

Comparison of two spectrophotometric methods of chlorophyll "a" determination in the sea water samples

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Concentration of chlorophyll "a" in sea water is an important parameter in numerous oceanological investigations. There are many spectrophotometric and fluorometric methods of chlorophyll "a" determination (Konovalov, Mordasova, 1970; Shibata *et al.*, 1954). A spectrophotometric method based on the measurements of absorption of water/acetone extracts of samples was accepted as a standard one for chlorophyll "a" determination (UNESCO, 1966; Edler, 1977).

There is, however, a possibility of applying a non-extractive method, first proposed by Yentsch (1957) and modified by Konovalov (Konovalov, Bekasova, 1969; Konovalov, Mordasova, 1970). This method was used in many research cruises organized by the Institute of Oceanology, Academy of Sciences of the USSR. This method does not require extraction of the material settled on a filter, and due to this fact all the possible errors related to this process can be avoided. The method is based on direct measurements of absorption spectra of the material settled on a filter. For this type of measurements a special type of a spectrophotometer, equipped with an integrating sphere, is required.

A comparison of concentrations of chlorophyll "a" measured with the use of both extractive and non-extractive methods is presented in this paper. In both cases, the obtained results are a sum of chlorophyll "a" and a pheophytin. 80 samples of sea water were collected at station Z (central part of the Gulf of Gdańsk) during the Experiment Sopot '87 and they were taken from the following depths: 0, 1, 2, 3, 5, 7, 10, 15, 20, and 30 m. Whatman GF/F (\varnothing 45 mm) filters with pores being 0.7 μ in diameter were used to filter the samples. Each filter was divided into two parts (3/4 of the filter was used in the extractive method and 1/4 in the non-extractive one). This procedure allowed to compare the results obtained from the same sample but by means of two different methods.

Measurements of absorbance of chlorophyll in acetone extracts were conducted in a SPECORD M 40 (Carl Zeiss, GDR) spectrophotometer.

In a case of the non-extractive method the absorption spectra were recorded with a spectrophotometer Sp-18 (ŁOMO, USSR). Concentration of chlorophyll "a" was calculated using the Konovalov equation (Koblenz-Mishke, Konovalov, 1974):

$$Ba^{ne} = 4.17 \left(D_{680} \cdot \frac{S}{V} \right)^{1.28},$$

where:

D_{680} —absorption of a filter with the "sediment" measured at $\lambda = 680$ nm,

s —surface of the filter (in $[cm^2]$),

V —volume of sample (in $[dm^3]$).

Concentrations of chlorophyll in the water samples used in this experiment (measured with the extractive method) varied from 2 to $30\text{ mg} \cdot m^{-3}$. The equation of linear regression (Fig. 1) was calculated on the basis of 80 simultaneous

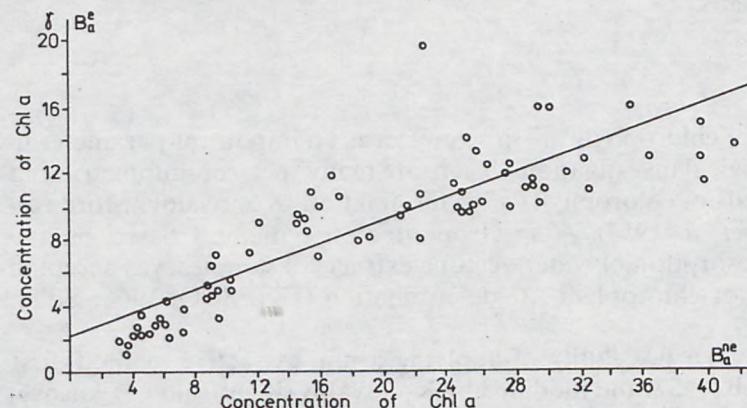


Fig. 1. Correlation between the chlorophyll "a" concentrations obtained with the extractive (Ba^e) and non-extractive (Ba^{ne}) method [$\text{mg} \cdot m^{-3}$]

(completed with the two methods) measurements of chlorophyll "a" concentration.

$$Ba^{ne} = 0.72 Ba^e + 3.28,$$

where:

Ba^{ne} —concentration of chlorophyll "a" measured by the non-extractive method,
 Ba^e —concentration of chlorophyll "a" measured by the extractive method.

A correlation coefficient was equal to 0.871. The mean values were equal to $\langle Ba^{ne} \rangle = 12.9$, $\langle Ba^e \rangle = 13.32$ and the standard deviations for Ba^{ne} and Ba^e were equal to 6.34 and 7.64, respectively.

