# THE MEANING OF SOME COMMON TERMS USED IN SAMPLING TOXIC PHYTOPLANKTON

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## Introduction

Newspapers, radio and television keep us informed about the sudden appearance of phytoplankton blooms, consisting of cyanobacteria (blue-green algae) and other microalgae, emphasising their harmful effects and the need for care and vigilance. Biologists who are directly involved in the study of these blooms talk about phytoplankters with unfamiliar names and refer to toxic samples, toxicity tests and peculiar toxins. For those who have to respond to this information, but have little or no experience in field studies or work related to these nuisance organisms, it is often hard to understand not only the dimensions and distribution of blooms but also the kind and significance of the samples and tests being used. In this brief note I shall try to explain some aspects of sampling phytoplankton blooms and the evaluation of results obtained from different methods. However, I shall not attempt to cover the entire field of blooms and methods of sampling that are available. Rather, my comments reflect some of the problems which I have encountered during field work and in the laboratory, when dealing with the public and newspapers, and, of course, when discussing matters with colleagues who have a background different from mine. Hopefully, some of these comments will be of use to the general reader and, perhaps, stimulate further discussion.

## Dimensions and distribution of blooms

Marine and freshwater phytoplankton communities may contain hundreds of species of microalgae (often called protists) and cyanobacteria. Some of these are abundant and dominant, numbering thousands of cells per millilitre of water, while others are relatively rare, perhaps less than ten cells per cubic metre of water. Some species tend to form surface or subsurface maxima, either actively or passively. Remarkable phytoplankton maxima, in which organisms are highly concentrated, are usually called blooms, and these are often almost monospecific. They may form very local discoloured patches occupying a

few square metres of a lake, frequently accumulating in sheltered bays along the shore, but they can extend over huge areas of oceans, covering hundreds of kilometres. Some blooms, notably the surface scums formed by cyanobacteria (formerly called blue-green algae), may suddenly appear for brief periods lasting for only a few hours, but others develop and persist over long periods of weeks and months. Some bloom events are recurrent and predictable, but most are episodic and their appearance may even depend on exceptional weather conditions.

Frequently one or several species in a bloom may produce substances or effects which are harmful or toxic to some or most other organisms (including other kinds of algae). Examples are given in Table 1 and some were mentioned by Reynolds (1991). Examples concerning the occurrence of toxic cyanobacteria and toxins in lakes are also found in, for example, Lindholm et al. (1989), Watanabe et al. (1992), and Kotak et al. (1993). Examples of marine phytoplankton blooms and shellfish poisoning events are reviewed by Smayda (1990), Shumway (1990) and Todd (1993). Carmichael (1994) summarises toxins and their effects.

Table 1. Examples of the types of lethal toxins and organisms producing them in phytoplankton blooms. Toxins from cyanobacteria may also contaminate drinking waters. Four main types of potentially lethal phytoplankton-related shellfish poisonings (SP) occur: paralytic (PSP), diarrhetic (DSP), neurotoxic (NSP) and amnesic (ASP).

| *Type of organism* | *Occurrence* | *Main type of toxins* | *Cases of lethal toxicity* |
|---|---|---|---|
| Dinoglagellates (e.g. *Alexandrium, Dinophysis*) | Red tides in marine, coastal waters | Saxitoxins, okadaic acid, neurotoxins | Toxins accumulating in mussels; PSP, DSP, NSP in humans |
| Prymnesiophytes (e.g. *Prymnesium, Chrysochromulina*) | Blooms in marine waters, fjords and lagoons | "Prymnesins"; various complex toxins | Toxins released to the water; marine and fish mortality |
| Cyanobacteria (e.g. *Microcystis, Anabaena, Nodularia*) | Surface scums in lakes, rivers and brackish waters | Hepatotoxic peptides and neurotoxic alkaloids | Toxic bloom material ingested; cattle, dog and wildlife mortality |
| Diatoms *(Pseudonitzschia)* | Blooms in marine waters, fjords, estuaries | Domoic acid | Toxin in mussels, fish; human poisoning (ASP), bird mortality. |

Most phytoplankton species are grazed upon by one or several zooplankton animals, which may migrate vertically and often occur in swarms or distinct layers. Therefore, when discrete samples of water and/or plankton are taken at a depth of about 1 metre, as frequently happens, they may or may not be entirely representative of the local

water mass. Occasionally a considerable amount of the total phytoplankton biomass and amount of toxin may be contained in the guts of fast-swimming zooplankters, which easily escape the sampler. Below I will briefly mention some of the different kinds of samples that are often encountered in work on toxic phytoplankton.

