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Optical characteristics of natural water samples collected in 2015-2017 in the supralittoral zone of the Alaid Volcano, the Kuril Islands

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ABSTRACT

The work presents the results of spectroscopic study of the natural water with microorganisms from the splash baths in the Atlasov Island (the Alaid Volcano). The Alaid Volcano is the highest, northernmost and one of the most active volcanoes of the Kuril Islands, the unique object for interdisciplinary research. In 2015 within the supralittoral zone of the island the several splash pools filled with colored marine water were discovered for the first time. The paper presents the results of spectral measurements and microbiological studies performed on natural water samples taken in 2015-2017 from the supralittoral zone of the Alaid Volcano, the Kuril Islands, as well as the photosynthetic microorganisms cultivated from those samples in laboratory. The absorption spectra of the native samples and the cultures of photosynthetic bacteria cultivated from original water demonstrated pronounced maxima at 380, 590, 806, 854 nm wavelengths and the shoulders peaked at 510, 590 and 890 nm. The observed absorption maxima revealed the presence of bacteriochlorophyll *a*, the main photosynthetic pigment of purple sulfur bacteria, and additionally the cells of purple sulfur bacteria *Thiocystis* and *Thiorhodococcus* morphotypes were detected in water under microscope. Observation of water colored by photosynthetic microbes in different parts of the Atlasov Island within several summer seasons in 2015-2017 allowed us to conclude that the development of the purple phototrophic bacteria in the splash baths above the littoral zone is not an accidental event, but the stable phenomenon. On the other Kuril Islands similar splash baths have not been encountered yet.

Keywords: fluorescence spectra, absorption spectra, natural water samples, photosynthetic pigments, algobacterial communities, purple sulphur bacteria, the Alaid Volcano, the Kuril Islands

1. INTRODUCTION

Due to massive development of photosynthetic microorganisms, natural water could get marvelous spectrum of colors. Typically, chlorophyll-containing algal or cyanobacterial cells give water blue-green tunes, while anoxygenic phototrophic bacteria provide other colors, from orange to purple due to presence of specific carotenoids. Distinguishing of photosynthetic microbial communities in water is necessary for many ecological applications. Fast detection of photosynthetic pigments in living cells is possible by optical methods *in situ*. While fluorescence spectroscopy is often used in ecological monitoring of phytoplankton and cyanobacteria in natural water [1-2], the tracking and diagnostics of anoxygenic phototrophic bacteria are rare in the field studies [3-5].

In this paper we present the results of spectral measurements performed on natural water samples with photosynthetic algo-bacterial communities collected in 2015-2017 within the supralittoral zone of the Alaid Volcano. The Alaid Volcano is the symmetrical stratovolcano which rises from depths of 750-800 m up to 3000 m from the sea floor and

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forms the Atlasov island (also called as Alaid Island), the northernmost island of the Great Kuril Islands. The Atlasov Island is located at the northeast end of the Great Kuril Ridge. The Atlasov is the highest island of Russia and 49th in the world. The Alaid Volcano is the most active volcanoes of the Kuril Islands, and it is the unique object for interdisciplinary research (Figure 1a) [6-7] The Alaid volcano together with the Grigoriev submarine volcano represent a single volcanic massif Alaid [8]. The last central crater eruption occurred on Alaid in April 1981, it was one of the largest in the Kuril Islands in historical times. In October 2012 a steam and gas plume reached 200 m above the crater at Alaid volcano.

