Bioindicators and biomonitors
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Chapter 9
Algae as ecological bio-indicators

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Abstract

The value of algae as bio-monitors and bio-indicators has already been recognised in the mid 19th century: The first concept which has been developed was the system of saprobity. It was mainly designed for organic pollution of streams and rivers. This system was altered, modified and expanded over the years by several authors. Because saprobity is defined as the intensity of heterotrophic activity, all photoautotrophic species were finally excluded from the saprobic system when problems of inorganic nutrient load to rivers became increasingly important. Parallel to the progress in running waters, the trophic classification system has been developed for lakes which is based on inorganic nutrients and their loading from the catchments. The basis of both this concepts and other systems is the belief that the presence, absence or abundance of species or species assemblage readily reflects the character of the habitat within which they are found. Those species are usually identified as bio-indicators. This concept of indicators can be extended beyond presence/absence by relating abundance, biomass or growth of algal species to environmental impacts in general or specific stress symptoms in particular. The indicator species then becomes a 'bio-sensor' for the bioassay of environmental contamination. Another concept associates indicator species with organisms accumulating substances from the surrounding environment so as to reflect natural levels and exposure to these substances Such species are 'bio-accumulators ' which are especially useful when concentrating very low levels of a substance.

In this chapter, principles of algal bio-indication and bio-monitoring in the environment is outlined for streams and rivers, lakes and reservoirs, as well as for marine ecosystems. Both pelagic and benthic algal groups and species are considered. Field and laboratory bioassay procedures and techniques are described and discussed for both natural assemblages and laboratory cultures. Aspects of sediment testing are included. Since environmental contamination and pollution has severely expanded in the recent past, ecotoxicological methods became increasingly important. More integrative new approaches such as 'ecosystem health' and 'environmental integrity ' are briefly discussed.

Keywords: algal bio-indicators, rivers, lakes, ecotoxicology, bioassay, saprobity, trophic-system

1. Introduction

The value of algae as bio-monitors for fresh waters has already been recognised in the mid 19th century (Cohn, 1853). The first attempt to classify aquatic organisms as indicators of water quality was made by Cohn (1870), later modified by Mez (1898). The relation of organisms to the quality of water was more clearly defined by Kolkwitz and Marsson (1902, 1908, 1909) who also created the name '*saprobic organisms*'.

The system of saprobity, a term to describe the biotope introduced by Šrámek-Hušek (1956), was further developed and revised by Kolkwitz (1950) and Liebmann (1962). Several simple and more elaborated definitions of saprobity by various authors are listed by Sládeček on page 27 in his comprehensive overview of 1973. Because saprobity is defined as the intensity of heterotrophic activity, all photoautotrophic species were excluded from the saprobic system in a revision (Friedrich, 1990) to avoid overlapping with trophic indication In short, the presence, absence or abundance of species or species assemblage readily reflects the character of the habitat within which they are found. Those species are usually identified as bio-indicators.

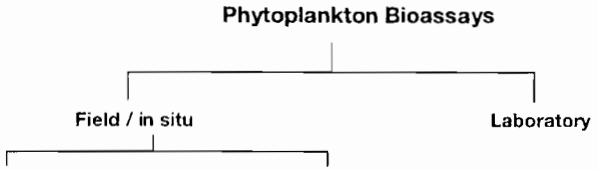
Since then, various elaborated systems deducing water quality from observations of indicator organisms have been developed, evolved, and diversified both in the field and in the laboratory as bioassay. The terms "indicator", "bio-indicator" or "indicator species" may be used and understood, however in several different ways. As a prerequisite for an alga to become an indicator we need to know the requirements of that species with regard to one or more environmental variables. The presence of such a species in a given habitat will then indicate that one or more parameters are within the tolerance limits of that species. This concept of indicators can be extended beyond presence/absence by relating abundance, biomass or growth of algal species to environmental impacts in general or specific stress symptoms in particular. The indicator species then becomes a "bio-sensor" for the bioassay of an environmental contamination. Another concept associates indicator species with organisms accumulating substances from the surrounding environment so as to reflect natural levels and exposure to these substances Such species are "bio-accumulators" which are especially useful when concentrating very low levels of a substance.

Algae are most useful as indicators in the context of eutrophication but have been employed as well to detect organic pollution because of their well documented tolerance (e.g. Palmer, 1969). Their value as bio-accumulators of e.g. pesticides or heavy metals is limited. Some species, such as *Selenastrum capricornutum*, are used as biosensors in laboratory bioassays while natural phytoplankton assemblages are often used for *in situ* bioassays (Schelske, 1984). Ecotoxicology is another field in which algae have been applied. Some common phytoplankton bioassay techniques mentioned later in the text are summarised in Figure 1.

In a wider concept, organisms are seen as fundamental sensors that respond to any stress affecting the system in which they live (Loeb and Spacie, 1994). Any stress, physical, chemical or biological, imposed on an aquatic system manifests its impact on the organisms living within that ecosystem through their health. The health of an aquatic ecosystem is affected when its capacity to absorb stress is exceeded. The concept proposes that the environmental health of aquatic ecosystems can be assessed by biological monitoring using organisms as diagnostic tools.

2. Bio-indication and bio-monitoring in the environment

Field assessment of environmental quality usually uses algae which are either planktonic or attached to surfaces. True planktonic forms are confined to lakes and large,



	Algal cultures	Natural assemblages
Indices to describe assemblage structure	Batch cultures	Batch cultures
Bottles / Moorings	Continuous cultures (Turbidostat / Chemostat)	Size spectra
Flow-through bottles	Microplate algal assay	Algal fractionation bioassay (AFB) (^{14}C-uptake / Biomass / Pigments)
Dialysis bags	*Selenastrum capricornutum* (^{14}C-uptake)	Autotrophic picoplankton assay (^{14}C-uptake / Abundance)
Microcosms / Mesocosms	*Ankistrodesmus bibrianus* (two chamber device)	Biosensors
In situ plankton cages	Continuous flow-through systems	Flow-cytometry

Figure 1. Phytoplankton bioassays commonly employed in laboratory and field/in situ situations (modified from Munawar et al., 1989.

slow flowing rivers. They are simple and inexpensive to collect by conventional limnological water-samplers. Attached algae may be removed in the field by scraping or brushing of definite areas when quantification is attempted. In order to overcome several deficiencies of detachment techniques, artificial substrates of various kinds have been tried with some success (Hellawell, 1986). Detailed description of methodology and statistical evaluation of benthic algae used as bio-monitors is included in Lowe & Pan (1996).

2.1. Rivers

Historically the concept of saprobity included autotrophic and heterotrophic organisms (Sládeček 1977). Therefore several indices have been developed and used for both. As organic pollution in rivers decreased due to restoration measures, trophic problems became more pronounced resulting in the development of separate trophic bio-indices (Table 3). Ultimately all autotrophic organisms were treated separately from the saprobic system (Friedrich, 1990). General indices of water pollution can be found in Abel (1989).

Trophic classification of rivers from phytoplankton and periphyton structure and abundance is now included in the EC-Water Framework Directive (2000) permitting biology based quality estimation. While the usage of benthic algae for the classification of running waters has quite a long tradition, phytoplankton has widely been neglected which is quite the opposite situation compared to lakes.

Table 1. Criteria for trophic classification of plankton-dominated rivers (modified from Schmitt, 1998).

Trophic classification		Primary productivity	Chlorophyll-a 90-percentiles [μg l^{-1}]	Chlorophyll-a Average [μg l^{-1}]
I	Oligotrophic	Very low	3–8	<1–4
I–II	Mesotrophic	Low to moderate	8–30	3–8
II	Eutrophic	Moderate	20–100	7–30
II–III	Eu- to polytrophic	Moderate to high	70–150	25–50
III	Polytrophic	High	120–250	50–100
III–IV	Poly- to saprotrophic	Very high	200–400	>100
IV	Saprotrophic	Extremely high		>400

2.1.1. Phytoplankton

Long-term changes in rivers using plankton biocoenoses are relatively easy to detect because methods are similar to lakes but standard protocols for surveys are jet to be developed. River plankton assemblages are most often dominated by diatoms. Green algae and Cryptophyceae appear in summer (Dokulil, 1991, 1996). At reduced flow rates Cyanobacteria can grow and sometimes produce short-lived blooms because of improved light conditions and less turbulence (Steinberg and Hartmann, 1988). In general, the plankton flora of rivers is far less diverse than those of lakes and is often dominated by centric diatoms (Rojo et al., 1994; Reynolds and Descy, 1996). Species which might be used as indicators are *Aulacoseira granulata, Actinocyclus normanii, Stephanodiscus neoastrea, Cyclotella meneghiniana* among many others. The usefulness of river plankton for bio-indication is, however hampered because of the wide ecological tolerance of most species (Lange-Bertalot, 1978, 1979).

A general trophic classification of plankton-dominated rivers (Schmitt, 1998) uses the 90th percentiles of the chlorophyll-a concentrations from the growing season, March–October (Table 1). Peak and average values may be used in addition. Ranges indicate the changing chlorophyll content of algal biomass with varying algal composition which increases according to Behrendt and Opitz (1996) from Bacillariophyceae (diatoms) to Cyanobacteria (Cyanoprokaryota) to Chlorophyta (green-algae).

A similar trophic system, including algal abundances and primary production, has been published by Felföldy (1987) for Hungarian rivers and lakes (Table 2). This system unifies trophic categorization for all types of surface waters as discussed by Hamm (1996).

2.1.2. Phytobenthos

In contrast to plankton species, the algal periphyton in running waters include many of the requirements for an excellent monitoring system because they occur ubiquitously from clean springs to highly polluted river sections (Patrick, 1994). Macro-

Table 2. Parameters for the trophic characterization of rivers and lakes (modified from Felföldy, 1987).

Trophic level		Algal abundance [10^6cells l^{-1}]	Chlorophyll-a [$\mu g\ l^{-1}$]	Primary production [mg C m^{-2} d^{-1}]	Primary production [g C m^{-2} a^{-1}]
1	Ultra-oligotrophic	<0.01	<1	<5β	<10
2	Oligotrophic	0.01–0.05	1–3	50–125	10–25
3	Oligo-mesotrophic	0.05–0.1	3–10	125–250	25–50
4	Mesotrophic	0.1–0.5	10–20	250–500	50–100
5	Meso-eutrophic	0.5–1.0	20–50	500–900	100–175
6	Eutrophic	1–10	50–100	900–1,500	175–300
7	Eu-polytrophic	10–100	100–200	1,500–2,500	300–500
8	Polytrophic	100–500	200–800	2,500–4,000	500–800
9	Hypertrophic	>500	>800	> 4,000	>800

scopic conglomerations of algae are sampled and evaluated from transects or squares. Evaluation of microscopic periphyton is done after scraping off from the natural habitat or uses artificial substrata such as glass slides, styrofoam, plexiglass or tiles.