**Qualitative samples of organisms (dip and net samples)**

Samples taken by "dipping" with a container (e.g. bottle or bucket), or caught by sweeping the water with a net, may be highly selective and should not be considered as quantitative. A net with a large mesh size (mesh size larger than ca. 50 urn) will of course collect the large and robust species. A fine net (ca. 25 urn) retains a broader spectrum of organisms but fragile ones may be missing. In most cases net sampling fails to catch the picoplankton and nanoplankton (smaller than 20 |im by definition). Even some filamentous, gliding cyanobacteria like *Planktothrix agardhii* easily pass through the net. However, a net drawn through a large volume of water may provide relatively large amounts of the less abundant (and presumably less important) organisms which are seldom seen in discrete samples. As all net samples are extremely concentrated, the organisms caught in them are frequently damaged, and they usually deteriorate in a matter of hours. Such samples are usually taken for provisional inspection and identification of the main organisms present. Extracts of freeze-dried net samples may be used for toxicity tests with mouse bioassays and, for example, for the purification of toxins to be used as toxin standards.

**Quantitative (discrete) samples of water and plankton in blooms**

Water samples are usually obtained by filling bottles of various shapes and sizes, including sampling devices that have been specially developed for opening and shutting at various defined depths. A single sample of water and the organisms in it is usually called a "quantitative sample" because the results of chemical analyses and counts of organisms in the sample are expressed as quantities per volume, usually a litre of water. Nevertheless, strictly speaking a single sample is only a semi-quantitative or relative estimate (orders of magnitude) of a population occupying a column of water (Table 2). Fully quantitative sampling (e.g. Heaney 1976; Irish 1980) is rarely undertaken, as this involves taking a series of replicated samples (of known volume) both across and down through the water column containing the bloom (Table 2).

As stated above, a single "quantitative" sample is unlikely to be truly representative of any but the smallest bloom. Even if a toxic species is

present in the water, the amount of toxin collected in a single sample is likely to be relatively low, unless the water is very strongly "green" in colour, due to very large numbers of algal cells. Therefore it may be necessary to collect large volumes of water in order to concentrate the toxins for chemical analysis and/or toxicity tests.

Table 2. Main categories of samples taken to estimate chemical constituents and the density of organisms (numbers per litre of lake water) in phytoplankton blooms.

*QUALITATIVE SAMPLES*
"Dip" samples taken with containers, handnet and towed nets. Used to collect organisms for identification and chemical analyses.

*SEMI-QUANTITATIVE SAMPLES*
1 (or 2) samples taken with a bottle or container of known volume. Used to identify chemical constituents in the water and estimate their concentrations; also for estimating the density of organisms.

*QUANTITATIVE SAMPLES*
Replicated samples, ideally taken in both the horizontal and vertical planes within the bloom, using sampler(s) of known volume. At least 5 replicates (preferably more within the bloom) are required to estimate a mean and variance for the natural population of organisms.

SUBSAMPLES IN THE FIELD OR LABORATORY
One or several subsamples of known (small) volume may be taken from each field sample or from each series of replicate field samples. The subsamples are used to estimate mean quantities in each original container (sampler); the means then are used to calculate mean values for the natural population forming the bloom.

(A single subsample from a container gives an unreliable estimate of the number of organisms in the container; several subsamples from the same container give a more reliable estimate of the mean numbers present, and the variance is a measure of the reliability (precision) of the subsampling procedure. The mean number is then frequently used as an (unreliable) estimate of the mean density of the natural population; the estimate may suffice where density is being studied in terms of orders of magnitude, which is often good enough for dense blooms.)

To obtain a reliable estimate of the natural population density at least one subsample from each replicated field sample is required. The overall calculated mean of these subsample counts is a fully quantitative estimate of the natural population mean, and the variance is a measure of the precision (standard deviation or fiducial = confidence limits) for that estimated mean.

"Clean" water samples can usually be stored for several days for species identification if they are kept cool. Cysts (resting stages) can sometimes survive for months in cold conditions and may be of use for identifying some toxic species. As most phytoplankton samples change or are spoiled in a relatively short time, it is preferable and wise to take subsamples immediately in the field, and preserve subsamples with a fixative such as Lugol's iodine solution (e.g. Wetzel & Likens 1979).

