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INFLUENCE OF THE CONDITIONS OF PRESERVING WATER SAMPLES AND THEIR DELAYED PROCESSING ON THE LIGHT ATTENUATION COEFFICIENT SPECTRA AND THE CONCENTRATIONS OF WATER CONSTITUENTS

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Abstract. The influence of the conditions of preserving water samples and their delayed processing on the results of measuring water properties were studied using the data obtained for three Estonian lakes, the Pärnu River, and Pärnu Bay. The following characteristics were under investigation: spectral values of the beam attenuation coefficient of filtered and unfiltered water (the former shows the contribution of yellow substance to the light attenuation in water bodies), and concentrations of chlorophyll *a* and suspended matter. The results show that for the determination of the beam attenuation coefficient and suspended matter the water samples have preferably to be preserved in cold and dark, but unfrozen. The "errors of delay" for a warm room exceeded those for a cold room by a factor of two or even three. For determining the concentration of chlorophyll *a* preservation of water samples at room temperature and light was found to be the most unsuitable (after 10 days the results decreased 2–7 times), it is better to freeze the water sample and to melt it quickly before analysis.

Key words: water properties, water constituents, measuring error.

INTRODUCTION

Growing human impact on water bodies, as well as their natural eutrophication, brings about the need to investigate and predict the ecological state of seas and lakes. For this purpose one has to carry out hydrophysical, hydrochemical, hydrobiological, and optical measurements in the water. An important part of these measurements is the determination of water properties and concentrations of

various constituents in water samples in the laboratory. To obtain authentic results, the processing of the water samples has to be performed immediately after taking them from a water body. However, this is sometimes impossible, e.g. during expeditions on lakes and in coastal regions of seas on board a small ship or boat without a moving laboratory on the shore. In these cases one has to preserve the water samples (or filtrates) till the end of the expedition when the materials are taken to a laboratory and their processing can begin. Naturally, some errors in the results of measurements are expected due to this delay. In this paper we present some estimations of the influence of this delay, trying to find out the change in the results for the following water characteristics: (1) spectral values of the beam attenuation coefficient; (2) spectral values of the beam attenuation coefficient of filtered water (showing the contribution of yellow substance to the light attenuation in the water); (3) concentration of chlorophyll *a*; and (4) concentration of suspended matter.

The natural water has to be considered as a multicomponental system. Its constituents may be divided by their sizes into three groups: dissolved matter (size of particles $< 10^{-6}$ mm), colloids (10^{-6} to 10^{-4} mm), and suspended matter ($> 10^{-4}$ mm). The main constituent of the first group is dissolved organic matter or yellow substance. The colloids are of organic or mineral origin. The colloid systems are rather stable in natural waters, because their sedimentation is very slow due to the small size of the particles. The suspended matter consists of phyto- and zooplankton, particles of sand and mud, poorly soluble hydroxides of metals, and some organic substances.

One can expect that the composition of water samples can vary depending on the time and conditions of their preservation, on the values of pH, and the type of water.

MEASUREMENTS AND METHODS

The beam attenuation coefficient spectra determined from water samples are of rather great interest. However, the measurement of this coefficient is complicated. Theoretically, the beam transmittance should contain no contribution from scattering, but in reality small-angle forward scattering does reach the detector. That is why the measured transmittance exceeds the theoretical value and the attenuation coefficient determined from the measured transmittance is less than its true value. Some estimations of the relationship between the real and measured values of the beam attenuation coefficient are presented in the papers by Zaneveld et al. (1992) and Bricaud et al. (1995). At the detector acceptance angle (0.9°) used in the Sea-Tech transmissometers, it was found that the difference between actual and measured beam attenuation coefficients ($c - c_m$) was 4–10% of the total scattering coefficient (b) for various volume scattering functions, i.e.:

$$c = c_m + 0.07(\pm 0.03)b. \qquad (1)$$

Therefore a specially designed device and the corresponding data processing techniques are necessary for measuring the beam attenuation coefficient of some light absorbing and scattering substance. We have at our disposal only a commercial spectrophotometer Hitachi U1000. It is designed for measuring the spectral absorption coefficients of solutions that practically do not scatter the light. The results of the measurements give us the difference between the absorption coefficient of the solution and that of distilled water. By treating the lake water samples we get the value $c^*(\lambda)$:

