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1. Introduction

Phytoplankton is a plant plankton which cannot move actively and changes location depending on the movement of water. Phytoplankton communities are widely spread from aquatic to terrestrial lands. Plankton form the first ring of food chain in aquatic environment effecting the efficiency of this environment. Daily, seasonally and yearly changes are important for calculating efficiency in aquatic fields. Phytoplankton composition is a trophic indication of the water mass. In addition, phytoplankton species are used as an indicator for determining the nutrient level which is the basis for preparing and monitoring the strategies of the lake management in the lakes. Using phytoplankton communities or other aquatic organisms for evaluating water quality is based on very ancient times. Saprobik and trophic indicator types are used in many researches [1-3]. In addition, various numerical indices have been developed [1-2]. However, none of them have been accepted extensively. This is caused by several reasons. Those reasons are:

a. Differences in phytoplankton groups and group concept
b. Dynamic properties of phytoplankton groups
c. Habitat diversity of freshwater ecosystems
d. Phytogeographical differences [4].

Phytoplankton communities are influenced by significant changes every year. The competitive environment known as seasonal succession has been changing [5]. If the conditions don’t change, this process results in the choice of communities dominated by one or more species. Phytoplankton responds rapidly to the condition changes. Conditional changes will result the formation of high compositional diversity [6].

The first approaches to classify algal communities don’t have wide range of application in determining water quality. Pankin’s approaches to classify algal communities in 1941 and 1945 weren’t generally accepted [4]. Reynold’s [7-8] applied a classic phytosociological
approach to phytoplankton data obtained from the lakes in northeast England and classified them into various communities. Sommer [5] found high similarities among the compositions of species and seasonal succession of Alpine lakes. Mason [9] reported that oligotrophic and eutrophic lakes have the communities of characteristic phytoplankton.

There are qualitative differences among phytoplankton communities in oligotrophic and eutrophic lakes. The composition and the amount of phytoplankton communities are affected by environmental conditions. For example, a numerical decrease is observed in *Anabeana* and *Aphanizomenon* species from heterosis blue-green algae found in mesotrophic lake layers with a decrease of nitrogen saturation in the lakes. In addition, there are differences among the environmental conditions preferred by *Diatoms*, *Dinoflagellate* or *Cosmarium*, *Pandorina* and *Gemellicystis* species, even though they can be found in the lakes with the same level of nutrients [10].

In the works related with phytoplankton communities used for predicting the ecological structure of aquatic systems, it has been tried to develop functional groups by improving the systematic investigation. Furthermore, some indices were developed according to numerical and biovolume values of phytoplankton (Palmer Index (1969), Descy Index (1979), TDI Index (1995) etc.). HPLC pigment analysis is used for the diagnosis of phytoplankton species in recent years [11].

The methods we will mention for examination of plankton in aquatic environments are summarized by compiling researchers’ methods and techniques. The main target of these suggested methods is to obtain values close to the actual volume and weight of plankton in freshwater and to calculate the volume and weight (biomass) of these organisms by this method.

Besides, the methods and techniques in this subject are sensitive, determining ecological parameters which are characterizing the aquatic environment are important for the researches. Sampling error in this review may cause errors far beyond the susceptibility of calculations. In addition, vertically and horizontally distributions of plankton may show big changes against the effects of wind and light. For this reason, evaluating various samples collected as vertically and horizontally causes to get more reliable results from each sample.

Many techniques have been developed depending on the number, volume and cell structures of freshwater phytoplankton. In this section, studies conducted by calculating biovolume of phytoplankton used for estimating ecological characteristics of freshwater ecosystems will be summarized.

