

Absorption and fluorescence of hydrophobic components of dissolved organic matter in several Karelian lakes with stratified structures

Daria A. Khundzhua^a, Anastasia V. Kharcheva^a, Elena D. Krasnova^b,

Olga M. Gorshkova^c, Kira A. Chevel^c, Viktor I. Yuzhakov^a, Svetlana V. Patsaeva^a

^aFaculty of Physics, Lomonosov Moscow State University, Moscow, Russia, spatsaeva@mail.ru;

^bN.A.Pertsov White Sea Biological Station, Lomonosov Moscow State University, Karelia, Russia;

^cFaculty of Geography, Lomonosov Moscow State University, Moscow, Russia

ABSTRACT

Abstract: Hydrophobic components of chromophoric dissolved organic matter (CDOM) extracted from water samples and sediments taken in several relic basins located on Karelian shoreline of the White Sea were analyzed using spectroscopic techniques. Those water reservoirs exist at various stages of isolation from the White Sea and represent complex stratified systems of fresh and marine water layers not completely mixing through the year. Basins separating from the White Sea are the unique natural objects for investigations of properties CDOM, its transformation in the process of turning the marine ecosystem into freshwater environment. CDOM occurring in all types of natural water represents a significant reservoir of organic carbon and plays a key role in the carbon cycle on the Earth. However, aquatic CDOM and nonliving organic matter in sediments from relic separating basins still have not been studied. The target of this work was to study absorption and fluorescence spectra of hydrophobic components of aquatic CDOM from different water depth and sediments in several separated basins of the Kandalaksha Gulf of the White Sea located near the N.A. Pertsov White Sea Biological Station.

Keywords: chromophoric dissolved organic matter (CDOM), separating basins, hexane extractions, hydrophobic components, absorption spectra, fluorescence spectra, emission wavelength.

1. INTRODUCTION

Humic substances are complex organic polymeric materials of the irregular structure of high molecular weight found in soils, sediments, as well in aqueous ecosystems as a result of transformation of dead biomass.¹⁻² Industrially produced humic acids or their salts (humates) have been studied for multiple technological applications. For instance, it was found that humic acids could enhance the removal of arsenic and co-existing heavy metals from mine tailings.³ Modified with ethylenediamine and triethylenetetramine the commercially produced and natural Algerian humic acids were utilized in electrochemical sensors for determination of nitrite in water.⁴ The Suwannee River humic acid was shown to control the behavior of the suspension of ZnO nanoparticles where it induced disaggregation of nanoparticles and decreased the aggregate size.⁵ The agglomeration and sedimentation of Al₂O₃, SiO₂, and TiO₂ nanoparticles were evaluated after being treated with bovine serum albumin and a commercial humic acid. It was shown that the BSA treatments retarded the sedimentation in most situations; however, HA treatments accelerated the sedimentation greatly in CaCl₂ electrolyte.⁶

Natural humic substances have the ability to emit fluorescence when exposed to the UV radiation⁷⁻⁹. The wavelength of emission maximum depends on the excitation wavelength.¹⁰⁻¹¹ Aquatic CDOM or humic substances of soil origin demonstrate a maximum of fluorescence emission located around 450-460 nm upon excitation at 270 nm. Along with changing the excitation wavelength from 270 to 310 nm the emission band maximum shifts towards shorter wavelengths.¹⁰⁻¹² Such a shift of the emission spectra with increasing excitation wavelength is called the "blue shift".¹³ Studies of industrial humates have shown that the analysis of the fluorescence spectra can be the first step in identifying their structure because the features and characteristics of the fluorescence bands contain information on the composition, structural features and genesis.¹⁴