$$c^*(\lambda) = c(\lambda) - \Delta b(\lambda) - c_d(\lambda), \qquad (2)$$

where $c(\lambda)$ is the real beam attenuation coefficient, $\Delta b(\lambda)$ is the contribution of the small-angle forward scattering to the measured radiation, and $c_d(\lambda)$ is the attenuation coefficient of distilled water (all in m^{-1}). The correction $\Delta b(\lambda)$ is not automatically equal to $0.07(\pm 0.03)b$ (Eq. 1), because our measurement device is different and the value of this correction may depend on the turbidity of water. The ratio $\Delta b(\lambda)/c^*(\lambda)$, which characterizes the underestimation of $c(\lambda)$, is not constant, but depends on the scattering properties, concentration and size of the scattering particles in water. To estimate the $\Delta b(\lambda)$ value, special investigations and apparatus are needed. However, our measurement results using the Hitachi spectrophotometer show that the $c^*(\lambda)$ spectra are rather good indicators of water transparency and quality. Therefore we decided to use these spectra as one characteristic to describe the properties of different lakes. We named it "the spectrometric attenuation coefficient" (if $\Delta b \approx 0$, then its value equals the real beam attenuation coefficient, $c(\lambda)$).

Since it is extremely difficult to determine individual organic compounds of yellow substance, the optical determination has distinct advantages over chemical analytic techniques (Dera, 1992). Using the optical method, we have to measure the absorption spectra of filtered water. The value of the absorption coefficient (in m^{-1}) at some reference wavelength is often considered as a characteristic of the yellow substance concentration in the water. The water samples were filtered through cellulose acetate filters (pore size 0.45 μm) and the corresponding spectra were determined with a Hitachi U1000. Since the scattering of light by filtered water is rather small, it may be assumed that the results of Hitachi measurements give approximately the spectra of absorption coefficients for filtered water samples. The errors caused by possible influence of the (weak) scattering by colloids are discussed in the papers by Bricaud et al. (1981), Davies-Colley & Vant (1987), and Mäekivi & Arst (1996). For estimating the yellow substance in the water we used these absorption values at the wavelength 350 nm.

For determining the chlorophyll *a* concentrations (C_{chl}) the water sample was filtered through the Whatman GF/F or GF/C filter (Ø 47 mm, pore size 0.70 or 1.2 μm) and fixed by adding 1% $MgCO_3$. After that chlorophyll was extracted by 90% solution of acetone or ethanol and the extract was analysed with a Hitachi

U1100 or U1000 spectrophotometer. The value of C_{chl} was then computed from the absorption at 630, 647, and 665 nm (acetone solution) or from the absorption at 665 nm (ethanol solution). The concentration of suspended matter (C_S) was determined by its dry weight after the filtration of the water through cellulose acetate filters (Millipore, Ø 25 mm, pore size 0.45 μm).

RESULTS AND DISCUSSION

For the estimation of the change of $c^*(\lambda)$ spectra with time we have data of measurements performed in lakes Nohipalu Valgjärv, Verevi, and Ülemiste. Some results of $c^*(\lambda)$ measurements with water samples processed immediately and after seven days are presented in Figs. 1 and 2. The water samples were preserved in a refrigerator (cold and dark room). For clear-water Lake Nohipalu Valgjärv (relative transparency z_{SD} = 5 m) the variation of $c^*(\lambda)$ spectra was in the limits of measurement errors. For Lake Verevi (z_{SD} = 2.4 m) the differences were bigger, especially for the surface layer. Table 1 presents the values of $c^*(\lambda)$ averaged over the PAR range (400–700 nm) for four stations of Lake Ülemiste (z_{SD} = 0.75–1.0 m). The delay of processing was 9 days, part of the water samples were preserved in a refrigerator (at 2–5 °C), the other part in a closet (20–22 °C).