In principle, the presence of any species whose environmental limits are clearly understood could be used as an indicator. In practice, its ecological range is often to broad or too little is known to be of any use. Healthy growth of a species often is a much better indicator than just its presence. *Cladophora glomerata*, for example appears in almost all streams but large growth is only found when nutrient levels are high (Whitton, 1979). Several lists of individual species do exist classifying species according to their reaction to one or the other type of pollution or contaminant (Mauch, 1976; Rott et al., 1997, 1999).

The most objective accounts of the tolerance of individual species have been made for diatoms. Continuous long-time monitoring of rivers using diatometers (Patrick and Hohn, 1956) clearly show that algal assemblages on glass-slides reflect well perturbations such as increase in pollution, building of dams or small amounts of toxic pollution (Patrick, 1976). Winter and Duthie (2000) evaluate for instance in-stream nutrient concentration from patterns of epilithic diatom distribution. From a combination of chlorophyll-a measurements and an analysis of benthic algal assemblages Biggs (2000) constructed a nomograph relating nutrient concentrations and days of accrual to trophic conditions (Fig. 2). These and many other observations and elaborations have led to the formulation of many different indices for algal communities, especially diatoms by a large variety of authors, summarised in Table 3. Besides saprobic and trophic indication, diatoms are used for many other indications such as salinity, acidity, pH-value, Al-concentration, dissolved organic carbon (DOC) and humic substances (Schönfelder, 2000). In palaeolimnology, water-temperatures, pH-values or phosphorus concentrations from the past are reconstructed from the analysis of diatom frustuls found in the sediments.

Some of the more recently developed diatom indices are often based on experimental investigations such as those by Reimann and Hamm (1996), who analysed

Table 3. Indices for the assessment of running waters based on algal biocoenoses of the natural environment (updated from Ghetti & Ravera, 1994 and DePauw et al., 1992).

Indices	Communities	References
Saprobic indices		
Biol. Effect of Org. Load (BEOL)	PA	Knöpp 1954
Relative Purity	PA	Knöpp 1954
Saprobic Index (S)	PA	Pantle and Buck 1955, DIN 38-410
Saprobic Index (S)	PA	Zelinka and Marvan 1991
Saprobic Index (S)	D	Sladecek 1986
Saprobic Index (SI)	D	Kobayasi and Mayama 1989
Saprobic Index (SI_{MI})	AD	Rott et al. 1997
Saprobic quotient (SQ)	P	Dresscher and Van der Mark 1976
Biotic indices		
Cemagref diatom Index (IDC)	PAD	Cemagref 1984
Diatom Index (IDD)	AD	Descy 1979
Diatom Index (IILB)	AD	Lange-Bertalot 1979
Diatom Index (IPS)	AD	Cemagref 1982
Diatom Index (ILM)	AD	Leclerq and Maquet 1987
Diatom Index (CEC)	AD	Descy and Coste 1991
Diatom assembl. Index (DAIpo)	D	Watanabe et al. 1986
Geueric diatom Index (GDI)	AD	Rumeaux and Coste 1988
Median diatomic Index (MI)	AD	Bazerque et al. 1989
Trophic diatom Index (TDI)	D	Schiefele and Kohmann 1993,
Trophic diatom Index (TDI)	D	Kelly and Whitton 1995; Kelly 1996
Eutrophic Pollution Index (E/P-I)	D	Dell 'Uomo 1996
Trophic Index (BRB)	D	Schönfelder 1997
Trophic Diatom Index (TDI)	D	Coring et al. 1999
Trophic Index (TI and TI_{DIA})	AD	Rott et al. 1999
Specific diversity indices		
Equitability	D	Lloyd and Ghelardi 1964
Log-normal distribution	D	Preston 1948
Number of individuals per taxon	PA	Helawell 1986, Plafkin et al. 1989
Sequential Comparative Index (SCI)	A	Cairns et al. 1968
Taxa richness (S)	PA	Helawell 1986, Plafkin et al. 1989
Total number of individuals (N)	PA	Helawell 1986, Plafkin et al. 1989
Comparative indices		
Fluctuation Index (D)	D	Dubois 1973

P = Plankton, A = Periphyton (Aufwuchs), D = Diatoms.

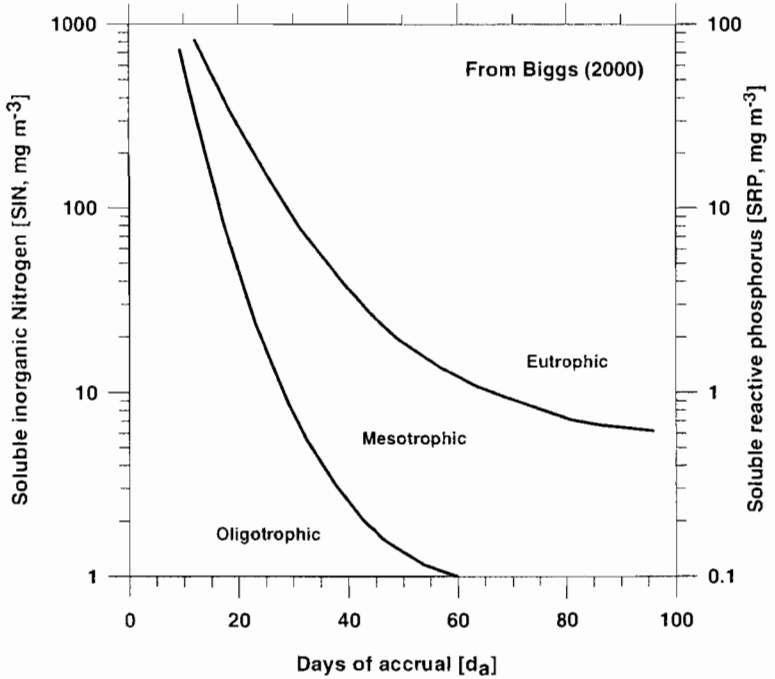


Figure 2. Nomograph predicting maximum benthic algal biomass as chlorophyll-a indicative of oligotrophic, mesotrophic, and eutrophic conditions from mean monthly soluble inorganic nitrogen (SIN, left *y*-axis), soluble reactive phosphorus SRP, right *y*-axis) and days of accrual (d_a, *x*-axis). Boundaries were set to 60 mg m^{-2} chlorophyll-a to separate oligotrophic from mesotrophic, and to 200 mg m^{-2} for mesotrophic to eutrophic (modified from Biggs, 2000).

periphytic diatoms in artificial field and laboratory mesocosms. Based on the concept of differentiating species (see below) and intensive field investigations throughout Germany Schiefele and Kohmann (1993) developed a weighted trophic diatom index (TDI):

$$TDI_{SS} = \frac{\sum_{i=1}^{n} Y_i \, TDI_i W}{\sum_{i=1}^{n} Y_i W_i}$$

with TDI_{SS} = Trophic diatom index for sampling site (*SS*)
Y_i = relative abundance of species $i = 1$ to n at the sampling site
TDI_i = Index based on either phosphorus or phosphorus and nitrogen for species 1 to n
W_i = Weight $i = 1$ to n for species 1 to n

This formula is mathematically equivalent to the saprobic index by Pantle and Buck (1955) or Zelinka and Marvan (1961).

Table 4. Relation of trophic levels to the Trophic Diatom Index (TDI), trophic condition and nutrient load (modified from Schiefele and Kohmann, 1993).

Trophic level	TDI	Trophic condition	Nutrient load
1	1.0–1.4	oligotrophic (o)	natural
1.5	1.5–1.8	oligo-mesotrophic (om)	low
2	1.9–2.2	mesotrophic (m)	moderate
2.5	2.3–2.7	meso-eutrophic (me)	critical
3	2.8–3.1	eutrophic (e)	significant
3.5	3.2–3.5	eu-hypertrophic (eh)	high
4	3.6–4.0	hypertrophic (h)	very high

The Trophic Diatom Index (TDI) characterises the trophic level of streams and rivers using seven levels similar to the saprobic system (Table 4). Both classifications are independent and their levels unequal. The value of the TDI lies mainly in its ability better to classify nutrient loads than some of the commonly used saprobic indices It is best applied to neutral or slightly alkaline, meso- to hypertrophic waters The weighted trophic diatom index of Schiefele and Kohmann (1993) for running waters is similar and comparable in methodology to the index developed for lakes by Hofmann (1993, see below Section 2.2).

Besides indices, several authors have attempted to describe community structure with elaborated differential concepts (Lange-Bertalot, 1978, 1979; Schiefele, 1987; Steinberg and Schiefele, 1988). These authors finally defined five groups of different tolerance levels against pollution and two groups describing nutrient conditions:

- most tolerant species (mt); reproduce even in polysaprobic areas
- highly tolerant species (ht) which occur up to the α-meso-polysaprobic level
- tolerant species (t) tolerating α-meso-saprobic conditions
- sensitive species (s) which are sensible against pollution but tolerating β- to α-meso-saprobic situations
- highly sensitive species (hs) which avoid saprobities greater than β-meso-saprobic
- oligotraphentic species (ol) indicating low nutrient concentrations
- eutraphentic species (eu) preferring high nutrient levels.

Using this system, running waters can be classified into three levels of pollution and four classes of trophy from relative species abundances according to the scheme given in Table 5. Examples of applications to running waters of various types include e.g. Dokulil et al. (1997) and Pipp and Rott (1994).

Other authors have used ordination techniques and weighted-averaging regression-calibration models for inferring stream water conditions from diatom patterns versus nutrients (e.g. Schönfelder, 2000; Winter and Duthie, 2000).

In some cases, phytosociological techniques were applied to bioindication of water quality using planktonic and benthic algal species (Möller and Pankow, 1981; Täuscher, 1999).

Table 5. Bioindication of trophy and pollution according to the system of differential diatom species (from Steinberg and Schiefele, 1988). ol = oligotraphentic species, eu = eutraphentic species, hs = highly sensitive species, s = sensitive species, t = tolerant species, ht = highly tolerant species, mt = most tolerant species. For more details refer to the text.