Although the optical properties of terrestrial humic substances¹⁵⁻¹⁷ and aquatic chromophoric dissolved organic matter (CDOM)¹⁸⁻¹⁹ have been investigated for decades, the structural basis of these properties remains unclear.²⁰⁻²¹ To date, there are two different views on the occurrence of such optical properties of terrestrial and aquatic humic substances. The

first postulates that the absorption (and emission) spectrum results from a simple linear superposition of the absorption (emission) spectra of an ensemble of chromophores (superposition model). In contrast, the second postulates the presence of only a few distinct chromophores or closely related classes of chromophores, which through electronic interaction produce new optical transitions that are responsible for the exponentially decreasing, long wavelength absorption tail (interaction model). Previous spectroscopic results indicate that the fluorescence spectra cannot result solely from a simple linear sum of the spectra of numerous independently absorbing and emitting chromophores, but instead that intramolecular electronic interactions between chromophores must also be playing an important role. The optical properties of humic substances and CDOM were proposed to result in part from intramolecular charge-transfer interactions between hydroxy-aromatic donors and quinoid (or other) acceptors formed through the partial oxidation of lignin and possibly other polymeric hydroxy-aromatic precursors.

Basins separating from the sea are the unique natural objects for investigations of natural organic matter, its form and transformation in the process of turning the marine into fresh water environment. Those water reservoirs exist at various stages of isolation from the White Sea and represent complex stratified systems of fresh and marine water layers not completely mixed through all the year.²¹⁻²² These reservoirs are called as "separating basins" and some of their hydrological and physico-chemical parameters have been described previously.²³⁻²⁶ It was shown that these separating from the White Sea water bodies are complex systems with conditions changing along the vertical profile from oxidative aerobic to anaerobic reductive conditions and representing specific microbial communities around the redox zone.²⁷⁻³¹

Dissolved organic matter (DOM) occurs in all types of natural water giving a significant reservoir of organic carbon and playing a key role in the carbon cycle on the Earth. However, aquatic DOM and nonliving organic matter naturally occurring in water and sediments in the separating basins still have not been studied. The target of this work was to study optical properties of hydrophobic components for aquatic CDOM at different depth layers and sediment organic matter found in relic water basins of the Kandalaksha Gulf of the White Sea.

2. MATERIALS AND METHODS

Hydrophobic components of chromophoric dissolved organic matter (CDOM) extracted from water samples and sediments from several water basins located on the Karelian shoreline of the White Sea were analyzed using spectroscopic techniques. The sampling of water and bottom sediments was performed from a number of reservoirs near the N.A. Pertsov White Sea Biological Station (WSBS) of M.V.Lomonosov Moscow State University, located on the Kandalaksha Gulf of the White Sea.

During the expedition in August 2013 water sampling from each of the studied basins was performed from every 0.5 m from the surface to a maximum depth using a submersible pump, and characteristics like water temperature, salinity, pH and dissolved oxygen concentration were measured in the field. Surface water in the studied lakes has subalkali pH due to good aeration of the lake and the processes of photosynthesis. Deep and bottom waters are sub-acid pH resulting from accumulation of hydrogen sulfide and organic acids as caused by fermentation of organic matter. The exception is the bottom water of Verkhnee Lake which pH is neutral.

For investigation of the hydrocarbon concentrations of hydrophobic components of water dissolved organic matter (DOM) and sediments organic matter (OM) the corresponding hexane extracts were obtained. DOM hydrocarbons were extracted from 100 ml of water by 10 ml of first grade hexane "Kriokhrom" (high purity) with UV absorption at 200 nm of 0.5 r.u./cm. Thus, together with hydrocarbons extraction (as the hydrophobic DOM components), it was their ten-fold concentration from natural waters. The extraction of OM from sediments was carried out at the preliminary preparation of the samples. Sediments previously air-dried, triturated and sieved through a sieve with a pore diameter of 0.5 mm. Next, the sieved sample was brought to constant weight in a SNOL drying oven at 500°C and was extracted by 10 ml of hexane from approximately 0.5 g of the sample. For quantitative characterization of the hydrophobic components of water DOM and bottom sediments OM was determined the concentration of total hydrocarbons for water samples and bottom sediments in the spectrofluorimeter "Fluorat-02-3M". As the standard was used state standard sample: SO Lum-NPG –turbine oil T22 solution on GOST 32-74 in hexane.