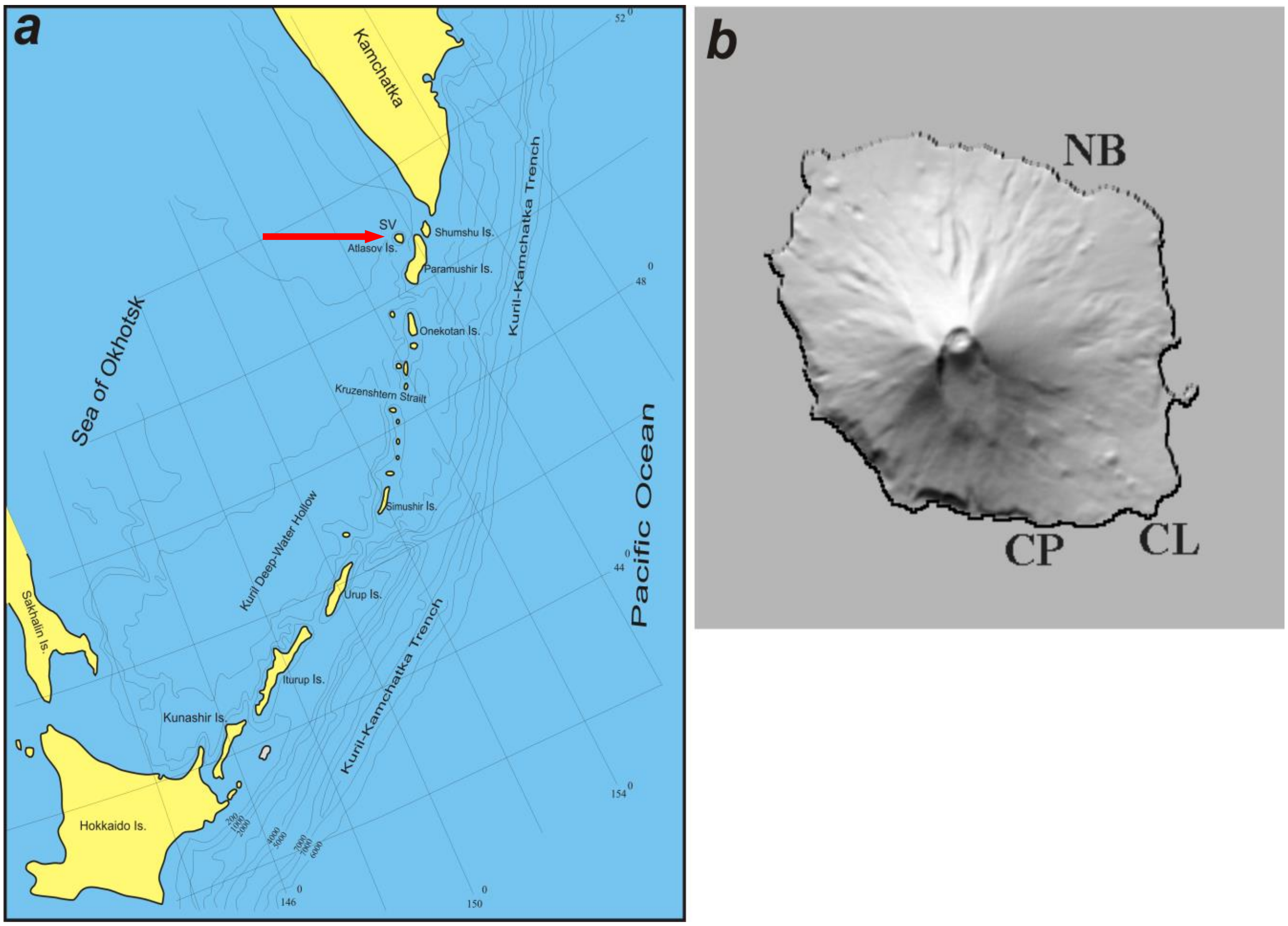

Figure 1. The location of the Atlasov Island (Alaid volcano) in the Kuril Island Ridge (a) and the locations of rock baths filled with colored water found on the Atlasov Island (b). SV – Grigoriev submarine volcano, NB - North Bay, CP - Cape Pologiy, CL – Cape Lava.

2. OBJECTS AND METHODS

2.1 Location of the sampling sites in the Alaid volcano

During the complex expedition in 2015 several water pools filled with colored (magenta, red, yellow or green) water due to the presence of well-developed algobacterial phototrophic communities were first discovered in three places within the supralittoral zone of the Atlasov island (Figure 1b) [9].

During 2016 and 2017 the colored water pools were found again, in the old or new locations (Table 1) [10-11]. The Figure 2 shows the splash baths in the area of Cape Pologiy with colored water on lava flows photographed in 2015 and 2017. Observation of water colored by photosynthetic microbes in different parts of the Atlasov Island within several summer seasons in 2015-2017 allowed us to conclude that this stable phenomenon is not an accidental event on the Atlasov Island. Interesting to mention that on the other Kuril Islands similar splash baths with massive development of microorganisms giving red-purple color to water have not been encountered yet.

Table 1. The list of samples taken from the splash baths in which the water had a pronounced color in 2015–2017.