### 2. Changes in biomass of phytoplankton in lakes

The composition and biomass of phytoplankton are very important parameters for understanding the structure and trophic level of aquatic systems. Phytoplankton cell size, carbon content and functional structure are investigated by many researchers. Phytoplankton communities can have cell size from a few microns to a few millimeters depending on the groups they belong to. Biovolume measurements are estimated by
automatic or semi-automatic methods. For example, morphometric methods, holographic method etc. In addition to these, the commonly used one is geometric method [12]. In addition to these methods, the most common method for calculating biomass is measuring chlorophyll a value. Chlorophyll a is an important photosynthetic pigment for plant organisms. Environmental factors affect the amount of ambient phytoplankton and chlorophyll a value changes depending on the amount of phytoplankton [13].

Coulter Counter method is based on measuring electrical conductivity among cells. Electric current flowing through the cells placed in physiological saline varies depending on the cell size. Thus, the cell size is determined.

Morphometric methods is used in determining the quantitative properties of cells. With this method, cell density is calculated depending on cell wall, chloroplast, vacuole, depot material and cytoplasmic content [14].

Holographic scanning technology which is used in conjunction with one curved mirror to passively correct focal plane position errors and spot size changes caused by the wavelength instability of laser diodes [15].

Geometric method is based on estimating biomass of phytoplankton via geometric shapes and mathematical equations. This model was found by Kovada and Larrance. 20 geometric models developed by Hillebrand et al. [16] are used for calculating biomass of algae. Each model was designed depending on cell structure with the shapes like sphere, cone, triangle etc. This method was applied to phytoplankton species found in sea waters in China and 31 geometric models were developed in this study [12].

Phytoplankton communities show vertical changes from time to time. Chlorophyll a is a pigment used for estimating biomass of phytoplankton. Seasonal changes cause variation in chlorophyll a value. For this reason, water affects the production of column light transmittance, hence, the value of chlorophyll a [18]. In determining chlorophyll a, fiber glass filter papers used for filtering water samples are waited for 3-4 hours then they are decomposed and kept in 10 ml 90% acetone one night, centrifuged and optical density of the extract is made by reading from spectrophotometer with 630, 645 and 665 nm wavelength [17].

The first step for calculating phytoplankton biomass is to store and protect the phytoplankton samples.

3. Storing and protecting phytoplankton samples

Before collecting phytoplankton samples from the lake, turbidity and temperature of the water were measured by Secchi disk. Phytoplankton samples have to be stored in 100-150 ml glass or polyethylene containers with 2% Lugol’s solution or 4% Buffere formalin solution [32].

4. Identification of phytoplankton

Water samples taken by phytoplankton scoop were identified according to world literature [19-29].
5. Phytoplankton counting

Water samples put into hydrobios plankton counting chambers depending on phytoplankton density, after standing overnight by dropping lugol’s solution, counting phytoplankton were made by using inverted microscope [30-31].

Following formula was used to calculate the number of phytoplankton [31]:

\[
\text{Number of phytoplankton (piece/ml)} = \frac{C \times TA}{F \times A \times V}
\]

Here;
- \(C\) = The number of organisms found by counting (number),
- \(TA\) = Bottom area of the cell count (mm\(^2\)),
- \(F\) = Counted field number (number),
- \(A\) = Field of view of the microscope (mm\(^2\)),
- \(V\) = Volume of precipitated sample (ml).

6. Estimation of phytoplankton biomass

Phytoplankton analysis is possible by a simple Kolkwitz chamber. Except deposited plankton in sample bottle, liquid at the top pour a few millilitres and centrifuged and their volumes are measured in sedimentation tubes then they are transferred to Kolkwitz chamber for analysis. In this method, phytoplankton analysis is the first, semi-field analysis of Kolkwitz chamber is the second and the third one is the analysis of the various fields.

Biovolume was estimated in the measurement of phytoplankton biomass. Phytoplankton were emulated to the geometric shapes like sphere, cylinder and cone and necessary measurements were taken from the phytoplankton while counting [32]. Geometric shapes and calculations used for calculating biovolume was done according to the formulas (Table 1) stated by [12] and [16]. After calculating average volume of every species, total volume were calculated by multiplying with the number of species. Following formula was used to calculate total cell volume of phytoplankton [31]:

\[
HH = \sum_{i=1}^{n} (HNixSHi)
\]

Here;
- \(HH\) = Total biovolume of plankton (mm/l),
- \(HNi\) = the number of organisms belongs to i. species /l,
- \(SHi\) = Average cell volume of i. species.