To study fluorescence properties of hydrophobic components NOM of the water and bottom sediments were prepared hexane extractions from surface water, from the water column with different depths and samples of bottom sediments. Hydrophobic components of DOM were extracted from 100 ml of water by ten ml of 1st grade hexane "Crogram" with the UV absorbance at 200 nm equal to 0.5 r.u./cm. Thus, with the extraction selection the DOM hydrophobic component grew tenfold concentration of natural waters. Extraction of NOM from bottom sediments was conducted during the

preliminary sample preparation. Samples of bottom sediments was pre-dried in air, ground and sieved through a sieve with a pore diameter of 0.5 mm. Further, the sieved sample was brought in a drying camera to constant weight at 50 °C and extraction was performed with 10 ml of hexane from 0.5 g of the sample using ultrasonic bath.

For the quantitative characterization of hydrophobic components of NOM of the water and bottom sediments the concentration of hydrocarbons (HC) was determined for all water samples and bottom sediments on “Fluorat-02-3M”. “State standard sample” was used as the standard: solution of turbine oil T22 in hexane according to GOST 32-74. Concentrations of hydrocarbons in water samples and sediment are given in the table 1.

3. RESULTS AND DISCUSSION

Hydrocarbon concentration in water samples and bottom sediments are presented in Table 1.

Table 1. Hydrocarbons (HC) in surface, near-bottom waters and bottom sediments of water bodies near the BBS.

Sample	HC in surface water	HC in near-bottom water	HC in bottom sediments
Kislo-Sladkoe lake	-	-	93,12±9,31
Biofiltrov bay , 11m	0,015±0,002	-	28,85±2,89
Biofiltrov bay, 14 m	0,056±0,006	-	-
N.Ershovskoe lake	-	0,057±0,006	94,87±9,49
Verhnee lake	-	0,049±0,005	48,47±4,85
Zeleniy Mis lagoon	0,022±0,002	0,061±0,006	43,85±4,39
Trehcvetnoe lake	0,015±0,002	0,066±0,007	133,40±13,34
Elovoe lake	0,013±0,001	0,041±0,004	83,54±8,35
Vodoprovodnoe lake	0,012±0,001	-	33,97±3,40

Absorption and fluorescence spectra for hexane extracts of natural water DOM and related sediments were measured using UV spectrophotometer Unico and fluorescence spectrometer Solar CM2203. Unlike typical absorption spectra of natural water, where distinct peaks in the UV region are absent, absorption spectra of hexane extracts from eutrophic water horizons and bottom sediments of separating basins showed peaks at wavelengths 223, 264 and 280 nm. In addition to the absorption in the UV region, for hexane extracts from brightly colored eutrophic transitional water layers with high biological productivity the absorption bands of carotenoids in the visible spectrum range from 400 to 500 nm (Fig. 1) were observed.