These data show that water samples should not be preserved at room temperature, even in dark. Preservation in a cool and dark room practically did not change the values of $c^*(\lambda)$ for clear-water lakes; however, errors connected with delay would probably grow with increasing water turbidity. To find out the

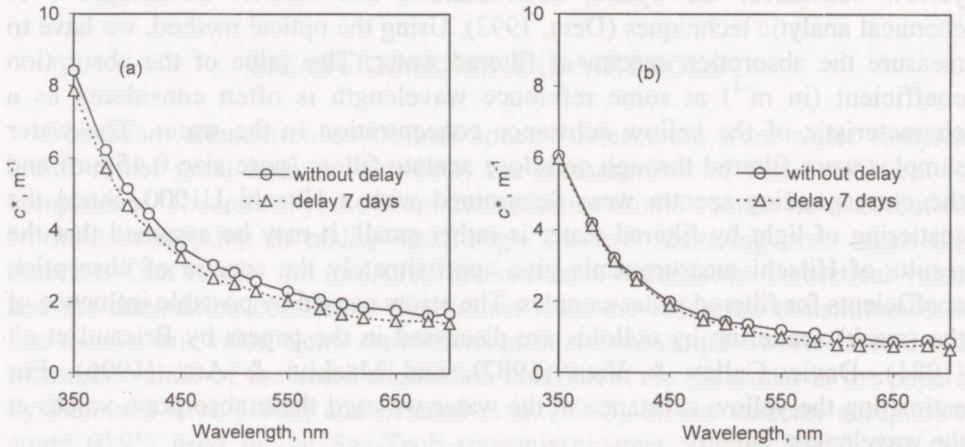

Fig. 1. The influence of one-week delay in the processing of water samples on beam attenuation coefficient (c^*) for Lake Nohipalu Valgjärv (Sept. 1996, Secchi depth 5 m): a, depth 0.5 m; b, depth 3 m. The water samples were preserved in dark at about 2–5 °C.

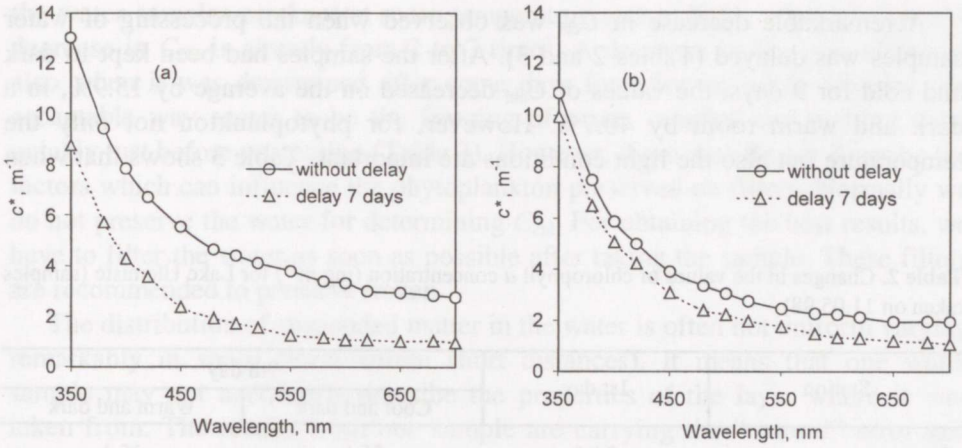

Fig. 2. The influence of one-week delay in the processing of water samples on beam attenuation coefficient (c^*) for Lake Verevi (Sept. 1996, Secchi depth 2.4 m): a, depth 0.5 m; b, depth 2 m. The water samples were preserved in dark at about 2–5 °C.

Table 1. Changes in the value of $c^*(400–700)$ (in m^{-1}) caused by the delay in the processing of water (stations in Lake Ülemiste, Secchi depth 0.75–1.0 m, samples taken on 11.05.98)

Station	1st day	9th day	
		Cool and dark	Warm and dark
Plant	7.1	6.8	4.0
Centre	6.7	6.3	4.2
Kurna	6.6	5.5	4.0
Loodus	6.6	4.4	4.1

exact relationship between the "delay errors" and water type, preserving time and conditions, additional experiments are needed. It must be also noted that each measurement result (even without delay) includes a "natural" measurement error. By our estimations the "natural" instability of $c^*(\lambda)$ spectral curve is 5–10%; for very small $c^*(\lambda)$ values it may be 20–40%.

As told above, the concentration of yellow substance is estimated through its optical influence by measuring the absorption spectra at the wavelength 350 nm. Our experiments show that the values of the absorption coefficient $a_y(350)$ determined from filtered water practically do not depend on the temperature of preservation, but they change with time. Results for Lake Ülemiste (water samples taken on 25.06.97 and 11.05.98) show a 1.02 to 1.5 times growth of $a_y(350)$ during a week.