Biovolume is calculated by assuming cell volume is equal to 1mg age weight/m\(^3\) algal biovolume for 1mm/ m\(^3\) [33].
<table>
<thead>
<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
</table>
| ![Shape 1](image1) | \( V = \frac{\pi}{6} \cdot a^3 \) | Crucigeniella apiculata  
Gomphosphaeria sp.  
Anabaena sp. |
| ![Shape 2](image2) | \( V = \frac{\pi}{6} \cdot b^2 \cdot a \) | Coelastrum microporum  
Actinastrum hantzschii  
Dinobryon divergens  
Cryptomonas sp.  
Pandorina sp. |
| ![Shape 3](image3) | \( V = \frac{\pi}{6} \cdot a \cdot b \cdot c \) | Trachelomonas caudata  
Peridinium sp.  
Botryococcus braunii  
Cocconeis placentula  
Phacus tortus |
| ![Shape 4](image4) | \( V = \frac{\pi}{4} \cdot a^2 \cdot c \) | Cyclotella sp.  
Mougeotia sp. |

Table 1. Geometrical shapes and formulas for calculating biovolume (continued)
<table>
<thead>
<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Biomass Now – Cultivation and Utilization" /></td>
<td><img src="image" alt="Shape" /></td>
<td><img src="image" alt="Biovolume" /></td>
</tr>
<tr>
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</tbody>
</table>

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<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>[ V = \frac{\pi}{4} \cdot b^2 \cdot a ]</td>
<td>Chroomonas sp.</td>
</tr>
</tbody>
</table>
| ![Image](image2.png) | \[ V = a \cdot b \cdot c \] | Asterionella sp.  
Syndra sp.  
Merismopedia sp.  
Epithemia zebra var. saxonica |
| ![Image](image3.png) | \[ V = \frac{\pi}{4} \cdot a \cdot b \cdot c \] | Pediastrum sp.  
Navicula sp. |
| ![Image](image4.png) | \[ V = \frac{\pi}{4} \cdot a \cdot b \cdot c \] | Cymatopleura sp. |
| ![Image](image5.png) | \[ V = \frac{1}{2} \cdot a \cdot b \cdot c \] | Nitzschia sp. |
| ![Image](image6.png) | \[ V = \frac{\pi}{4} \cdot a \cdot b \cdot c \] | Phaeodactylum sp. |

**Table 1.** Geometrical shapes and formulas for calculating biovolume (continued)
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<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Biomass Now – Cultivation and Utilization 336" /></td>
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<td><img src="image" alt="Biomass Now – Cultivation and Utilization 336" /></td>
</tr>
</tbody>
</table>

- **Monorophidium sp.**
- **Eunotia sp.**
- **Cymbella sp.**
- **Amphora ovalis**
- **Epithemia sp.**
- **Rhopalodia gibba**
- **Hydrosera sp.**
- **Tetradinium**
### Table 1. Geometrical shapes and formulas for calculating biovolume (continued)