Location	Period	Bath No	Water color	Sample name
Cape Pologiy	August 2015	CP-1	red	CP -2015
	August 2017	CP-1	transparent	-
	August 2017	CP-2	red	CP -2017 (red)
		CP-3	green (purple after 12 days of storage)	CP -2017 (green)
North Bay	August 2016	NB-1	red (discolored within 10 days)	NB -2016 (red)
		NB-2	yellow (discolored within 10 days)	NB -2016 (yellow)

Figure 2. Photographs of the splash baths in the area of Cape Pologiy with "colored water" on lava flows (left – bath CP-1 in August 2015, right – bath CP-2 in August 2017).

2.2 Fluorescence and absorption measurements

The naturally colored water samples were collected in 2015-2017 and the microbial cultures grown were from these water samples on medium for anoxygenic phototrophic bacteria in laboratory conditions. The absorption spectra were measured with a Solar PV1251 spectrophotometer in the spectral range from 315 to 995 nm. The absorption spectra of water samples or cell suspensions in water were recorded with respect to distilled water, the absorption spectra of acetone-methanol extracts with respect to a mixture of acetone-methanol-water. Fluorescence emission spectra were recorded using a Solar CM2203 fluorimeter at an excitation wavelength of $\lambda ex = 390$ nm. All spectral measurements were carried out in standard quartz cuvettes with an optical path length of 1 cm.

2.3 Preparation of extractions of photosynthetic pigments

To obtain pigment extracts from water samples, the volume of 4 ml of a mixture of acetone and methanol in proportion 7:2 was added to 1 ml of a water sample. A bottle with the obtained extract in a volume of 5 ml was wrapped in foil (to exclude the access of light) and left for several days in the refrigerator.

2.4 Cultivation of photosynthetic microorganisms and microbiological studies

To obtain accumulative cultures of phototrophic bacteria, the samples of native water with living microbial cells were placed in hermetically sealed glass bottles of 30 ml volume filled with a nutrient medium. Cultivation was carried out anaerobically for 2 weeks with illumination of 2000 lux and a temperature of 20-25 °C. For the cultivation of phototrophic sulfur bacteria, the modified Pfennig medium of the following composition (g / l) was used: KH_2PO_4 – 0.7; NaCl – 30; $MgSO_4·7H_2O$ – 0.5; NH_4Cl – 0.7; KCl – 0.33; $NaHCO_3$ – 1.5; $CaCl_2·6H_2O$ – 0.1; $Na_2S·9H_2O$ – 0.7; $Na_2S_2O_3·5H_2O$ – 1, Na-acetate·$3H_2O$ - 0.5; Na-pyruvate - 0.5; yeast extract - 0.1; vitamin B12 - 20 microgramm / L; trace element solution - 1 ml [12], pH 8.

Micrographs of cells were obtained using a light microscope at a magnification of 1200 (90x objective) with an immersion system and phase contrast. Microorganisms were identified by morpho-physiological characteristics.

3. RESULTS AND DISCUSSION

3.1 Absorption spectra of native water samples and pigment extracts

Absorption spectra of native water samples showed no significant absorption peaks from chlorophyll (at 660–663 nm), which indicates that little amounts of eukaryotic algae cells remained in the samples after prolonged transportation from the place of sampling to the laboratory.

The absorption spectra of natural water and of photosynthetic bacteria cultures grown from these samples demonstrated pronounced maxima at 380, 590, 806, 854 nm wavelengths and the shoulders peaked at 510, 590 and 890 nm. Figure 3 shows absorption spectrum of enriched purple bacteria culture grown from the rock baths of the Cape Pologiy water sample. According to the literature data, the observed maxima (805 nm, 854 nm) are due to the presence of bacteriochlorophyll (BChl) *a* [13], the main photosynthetic pigment of purple bacteria. The shoulders at 510, 590 and 890 nm can be explained by the presence of carotenoids. Carotenoids are intensively absorbed in the blue-green region of the spectrum, thereby providing a purple color to bacterial cells. However, the peaks of the individual carotenoid compounds are indistinguishable against a wide background of absorption of other organic compounds and cell scattering. In vivo culture absorption spectra, in contrast to extracts in organic solvents, rather intense light scattering by cells is present. The scattering contribution increases with decreasing light wavelength and prevails over the absorption of cell pigments in the UV region.

