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Red-colored water in the supralittoral zone of the Alaid Volcano: long-term spectroscopic observations

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ABSTRACT

The paper presents the results of a spectroscopic study of the "colored" water detected in the splash baths in the supralittoral zone of the Alaid Volcano (the Kuril Islands) from 2015 to 2019. The splash baths with magenta, red and red-yellow water located in different parts of the island in various summer seasons. The analysis of fluorescence and absorption data of the water samples revealed the presence of a large amount of bacteriochlorophyll *a* in the water, which is the main photosynthetic pigment of purple sulfur bacteria. The assumption of the presence of cells of microorganisms was confirmed by observations under a microscope. To determine the morphotype of microorganisms the acetone-methanol extracts from the samples were prepared and studied using spectral methods. The characteristic absorption lines of carotenoids and the monomeric form of bacteriochlorophyll identified cells as *Thiocystis* and *Thiorhodococcus* morphotypes of purple sulfur bacteria. The regular observation of water samples with microorganisms in the supralittoral zone of the Alaid Volcano leads to the conclusion that the development of the purple phototrophic bacteria is not an accidental event, but a typical phenomenon in this area. The fact that each summer season colored water were found in different places indicates a cyclical changes in living conditions of microorganisms from favorable to unfavorable habitats.

Keywords: absorption spectra, fluorescence spectra, red-colored water, bacteriochlorophyll *a*, photosynthetic pigments, purple sulfur bacteria, the Alaid Volcano, the Kuril Islands

1. INTRODUCTION

The color of natural water depends on various factors: an illuminance, a concentration of organic and inorganic compounds, a presence of pigments of algae, cyanobacteria and other microorganisms. In general algal or cyanobacterial chlorophyll-containing cells turn water in green color, while water with purple bacteria can take a number of colors from orange to purple due to the presence of carotenoids. Spectral methods are widely used in ecological monitoring thanks to some advantages as a high sensitivity, a possibility of observations of photosynthetic pigments in situ and fast detection¹⁻⁴. Spectral properties of pigments of phototrophic bacteria have not been fully studied yet, but their study is of considerable interest. According to currently available data, the evolution of photosynthesis included a transition from anaerobic bacterial organisms (that use sulfur, nitrogen, and simple organic compounds as electron donors) to cyanobacteria, algae, and higher plants (that use water - the electron donor with a lower degree of recovery). There is a hypothesis that the evolutionary transition from anoxygenic photosynthetic bacteria to ancient oxygenic microorganisms could occur through an intermediate purple bacterium^{5,6}. In this way an optical research of purple bacteria, as well as detection of their habitats, can be useful to study the evolution process.

This work continued and summarized the spectral studies of natural water sampled from the splash baths of the Alaid Volcano in 2015-2019⁷. The Alaid Volcano forms the Atlasov Island. It is the northernmost, the highest and one of the most active volcanoes in the Great Kuril Islands (Fig. 1), which rises from depths of 750-800 m up to 3000 m from the sea floor. The last central crater eruption of the Alaid Volcano occurred in April 1981, it was one of the largest eruptions in Kuril Islands. The last activation of the volcano was in October 2012, a steam and gas plume reached 200 m above the crater. Nowadays Atlasov Island is the unique object for interdisciplinary research⁷⁻¹². The study of features of

the island using spectral data will contribute to a better understanding of the nature of volcanic factors and the response of ecosystems to them¹³.