Relative abundances of the differentiating algal groups				
Trophic level				
I	ol ≥ 50%	hs ≥ 10%	eu < 10%	t + ht + mt + s < 10%
II	ol ≥ 10%	hs ≥ 10%	eu < 50%	t + ht + mt + s < 10%
III	ol < 10%	hs ≥ 10%	eu ≥ 50%	t + ht + mt + s < 10%
IV	ol < 10%	hs < 10%	eu ≥ 50%	
Pollution class				
1	ol + hs <10%		eu < 50%	t + ht + mt + s ≥ 10%
2	ol + hs <10%		eu < 50%	t + ht + mt + s ≥ 50%
3	ol + hs <10%		eu < 10%	t + ht + mt + s ≥ 50%

Attached algae other than diatoms are additional valuable indicators of conditions in flowing waters (e.g. Backhaus, 1973). Macroscopic and microscopic sessile green algae, although difficult to identify, are often the most common species present in river beds during summer. John and Johnson (1991) developed a field and laboratory protocol to enable the use of these species for detection of response to heavy metals, nutrient enrichment and other types of pollution. In conjunction with the EC Directive on the ecological quality of waters, many countries develop protocols, standards and lists of indicator species for the assessment of river water quality (e.g. Jarlman et al., 1996; Rott et al., 1997, 1999).

The indices developed by Rott el al. (1997, 1999) include saprobic as well as trophic indices. The latter is either based on all algal classes or solely on diatoms. Mathematically it is similar to the trophic index of Schiefele and Kohmann (1993):

$$TI = \frac{\sum_{i=1}^{n} TW_i G_i H_i}{\sum_{i=1}^{n} G_i H_i}$$

with TI = trophic index of all algal groups or diatoms only (TI_{DIA})
TW_i = trophic value of species i (tabulated in Rott et al., 1999)
G_i = Weight given to species i (tabulated in Rott et al., 1999)
H_i = relative abundance of species i in %

Biotic integrity of rivers is estimated with a new index of biological integrity (PIBI) developed from periphyton assemblages using a wide variety of estimators such as algal genera richness, relative abundance of diatoms, various types of acidophilic, eutraphentic or motile dominant diatom genera, cyanobacteria, chlorophyll and ash-free dry biomass (Hill et al., 2000).

Biomass per unit area or ratios of different components have sometimes been used as indicators of water quality. For instance, Weber and McFarland (1969), quoted from Whitton (1979), proposed an index of ash-free dry weight of periphyton to their respective chlorophyll-a content, both in g m^{-2}. This index should be higher in polluted areas that contain a larger proportion of heterotrophic organisms.

The response of photosynthesis and respiration to factors such as nutrient enrichment or a pollutant can be used to evaluate water quality. Of especial importance is the P/R-ratio which is: <1 in septic zones, increases rapidly and reaches values >1 in the recovery zone. Further downstream the P/R ratio approaches one. River primary production is often estimated from continuous upstream-downstream recordings of oxygen and other parameters. Water quality is deduced from these measurements (Kelly et al., 1976).

2.2. Lakes and reservoirs

Several techniques, indices and indicator species have been proposed by a variety of authors for the trophic classification of lakes and reservoirs with natural phytoplankton assemblages. The phytobenthos (periphyton, Aufwuchs) in lakes has attracted much less attention, especially when compared to river benthos. In some cases differences at higher taxonomic levels (algal groups) were used to characterise trophic levels of lakes.

2.2.1. Phytoplankton

2.2.1.1. Indices using algal groups

- Chlorococcal – Desmid Quotient (Thunmark, 1945)

Trophic levels are characterised by the relationship of the number of species found in a sample according to

$$Q = \frac{\text{Chlorococcal species number}}{\text{Desmid species number}}$$

Oligotrophic lakes have values <1, usually between 0.2 and 0.7; eutrophic waters are characterised by $Q \geq 1$ (1–3); hypertrophic lakes may reach values as high as 14.

Other authors could not validate this quotient and reported high variability.

- Algal quotients according to Nygaard (1949)

In addition to Thunmark's index, Nygaard developed further indices based on various algal groups:

$$\text{Myxophyceae Quotient} = \frac{\text{Myxophyceae}}{\text{Desmids}}$$

$$\text{Diatom Quotient} = \frac{\text{Centrales}}{\text{Pennales}}$$

$$\text{Euglenophyceae Quotient} = \frac{\text{Euglenophyceae}}{\text{Myxophyceae} - \text{Chlorococcal greens}}$$

$$\text{Compound Quotient} = \frac{\text{Myxophyceae} - \text{Chlorococcales} + \text{Centrales} + \text{Euglenophyceae}}{\text{Desmids}}$$

Characteristic values of trophic levels are:

Dystrophic	0–0.3
Oligotrophic	<1
Mesotrophic	1–2.5
Eutrophic	3–5
Hypertrophic	5–20
Polytrophic	10–43

Again, the compound index, as all others proved to be of rather limited value.

- E:O und EV:EO ratios according to Järnefelt et al. (1963 cit. acc. to Heinonen, 1980)

The ratio of eutraphentic to oligotraphentic species (E:O) and the quotient of the total biomass of eutraphentic species to the biomass of oligotraphentic species EV:OV) is defined on species level:

$$\frac{E}{O} = \frac{\text{Number of eutraphentic species}}{\text{Number of oligotraphentic species}}$$

$$\frac{EV}{OV} = \frac{\text{Total biomass of eutraphentic species}}{\text{Total biomass of oligotraphentic species}}$$

According to Heinonen (1980) the E/O index fits better at higher trophic levels while the biomass based quotient is very variable. Moreover, application is restricted due to the limited number of oligotraphent indicator species.

- Algal quotient according to Stockner (1971)

The index is based on the ratio of the two diatom groups Araphidineae/Centrales. Originally, it was developed for diatom frustules in recent sediments. The author proposed the following classification:

A/C ratio	Lake type
0.0–1.0	Oligotrophic
1.0–2.0	Mesotrophic
> 2.0	Eutrophic

2.2.1.2. Classification based on indicator species

Some of the above mentioned authors as well as several others have tried to classify lakes using the indicator species concept. A pre-requisite for defining indicators is a good knowledge of the algal species specific taxonomy and their related environmental requirements (e.g. Teubner, 1995).

- Indicative species according to Thunmark (1945), Nygaard (1949), Järnefelt (1952) or Teiling (1955)

All these authors proposed various lists of algal species which are either indicative for specific trophic situations, are indifferent or have no indicator value for lakes. For more details one must consult the original reference because the listings are voluminous. These approaches are of limited regional importance because most information originated from Scandinavian countries.

- Dominant limnetic algae according to Rawson (1956)

The author proposed a list for Western Canada in which the dominant algal species are placed in approximate sequence from oligotrophic to eutrophic occurrence (Table 6). Dominance is defined as a high percentage of the species in phytoplankton counts over much of the summer season. It should be made clear that this list shall only be used in Canada. Lakes in different regions of the world may need different species list (see further down).

- Qualitative characterisation according to Heinonen (1980)

Classification was based on qualitative phytoplankton analyses and on a differentiation of lakes based on their total plankton biomass. Lakes with a biomass ranging from

Table 6. Approximate trophic distribution of dominant algae in lakes of Western Canada (from Rawson, 1956).

Oligotrophic	*Asterionella formosa*
	Aulacoseira islandica
	Tabellaria flocculosa var. fenestrata
	Tabellaria flocculosa
	Dinobryon divergens
	Fragilaria capucina
	Stephanodiscus niagarae
	Staurastrum spp.
	Aulacoseira granulata
Mesotrophic	*Fragilaria crotonensis*
	Ceratium hirundinella
	Pediastrum boryanum
	Pediastrum duplex
	Coelospherium naegelianum
	Anabaena spp.
	Aphanizomenon flos-aquae
	Microcystis aeruginosa
Eutrophic	*Microcystis flos-aquae*

0.01–0.50 mg l^{-1} are considered oligotrophic those with B >2.5 mg l^{-1} are called eutrophic. Indicator species dominant in one or the other lake type are listed. Comparison to the species listed by Järnefelt et al. (1963, cited in Heinonen 1980) substantiated the indicative value of species such as *Arthrodesmus incus, Dinobryon cylindricum* and *Mallomonas borgei.* Many of the so called oligotraphentic species, however were often found in eutrophic lakes.

- Trophic Lake Index (Hörnström, 1981)

Hörnström postulates that the composition of the phytoplankton reacts more slowly to changing trophic conditions (>1 year) while total biomass readily reflects the nutrient situation. Based on these assumptions, he devleoped a Trophic Lake Index (I_L) which is calculated from

$$I_L = \frac{\sum (f * I_S)}{\sum f}$$

with I_S = Trophic Index of the species (range 0–100)
f = frequency as log Biovolume in μm^3 ml^{-1} (modification by Tremel, 1996).

The indicator valence of an algal species, ranging from 0 to 100 with increasing trophic state, is estimated from calculating median biomass for all the lakes in which a species occurs relative to the highest median observed (Fig. 3). This index is interesting for classification because it is based on relative frequencies which should remain more stable than absolute occurrence in case of zooplankton grazing.

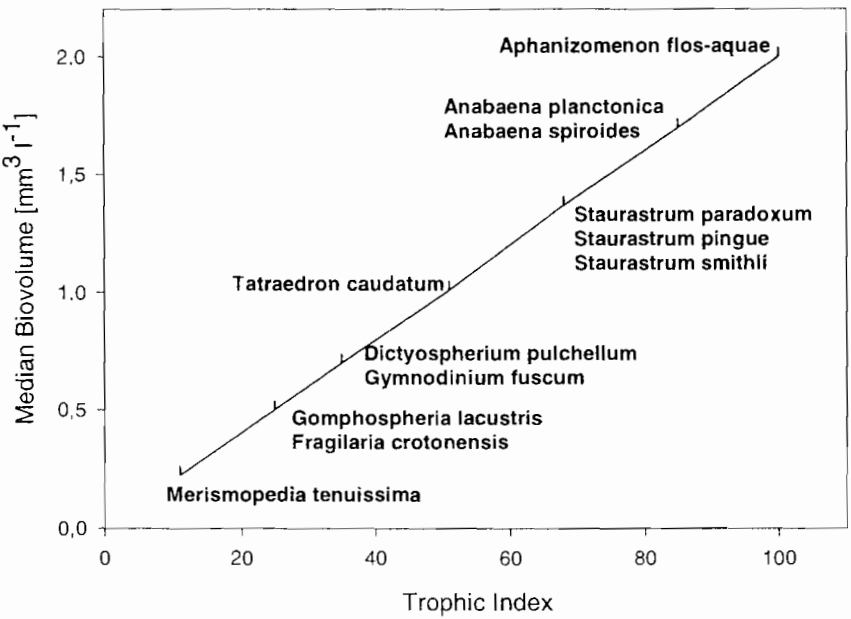


Figure 3. Relation between median volume and trophic index of phytoplankton species (modified from Hörnström, 1981).