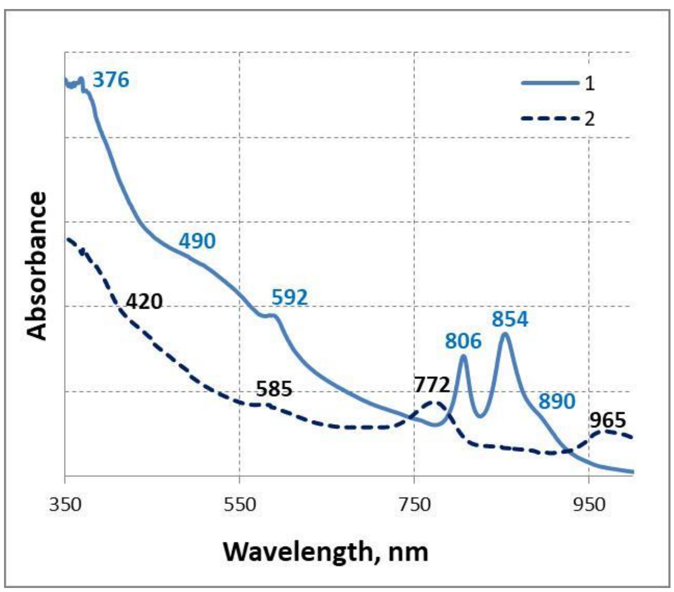

Figure 3. Absorption spectra of enriched culture of purple bacteria cultivated from the rock baths of the Cape Pologiy (solid lines) and the pigment extraction prepared from that sample (dashed lines).

To specify the content of different types of bacteriochlorophylls in the cells the pigment extracts were prepared from the natural water samples and the phototrophic bacterial cultures, and the spectral analysis of them was undertaken. The absorption spectra of acetone extracts had a narrow band with a maximum in the region of 770 nm and a broad band in the range 340–540 nm (BChl *a* of purple sulfur bacteria [14]). It was confirmed by absorption spectra of extracts and by microbiological studies that the anoxygenic phototrophic bacteria containing the BChl *a* and the carotenoid rhodopinal colored the water bright red and purple.

3.2 Fluorescence spectra of native water samples

The fluorescence spectra of all natural water samples (even discolored during transportation) and storage cultures, when excited by light at a wavelength of 390 nm, have two pronounced fluorescence bands at 612 and 675 nm (Fig. 4).

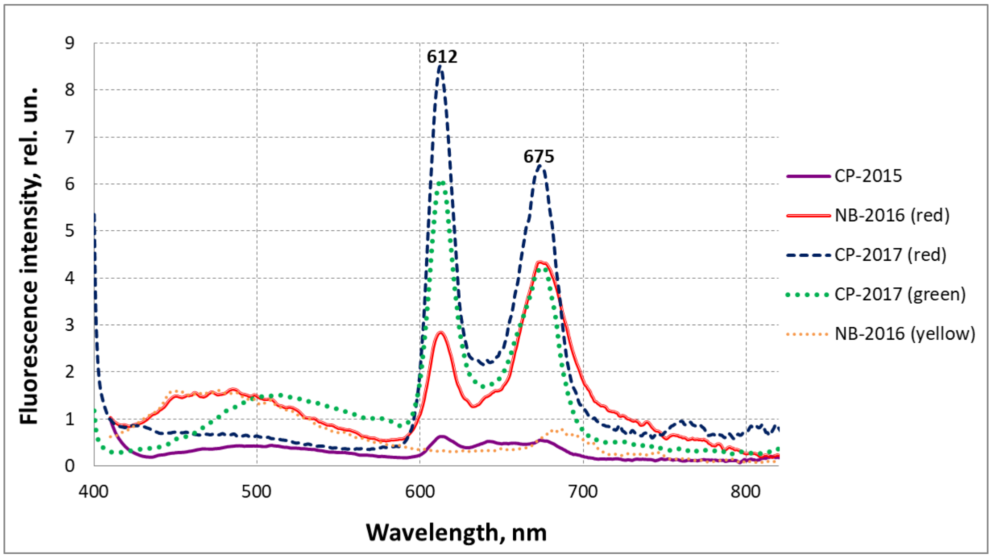

Figure 4. Fluorescence emission spectra excited by light at a wavelength of 390 nm for water samples from the 2015 and 2016 years and accumulative cultures of bacteria (samples 2017) from the rock baths of the Cape Pologiy and North Bay.

BChl fluorescence in bacterial cells with a whole intact photosynthetic apparatus falls on the infrared region of the spectrum (with wavelengths longer 1000 nm), therefore it cannot be seen in the visible range. In the visible range of the two above maxima for all samples of microorganisms, the authors of this article refer to the luminescence of bacteriochlorophyll *a* monomers formed during the destruction of antenna complexes of the photosynthetic apparatus of purple bacteria, in which bacteriochlorophyll is contained in a highly aggregated state.