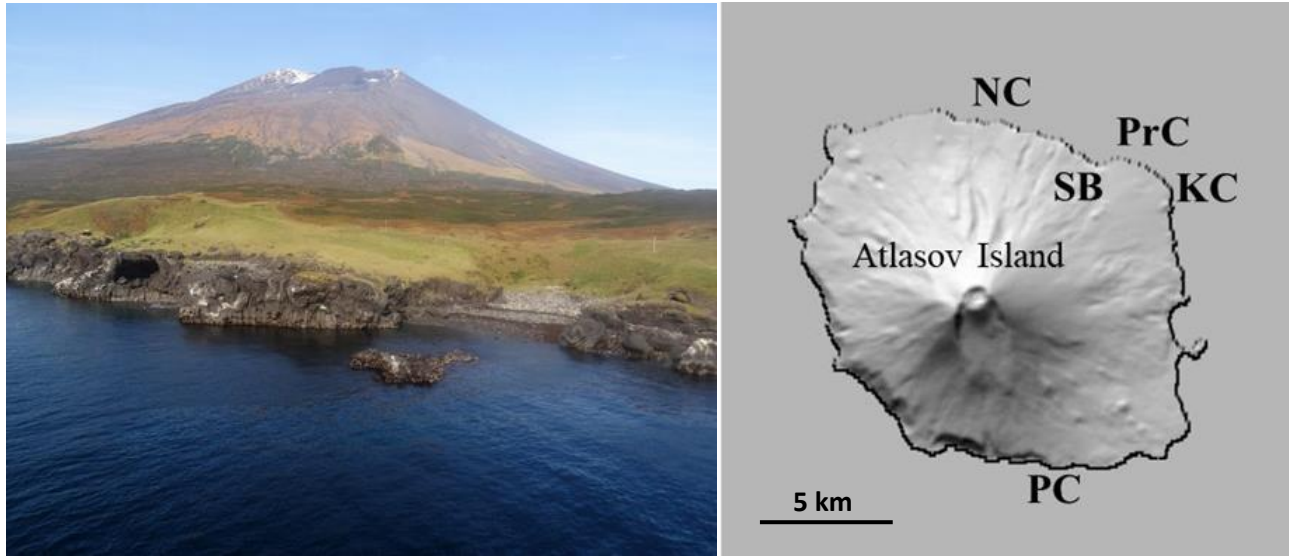


Figure 1. The Alaid Volcano (Atlasov Island) in 2020 (left) and the locations of the splash baths with colored water detected in the Atlasov Island (right). NC – Nochnoy Cape, SB – Severnaya Bay, PrC – Praviy Cape, KC – Khitriy Cape, PC – Pologiy Cape.

2. OBJECTS AND METHODS

2.1 Locations of the water samples

The unique "red water" was detected for the first time during the complex geological and geophysical studies of the Alaid Volcano in August 2015. The water was sampled in the area of Pologiy Cape (Fig. 1) and transported to the laboratory in Moscow⁸. Over the next five years similar splash baths were found at other locations in various parts of the island. During 2016 and 2017 the colored water was observed in Severnaya Bay and Pologiy Cape. It should be noted, that in 2017 the splash bath (containing colored water in 2015) was filled with ordinary sea water with no signs of microorganisms^{9,10} (Fig. 2).













Figure 2. Photographs of the splash baths in the area of Pologiy Cape: bath PC-1 in August 2015 (left), bath PC-1 in August 2017 (right).

In 2018 splash baths with colored water were found in the area of the Nochnoy Cape, where the scientists worked¹¹. The phenomenon was observed again in the following year, the water samples were detected in the northern part of

the island between the Praviy Cape and Khitriy Cape¹². The Table 1 shows the location, the date, the color of water and the mark of each sample.

Table 1. The list of the samples from the splash baths detected in 2015–2019 in the supralittoral zone of the Alaid Volcano.

Location	Date	Bath №	Water color		Sample name
Pologiy Cape	August 2015	PC-1	red		PC-1 (2015)
	August 2017	PC-1	transparent		PC-1 (2017)
		PC-2	red		PC-2 (2017)
Severnaya Bay	August 2016	SB-1	red (discolored within 10 days)		SB-1 (2016)
Nochnoy Cape	August 2018	NC-1	green		NC-1 (2018)
		NC-2	red (discolored within 10 days)		NC-2 (2018)
Praviy Cape-Khitriy Cape	August 2019	PKC-1	yellow		PKC-1 (2019)
		PKC-2	ochre		PKC-2 (2019)
		PKC-3	milky white		PKC-3 (2019)
		PKC-4	red		PKC-4 (2019)

2.2 Analysis of fluorescence and absorption data

The naturally colored water samples were transported to the laboratory at Moscow, where analysis of fluorescence and absorption data was conducted. 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 were recorded with respect to distilled water, the absorption spectra of acetone-methanol extracts were recorded with respect to a mixture of acetone-methanol-water. A Solar CM2203 fluorimeter was used for registration fluorescence emission spectra at an excitation wavelength 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 influence of light) and left in a cold storage for several days.