Table 7. Algal bioindicators for trophic levels (from Kümmerlin, 1990). Species which are common but have no indicative value are listed under 'eutraphent'.

Trophic level	Algal group	Algal species
Oligotrophic	Bacillariophyceae	*Cyclotella bodanica*
	Chrysophyceae	*Chromulina erkensis*
		Chromulina rosanoffii
	Xanthophyceae	*Istmochloron trispinatum*
	Cryptophyceae	*Cryptomonas obovata*
Oligo-mesotrophic	Cyanophyceae	*Microcystis wesenbergii*
	Cryptophyceae	*Cryptaulax vulgaris*
Mesotrophic	Bacillariophyceae	*Tabellaria fenestrata*
Eutrophic	Cyanophyceae	*Microcystis aeruginosa*
		Aphanizomenon flos-aqae
		Anabaena planctonica
	Bacillariophyceae	*Stephanodiscus hantzschii*
		St. astrea
		St. binderanus
	Conjugatophyceae	*Mougeotia thylespora*
Eutraphent (euryök)	Bacillariophyceae	*Asterionella formosa*
		Cyclotella radiosa
	Dinophyceae	*Ceratium hirundinella*
	Cryptophyceae	*Rhodomonas minuta*
		Cryptomonas ovata

- Indicator species and indicator group study by Rosén (1981)

From a large data-set of medium and small sized Swedish lakes, algal species with clear environmental characteristics were defined from distribution functions. Results indicate that blue-green and green planktonic algae, besides well defined eutrophic species, comprise types indicative of clear lakes or low or high humic content. Chrysophyceans often dominate in nutrient poor waters. Diatoms are absent from ultra-oligotrophic lakes. Dinoflagellates and Cryptophyceans are confined to certain lake types. Within the Chloromonadophyceans, *Gonyostomum semen* is an excellent indicator for humic lakes. The study contains detailed lists of the various species and their indictor value with respect to several limnological important variables.

- Algal Bioindicators according to Kümmerlin (1990)

Indicator species are deduced from long-term observations on Lake Constance, Germany (Table 7).

- Algal Bioindicators and Trophic Index by Brettum (1989)

The system used by Brettum (1989) is an extension and elaboration of the method earlier developed by Hörnström (1981, see above). More than 120 species are assigned

to seven trophic categories (ultra-oligotrophic to hyper-eutrophic) according to the probability of their highest appearance calculate from

$$p = \frac{n_i}{N_i} \times V_i$$

with N_i = total number of algal species within a trophic class
n_i = number of a specific species (i) per group
V_i = percentage contribution of species i to total biovolume

These values are normalised to the interval at which the species contributes most (=100) which results in a numeric distribution of all species in the seven trophic classes which are summarised in Brettum's study. A compound index is finally calculated from the individual species indices:

$$I_T = \frac{\sum (\nu_i * i_{iT})}{\sum \nu_i}$$

with I_T = index for the trophic level T
ν_i = total biovolume of species i
i_{iT} = index value of species i for the trophic category T

This index has the advantage that it uses volumes rather than relative abundances. Similar to the study by Rosén (1981), distribution of species is related to several environmental variables

- BRB-Index (Schönfelder, 1997, 2000)

Bioindication with the BRB-index was developed for *b*icarbonate-*r*ich waters in *B*randenburg, Germany and is therefore restricted to this and similar types of waters but can be used for plankton and benthic diatoms both in streams and lakes. The concept of Schönfelder (1997) is based, similar to many other approaches, on the optimum and the tolerance range of diatom species to total phosphorus concentrations which are calculated from:

$$\ln TP - \text{Optimum}_k = \frac{\sum_{i-1}^{S} \ln TP_i * d_{k,i}}{\sum_{i-1}^{S} d_{k,i}}$$

with $d_{k,i} = n_{k,i}/n_i$

where TP = total phosphorus
k = taxon for which TP is estimated
i = sample number
s = number of samples
$d_{k,i}$ = dominance of taxon k in sample i
$n_{k,i}$ = abundance of taxon k in sample i
n_i = abundance of all species in sample i

The tolerance range of the individual species is estimated from the standard deviation of ln *TP*:

$$t_{\ln TP,k} = \left(\frac{\sum_{i=1}^{S} d_{k,i} * (\ln TP - \text{Optimum} - \ln TP_i)^2}{\sum_{i=1}^{S} d_{k,i}} \right)^{1/2}$$

These tolerance values are then converted to integer TP-factors from:

$$SF_{TP,k} = 3.4999 - 3.333\ t_{TP,k}$$

Mathematically negative results are considered to be zero. These factors are inversely proportional to the indication of TP by the species ($SF_{TP,k} = 0$ equals wide ecological tolerance, $SF_{TP,k} = 3$ little tolerance).

The trophic index is finally calculated from the dominance, the TP-factors and TP-optima of all the species (m) from:

$$\text{BRB - Index} = \frac{\sum_{k=1}^{m_i} d_{k,i} * SF_k * \ln TP - \text{Optimum}_k}{\sum_{k=1}^{m} d_{k,i} * SF_k}$$

The calculated index is calibrated against the natural-logarithms of the measured TP-concentrations which are related to 11 trophic conditions (Table 8). The TP factors for a large number of benthic and planktonic species can be found in Schönfelder (1997).

- Phytoplankton Indicators (Lepistö and Rosenström, 1998; Lepistö, 1999)

Most recent collection of extensive lists of indicator species for various types of trophic conditions. Indication based on an evaluation of references and own observations.

2.2.1.3. Classification from biomass or biovolume

Phytoplankton biovolume or biomass has been used by several authors for the trophic classification of lakes. The systems of Rosén (1981) and Rott (1984) are identical. The Norwegian (Brettum, 1989) and the Swedish classifications (Willén, 2000) are both based on either mean or maximum values. Four systems are compared in Table 9 from which it becomes evident that greatest discrepancies among delineation by authors are between Heinonen (1980) and all others. The main differences lie in the number of trophic categories considered. Brettum's classification is the most differentiated one within this comparison while those of Rosén (1981) and Rott (1984) have overlapping values but consider only three trophic levels. Categorisation and delineation using algal biomass by different authors is graphically summarised in Figure 4.

2.2.1.4. Classification based on seasonal phytoplankton associations

Detailed analyses of phytoplankton succession and seasonal development culminated in the description of 26 provisional associations by Reynolds (1997) which in his view

Table 8. Concentrations of total phosphorus (TP) for the trophic categories as defined by Schönfelder (1997).

Trophic status	Range of TP [$\mu g\ l^{-1}$]
Ultraoligotrophic	<4.3
Ultra- to oligotrophic	4.3–7.0
Oligotrophic	7.0–11.6
Oligo- to mesotrophic	11.6–19.1
Mesotrophic	19.1–31.5
Meso- to eutrophic	31.5–51.9
Eutrophic	51.9–85.6
Eu- to polytrophic	85.6–141.2
Polytrophic	141.2–232.8
Poly- to hypertrophic	232.8–383.8
Hypertrophic	>383.8

Table 9. Comparison of trophic delineation from phytoplankton fresh-weight biomass according to various authors.

Trophy	Average fresh weight biomass [$mg\ l^{-1}$]			
	Heinonen (1980)	Rosén (1981), Rott (1984)	Brettum (1989)	Willén (2000)
Ultra-oligotrophic	<0.2		<0.12	<0.1
Oligotrophic	0.21–0.5	0.1–1.0	0.12–0.40	0.1–0.5
Oligo-mesotrophic	0.5 –1.0		0.4 –0.6	
Mesotrophic	1.0 –2.5	0.5–5.0	0.6 –1.5	0.5–1.5
Meso-eutrophic			1.5 –2.0	1.5–2.5
Eutrophic	2.5 –10	>2	2.0 –5.0	2.5–5.0
Polytrophic			2.0 –5.0	
Hypertrophic	>10		>5	>5

are different vegetation types recognisable within freshwater phytoplankton (Table 10). The number of entries and the species associated with are seen as an open, changeable system by Reynolds. Associations are not defined via species but through functional algal groups. Adaptations to limiting factors can result in the preference of certain morphotypes, such as colonial or filamentous forms when grazing pressure increases, independent from their taxonomical position. Although Reynolds (1997) never uses the term 'indicator' about 60% of the species and genera mentioned in the broad description of his associations can be assigned to different trophic categories.

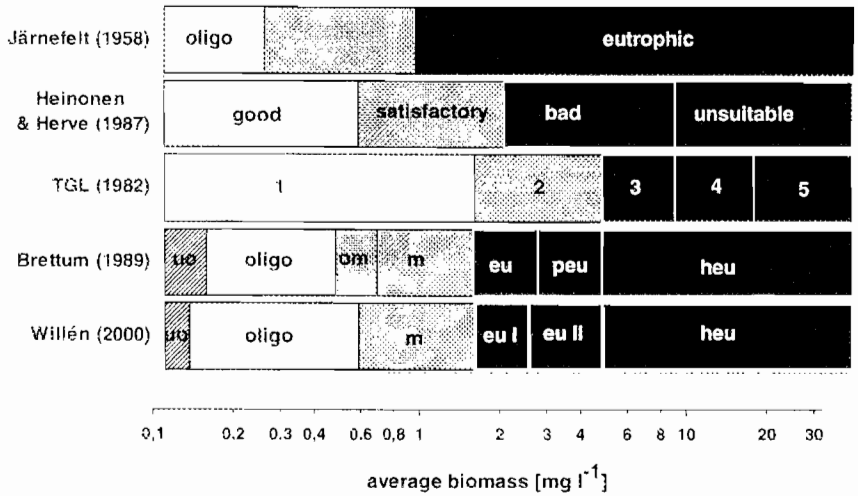


Figure 4. Comparison of five classification systems for lakes using average phytoplankton biomass

2.2.1.5. Other Approaches

Palaeolimnological investigations can provide background information of the recent past of lake ecosystems to be monitored (Simola et al., 1996). This approach is discussed in more detail in e.g. Charles et al. (1994) which came to the conclusion that palaeolimnological investigations can significantly enhance the usefulness and applicability of monitoring data by extending the temporal record of ecosystem conditions for a considerable time into the past, and providing a context for evaluating more recent measurements.