<table>
<thead>
<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Triangle" /></td>
<td>$V = \frac{a \cdot b}{4} \left[ a + \left( \frac{\pi}{4} - 1 \right) \cdot b \right]. \quad a \sin \left( \frac{c}{2a} \right)$</td>
<td>Gomphonema constrictum</td>
</tr>
<tr>
<td><img src="image2" alt="Cylinder" /></td>
<td>$V = \frac{\pi}{3} \left( a_1 + a_2 \right) \cdot b_2^2 + \frac{\pi}{4}. \quad \left( a_2 + b_2 \right) \cdot b_2^2 + \frac{\pi}{12} \cdot a_2 \cdot b_1 \cdot b_2$</td>
<td>Euglena sp.</td>
</tr>
<tr>
<td>Shape</td>
<td>Biovolume</td>
<td>Samples</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Climacodium sp.</td>
<td>$V = \frac{\pi}{4} \cdot a_1 \cdot b_1 \cdot c_1 + \frac{\pi}{3} \cdot a_2 \cdot b_2^{\frac{3}{2}}$</td>
<td></td>
</tr>
<tr>
<td>Caloneis sp.</td>
<td>$V = \frac{\pi}{4} \cdot a \cdot b \cdot c$</td>
<td></td>
</tr>
</tbody>
</table>
| Staurastrum sp. | $b_2 = b_1 = b_4$  
$V = \frac{\pi}{4} \cdot a_2 \cdot b_2^{\frac{3}{2}} + \frac{\pi}{12} \cdot (a_3 + a_4) \cdot b_2^{\frac{3}{2}} + \frac{\pi}{6} \cdot a_1 \cdot b_1 \cdot b_2$ | |
| Cosmarium sp. | $V = \frac{\pi}{12} \cdot a^3$ | |

Table 1. Geometrical shapes and formulas for calculating biovolume (continued)
<table>
<thead>
<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature Ceratium hirundinella</td>
<td>$V = \frac{\pi}{4} \cdot a_1 \cdot b_2^2 + \frac{\pi}{2} \cdot a_3 \cdot b_3^2 + \frac{\pi}{12} \cdot a_1 \cdot \left( b_1^3 + b_1 \cdot b_2 + b_2^2 \right)$</td>
<td>Ceratium hirundinella</td>
</tr>
<tr>
<td>Mature Ceratium hirundinella</td>
<td>$V = \frac{\pi}{4} \cdot a \cdot b^2 \cdot c$</td>
<td>Pleurosira sp.</td>
</tr>
<tr>
<td>Immature Pleurosira sp.</td>
<td>$V = \frac{\pi}{4} \cdot b^2 \cdot a$</td>
<td>Pleurosira sp.</td>
</tr>
<tr>
<td>Fragilaria crotonensis</td>
<td>$V = \frac{\pi}{4} \cdot a \cdot b \cdot c$</td>
<td>Fragilaria crotonensis</td>
</tr>
<tr>
<td>Immature Ditylum sp.</td>
<td>$V = \frac{\sqrt{3}}{4} \cdot a \cdot b^2$</td>
<td>Ditylum sp.</td>
</tr>
</tbody>
</table>

*Table 1.* Geometrical shapes and formulas for calculating biovolume (continued)
There is a significant correlation between biomass of phytoplankton with the concentration of phosphorus. Changes are seen in phytoplankton biomass or production rate with the changes of the concentration of phosphorus in the lakes. It has been showed in the field studies that light and temperature play a significant role in the relationship between the extinction rate and biomass and carrying capacity of the composition of species [34].

In a study conducted in Lake Erie, samples have been collected from three different points in spring, summer and fall season for five years; 49 species were determined at the end of the study [35].

In a lake with 25000 km$^2$ surface area, phytoplankton biomass showed local changes and it was determined as 1.88 ± 0.12 g/m$^3$, 1.04 ± 0.07 g/m$^3$ and 0.63 ± 0.071 g/m$^3$ in west, mid and east part of the lake respectively. It was determined that algal biomass decreased and biomasses of *Aphanizomenon flos-aquae*, *Stephanodiscus binderounus*, *S. niagarae*, *S. tenuis*, *Rhodomonas minuta* decreased in the rate of 70-98% from 1970 to 1983-1987 [35].

Phytoplankton communities and distributions were investigated from the samples taken weekly from two dam lake with different nutrient levels in Sicily. Lake Arancio is a shallow eutrophic lake and Lake Rosamarina is a deep mesotrofik lake. It was stated that the increment in the concentration of nutrients in Lake Arancio doesn’t change the composition of phytoplankton but increase biomass of phytoplankton [36].