3. RESULTS AND DISCUSSION

3.1 Absorption spectra of natural water samples and their extracts

Absorption spectra of majority of natural water samples showed pronounced maxima at 380, 590, 806, 854 nm wavelengths and the shoulders peaked at 510, 590 and 890 nm. In accordance with an information in literary sources, the recorded maxima (805 nm, 854 nm) can be explained by the presence of bacteriochlorophyll *a* (BChl *a*)¹⁴, which is the main photosynthetic pigment of purple sulfur bacteria. The shoulders at 510, 590 and 890 nm confirmed the presence of carotenoids, which absorb light in the blue-green region of the spectrum, thereby providing a purple color to cells of bacteria. In addition to carotenoids other organic compounds and cell scattering create a wide background of absorption in the ultraviolet and visible parts of the spectrum, thus the peaks of the individual carotenoid compounds are indistinguishable.

The absorption measurement of the water sample NC-1 (2018) revealed the characteristic absorption line at 670 nm. This fact demonstrated the presence of chlorophyll-containing microorganisms (algae or cyanobacteria) in it, providing the bright green color of water. Some samples (SB-1 (2016), PKC-1 and PKC-4 (2019)) did not show any pronounced maxima on absorption spectra (Fig. 3). We suggested that this phenomenon can be explained by prolonged transportation from the place of sampling to the laboratory during which the cells died. It is supported by discoloration of some samples after transportation.

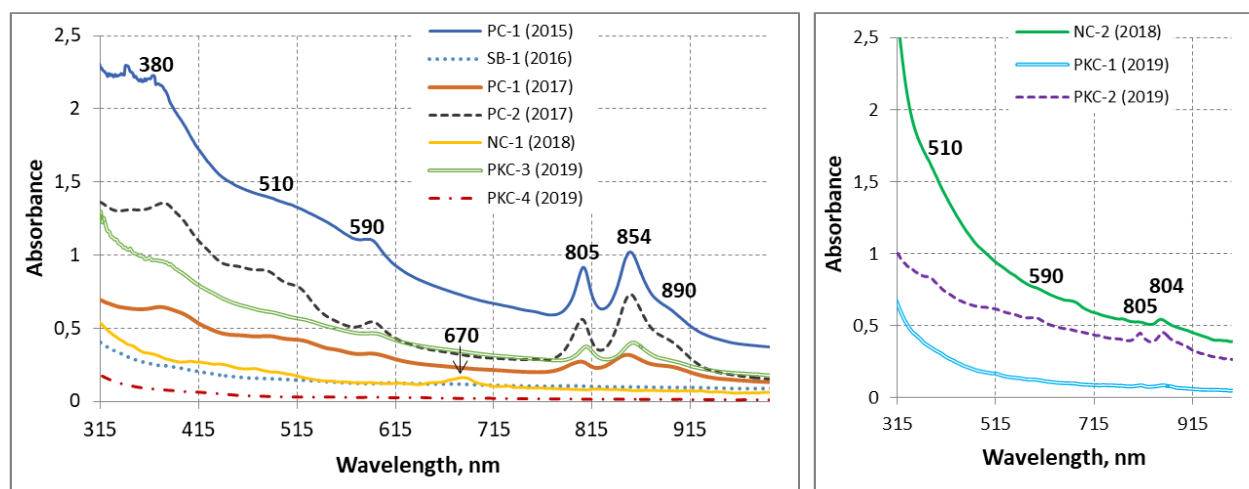


Figure 3. Absorption spectra of water samples of the splash baths detected in the Atlasov Island.

For more detailed determination of internal composition of purple bacteria the acetone-methanol extracts from the samples were prepared and studied using absorption analysis. Figure 4 demonstrates absorption spectra of the monomeric bacteriochlorophyll and pure carotenoids in contrast to absorption spectra of aggregated bacteriochlorophyll in living cells.