A remarkable decrease in C_{chl} was observed when the processing of water samples was delayed (Tables 2 and 3). After the samples had been kept in dark and cold for 9 days, the values of C_{chl} decreased on the average by 15.9%, in a dark and warm room by 40.7%. However, for phytoplankton not only the temperature but also the light conditions are important. Table 3 shows that when

Table 2. Changes in the values of chlorophyll *a* concentration (mg m^{-3}) for Lake Ülemiste (samples taken on 11.05.98)

Station	1st day	9th day	
		Cool and dark	Warm and dark
Centre	34.6	25.1	15.1
Kurna	33.1	32.1	23.0
Loodus	30.3	26.7	23.0
Pirita	22.6	19.4	13.7
Plant	29.3	22.6	13.7

Table 3. Changes in the values of chlorophyll *a* concentration (mg m^{-3}) for the Pärnu River and Pärnu Bay

Sampling date	Preserving conditions*	Delay in days								
		0	2	4	8	10	15	17	22	25
Experiment 1: water from the Pärnu River										
24.04.98	Room temperature	2.72	–	–	–	1.33	–	1.06	–	0.94
	Frozen and quickly melted	2.72	–	–	–	2.00	–	2.44	–	–
	Frozen and melted 24 h	2.72	–	–	–	1.34	–	–	–	–
11.05.98	Room temperature	2.38	1.51	1.63	1.10	–	0.38	–	0.55	–
	Frozen and quickly melted	2.38	–	–	1.86	–	1.86	–	1.68	–
	Frozen and melted 24 h	2.38	–	–	1.50	–	2.19	–	1.34	–
Experiment 2: water from Pärnu Bay										
24.04.98	Room temperature	14.95	–	–	–	2.13	–	1.49	–	0.81
	Frozen and quickly melted	14.95	–	–	–	5.55	–	8.98	–	–
	Frozen and melted 24 h	14.95	–	–	–	2.42	–	–	–	–
11.05.98	Room temperature	6.0	4.46	4.13	1.63	–	1.61	–	1.36	–
	Frozen and quickly melted	6.0	–	–	4.79	–	4.56	–	–	–
	Frozen and melted 24 h	6.0	–	–	3.05	–	4.16	–	3.9	–

* All samples preserved at room temperature were kept in normal daylight conditions in the laboratory;
– not analysed.

the water samples are kept at room temperature and in light, after 10 days the decrease in C_{chl} is already from 2 to 7 times. A decrease in C_{chl} was observed also when it was determined after some days from frozen water samples. An acceptable way seems to be the freezing of water samples and melting them quickly just before processing (Table 3). However, these data do not describe the factors which can influence the phytoplankton preserved on filters. Normally we do not preserve the water for determining C_{chl}. For obtaining the best results, we have to filter the water as soon as possible after taking the sample. These filters are recommended to preserve frozen.

The distribution of suspended matter in the water is often not uniform varying remarkably in space (even within short distances). It means that one water sample may not adequately describe the properties of the layer where it was taken from. The results from one sample are carrying the "natural" error and also a possible error due to spatial variation of substances in the water body. This is true also for chlorophyll content determined from phytoplankton in water samples. According to the estimates by the Pärnu Laboratory of the Estonian Marine Institute the measurement error should not exceed 13% for big values of C_S ($> 30\ mg\ L^{-1}$), but for small values of C_S it can be remarkable (if $C_S < 3\ mg\ L^{-1}$, then its relative error can be even more than 100%).

We have some indirect data for estimating the instability of results for C_{chl} in the same water body. Figure 3 presents the comparison of C_{chl} values for five Finnish lakes measured in the laboratories of the Estonian Marine Institute and the Finnish Environment Institute. The water samples were taken from the same lakes on the same day, but at a distance of 10–200 m from one another. These data show that simultaneous C_{chl} measurement results could be even 1.5 times different for the same water body. However, we cannot be sure that these differences reflect only the spatial change of chlorophyll in lakes, but they can be (to some extent) caused also by "laboratory" errors.

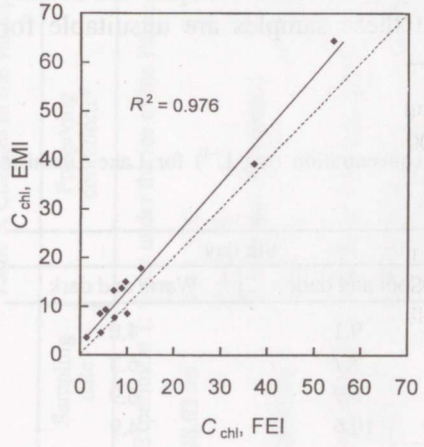

Fig. 3. Comparison of C_{chl} values measured in water samples taken from five Finnish lakes in two replicates (different boats, distance 10–200 m, time difference 0.1–3 h). Measurements were performed in summer 1997 by the Estonian Marine Institute (EMI) and the Finnish Environment Institute (FEI).

