Methods and comments

In the course of time, beginning from the 1960s, methods of sampling, counting and microscoping have changed. More complete handbooks have become available and most importantly, inexperienced students have gradually developed into specialists. Many determinations of species have been questioned several decades later. Important changes in counting methods took place in 1999 when the invert microscope was taken into use and the Utermöhl (1958) method was introduced.
A large number of tiny unicellular organisms became visible and enumerated.
Macroscopic colonies of *Gloeotrichia echinulata* were enumerated visually in 500 ml measuring cylinder.

Methods until 1999, samples were in most cases concentrated by precipitation up to 15 ml. Counting was made on a striped microscope slide within volume 0,1 ml. Microscopes: MBI-3 (magnification 15x20 and 15x40) and Jenaval (7x40).
Up to 1988 the samples were preserved with formaldehyde (not neutralised), and lots of samples were spoiled: sample sediment became flaked, stuck together, or rusty. By this reason, a number of results of countings are not representative.

Beginning from 1999, phytoplankton samples were preserved in Lugol's (acidified iodine) solution and counted under an inverted microscope (Utermöhl, 1958). 3 ml of preserved sample was settled overnight and counted in random fields or transects. Biovolumes of algal cells, colonies and/or filaments were calculated using assigned geometric shapes dimensions, and converted to biomass assuming the specific density of 1 g cm^{-3} in accordance with Edler (1979).

Counting units
Counting units are independent (single) algal cells, colonies or filaments/trichomes. One species or taxon may be present in a sample in the form of different counting units, or it may be counted at different magnifications.
For example, *Microcystis* colonies are counted in the whole chamber or transect but individual *Microcystis* cells (which may be present if colonies are disintegrating) are counted in random fields.
Similarly, *Dinobryon* colonies may be counted in whole chamber or diameter transects, but single *Dinobryon* cells often need to be counted in random fields. In the case of *Dinobryon*, only monads are measured.

Other examples of counting/algal units include:
colonies e.g. *Aphanocapsa, Aphanothece, Coelomoron, Coelosphaerium, Cyanodictyon, Cyanonephron, Gomphosphaeria, Microcystis, Radiocystis, Snowella, Woronichinia, Coelosphaerium, Planktosphaeria, Sphaerocystis.*
Algal cells which can occur as single cells but also form colonies or filaments, e.g.
Aulacoseira, Fragilaria, Dinobryon, Melosira, are counted as cells.

Colonies and coenobia which have more or less permanent cell numbers, e.g. *Desmodesmus/Scenedesmus Oocystis* (2, 4 or 8 cells), *Pandorina* (16 cells), *Crucigenia* (4 cells); were measued as whole;
Single cells with a high variation of size were divided in size-classes (e.g. Cryptomonadales <16 μm, 16-26 μm, >26 μm).

Filaments with the different number of cells (most Oscillatoriales, Nostocales etc) are measured as cylinder with different length.

References of methods accepted

Approved by CEN on 14 July 2006 “Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)” (CEN 15204, 2006)

European Standard EN 15204:2006

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Edler, L. (ed.), 1979. Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. Baltic Marine Biologists WG 9.

(13) Biovolume calculation for pelagic and benthic microalgae | Request PDF. Available from: https://www.researchgate.net/publication/220031275_Biovolume_calculation_for_pelagic_and_benthic_microalgae [accessed Oct 29 2018]. The most commonly used traditional biomass estimate for microalgae is cell biovolume, which is calculated from microscopically measured linear dimensions (Steinman et al. 1991, Snoeijs 1994, Sommer 1994, 1995, Hillebrand and Sommer 1997).

Comments to genera

Some genera in different groups need special methods of processing and microscoping to be identified. Inexperience, lack of skills and uncertainties have led to doubtful identifications.
For this reason, a number of genera were not identified up to the species, and some genera were classified under a larger taxon (family or class).
The centric diatoms *Cyclotella*, *Stephanodiscus, Cyclostephanos, Actinocyclus* are joined under the genus *Cyclotella* and/or *Stephanodiscus*.
The narrow filaments of Oscillatoriales *Planktolyngbya*, *Heteroleibleinia*, *Limnothrix*, *Geitlerinema*, *Jaaginema*, *Pseudanabaena* are joined under the group of Filament.
Under the group of Varia there are placed some cysts of unknown origin and some cells that are possibly not algae.

Handbooks used in 1960s-1980s

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Korshikov, A.A. (1953). *Viznachnik prisnovodnikh vodorosley Ukrainsykoi RSR [Vyp] V. Pidklas Protokokovi (Protococcineae). Bakuol'ni (Vacuolales) ta Protokokovi (Protococcales)* [The Freshwater Algae of the Ukrainian SSR. V. Sub-Class Protococcineae. Vacuolales and Protococcales]. pp. 1-439. Kyjv [Kiev]: Akad. NAUK URSR.

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Мошкова Н.А., Голлербах М.М. 1986. Определитель пресноводных водорослей. Вып. 10(1). Зеленые водоросли. Класс Улотриксовые (1). Изд. Наука, Ленинград. 360 стр.

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Huber-Pestalozzi, G. 1961. Das Phytoplankton des Süsswassers. 5. Teil. Chlorophyceae. Volvocales. Stuttgart. 844. S.

Key books added later, most representative

Huber-Pestalozzi, G., Komarek, J., Fott, B. 1983. Das Phytoplankton des Süsswassers. 7(1). Chlorophyceae. Chlorococcales. Stuttgart. 1044. S.

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