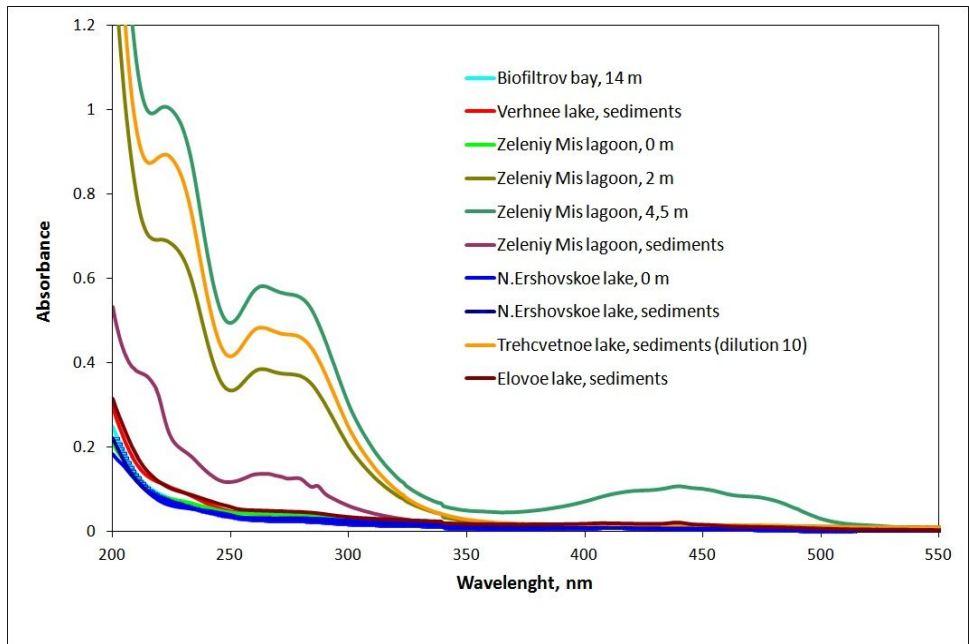


Figure 1. Absorption spectra of hexane extracts of DOM hydrophobic components and sediment in different basins.

On Figure 1 it should be noted Zeleniy Mis lagoon, where with change in depth increases the absorption of DOM hydrophobic components and bottom sediments OM in the 250 – 300 nm area and an absorption band at 450 nm (carotenoids) appears.

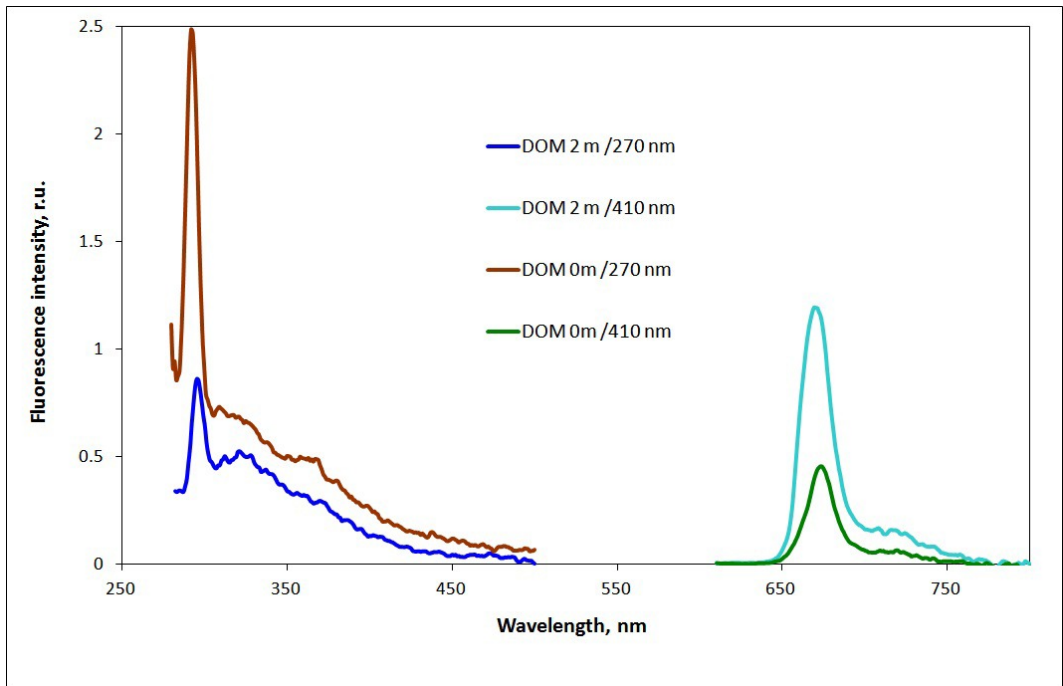


Figure 2. Fluorescence spectra of hexane extractions of hydrophobic components DOM from different depths in the Zeleniy Mis lagoon at the excitation wavelength of 270 and 410 nm.

