island of Mljet, appears to be somewhat similar to the appearance of red water i the Swiss lake of Rito for which Düggeli (op. cit.) sustained that it is caused by a species of *Chromatium* sp. which made its appearance in that lake at the frontier betwween the upper water with a normal density of oxygen and the lower water which contains fairly big quantities of sulphur-hydrogen. However a more detailed comparison is rendered difficult since the lake Rito has fresh water, whereas in our case the question is about a salt water lake.

According to the hydrographic conditions prevailing on the »Malo jezero«, on the island of Mljet, at the moment when the red water appeared at a depth of 20 m. and according to our laboratory experiments, we can reconstruct in its main lines the way and the conditions of the appearance of red water in this locality.

In 1953, winter and vernal rains drifted from the ground into the lake a great deal of organic substances which deposited in the bottom. Here occurred a destruction of the organic substances by the proteolitic and other bacteria, in consequence of which, besides phosphates and other substances, was created sulphur-hydrogen in remarkable quantities. Together with the appearance of sulphur-hydrogen in the water ensues also a lessening of pH water, enabling thus formation of lactic acid from the vegetable substances which under anaërobic conditions results as a final product of glycolisis and consequently as a dissimilating and in

no way as an assimilating product. When in the month of May 1953, when the water was tampered, also the surface of the water layer was upset at a depth of 20 m. — the sulphurhydrogen was lost the oxygen appeared and the pH of the water rose. The water of »Malo jezero« contains enough calcium carbonate, a fact which may be presumed from the recent sediments of the bottom, which were found to amount to 70% (V u l e t i ć, op. cit.). From what has been exposed we may conclude that owing to the rise of temperature and salinity, the drift of lactic acid and a good deal of organic substances, as well as of calcium carbonate, all the conditions for the most favourable multiplication of the strains O and P were practically fulfilled. The rising of the sulphur-hydrogene which deals as a stimulant for the development of the culture of the strains O and P at the starting stage, — and particularly on the intensity of the pigment, is not fully explainable. The question arises whether the H_2S exercises in the generation of the »red water«, i. e. in creating the conditions for the most favourable development of the strains O and P, the mere role of factor which partakes in lowering or rising the concentrations of H-ions, or — as it is more probable — in another sense, it stimulates the development of the bacterial cultures of *Rhodopseudomonas* sp. — Very likely we have to do here with a process which looks like the one established by U m b r e i t (1954) and before him by some authors for the *Thiobacillus thiooxidans,* which turns the energy received from the oxidization of sulphur into an energy of phosphorylation. — In any case particular attention should be paid to this question.

SUMMARY

It has been ascertained that the apparition of »red water« at a depth of twenty meters in the »Malo jezero« (The Small Lake) on the island of Mljet during the months of May, June and August 1953, has been provoked by two bacterial strains (*Rhodopseudomonas* sp.).

In the laboratory experiments it has been shown that these bacterial strains require for their most favourable development enough organic substances, lactic acid and calcium carbonate and that at the time when the »red water« appeared the hydrographic and other conditions of the water in »Malo jezero« did practically fulfil such conditions and thereby enabled the most favourable development of *Rhodopseudomonas* sp., from the cells' pigment of which the water derives its red-pinkish color.

It has been determined that the pigment of the bacterial strain *Rhodopseudomonas* sp. are of a carotenoidis nature.

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CRVENA VODA U MALOM JEZERU (OTOK MLJET)

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Kratak sadržaj

U mjesecima maju, junu i augustu 1953. godine pojavila se na 20 m dubine u Malom jezeru otoka Mljeta voda ružičasto-crvene boje. Ustanovljeno je, da su ovu boju vode izazvala dva bakterijska soja (*Rhodopseudomonas* sp.).

Laboratorijski eksperimenti su pokazali, da ovi bakterijski sojevi trebaju za svoj optimalni razvoj dosta organske tvari, mliječne kiseline i kalcijskog karbonata.

Hidrografska ispitivanja, izvršena u vrijeme pojave crvene vode, pokazala su, da je u to doba na 20 m dubine u Malom jezeru bio maksimum slobodnih fosfata i željeza, da je bio minimum kisika, ili je utvrđen sumporovodik u vodi, a ostali faktori, kao temperatura, salinitet i koncentracija H-iona bili su uglavnom normalni.

Na temelju laboratorijskih eksperimenata i nalaza hidrografskih prilika, u doba pojave crvene vode, izveden je zaključak, da su u mjesecima maju, junu i augustu 1953. godine bili ispunjeni uslovi za optimalni razvoj nađenih bakterijskih sojeva, (*Rhodopseudomonas* sp.), čiji je crveni pigment izazvao pojavu crvene vode.

Data je evidencija da nađeni sojevi stvaraju crveni pigment samo u aerobnim uslovima, te da je taj pigment karotenoidne naravi.

Tiskanje završeno 31. VIII. 1955.

Tisak: Novinsko-izdavačko poduzeće „Slobodna Dalmacija“ - Split