Use of Plants to Monitor Heavy Metals in Freshwaters 1991

Methods for the Examination of Waters and Associated Materials

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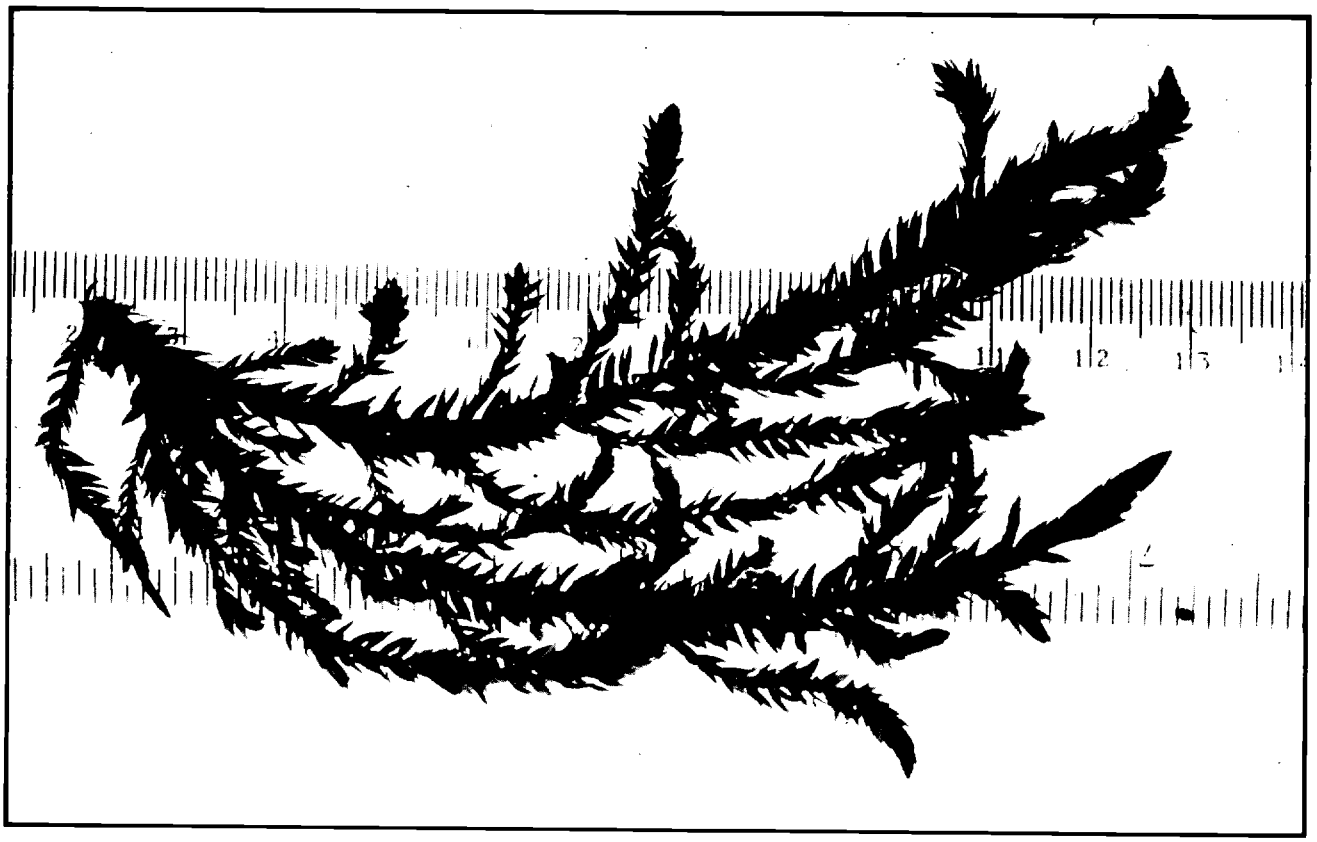

Use of Plants to Monitor Heavy Metals in Freshwaters 1991

B. A. Whitton, M. G. Kelly, J. P. C. Harding and P. J. Say

Methods for the Examination of Waters and Associated Materials

This booklet contains methods for using aquatic plants to monitor heavy metals in freshwaters, especially rivers and streams. The principal method depends on the measurement of metal concentrations in particular parts of plants in order to assess the concentrations of metals in the water during hours or days previous to the period of sampling. The development of methodology specifically for this book was carried out by B. A. Whitton and M. G. Kelly (Department of Biological Sciences, University of Durham), J. P. C. Harding (National Rivers Authority, Severn-Trent Region) and P. J. Say (Northern Environmental Consultants Ltd, Consett). The booklet begins with general notes on safety.

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Contents

Note: There may sometimes be legal constraints on the use of these methods in certain areas, but these can usually be complied with if permission is obtained in advance.

The provisions of the Wildlife and Countryside Act 1981, the Health and Safety at Work etc Act 1974, the corresponding Scottish and Northern Ireland legislation, all National and Local Safety Regulations and all laws and regulations with regard to trespass and rights of access must be observed.

Care needs to be taken if plants are removed, not only to comply with the law and with local requirements, but also not to cause permanent denudation or erosion of the bed, or to disturb breeding fauna.