3.3 Data from microbiological studies

The cells of green algae and purple sulfur bacteria were found in water samples from the baths No 2 and 3 in the area of Cape Pologiy in 2017 (Figure 5). During the 12-day transportation, the green water from the bath No 3 gradually turned purple, and visually almost did not differ in color from the water of the bath 2. However, we can clearly see the difference under microscope.

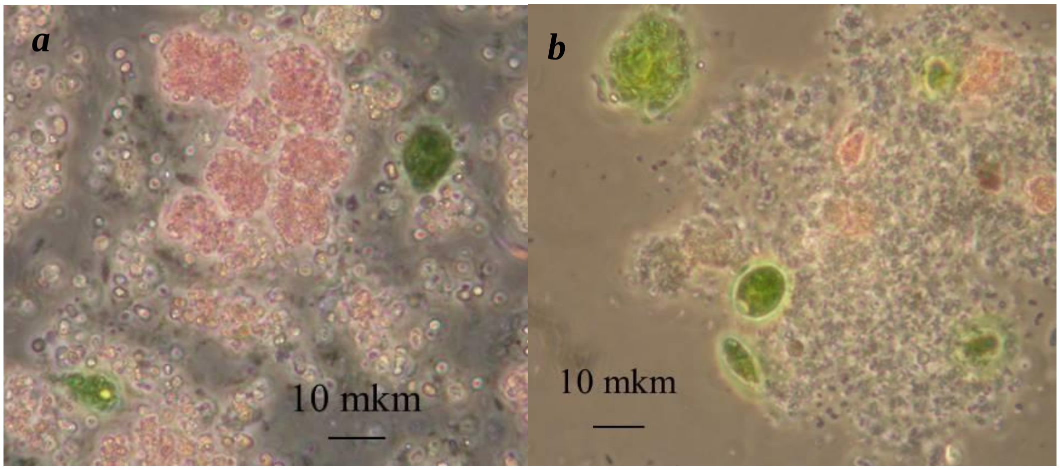

Figure 5. The microbial cells under microscope from native water sampled in the rock baths of the Cape Pologiy in August 2017: unicellular green alga (cells of green color) and *Thiocystis-Thiorhodococcus* morphotype bacterial cells (purple cells). a – red water from the bath No 2; b – green water from the bath No 3.

Microscopic analysis of the water brought in in both samples (bath No 2 and No 3) revealed small groups and single cells of eukaryotic algae, many of which were similar to euglena, and the remains of destroyed silicon shells of diatoms were also found. Bacteria of various morphotypes were observed: thin filamentous, small cocci, motile spirils, rods, spindle-shaped cells with pointed ends, etc., but the same purple sulfur bacteria prevailed in both samples, which turned out to be similar to the bacteria of the genus *Thiorhodococcus*.

The cells of the dominant purple sulfur bacteria were of round shape, 0.7-1.0 × 1.5-2 μm in diameter, motile, contained sulfur droplets (Figure 5), and, as can be seen from the spectra of the water sample (Figure 3), their main pigments there were BChl *a* and the carotenoid rhodopinal. According to the combination of morpho-physiological properties[15], the dominant species most closely resembled *Thiorhodococcus* minor.

4. CONCLUSIONS

Observation of water colored by photosynthetic microbes in different parts of the Atlasov island within several summer seasons in 2015-2017 allowed us to conclude that the development of the purple phototrophic bacteria in the splash baths above the littoral zone is not an accidental even, but the stable phenomenon. The work presents the results of spectroscopic study of the natural water with microorganisms from the splash baths in the island. It was confirmed by absorption spectra of extracts and by microbiological studies that the anoxygenic phototrophic bacteria containing the bacteriochlorophyll *a* and the carotenoid rhodopinal colored the water bright red and purple. Under microscope the purple sulfur bacteria of *Thiocystis* and *Thiorhodococcus* morphotypes were detected as prevailing in the water samples. According to the combination of morpho-physiological properties and photosynthetic pigment attribution from absorption spectra, the dominant species most closely resembled *Thiorhodococcus minor*.

We assume that in the future such multidisciplinary studies (or, at a minimum, initial processing of the samples) should be carried out at the sampling site; otherwise, the results may be distorted and cannot be used to judge about native algobacterial communities and their changes during the summer period.

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