Environmental changes over longer time periods can be monitored using algal microfossils preserved in freshwater sediments (Dixit et al., 1992). Among many other potential indicators, the morphological remains of diatom frustules, chrysophyte scales and cysts are usually abundant in lake sediments, and they often form essential parts of palaeolomnological studies. Correlation of diatom and chrysophyte changes with specific lake water variables allow acidity, trophic and salinity reconstruction. The most effective way of studying algal populations with respect to lake water quality is to analyse the microfossils present in surface sediments from a set of lakes with known water chemistry. These diatom and chrysophyte training or calibration sets, integration over time and space, contain enough autecological and synecological information to enable deduction of environmental conditions from species composition of samples (Wunsam and Schmidt, 1995; Kamenik and Schmidt, 2001). Recent developments in ordination analysis has greatly improved our understanding of the relations between species distribution and environmental variables. Moreover, as a result of recent refinements in methodology of sediment coring and sectioning procedures detection of lake water quality changes of the last 5 to 10 years is now possible. The rapidly increasing data sets suggest that a number of environmental variables such as Secchi-depth, conductivity, several chemical elements etc. can be monitored using sedimentary algal remains.

Table 1. Associations of freshwater phytoplankton from Reynolds (1997). Trophic status and description of assemblages added from the text therein

	Trophic status	*Typical taxonomic units*	Description of plankton assemblage
A	Oligotrophic	*Urosolenia spp., Cyclotella comensis*	Oligotraphentic diatom dominated
B	Oigotrophic	*Asterionella spp., Aulacoseira italica*	Oligotraphentic spring-diatom s
C	Etrophic	*Asterionella, Stephanodiscus rotula, Aulacoseira ambigua*	Oligotraphentic spring-diatom
D	Hertrophic	*Stephanodiscus hantzschii*	Diatoms in hypertrophic shallow lakes
E	Mesotrophic	*Dinobryon, Chrysosphaerella*	Mesotraphentic Chrysophycean dominated
F	Oligotrophic	*Sphaerocystis, Botryococcus*	Oligotraphentic green algae
G	Eutrophic	*Eudorina, Pandorina*	Eutraphentic green algae
H	Eutrophic	*Anabaena*	N-fixing blue-green aggregates
J	Eutrophic	*Pediastrum, Scenedesmus, Oocystis borgei*	Eutraphentic green algae
K		*Aphanocapsa, Aphanothece*	Small sized blue-green aggregates
L_O	Oligo-mesotrophic	*Ceratium, Peridinium inconspicuum, Gomphosphaeria*	Oligotraphentic Dinoflagellates
L_M	Meso-eutrophic	*Ceratium, Microcystis*	Eutraphentic Dinoflagellates
M		*Microcystis*	*Microcystis*-dominated
N	Oligo-mesotrophic	*Cosmarium, Tabellaria*	Oligotraphentic desmid-diatom plankton
P	Eutrophic	*Staurastrum, Fragilaria.*	Eutraphentic plankton
R		*Planktothrix rubescens/mougeotii*	Deep-living blue-green algae
S	Eutrophic	*Planktothrix agardhii / Limnothrix redekei*	Eutraphentic, filamentous blue-green algae at low transparency
S_N	Eutrophic	*Cylindrospermopsis*	N-fixing, filamentous blue-green algae
T		*Geminella, Binuclearia, Tribonema*	Filamentous algae at high mixing
U		*Uroglena*	Early summer plankton at very low phosphorus concentrations
V		Phototrophic bacteria	Phototrophic bacterio-plankton
W		*Euglena, Synura, Gonium*	Plankton in ponds at high organic load
Xl	Eutrophic	*Chlorella, Ankyra, Monoraphidium*	Eutraphentic nanoplankton
X2	Eutrophic	*Rhodomonas, Chrysochromulina*	Eutraphentic mobile nanoplankton
X3	Oligotrophic	*Koliella, Chrysococcus*	Oligotraphentes nanoplankton
V	Meso-eutrophic	*Cryptomonas spp.*	Eutraphentic
Z	Oligotrophic	*Synechococcus, Chlorella minutissima*	Oligotraphentic picoplankton

In some cases algal-based models can help to predict trophic level changes (e.g. Dokulil and Frisk, 1993; Jørgensen, 1992.).

2.2.2. *Phytobenthos*

Periphyton is an important component of the litoral zone of lakes and reservoirs. Among the many algal groups which have been tried as bio-indicators for the lake litoral by several authors (e.g. Kann, 1978, 1986), the diatoms attracted particular attention because of their widespread distribution, high sensibility, good preservation and well developed indication techniques for both saprobity and trophy.

Most of the many investigations that deal with litoral diatoms are either systematically orientated or are interested in correlations to variables other than those responsible for eutrophication. As a consequence, information on species distribution and their environmental requirements within the trophic spectrum is limited (Lowe, 1974; Whitmore, 1989).

The trophic diatom index by Hofmann (1993, 1999) is one of the few examples of bio-indication using lake litoral diatoms. About 400 algal species from the epilithon, epiphyton and from artificial substrates were analysed for their requirements with special emphasis on total phosphorus because of its relevance for the trophic state. The organisms were assigned into five categories: a group containing all the ubiquitous, tolerant species found at all trophic levels and four indicative classes (oligotraphentic, oligo-mesotraphentic, meso-eutraphentic, and eutraphentic taxa). These four levels were combined with three weight-factors. The index is then calculated from the formula of Zelinka and Marvan (1961) with an equation similar to one of those already shown above. Classification is bases on five trophic classes from oligo- to hypertrophic.

2.3. Marine ecosystems

Most what has been outlined above for freshwater indicators and bioassays, equally applies to bio-indicators in the marine environment. Approaches and protocols for marine phytoplankton may be found in Maestrini et al. (1984). The most common approach for assessing the relation between phytoplankton and the nutritional environment is the classical descriptive one of drawing information from (i) concentrations of nutrients, (ii) phytoplankton biomass or (iii) phytoplankton biochemical activity. The complex nature of natural assemblages, however does not allow unequivocal answers. Nitrogen additions to coastal waters often result in increased phytoplankton productivity and perhaps biomass (Costa et al., 1992). Red and brown tides or the appearance of toxic algae is often linked to water quality (Watanabe 1983) but may also be considered as early indication of climatic change (Hinckley and Tierney, 1992).

Advantage is gained therefore from experimental approaches which use the response of organisms in bioassays. By definition, bioassays include a multitude of methods and techniques, such as in situ versus in vitro bioassays using either natural assemblages or unialgal cultures (for details refer to Maestrini et al., 1984, and Appendix in Maestrini et al., 1984).

Macroalgae or seaweeds in coastal marine waters are far more important as biomonitors than macrophytic algae in fresh-waters. Seaweeds have several intrinsic advantages as organisms for monitoring environmental impacts. Because of their sessile nature they are easily collected in abundance and can be used to characterise locations over time. Accumulation of compounds from the surrounding water make them idle as bioaccumulators. Sometimes the extent of benthic macroalgal distribution can be quantified by aerial mapping (Costa et al., 1992).

Investigations at the community level are time consuming and require expertise. Interpretation of results is difficult and detection of impacts often requires long-term studies. Alternatively, the responce of individual species to environmental conditions, measured as growth or productivity, can be used for biodetection under laboratory bioassay or field deployment conditions (Levine, 1984).

The diverse life history types among the algae offer a wide variety of approaches. Annual species reflect conditions over a well-defined period while perenniating algae integrate the milieu of several years past. Within-species assays are possible with the various stages in algal life cycles which can be expected to have different susceptibilities. The reproductive cells are of particular interest because they are usually most vulnerable.

Brown algae (Phaeophyceae), often dominating seaweed communities in the littoral and sub-littoral, are frequently employed for coastal monitoring. Members of the Fucales (*Fucus*, *Ascophyllum*) and Laminariales (*Laminaria*, *Macrocystis*) received most attention. Pollution assessment studies have also used red algae (Rodophyceae) which often make up the major biomass in subtidal communities. The life cycle of these algae involves three plant types: gametophytes, carposporophytes and tetrasporophytes. Any or all of these stages can be used for monitoring pollution effects.

Among the green seaweeds (Chlorophyceae), the genera *Ulva* and *Enteromorpha* have attracted considerable attention as biomonitors. The ability of gametes to develop parthenogenetically in *Ulva* is of particular interest, offering genotypically identical plants minimizing effects of genetic differences between experimental organisms.

2.3.1. *Size measurements*

All these plants grow by cell division and thallus elongation. The magnitude and rate of these processes can be measured simplest by the increase in dimensions. Alternative approaches include the determination of biomass and the estimation of rates of primary production.

In seaweeds, size is the most frequently used measurement for impact assessments. Some seaweeds have localized meristematic regions which can easily be used for growth determination. In the kelps, for instance, growth is primarily restricted to the base of the blades. Growth measurements are taken by punching a hole into the blade at a predetermined distance from the meristematic zone. After a certain period of growth, another hole is punched at the same location. The distance between holes is an estimation of growth. This approach has also been used in continuous flow culture systems of *Laminaria saccharina*. In addition, early developmental stages have been used by several authors to test effects of toxicants on seaweeds (for references ref. to Levine, 1984). Similar or more complicated techniques were developed for growth studies using a large number of red algal species, fucoid algae and *Ulva*.

Because sewage pollution in coastal waters is correlated with abundant *Ulva* growth several in situ and laboratory bioassays have been developed using discs cut from the thalli of *Ulva* or deploying genotypically identical *Ulva* plantlets (Levine, 1984).

2.3.2. Biomass

Another way of estimating growth and productivity in seaweed is through the determination of biomass. A complete over-view on sampling and quantitative procedures is given by Gonor and Kamp (1978). Bellamy et al. (1973) developed an approach to determine productivity from biomass estimations.

2.3.3. Photosynthesis and respiration rates

Seaweed productivity can be estimated from photosynthetic and respiration rates. Techniques commonly employed include CO_2-detection by infrared absorption, the oxygen evolution/consumption method and the carbon-14 technique. Rate measurements in situ or under laboratory conditions were used to detect effects of oil coating, sewage effluents, iron-ore dust, etc. on various types of marine algae used as bio-indicators.

2.3.4. Reproduction

The reproductive processes of seaweeds offers yet another way to investigate effects of pollutants or toxicants. Meiosis is a particularly sensitive phase in the life cycle of most organisms. Tropic responses, motility of reproductive cells and sexual processes offer a wide variety of possible test alternatives for pollutants such as oil, petroleum, iron-ore dust, detergents and other toxicants.