Although the results from semi-quantitative samples are expressed as amounts (concentrations) or numbers of organisms per litre, it should be remembered that nevertheless they are really estimates of relative abundance (orders of magnitude). This is often sufficient for most investigations on phytoplankton blooms, because the organisms and chemical components associated with them are present in large amounts and these probably fluctuate within quite wide limits in both space (especially in windy conditions in lakes) and short periods of time (hours).

**Filtration of samples in the laboratory**

Various filters can be used for semi-quantitative sampling of phytoplankton as, for example, in the case of routine analysis *of* amounts or concentrations of chlorophyll-a. Material collected on the filters can easily be stored deep-frozen and also be used for various chemical analyses. As long as the sampling and subsampling routines are appropriate and the filtered volume is known, material on the filters may be used to estimate the quantities of toxins present in the biomass of phytoplankton (toxins remaining in cells).

The filtrate, i.e. the water passing through the filter, contains excreted and dissolved substances (and fine material from broken cells). Free toxins in solution may be *of* interest, e.g. in drinking water treatment plants and in laboratories which use filtered sea-water or lake-water for experiments. Usually, rather low concentrations of toxins are likely to occur in filtered water samples, and they may have to be concentrated for analysis.

**Concentrating samples by freeze-drying**

Samples and/or subsamples may be concentrated in the laboratory by freeze-drying. Freeze-dried water samples may be used for toxicity tests. Large volumes of water collected for freeze-drying may contain representatives of all or most of the organisms and debris in the water column, but they are rarely "quantitative" samples. Freeze-dried material is, however, easy to store and useful for provisional screening for toxins and for certain chemical analysis. Freeze-dried samples of phytoplankton blooms that have been collected with a net are usually much more selective than freeze-dried samples of water (some toxins may even be absent).

**Samples from algal cultures in the laboratory**

Cultures of algae are usually used for research. Samples taken on different days from cultures and filtered, freeze-dried and homogenized are much more homogenous in content than natural water or bloom samples, but

nevertheless they can still be quite hard to precisely define. They may represent one or several strains and be in different growth phases. Axenic (sterile) cultures are monospecific and are not contaminated with bacteria.

**Toxins**

Certain organisms manufacture organic substances that are toxic to others (Table 1). The toxins are either excreted into the surrounding water or stored within the cell and released when it dies and decomposes. It is very difficult to distinguish toxins that are already present in the water from those held in the cells, because of difficulties in separating water from cells without damaging the latter. Usually the toxins present in a natural bloom are studied by extracting them from the algal cells with organic solvents such as methanol. Crude extracts are sufficiently pure for certain purposes (tests). The extract may contain either a fraction of a particular toxin or, in some cases, most of the toxin(s) present in, for example, a freeze-dried algal sample.

**What is a "pure" toxin?**

Separation of a certain toxin, which is present in a crude extract, may be done with various organic solvents and, for example, HPLC methods (high performance liquid chromatography). Pure toxins are useful tools for studying the chemical structures of toxins and their physiological effects at the cellular and organismic levels. For pure toxins it is relevant to talk about toxicity, if properly tested and the solvent used does not influence toxicity. Many of the toxins which are produced by phytoplankton are in fact supertoxic, with toxicities of about 100 ug per kg body weight in mouse assays, i.e. a dose of a few micrograms is enough to kill a mouse (usually intraperitoneal injection or i.p. is applied).

**Final comments**

A sample from a toxic bloom may, in fact, consist of anything between a bucket of water containing a large diversity of organisms, and a white powder of purified toxin. As different kinds of samples can have very different properties, be more or less concentrated, and be more or less representative, it is urgent that adequate descriptions of sampling procedures and treatments are provided. Some readers of a report may have to judge whether the toxic sample originated from a trivial, extremely local bloom or whether there is a real threat to the health of people or domestic animals. A single "positive" sample (perhaps even "amplified" during weeks in a culture medium), does not necessarily mean that a large lake must be closed! On the other hand, it is urgent to detect, respond to and inform others about toxic algal blooms that may be harmful.

The testing of toxicity and the units and vocabulary involved require a separate article to cover them adequately. This also applies to the complex topic of exposure to toxins in the field, which is difficult to evaluate and quantify. For example, a water-bird may swim in the middle of a toxic bloom without being lethally exposed to toxins in the water, but it may receive a lethal dose of toxins via food items like fish - even miles away from the toxic bloom which originally contaminated the fish.

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