A typical fluorescence spectrum of DOM hydrophobic components hexane extract consists of two broad overlapping bands: UV fluorescence with a maximum in the region of 300-350 nm (fluorescence of aromatic amino acids or phenolic compounds) and band in the visible spectral range with a maximum of 400-450 nm (humic compounds fluorescence).

The humic compounds fluorescence maximum depends on the wavelength of excitation. The emission maximum shifts toward shorter wavelengths with change of excitation wavelength from 270 to 310 nm (the so-called "blue shift" of the emission spectra). The value of the "blue shift" depends on the type of sample and varies for humic substances from river and sea water. For hexane extracts at short UV excitation wavelengths intensity of fluorescence depends on the concentration of hydrophobic components in the sample (Figure 2). For some samples the fluorescence of chlorophyll a is observed. The intensity of the chlorophyll a fluorescence is higher for the samples of bottom sediments.

4. CONCLUSIONS

1. The greatest amount of hydrocarbons was found in bottom sediments of Trehcvetnoe lake, and the lowest - in the bottom sediments of Vodoprovodnoe lake, Zeleniy Mis lagoon and Biofiltrov bay.
2. The comparison of hydrocarbon concentrations in surface water, bottom water and sediment shows that content of hydrocarbons, as the hydrophobic OM component, in surface water is negligible, but in near-bottom water samples their concentration increases in 3 - 4 times. In the sediments hydrocarbons deposits and their concentration can increase from 2000 to 9000 times. This is due to a large biomass of microorganisms in the bottom water and sediments.
3. Considering the change of oxidative conditions on recovery conditions during the transition from surface to near-bottom water and bottom sediments, it can be assumed that the deposited hydrophobic components are the products of microbiological fermentation, which redact oxidized DOM to the hydrocarbon.
4. Some spectral bands in the UV region with maximum at wavelengths 223, 264 and 280 nm have been observed in the absorption spectra of the hexane extracts of water samples from different depths, near-bottom water and bottom sediments. The intensity of absorption in this region increases with increasing depth.
5. Maximum absorption in the visible region of the spectrum, which is typical for carotenoids, was observed for brightly colored by phototrophic microorganisms transitional water layer (Zeleniy Mis lagoon, 4.5 m). This corresponds to the maximum concentration of photoautotrophic and chemoautotrophic microorganisms in the on the geochemical barrier.
6. Comparative analysis of the DOM hydrophobic components fluorescence spectra showed an increase in the fluorescence intensity of chlorophyll a in the transition from surface waters to bottom waters and bottom sediments.
7. The contribution of aromatic hydrocarbons in the composition of the DOM hydrophobic components (410 nm) increases with increasing depth.

ACKNOWLEDGEMENTS

The work has been supported by Russian Foundation for Basic Research (projects no 13-05-00241_a and 15-04-00525_a).

REFERENCES

- [1] Stevenson, F.J., [Humus chemistry: genesis, composition, reactions (2nd edition)], NY: John Wiley&Sons, 496 pages, (1994)
- [2] Tan, K.H., [Humic matter in soil and the environment: principles and controversies], CRC Press. 386 pages, (2003).
- [3] Wang, S. and Mulligan, C. N., "Comparing the Effects of a Biosurfactant and a Humic Acid on Arsenic Mobilization from Mine Tailings," *Journal of Environmental Engineering* 140(7), (2014).
- [4] Ramdane-Terbouche, C. A., Terbouche, A., Djebbar, S. and Hauchard, D., "Electrochemical sensors using modified electrodes based on copper complexes formed with Algerian humic acid modified with ethylenediamine or triethylenetetramine for determination of nitrite in water," *Talanta* 119, 214-225 (2014).
- [5] Omar, F.M., Aziz, H.A. and Stoll, S., "Aggregation and disaggregation of ZnO nanoparticles: influence of pH and adsorption of Suwannee River humic acid," *Science of the Total Environment* 468, 195-201 (2014).