As follows from Figure 1, the measuring points begin to deviate from the obtained regression line starting from the concentration $15\text{ mg} \cdot m^{-3}$ (according to the non-extractive method). This figure indicates that the concentrations of chlorophyll obtained by the non-extractive method are lower as compared with

those obtained by the extractive one. Taking the above into consideration it can be stated that the non-extractive method allows to obtain the results which are in agreement with the results of the extractive one but only when the absorption, measured at $\lambda = 680$ nm, of the seston on a filter does not exceed 1. Consequently, the volume of water to be filtered and the filtering surface should be chosen in such a way that the absorption would be not exceed 1.

Taking these remarks into consideration, the equation describing the regression line was recalculated and only those measurements for which $Ba^{ne} < 15 \text{ mg} \cdot \text{m}^{-3}$ were taken into account. The following equation was obtained:

$$Ba^{ne} = 1.13 Ba^e + 0.27.$$

The correlation coefficient was higher (0.913) and the mean values were equal to $\langle Ba^{ne} \rangle = 8.45$, $\langle Ba^e \rangle = 7.23$. The standard deviations for Ba^{ne} and Ba^e were equal to 4.69 and 3.70, respectively. For both methods and for each depth the average concentration obtained from all the measurements carried out at this depth was calculated. In this way, the mean vertical distributions for both methods (Fig. 2) were obtained. The vertical distributions of chlorophyll show its

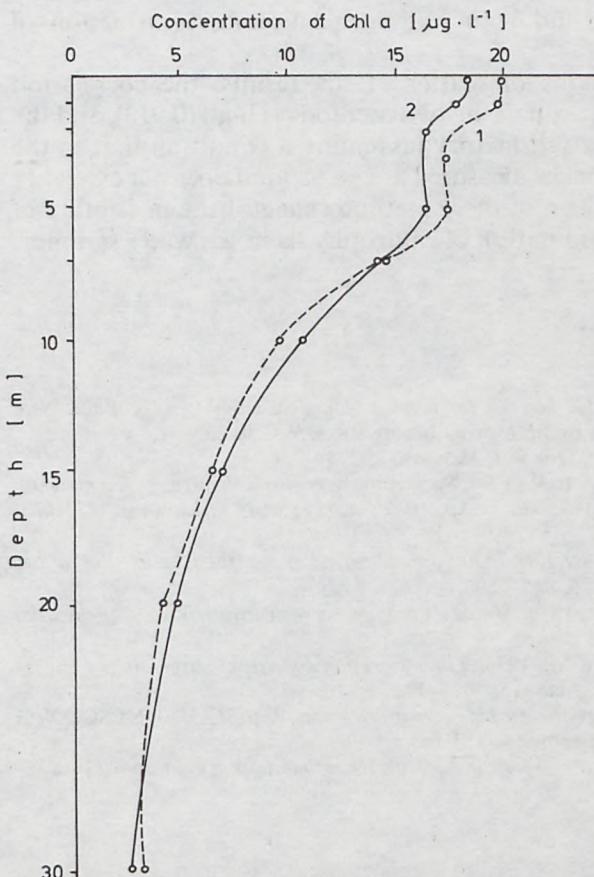


Fig. 2. Mean vertical profiles of chlorophyll "a" concentrations obtained by the two methods
1—extractive method, 2—non-extractive method

high concentrations in the surface homogeneous layer (0–5 m) and a decrease of chlorophyll concentrations with the depth (10–30 m). The vertical distributions of mean concentrations differ for both methods in the surface layer where the concentrations were higher than $15 \text{ mg} \cdot \text{m}^{-3}$.

In two cases (for depths of 2 and 3 m), the difference between concentrations measured with the two methods exceeded 20%. These cases were considered to be random errors and they were excluded from the analysis.

The error in comparison of the two methods can arise from the inaccuracy when dividing the filter and from an uneven distribution of the seston on the filter. However, it was estimated that this error did not exceed 5%.

Additionally, the results were affected by errors arising from each method itself. Due to the fact that the same filter was used for comparison, the error resulting from sampling and filtering could be eliminated. Other sources of errors in the non-extractive method are as follows:

- (i) uneven distribution of the seston on a filter,
- (ii) the fact that absorbance measured at 680 nm is also influenced by the physiological state of plankton, the composition of pigments in plankton, and the degree of aggregation (Govindjee, 1987).

In the extractive method, the errors are mainly connected with changes during storage of the samples and with homogenization and extraction of samples.

Despite the mentioned reasons of scatter of the results, the correlation coefficient obtained from comparison of the two methods is high (0.913), and the agreement between the results is satisfactory (assuming a condition that in the non-extractive method the absorbance measured at $\lambda = 680 \text{ nm}$ does not exceed 1). The obtained results of comparison of these methods suggest the possibility of using both methods for the determination of chlorophyll a in sea water samples.

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