To determine C_S by the dry weight method it is necessary to pump rather a large amount of water through the filter. This cannot be performed with a small vacuum pump, a stronger (and bigger) pump is needed. Therefore, often filtration can begin only after the field work. Consequently, the preserving conditions (temperature, light) of water samples are important. Tables 4 and 5 demonstrate the results of experiments where five different combinations of preserving conditions were used: (1) in a cold and dark room (refrigerator) at 2–5 °C; (2) in dark, but at room temperature; (3) at room temperature and in light; (4) frozen samples, melted quickly; (5) frozen samples melted slowly (during 24 h). As shown in Table 4, a nine-day delay brings about a decrease in C_S: in a cool and dark room by 10–25%, in a warm and dark room by 30–60%. The results obtained keeping the water samples in the light at room temperature (Table 5) are somewhat contradictory to those presented above: after 20–25 days the value of C_S had increased very slightly (experiment 3) or increased 1.35–2.34 times (experiments 1 and 2). This increase did not coincide with the results when the water samples were kept at room temperature but in dark (Table 4). Additional investigations are needed to determine how much different light conditions affect the results.

The values of C_S obtained after preserving the water samples at 1.5 °C (refrigerator) for 1–20 days varied rather little, from –16 to +5% during this period (Table 5, experiment 1). This does not coincide with data in Table 4, but is not necessarily contradictory. The conclusion is that the preserving of the samples in a cold and dark room brings about changes (mostly a decrease) not exceeding 25%.

Preservation of the water samples in frozen form brings about a serious increase in the results for C_S (Table 5). After one week the differences were within the limits of 88–400% (with one exception). The results depend also on the speed of melting. The general conclusion is that for the preservation of water samples for suspended matter analysis the most suitable conditions are 2–10 °C and a dark room. As known, for nutrients analysis the water samples are usually kept frozen. However, our results show that these samples are unsuitable for measuring the C_S values.

Table 4. Changes in the values of suspended matter concentration (mg L^{-1}) for Lake Ülemiste (samples taken on 11.05.98)

Station	1st day	9th day	
		Cool and dark	Warm and dark
Centre	10.4	9.1	4.0
Kurna	10.3	7.7	6.3
Loodus	10.2	8.9	6.9
Plant	11.8	10.6	4.9

Table 5. Changes in the values of suspended matter concentration (mg L^{-1}) for the Pärnu River and Pärnu Bay

Sampling date	Preserving conditions*	Delay in days												
		0	1	2	4	7	8	10	14	15	17	20	22	25
Experiment 1: water under the ice of the Pärnu River														
28.03.96	$t = 1.5$ °C	5.33	4.4	–	5.6	5.2	–	–	5.5	–	–	5.0	–	–
	$t = 21$ °C	5.33	6.0	–	5.6	5.6	–	–	11.0	–	–	12.5	–	–
	Frozen and then melted	5.33	–	–	–	27.2	–	–	13.4	–	–	–	–	–
29.03.96	$t = 17$ °C	5.00	–	–	5.6	4.5	–	–	6.5	–	–	4.5	–	–
Experiment 2: water from the Pärnu River														
24.04.98	Room temperature	9.0	–	–	–	–	–	11.0	–	–	12.0	–	–	13.1
	Frozen and quickly melted	9.0	–	–	–	–	–	31.0	–	–	14.0	–	–	–
	Frozen and melted 24 h	9.0	–	–	–	–	–	18.0	–	–	–	–	–	–
11.05.98	Room temperature	5.0	–	5.0	6.7	–	6.1	–	–	10.6	–	–	9.4	–
	Frozen and quickly melted	5.0	–	–	–	–	11.7	–	–	28.8	–	–	25.0	–
	Frozen and melted 24 h	5.0	–	–	–	–	9.4	–	–	21.3	–	–	23.0	–
Experiment 3: water from Pärnu Bay														
24.04.98	Room temperature	5.0	–	–	–	–	–	7.0	–	–	5.0	–	–	5.6
	Frozen and quickly melted	5.0	–	–	–	–	–	10.0	–	–	14.0	–	–	–
	Frozen and melted 24 h	5.0	–	–	–	–	–	5.5	–	–	–	–	–	–
11.05.98	Room temperature	5.6	–	7.0	8.0	–	6.5	–	–	14.4	–	–	13.1	–
	Frozen and quickly melted	5.6	–	–	–	–	14.8	–	–	18.8	–	–	–	–
	Frozen and melted 24 h	5.6	–	–	–	–	6.0	–	–	17.5	–	–	10.0	–