2.3.5. Bioaccumulation

Accumulation of polluting substances by marine macro-algae have received much attention because these attached plants reflect environmental conditions over prolonged time periods. Moreover, their sessile nature enables relatively easy collection. Seaweeds accumulate heavy metals, hydrocarbons, pesticides, PCBs, radionuclides and numerous other compounds from the water. The accumulation and release of these different compounds largely depends on their chemical properties and concentration in the environment but is strongly modified by several circumstances. The position of the plant on the shore or in the water column affects the degree of contamination since some contaminants are restricted to the surface layers while others sink relative rapidly to deeper layers or become associated with the sediment.

The principal mode for accumulation of substances by algae appears to be the process of adsorption. Frequently, the uptake involves two stages: (1) an initial passive accumulation by adsorption to the exterior surface, followed by (2) a slower uptake mediated by metabolic processes which in turn depend on external physical variables. The environmental regime at different localities, regions or seasons can therefore significantly influence the interpretation of bioaccumulation data.

In addition, structural differentiation must be considered. Many marine macroalgae accumulate substance with virtually every cell while in some of the more differentiated seaweeds uptake and accumulation varies in different portions of the plant. Generally the older plant parts have accumulated higher concentrations of pollutants. Many complications are involved, however, in the interpretation of such data (Levine, 1984).

2.3.6. Mutagenicity assays

Many man-made chemicals introduced into the environment are suspect to induce cancer. These compounds can be tested for cancerogenesis by their ability to induce mutations in microbial DNA, since carcinogenic and mutagenic chemicals are highly correlated. Application to water samples, however, is restricted owing to dilution and the large volumes needed therefore. This procedural bottleneck can be circumvented by using extracts derived from bioaccumulator species. Mutagens were detected within tissue extracts derived from *Porphyra umbilicalis, Fucus vesiculosus,* and *Enteromorpha* spp. by Barnes (1980). Due to its parenchymatous nature, lowest mutagenic activity was found in Fucus.

Mutagenic substances are either endogenously produced by seaweeds or accumulated from the external environment. For coastal monitoring, the character of mutagenic agents is of prime importance. Many compounds are present in all marine waters as a result of both natural production an anthropogenic inputs. Tissues of bioaccumulator species integrating environmental regimes in conjunction with mutagenicity assays provide reliable screening procedures for hazardous chemicals in marine ecosystems.

3. Bioassays

Theoretical principles, selection of organisms and their pre-cultivation in algal bioassays are extensively discussed in Marvan et al. (1979). Biotests with algae are carried out either in the natural environment or, under more 'standardised' conditions, in the laboratory with single cultivated algal strains, mixtures of them or natural assemblages. In general algal bioassays can be an important tool for the assessment of present or potential deterioration of water quality (Bellinger, 1979). A disadvantage of many algal bioassays, however, is that they do not consider nutrient recycling in the water-body. Results obtained therefor often refer to maximum attainable algal biomass rather than to total algal growth. Since such algal-bioassays only show what happens in the short term, longer-term bioassays become increasingly important.

3.1. Field approaches and in situ techniques

Field or *in situ* approaches do not use "standard conditions" of growth for incubations, but rely on available nutrient supply, temperature and light conditions prevailing at the time. The disadvantage is the infinite range of variables in nature. Field incubations of algae, usually phytoplankton or cultivated algal species used in the bioassay, are carried out using one of a variety of chambers or enclosures. There is an almost endless

variety of systems either closed or open, a multitude of experimental designs, species used and measurement parameters. The individual investigator must decide and choose which technique is the best for the problem to be solved (Trainor, 1984).

3.1.1. Types of enclosures

In situ enclosures may be divided into five types: bottles, bags, tubes, curtains, and specialised types of flow-through studies.

Approaches using *bottles* are probably the oldest. As early as 1891, Regnard used bottles to expose cress and radish seedlings to the underwater light gradient in an attempt to explore the growth and chlorophyll regulation (cit. acc. to Talling, 1984). Capacities range from 125 ml bottles to 'carboys' of 20 litres or more. Strickland and Terhune (1961) were the among the first to use *bags* or *spheres* to study the dynamics of phytoplankton. The size of these bags can range from about 120 m^3 to as small as 1 or 2 litres. *Tubes* have two important differences to spheres; they have a more or less fixed diameter and they can be open at the top or on both ends. When open, the tubes do not isolate the enclosed water mass from the atmosphere or the bottom sediment. A large range in size among different types of tubes has been employed for ecological studies. The first small *in situ* enclosures were probably the 'plankton-test-lots' used by Thomas (1964) for nutrient enrichment experiments. Other enclosures range from diameters of about 1 m through medium-sized 'limnocorrals' to the 45 m diameter 'Lund tube' (Lund, 1972). The largest enclosures are generally those used in marine studies (Schelske, 1984). Water masses within an aquatic system may be separated from one another by *curtains* extending from the surface to the bottom sediments effectively restricting the exchange of water between the two parts (Schindler, 1974). This type has since been used by many investigators. Specialised types of field enclosures include the *in situ* chemostat (deNoyelles and O'Brien, 1974), sacs constructed from dialysis membranes and devices using either membrane or similar filters or dialysis membranes to separate cultures or natural assemblages from the surrounding water.

There is no obvious advantage of one single type of enclosure. All types have shortcomings. Application to a particular experiment will depend on several factors such as the number of independent variables

3.1.2. Algal fluorescence

Similar to standardised laboratory bioassays (see below), the toxicity of chemicals, mixtures of chemicals or polluted waters is measured as their inhibitory effect on the photosynthesis of natural algal assemblages. Measurements continuously monitor chlorophyll-a fluorescence signals. Additional parameters include cell numbers and turbidity (Sayk and Schmidt, 1983; Noack, 1987). Other 'on line' systems use algal cultures (*Scenedesmus subspicatus, Chlamydomonas reinhardtii* or *Microcystis sp.*) which are added to the water as test organisms. The delayed fluorescence signal from darkened cells (DF-algal test) is measured every 30 minutes (Gerhardt and Putzger, 1992). Both techniques are cost intensive on installation, require regular servicing but offer quasi-permanent control abilities. Correct interpretation of results is sometimes difficult. In combination with other (semi)-automatic tests these techniques can be used

for alert systems. Further on line systems under development are summarised in Gunkel (1994, p. 356ff).

3.1.3. Oxygen-production and respiration rates in situ

Standard test: DIN 38412, L13, 1983a
Organisms: Natural phytoplankton
Oxygen concentrations are measured chemically or amperometrically in light and dark bottles at the beginning and at the end of an incubation period of up to 24 hours. Because of the rapid light attenuation in natural waters this test is usually performed at several different depths.

O_2 in light – O_2 in dark = Gross oxygen-production
Initial O_2 – O_2 in $dark_{24h}$ = Respiration

Sensitive, cost effective test with high ecological relevance because of multi-species approach. Not used in ecotoxicology. Methodological comparison to other techniques see e.g. Sakamoto et al. (1984).

3.1.4. Bioassays using picoplankton

A number of studies (Munawar et al., 1987; Munawar and Weisse, 1989; Weisse, 1991) indicate that photoautotrophic picoplankton are useful indicators of contaminant stress in marine and freshwater ecosystems. They are often a dominant component of oligotrophic, pelagic ecosystems (Weisse, 1993; Stockner et al., 2000). From recent *in situ* nutrient enrichment bioassays with picoplankton Schallenberg and Burns (2001) concluded that picocynaobacteria in oligotrophic lakes are sensitive to extremely small changes in nutrient availability and therefore highly useful as early indicators of nutrient enrichment.

3.1.5. Assessment of micro-'Aufwuchs' biocoenoses

Not standardised
Organisms: Aufwuchs-biocoenoses in running waters
The adaptation of the Aufwuchs organisms to ecological conditions are assessed through microscopical analysis, dry weight estimation, chlorophyll analysis or measurement of the oxygen production potential (see below) after an incubation period of 3–4 weeks.

Sensitive, ecologically relevant and integrative assessment at moderate costs. Large time lags and interpretation problems when specific parameters must be monitored.

Techniques for *in situ* algal assays with periphyton using bottles or artificial substrates are discussed by Sládečková (1979).

3.2. Laboratory tests and bioassays

In several countries tests have been developed to determine the algal growth potential, the AGP, of a water. Basically the experiment determines the maximum concentration

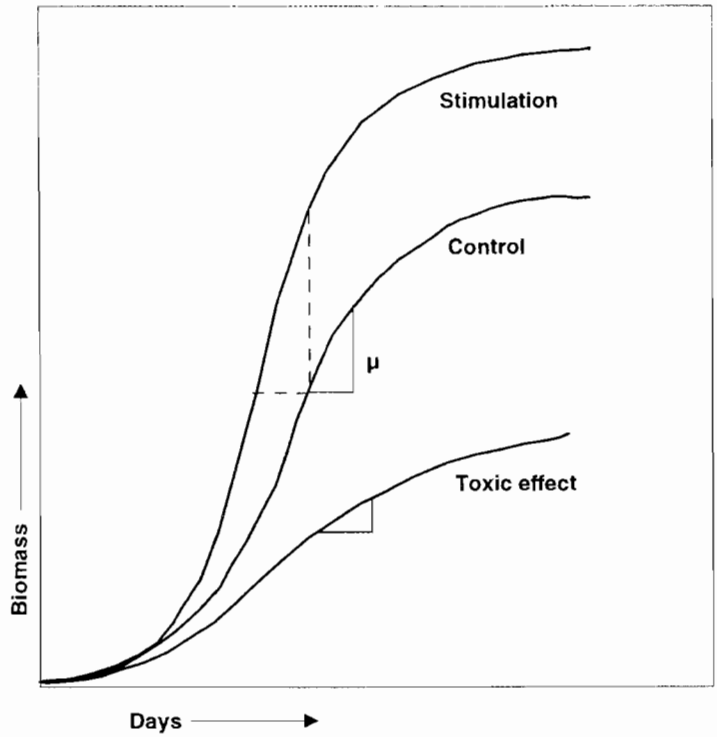


Figure 5. Schematic growth curve of algae in batch culture compared to stimulation and toxic effects of a test substance. μ = specific growth rate coefficient.

of algae that can grow in a water sample under standardized conditions. These tests are often used to judge:

- the degree of eutrophication of surface water (Thomas, 1953; Skulberg, 1964)
- the eutrophication potential of the effluent of sewage treatment plants (Forsberg, 1972)
- the possible effects of environmental measures on the degree of eutrophication of water systems (van der Does and Klapwijk, 1987).

Growth curves obtained by such tests or bioassays schematically are displayed in Figure 5 together with schematic effect-curves of a stimulating and a toxic substance. In some cases, these growth curves show a second exponential phase after a retardation phase. depending on the culture medium used (Bolier and Donze, 1989).