The absorption spectra of all extracts had a narrow band with a maximum in the region of 770 nm and a broad band in the range 350–550 nm, which represents a light absorption by monomers of BChl *a* and carotenoid rhodopal¹⁵. Microscopic analysis of the samples detected in 2017 revealed small groups and single cells of eukaryotic algae (similar to euglena), and the remains of destroyed silicon shells of diatoms. However the purple sulfur bacteria prevailed in the samples⁷. According to the combination of morpho-physiological and spectral properties, the dominant species most closely resembled *Thiocystis* and *Thiorhodococcus minors*¹⁶.

3.2 Fluorescence spectra of water samples

The fluorescence spectra of all natural water samples (not even showing any pronounced maxima on absorption spectra), when excited by light at a wavelength of 390 nm, have two pronounced fluorescence bands at 612 and 675 nm (Fig. 5). In some cases we detected the addition fluorescence band at 643 nm. In bacterial cells BChl fluorescence with a whole intact photosynthetic apparatus is located in the infrared range of the spectrum (at wavelengths exceeding 1000 nm), therefore a fluorescence of BChl *a* cannot be observed in a visible range. We suggested that observed bands at 612 and 675 nm refer to the fluorescence of bacteriochlorophyll *a* monomers. The monomers are formed during the destruction of

antenna complexes of the photosynthetic apparatus of purple sulfur bacteria, in which bacteriochlorophyll is contained in a highly aggregated state.