- [6] Hu, F.B., Lin, Y.F., Chen, R., Ding, L. and Jiang, W., "Effects of humic acid and bovine serum albumin on the agglomeration and sedimentation of oxide nanoparticles," *Journal of Zhejiang University SCIENCE A* 15(8), 643-652 (2014).
- [7] Patsayeva, S. V., Fadeev, V. V., Filippova, E. M. and Yuzhakov V.I., "Temperature and laser ultraviolet radiation influence on luminescence spectra of dissolved organic matter," *Moscow University Phys. Bull* 32 (6), 71-75 (1991).
- [8] Patsayeva, S.V., Fadeev, V.V., Filippova, E.M. and Yuzhakov V.I., "The fluorescence saturation effect of dissolved organic matter," *Moscow University Phys. Bull* 33 (5), 38-42 (1992).
- [9] Del Vecchio, R., Blough, N. V., "On the origin of the optical properties of humic substances," *Environmental Science and Technology* 38, 3885-3891 (2004).
- [10] Gorshkova, O.M., Milukov, A.S., Patsayeva, S.V. and Yuzhakov V.I., "Fluorescence of DOM nanoparticles in natural water," *Proc. SPIE Vol. 6263, Atomic and Molecular Pulsed Lasers VI*, 248-255 (2006).
- [11] Gorshkova, O. M., Patsaeva, S.V., Fedoseeva, E. V., Shubina, D. M. and Yuzhakov V. I., "Fluorescence of dissolved organic matter in natural water," *Water: chemistry and ecology* 11, 31-37 (2009).
- [12] Khundzhua, D. A., Patsaeva, S. V., Terekhova, V. A. and Yuzhakov V. I., "Spectral characterization of fungal metabolites in aqueous medium with humus substances," *Journal of Spectroscopy (Hindawi Publishing Corporation)*, (Article ID 538608) 1-7 (2013).
- [13] Shubina, D. M., Gorshkova, O. M., Patsaeva, S.V., Terekhova, V. A. and Yuzhakov V. I., "The "blue shift" of emission maximum and the fluorescence quantum yield as quantitative spectral characteristics of dissolved humic substances," *EARSel eProceedings* 9, 13-21 (2010).
- [14] Gosteva, O.Yu., Izosimov, A.A., Patsaeva, S.V., Yuzhakov, V.I. and Yakimenko, O.S., "Fluorescence of aqueous solutions of commercial humic products," *Journal of Applied Spectroscopy* 78(6), 884-891(2012).
- [15] Ma, J., Del Vecchio, R., Golanosky, K. S., Boyle, E. S. and Blough, N. V., "Optical Properties of Humic Substances and CDOM: Effects of Borohydride Reduction," *Environmental Science and Technology* 44, 5395-5402 (2010).
- [16] Boyle, E. S., Guerriero, N., Thiallet, A., Del Vecchio, R. and Blough, N. V., "Optical Properties of Humic Substances and CDOM: Relation to Structure," *Environmental Science and Technology* 43, 2262-2268 (2009).
- [17] Green, S. A. and Blough, N. V., "Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters," *Limnology and Oceanography* 39(8), 1903-1916 (1994).
- [18] Blough, N.V. and Del Vecchio, R., [Hansel, D.A., Carlson, C.A. (Eds.)], *Biogeochemistry of Marine Dissolved Organic Matter*, "Chromophoric DOM in the coastal environment," Academic Press, San Diego, California, 509-546 (2002).
- [19] Trubetskoj, O.A., Trubetskaya, O.E. and Richard, C., "Photochemical activity and fluorescence of aquatic humic matter," *Water Resour* 36, 518-524 (2009).
- [20] Piccolo, A., "The Supramolecular Structure of Humic Substances: a Novel Understanding of Humus Chemistry and Implications in Soil Science," *Advances in Agronomy* 75, 57-134 (2002).
- [21] Krasnova, E.D. and Pantylin, A.N., "Sweet-and-sour lakes full of wonders," *Priroda* 2, 39-48 (2013).
- [22] Shaporenko, S., Korneeva, G., Pantyulin, A. and Pertsova, N., "The features of ecosystems of separating water basins in the Kandalaksha Gulf of the White Sea," *Water resources* 32(5), 517-532 (2005).
- [23] Krasnova, E.D., Pantyulin, A.N., Belevich, T.A., Voronov, D.A., Demidenko, N.A., Zhitina, L.S., Ilyash, L.V., Kokryatskaya, N.M., Lunina, O.N., Mardashova, M.V., Prudkovsky, A.A., Savvichev, A.S., Filippov, A.S. and Shevchenko, V.P., "Multidisciplinary studies of the separating lakes at different stage of isolation from the White Sea performed in March 2012," *Oceanology* 53 (5), 714-717 (2013).