Growth tests are performed in batch cultures under defined nutrient, CO_2, pH and light conditions. Biomass development over time is estimated from microscopic or electronic cell counts, chlorophyll-a concentration, ATP-, DNA-content or similar parameters. Turbidity measurements may overestimate biomass when bacterial contamination is high. Test results are analysed from algal growth curves against an untreated control (Gunkel, 1994). Laboratory tests used in eco-toxicology are summarised in Steinberg et al. (1995).

3.2.1. Algal growth inhibition test

Standard test: OECD Guideline for testing of chemicals 201, 7.1984
Organisms: unicellular green algae (*Ankistrodesmus bibraianum, Scenedesmus subspicatus* or *Chlorella vulgaris*)
Dilution series between NOEC and concentration >LC 50. Growth is followed by cell counting for 72 hours.
End point: EC 50 and NOEC

3.2.2. Inhibition of green-alga by water contaminants (Scenedesmus growth-inhibition test)

Standard test: DIN 38 412, L9, 1989 (ISO/DIS 6862:06.87).
Organism: *Scenedesmus subspicatus* CHODAT, a unicellular green alga
Dilution series of the substance or water to be tested run in 100 ml Erlenmayr-flasks with culture media at 23°C, 8 000 Lux continuous light for 3 days.
Biomass must at least be estimated after 24, 48 and 72 hours.
End point: EC 10 and EC 50 after 72 hours

3.2.2. Measurement of non-toxic effects of water contaminants on green algae (Scenedesmus-chlorophyll-fluorescence test) in dilutions

Standard test: DIN 38 412, L33, 1991
Organism: *Scenedesmus subspicatus* CHODAT, a unicellular green alga
Dilution series are incubated as above. Fluorescence is measured at the end at 685 nm from all dilutions relative to the untreated control. Toxic effects are present if fluorescence is inhibited by 20%.

All the above mentioned tests can largely be automated.

Typical results of algal growth potential (AGP) tests are shown in Figure 6 from an intensive study in the Netherlands (Klapwijk et al., 1989) and from a deep alpine lake (Dokulil, unpublished). Both observations indicate that phosphorus was the substance most likely limiting growth. Seasonal varying nutrient limitation was found in English lakes using laboratory bioassays with *Asterionella formosa* and *Rhodomonas lacustris* as test organisms (Barbosa, 1989). Phosphate was the major element limiting both species throughout the year, except during spring diatom development when dissolved silica became limiting. Chelated iron increased growth, particularly in combination with phosphate. Comparison of AGP-bioassay and phosphate uptake kinetics with natural phytoplankton, however gave somewhat inconclusive results as reported by Van Donk et al. (1989).

3.2.3. Bioassays with macroalgae

Macroalgae may also be used as test-organisms. Inhibition of trichom movement in the blue-green *Phormidium autumnale* (cyanobacteria) is used to screen for toxic substances (Noll and Bauer, 1973; Breitig and Tümpling, 1982). Håglund et al. (1990) use the red-alga *Gracilaria tenuistipitata* (Rodophyta) to test marine and brackish waters for toxic pollutants. Effects of tributyltin (TBT) on community metabolism

Figure 6. Two typical examples of algal growth potential (AGP) bioassay experiments with nitrogen and phosphorus enrichment. The left hand panel, modified from Klapwijk et al. (1989), shows yields (± st. dev.) as optical density for laboratory batch experiments with water from the Reeuwijk lakes in The Netherlands using the alga *Scenedesmus quadricauda* as test organism. In the right hand panel, yields are expressed as chlorophyll-a for an in situ AGP bioassay using natural phytoplankton from Mondsee, Austria (Dokulil, 1989). Both experiments were presumably limited by phosphorus.

dominated by *Fucus vesiculosus* were measured with a portable continuous flow-through system in a study by Lindblad et al. (1989).

3.2.4. Bioprobes

Bio-electrodes are currently developed which use the electron flow produced by photosynthesis or respiration of the cyanobacterium *Synechococcus*, embedded in alginate and fixed to the tip of the electrode, as a measurement signal (Steinberg et al., 1992).

3.2.5. Ataxonomic bioindication

Because size distribution of pelagic organisms is continuous in undisturbed systems (Gaedke, 1992), biomass spectra may be used to evaluate environmental disturbances. Optimal size spectra can most easily by obtained by modified flow-cytometry (Steinberg et al., 1999).

3.2.6. Oxygen-production potential – light-dark bottle laboratory test (DIN 38 412, L14, 1983b)

This test estimates the production potential as well as the respiration of freshwaters. Both rates are essential for the overall oxygen budget.

Six oxygen bottles are filled with the test-water. Two bottles are used to estimate the initial oxygen concentration, two are incubated under constant light in the laboratory at 20°C for 24 hours, and the remaining two are completely darkened. At the end of the incubation period the oxygen levels in the bottles are measured. Results are expressed as:

O_2 in $light_{24h}$ – O_2 in $dark_{24h}$ = Gross-potential production
Initial O_2 – O_2 in $dark_{24h}$ = Respiration-potential

Cheap, sensitive and easy assessment of potential multi-species production and destruction rates. Rarely used for effect monitoring of contaminants.

3.3. Sediments

Many pollutants are associated with sediments in aquatic systems. Hazard assessment to establish sediment quality criteria require rapid, inexpensive screening test. Direct bioassay with algae have proven to be very sensitive indicators of contaminant stress.

Numerous methods are available for the assessment of environmental impacts of sediment-associated contaminants Ahlf and Munawar (1988). Effects of sediment elutriates on algae are measured as the amount of inhibition in photosynthetic ^{14}C assimilation of e.g. *Selenastrum capricornutum* under laboratory conditions (Ross et al., 1988). Approaches using natural phytoplankton were developed by Munawar and Munawar (1987). Effects of increasing concentrations of sediment elutriates on photosynthetic rates of natural phytoplankton under controlled laboratory conditions are tested in the short-term "algal fractionation bioassay" (AFB). Carbon-uptake of size fractions (>20 μm – netplankton ; 5–20 μm – nanoplankton ; 1–5 μm – ultraplankton ; < 1 μm – picoplankton) is estimated against an untreated control. Similarly,

long-term effects of sediment elutriates can be evaluated from 4 day bioassays in 5-litre bottles using natural phytoplankton or a mixed culture of *Ankistrodesmus braunii* (Naeg.) Brunnthaler and *Chlorella vulgaris* Beyerinck. This test may be expanded to include the solid phase of the sediment allowing differential bioassay of the effects of both solid- and liquid-phase of sediment contaminants. In this case, the sediment compartment may be separated from the water/organism part by a membrane allowing exchange of substances but prevent mixing (Ahlf, 1985). The use of sediments directly in bioassays with algae is recommended over elutriates because a large number of toxic chemicals can not be extracted with water (Ongley et al., 1988).

All these bioassay techniques integrate the response of test organisms to contaminants and nutrients. They often give best results when combined with other assessment methods (Ahlf et al., 1989; Gregor and Munawar, 1989).

4. Ecotoxicology

In ecotoxicology biomonitoring is the accumulation of contaminants in cells or tissues of organisms without severe damage or even death. The contaminant and its quantity can only be evaluated after chemical analysis (*exposure-monitoring*). Effect-monitoring estimates the quality and quantity of a contaminant through analysis of the population structure (bioindication) because populations or assemblages change characteristically when impacted by polluting substances.

A contaminating substance (xenobiotica) must be biologically available to be of environmental relevance and hence be taken up by organisms in one way or the other. The ability of many plants and animals to accumulate exotic substances makes them idle for biomonitoring.

Criteria for effective biomonitors for organic contaminants include the following:

1. The organisms must accumulate the xenobiotic substances without being affected by environmental relevant concentrations
2. The organisms should preferably be sessile to be representative for the investigated area.
3. The organisms should either live everywhere in the area investigated or be tolerant to exposure in chambers, cages, etc.
4. The organisms shall be long lived to act as integrators of contaminations.
5. The organisms shall be of such a size that enough tissue for chemical analysis is available.
6. Collection and handling of the organisms should be easy.
7. A simple correlation should exist between the mean concentration of the contaminant in the environment and the content in the organism.
8. All individuals of a species used in biomonitoring must, under all circumstances, have the same relation to the concentration of the contaminant.

4.1. Measurement techniques

Acute toxicity is usually estimated from 72 hour growth tests using the green-algae *Scenedesmus subspicatus*. The no observed effect concentration (NOEC) is defined as

the concentration at which less than 20% of the organisms ($<EC_{20}$) are affected (Steinberg et al., 1999). A similar growth test is used to estimate the quality of sediments.

Alternatively, acute aquatic toxicity can be assessed by a practical and cost-efficient micro-bioassay using microplates with *Selenastrum capricornutum* as test organism following the standardized protocol developed by Blaise (1986) and Blaise et al. (1986).

Long-term sublethal toxicity is much more difficult to assess. Rhee (1989) used a two-stage continuous algal culture bioassay to investigate steady-state responses of a diatom (*Fragilaria crotonensis*), a green-alga (*Ankistrodesmus falcatus*) and a cyano-bacterium (*Microcystis sp.*) to organic pollutants (PCBs). Results showed a variety of sublethal effects such as enhancement of growth, photosynthesis and P-uptake as well as their inhibition, growth rebound and development of resistance.

4.2. Effects of inorganic nitrogen substances

A summary of acute and chronicle toxicity effects of ammonium and nitrite on algae, invertebrates and fish is provided by Schwoerbel et al. (1991). Phytoplankton species such as *Chlorella vulgaris* show acute toxic effects at concentrations (LC-50/5d) of 8.55 mg l^{-1} NH_3. Chronicle effects on CO_2-uptake of five species of green and blue-green algae were only observed at ammonia concentrations much higher than those commonly observed in running waters.

4.3. Uptake of organic contaminants by algae

Uptake and accumulation of a contaminant by algae follow a saturation curve where saturation must be seen as an equilibrium between adsorption and de-sorption. Mathematically these behaviours can be described by Langmuir's isotherms, the Michaelis-Menten equation or as equilibrium distribution according to Nernst.

Uptake of contaminants in algae is mediated by a variety of cellular processes which results in non-constant bio-concentration factors (BCF's). Quantitative correlation of accumulation in algae with storage products other than lipids or oils deviate from animals in the slope of the regression equation.