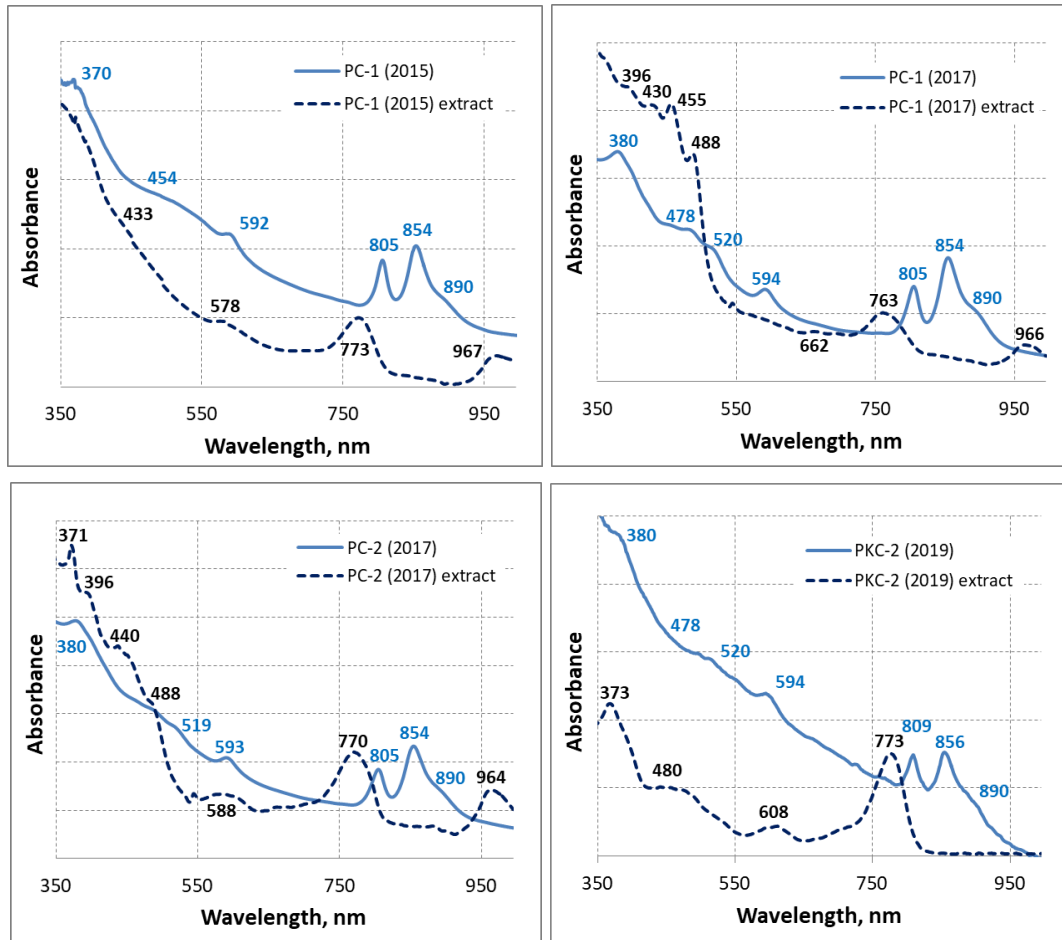


Figure 4. Absorption spectra of purple bacteria sampled from the splash baths (solid lines) and the pigment extraction prepared from the sample (dashed lines).

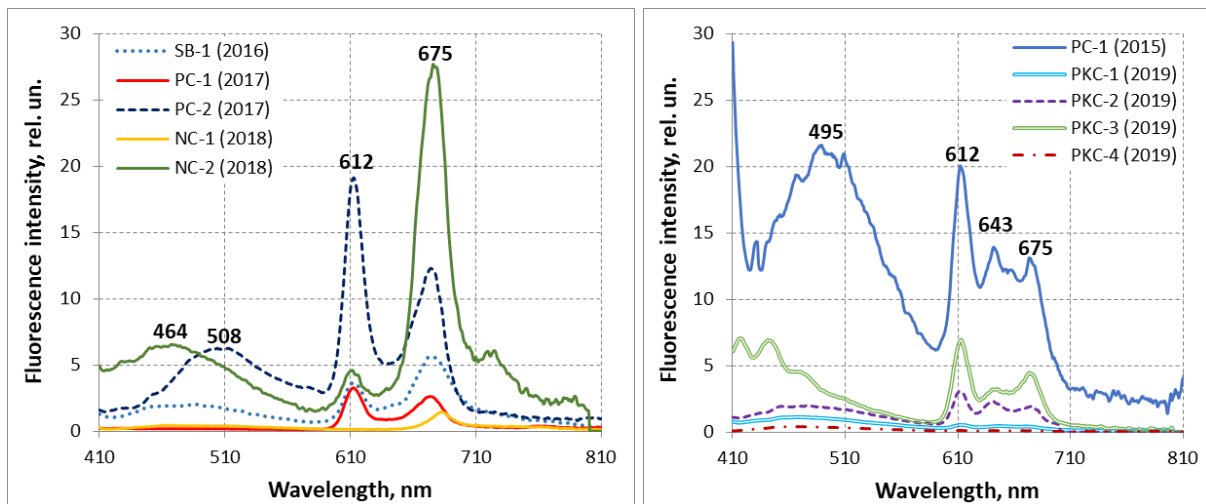


Figure 5. Fluorescence emission spectra excited by light at a wavelength of 390 nm for water from the splash baths sampled in 2015-2019.

The discoloration of some samples after transportation support this hypothesis.

4. CONCLUSIONS

In this work the spectral studies have shown that the bright red and purple color of water is due to the presence of purple sulfur bacteria closely resembled *Thiocystis* and *Thiorhodococcus* minors. We established that chlorophyll-containing algae and cyanobacteria give water green color. The presence of phototrophic microorganisms in the water sampled from the splash baths of Atlasov Island is of great interest for many reasons. Splash baths are special areas, contained bacterial and algae communities, that freeze out in winter and re-create in spring. The fact that each summer season colored water were found in them indicates a cyclical changes in living conditions of microorganisms from favorable to unfavorable habitats. Splash baths with microorganisms have not been observed in other islands of the Great Kuril Islands before. The observation of "colored" water during five summer seasons (but in different places of the island) allows us to conclude that the development of the purple phototrophic bacteria is not an accidental event, but a typical phenomenon in this area. This phenomenon needed further attention in the future. We hope this will contribute to a better understanding of the nature of volcanic factors and the response of ecosystems to them.

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