- [24] Kharcheva, A.V., Meschankin, A.V., Lyalin, I.I., Krasnova, E.D., Voronov, D.A., Patsaeva, S.V., “The study of coastal meromictic water basins in the Kandalaksha Gulf of the White Sea by spectral and physicochemical methods,” Proceedings of SPIE – The International Society for Optical Engineering 9031, (2014).
- [25] Krasnova, E., Voronov, D., Frolova, N., Pantyulin, A., Samsonov, T., “Salt lakes separated from the White sea,” EARSeL eProceedings 14 (S1), 8–22 (2015).
- [26] Vinogradov, D., Varlamov, S., Volovich, N., Kuznetsov, V., Grigoryeva, A., Mardashova, M., Krasnova, E., “Hydrological and spectrophotometry research on Kislo-Sladkoye lake,” EARSeL eProceedings 14 (S1), 55–62 (2015).
- [27] Krasnova, E.D., Pantyulin, A.N., Matorin, D.N., Todorenko, D.A., Belevich, T.A., Milyutina, I.A., Voronov, D.A., “Cryptomonat alga *Rhodomonas* sp. (*Cryptophyta*, *Pyrenomonadaceae*) bloom in the redox zone of the basins separating from White Sea,” Microbiology 83 (3), 270-277 (2014).
- [28] Savvichev, A.S., Lunina, O.N., Rusanov, I.I., Zakharova, E.E., Veslopolova, E.F., Ivanov, M.V., “Microbiological and isotopic geochemical investigation of Lake Kislo-Sladkoe, a meromictic water body at the Kandalaksha Bay shore (White Sea),” Microbiology 83 (1), 56-66 (2014).
- [29] Kharcheva, A.V., Krasnova, E.D., Voronov, D.A., Patsaeva, S.V., “Spectroscopic study of the microbial community in chemocline zones of relic meromictic lakes separating from the White Sea,” Proceedings of SPIE – The International Society for Optical Engineering 9448 (2015).
- [30] Krasnova, E.D., Kharcheva, A.V., Milyutina, I.A., Voronov, D.A., Patsaeva, S.V., “Study of microbial communities in redox zone of meromictic lakes isolated from the White Sea using spectral and molecular methods,” Journal of the Marine Biological Association of the United Kingdom, 95 (8), 1579-1590 (2015).
- [31] Lunina, O.N., Savvichev, A.S., Kuznetsov, B.B., Pimenov, N.V., Gorlenko, V.M., “Anoxygenic Phototrophic Bacteria of the Kislo-Sladkoe Stratified Lake (White Sea, Kandalaksha Bay),” Microbiology 82 (6), 815–832 (2013).
- [32] Kapitsa, A. P., Krasnushkin, A. V., [Laboratory techniques to study and control environmental conditions.] (in Russian), Faculty of Geography MSU, Moscow, 180 pages, (2008).