An example of the variability of the BCF's is shown in Table 11 for the bio-accumulation of atrazin by the green coccoid algae *Scenedesmus acutus.*

The sorption capacity increases with increasing concentrations in the surrounding medium according to

$$BCF_F = Sk/c_w \qquad (1)$$

where BCF_F is the bio-concentration factor related to fresh-weight, Sk is the sorption capacity and c_w is the concentration of the xenobiotic substance in the medium.

The uptake of herbicides by algae has two steps: a *protein-specific binding* which follows a saturation function and a *unspecific binding* where distribution-equilibria with the lipid phase are important.

Steinberg et al. (1992) summarise BCFs by the green algae *Chlorella fusca* for more than 100 selected organic chemicals. These BCFs span several orders of magnitude

Table 11. Dependence of the bio-concentration factors (BCF) of *Scenedesmus acutus* from external concentrations of Atrazin (after Steinberg et al., 1992).

Atrazin concentrations [mg l^{-1}]	BCF-value [volume related]	Sorption coefficient (Sk) [mg kg^{-1} dry weight]
0.0012	51	0.36
0.012	27	1.97
0.100	10	6.80
1.100	6	44.20

from 10 for 2,6-dichlorbenzamid to 28,000 for methanol which is incorporated and metabolised like acetate or urea. Condensed aromatic substances have intermediate BCFs.

Eco-toxicological effects may be estimated from physico-chemical attributes of the (untested) substance by the Quantitative Structure Activity Relationship (QSAR). If the concentrating phase in the organism are lipids then the BCFs should have a simple relationship to the octanol/water partition coefficient K_{OW}. For green algae, such as *Chlorella fusca* or *Ankistrodesmus bibraianum,* these correlations deviate significantly from those observed for various animals (cit acc. to Steinberg et al., 1992). Uptake by algae, picoplankton and bacteria is a two-step process involving lipid independent adsorption to surfaces and later incorporation into the lipid phase (see Falkner and Simonis, 1982 and Steinberg et al., 1992 for further discussion).

Bioaccumulation and -magnification within the food chain depends on the partition coefficient K_{OW} of the contaminant. At values of log K_{OW} less than 5 accumulation is not important. Substances with values between 5 and 7 are strongly magnified within the food chain. At log $K_{OW} > 7$, effects will largely depend on assimilation and accumulation by phytoplankton (Thomann, 1989).

4.4. Heavy metals

Several algal species accumulate considerable amounts of metals and can thus be used as monitors for elements such as cadmium, copper or lead (Hellawell, 1986; Whitton, 1984). Both field and laboratory populations have been used with success. A detailed description of toxicity effects of various metals can be found in Moore and Ramamoorthy (1984). Most metals are slightly to highly toxic to algae, arsenic, copper, mercury and zinc having the greatest effect. Impacts on algae in natural waters is highly variable. Cyanobacterial strains reacted more sensitive to heavy metals in a comparative laboratory growth inhibition test than green algae (Kusel-Fetzmann et al., 1989). Because of its widespread occurrence, the filamentous green alga *Cladophora* has been assessed more than any other species except perhaps for *Chlorella* in the laboratory. Both species concentrate various metals proportionally to ambient concentrations. An example of pH-dependent Zn uptake by *Cladophora glomerata* (L.) Kütz.

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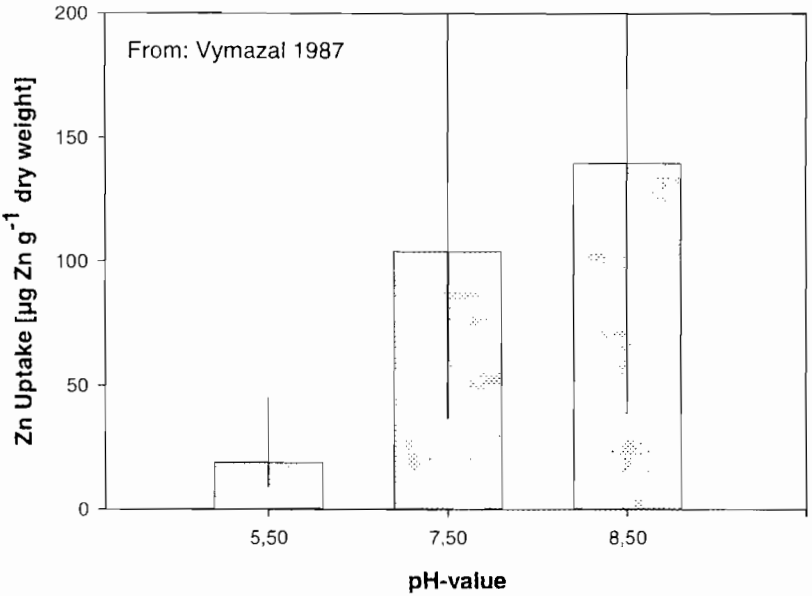

Figure 7. Relationship between Zn uptake in *Cladophora glomerata* and pH. Bars indicate maximum and minimum values (modified from Vymazal, 1987).

is given in Figure 7 (Vymazal, 1987). In contrast, enrichment ratios of the red-alga *Lemanea fluviatilis* decreased with increasing aqueous concentration (Fig. 8, Whitton, 1984).

4.5. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are usually mixtures of isomeres marketed under a variety of names. They are non-ionic, non-flammable compounds with extremely low water solubility but are highly lipophilic, and hence of significance to biota.

Algae as indicators of PCB-pollution are advantageous because they represent organism at the basis of the food chain. Marine phytoplankton for instance has an enormous capacity for accumulating organohalogen compounds such as polychlorinated biphenyls (Ramade, 1987). Concentrations of PCB in the range of 11 to 111 µg l^{-1} were reported to inhibit growth and photosynthesis in green algal species (see Steinberg et al., 1992 or Hellawell, 1986).

4.6. Pesticides

Several of the many different groups of pesticides can not be biomonitored with algae mainly because of their low bioaccumulation (e.g. urea-based pesticides, comp. Steinberg et al., 1992, p. 178 ff). Although nitrogen-based herbicides such as e.g. atrazin are strongly accumulated by phytoplankton and the coccoid green-algal species *Scenedesmus acutus* and *Chlorella fusca*, biomonitoring is not possible because of the

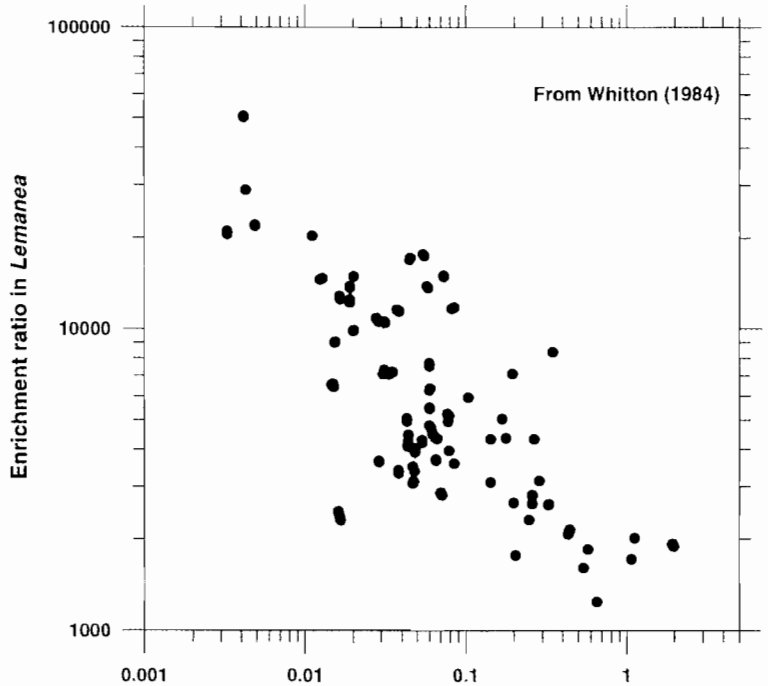

Figure 8. Relationship between enrichment ratio for zinc in *Lemanea fluviatilis* and total zinc concentration in water of stream and rivers ($r = -0.84$), modified from Whitton (1984)

high variability (Kusel-Fetzmann et al., 1989) and discrepancy of measured and calculated BCF-values. Side-effects of atrazin on aquatic ecosystems are reported, however for single species, communities and food chains (Lampert et al., 1989). In running water experiments, the composition and quantity of periphytic algae, especially *Rhopalodia, Phormidium* and *Cladophora* are affected at atrazin-levels of 1 mg l^{-1}. Adverse effects on diatoms become visible already at concentrations of 0.01 mg l^{-1}. Pre-incubation with the herbizide did not result in adaptation (Kosinski, 1984; Kosinski and Merkle, 1984). Tabulated data on accumulation and toxicity of selected pesticides by algal species are summarized in Steinberg et al. (1992).

The specific diversity of phytoplankton and biomass estimations via chlorophyll-a in ponds were used by Goacolou and Echaubard (1987) to evaluate in situ pesticide contamination. The biocoenotic structure, species richness and chlorophyll levels were significantly altered in ponds affected by pesticides.

4.7. Tensides

Toxicity from various tenside classes vary by four orders of magnitude within a single algal species. In general, however, kationic tensides are far more effective to algae than anionic or non-ionic tensides. The sensitivity of different algae to a single tenside varies by three orders of magnitude, depending on the species used, their physiology

Table 12. Mean concentration factors for various radioisotopes in marine algae (from Ramade, 1987).

Radionuclides	Conc. factor	Radionuclides	Conc. factor
^{3}H	0.9	$^{89,90}Sr$	50
^{7}Be	250	$^{90,91}Y$	500
^{14}C	4000	^{95}Zr, ^{95}Nb	1500
^{24}Na	1	$^{103,105}Ru$, ^{106}Rh	400
^{32}P	10^4	^{131}I	5000
^{45}Ca	2	^{133}Xe	1
^{46}Sc	1200	^{137}Cs	15
^{51}Cr	2000	^{140}Ba, ^{140}La	25
$^{54,56}Mn$	3000	$^{141,144}Ce$	700
$^{55,59}Fe$	2×10^4	$^{183,187}W$	5
$^{57,58,60}Co$	500	$^{203,210}Pb$	700
^{65}Zn	10^3	^{210}Po	1000
^{85}Kr	1	^{226}Ra	1000
		^{239}Pu	1300

and the overall test conditions. The cyanobacterium *Microcystis aeruginosa* for instance, is 10-times more sensible than the green *Ankistrodesmus bibraianum.* Toxicity values for tensids are tabulated by Lewis (1990).

4.8. Radioisotopes

According to the summary by Ramade (1987) the average concentration factor of radioisotopes by algae varies considerably depending on the isotope (Table 12).

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