* All samples preserved at room temperature were kept in normal daylight conditions in the laboratory;
– not analysed.

CONCLUSIONS

1. A delay of 7–10 days in processing water samples kept in a refrigerator without freezing (cold and dark room) has some influence on the light attenuation coefficient spectra and its mean value for the PAR region measured with a Hitachi U1000 spectrophotometer in laboratory. The spectra of $c^*(\lambda)$ from a clear-water lake show only a slight change during a week. The errors grow with increasing turbidity of water. Preservation of water samples in a warm room is even less suitable with "errors of delay" exceeding those for a cold room by a factor of two.

2. The errors for filtered water practically do not depend on preserving conditions. The values of the yellow substance absorption coefficient determined using filtered water at the wavelength 350 nm change slowly, in most cases showing an increase with time.

3. A few days delay in the determination the C_{chl} from water samples brings about a decrease in its values. The most unsuitable conditions for preserving water samples are at room temperature and in light (during 10 days C_{chl} decreased 2–7 times); the best way is to freeze the water sample and to melt it quickly before analysis.

4. The results for suspended matter concentrations were somewhat contradictory. In dark a decrease in C_S with time was observed (at 2–5 °C it was 10–25%, at 20–22 °C 30–60%). However, at room temperature and in the light the value of C_S stayed almost stable or a remarkable increase (after 20 days 1.3–2.3 times) was observed. Especially big and hard to prognosticate errors occurred when the water samples were preserved frozen: after one week the increase in C_S was 88–400%.

5. The analysis of data obtained shows that it is not an easy task to estimate the influence of delay in the processing of water samples, preserved in different conditions, on the results of the determination of $c^*(\lambda)$, $a_y(\lambda)$, C_S, and C_{chl}. Experiments give sometimes even contradictory results, which probably depend also on the properties of water under consideration. Continuation of the investigations, involving different water types and different preserving conditions, is necessary.

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VEEPROOVIDE SÄILITUSTINGIMUSTE JA NENDE ANALÜÜSI AJALISE VIIVITUSE MÕJU SUUNATUD KIIRGUSE NÕRGENEMISKOEFITSIENDI JA VEE KOOSTISOSADE KONTSENTRATSIOONI MÕÕTMISTULEMUSTELE

Helgi ARST, Ants ERM, Kalle KALLASTE ja Sirje MÄEKIVI

Kasutades mõõtmistulemusi kolme Eesti järve, Pärnu jõe ja Pärnu lahe kohta, uuriti veeproovide säilitustingimuste ja nende analüüsi ajalise viivituse mõju vee omaduste ja koostisosade hindamisel. Vaadeldi järgmisi näitajaid: 1) suunatud kiirguse nõrgenemiskoefitsient filtreeritud ja filtreerimata vees (esimene neist näitab kollase aine osatähtsust valguse nõrgenemisel veekogudes); 2) klorofüll *a* kontsentratsioon; 3) heljumi kontsentratsioon. Tulemused näitavad, et kiirguse nõrgenemiskoefitsiendi ja heljumi hulga määramiseks peaks eelistama veeproovide säilitamist pimedas ja jahedas ruumis (külmkapis), kuid mitte külmutatult. Toatemperatuuril säilitamisel võivad viivitusvead olla 2–3 korda suuremad kui jahedas ruumis säilitamise puhul. Klorofülli hulga määramisel on veeproovide säilitamiseks eriti ebasobiv toatemperatuur ja -valgustus (kontsentratsioon väheneb kümne päeva jooksul 2–7 korda), parem on külmutada veeproovid ja enne mõõtmist sulatada nad kiiresti. Uuringute jätkamine on kindlasti vajalik, et täpsustada seoseid veeproovide hoiutingimuste, ajalise viivituse ja veetüüpide vahel.