In a study examining 27 lakes in Russia; plankton biomass and total phosphorus concentration were investigated and it is stated that total phosphorus concentration changes between 10-137 mg/m$^3$ and biomass changes between 0.4-20 g/m$^3$. Total 160 phytoplankton species were identified and it was reported that most of those are belong to the blue-green algae and euglenophyceae classes. The lakes were determined to be hypertrophic and acidic [37].

In a study conducted with Lake Dorani, it was determined that the most common class in the lake was chlorophyceae followed by cyanophyceae. Total phytoplankton biomass is found similar to eutrophic lakes. While, nanoplankton biomass constitute 90% of total phytoplankton biomass in spring but it is 10% throughout the year. It is found that total biomass is high in summer, low in winter and changes between 0.43-30.30 mg/l [38].

### Table 1. Geometrical shapes and formulas for calculating biovolume (continued)

<table>
<thead>
<tr>
<th>Shape</th>
<th>Biovolume</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Shape Image]</td>
<td>$V = c \cdot (a_1 \cdot b_1 + \frac{\pi}{4} \cdot a_2 \cdot b_2)$</td>
<td><em>Climacosphenia sp.</em></td>
</tr>
</tbody>
</table>
Seasonal changes of phytoplankton communities in Lake Managua are investigated, it is reported that blue-green algae are dominant during the research period. Seasonal biomass are measured monthly for two years and the lowest phytoplankton biomass was found at the end of the rainy seasons (October, November). In short term studies (3-14 days), important changes in biomass were reported. Nutrient levels of the lake were estimated as hypertrophic according to chlorophyll $a$ value (79 µg/l yearly average, 1987-1988) [39].

During the research conducted with Lake Beysehir, diatoms and green algae are found dominant throughout the research. Aulacoseira granulata and Cyclotella meneghiniana from centric diatom, Asterionella formosa, Cocconeis placentula, Cymbella affinis and Ulnaria acus from pennate diatoms, Monoraphidium spp., Mougeotia sp. and Scenedesmus linearis from Chlorophyta, Dinobryon divergens from Chrysophyta and Cryptomonas marssonii, Rhodomonas lacustris from Cryptophyta, Merismopedia glauca from Cyanophyta are commonly found and partly numerical increases are observed. Phytoplankton biomass in the lake changes between 0.40±0.11 and 6.43±1.00 mg/l. The lake is in mesotrophic nutrients level according to average phytoplankton biomass (1.98±0.2 mg/l) and it is in good ecological quality class [40].

8. Results and suggestions

When the comparison was made between geometrical and other models, geometrical model is used more. Trials for other models in computer environment still continue. Techniques used for calculating biomass have some advantages and disadvantages. For example, Strombomonas gibberosa is phytoplankton with complex shape. For this reason, some different opinions arise for choosing proper geometric shape for calculating biovolume.

Three problem stands out in the estimation of biomass.

1. The shapes of phytoplankton cells has irregular and complex structure which makes it hard to measure them under microscope
2. Cell dimensions changes in the study of dead cells. In addition, it makes hard to determine chloroplast and vacuollarin.
3. Physiological state of a cell (light, temperature, nutrient) may affect cell height and intracellular volume [14].

Calculating biomass is important for determining ecological status of aquatic ecosystems. There is a relationship between cell structure of phytoplankton communities and many Physico-chemical parameters. For this reason, physical and chemical changes of water have to be considered in biomass calculations.

Since some species show physiological changes with the changes in environmental conditions, characteristics of phytoplankton groups should be well known. In some species in group of cyanobacteria, fringes observed depending on the increase of the value of nitrogen and phosphorus in the medium. This causes changes in cell dimensions. For this reason, it is suggested to support biomass usage for classifying freshwater ecological systems with physico-chemical parameters.
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Acknowledgement
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9. References