A few riverside plants are highly toxic such as Giant Hogweed and Hemlock Water Dropwort. It is wise to know what these few look like and treat them with care if met.

About This Series

This booklet is part of a series intended to provide recommended methods for determining the quality of water and associated materials. In addition short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare so that they were often partially out of date before they appeared in print. The present series is published as a series of booklets on single or related topics, thus allowing for the replacement or addition of methods as quickly as practicable without the need for waiting for the next edition. The rate of publication is also related to the urgency of the requirements for that particular method.

Although ideally, all methods published should be fully tested, this is not often possible without delay in publication. Furthermore, the limit of detection, range, precision and interference effects applying to instrumental methods can be dependent on the actual instrument used, as well as on sample type, reagent purity and operator skill, etc. Even methods tested in many laboratories have been known to acquire problems when new products appear (introducing new substances into effluents), changes in production methods affecting reagent quality, or the method used to analyse new types of sample (despite apparent similarity to samples already evaluated). As a guide, the following categories have been given to methods:

(i) tested, usually in five or more laboratories
– no grade indicated;

(ii) tested in one to three or four laboratories
– Tentative;

(iii) evaluated, but not fully tested, but publication is urgently required
– Note;

(iv) tested and found to be satisfactory by several laboratories, but in the opinion of experts requires a high degree of skill or has some other difficulty such that the method would be replaced if a better method were discovered.
– Provisional.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users and senior technical staff, to decide which method to use for the determination in hand. Whilst the attention of users is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has nine working groups, each responsible for one section or aspect of water quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage works control methods
9.0 Radiochemical methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press. A current list of publications can be obtained from the Secretary.

Every effort is made to prevent errors from occurring in the published text. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically. However, should any errors be found, please notify the Secretary.

Dr D WESTWOOD
Secretary

15 August 1991

Warning to Users

The analytical procedures given in this booklet should only be carried out by competently trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or workplace, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text, so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

The best safeguard is a thorough consideration of hazards and consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra-violet) use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Safe practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', issued by the Royal Society of Chemistry, London: 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Services Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient, give information on the nature of the injury especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

Safety while Sampling

Prior consideration must be given, especially when sampling in confined spaces or where access is difficult, to guard against suffocation, drowning, falls, and poisoning or infection by ingestion, inhalation, or skin contact.

Good Laboratory Practice

The Department of Health issue a booklet entitled:
Good Laboratory Practice; the United Kingdom Compliance Programme, 1989.
This can be obtained by writing to that Department in London. It deals chiefly with toxicity studies, but much can be applied to analytical chemistry.

Summary

This booklet describes methods by which plants can be used to monitor heavy metal concentrations in freshwaters. The principal method recommended involves the analysis of metal composition of the plants. Other methods reported briefly are the use of bioassays and toxicity tests, and measurement of genetic tolerance and community structure.

These methods are mostly intended to supplement chemical analysis of water and/or sediments. However, there are situations where plants can provide information not easily obtained by other means. Examples include qualitative evidence for occasional releases of metals days or even weeks before sampling, and more accurate estimates of aqueous metal concentrations which passed downstream some hours prior to the site being inspected. The high levels of accumulation shown by some species increase the sensitivity of detection and this accumulated metal gives a better indication than conventional chemical analysis of the fraction in the environment which is likely to affect aquatic biological systems.

The plants used for analysis may already occur at the site, but in some cases it is necessary to import material from elsewhere; this may be an alga or bryophyte attached to boulders or detached material of a robust moss species placed inside mesh bags ("moss-bag" technique), with the bag fixed in some way at the site.

Ten species are recommended for general use. In shallow rivers with moderate to high current velocities it is usually possible to find at least one of these species, but in deeper waters or lakes it may be necessary to import material. In some circumstances there may be advantages in using a species not included here, but for most of the ten there is a considerable body of data about their heavy metal composition, so one should be chosen wherever feasible.

The ten species recommended for metal analysis are four algae (*Lemanea fluviatilis*, *Cladophora glomerata*, *Enteromorpha flexuosa*, *Nitella flexilis*), four bryophytes (*Amblystegium riparium*, *Fontinalis antipyretica*, *Rhynchostegium riparioides*, *Scapania undulata*) and two flowering plants (*Elodea canadensis*, *Potamogeton pectinatus*). Among these, *Rhynchostegium riparioides* is overall the most useful, because of its especially widespread occurrence and the high concentrations to which it accumulates metals, but there are situations where each species has a particular advantage. In some species it is possible to use the whole plant for the final chemical analysis, but for the majority it is preferable to use only the apical region of the shoots. For mosses this is the terminal 2 cm.

The various stages in the preparation of the plant sample for analysis are essentially the same for Cr, Zn, Ni, Cu, Cd and Pb; the same digest may also be used for Al, Mn and Fe, which are not 'heavy metals'. However several modifications are needed for Hg.

The nature of the problem to be investigated will determine the most suitable method for using the plant material. For many purposes all that is required are samples to provide a comparison between sites; this is the situation, for instance, when there is a need to locate a source of intermittent pollution. For some purposes, however, a more quantitative approach is needed. An outline of how to achieve this is included, together with a guide to the literature. The most detailed information is available for Cu, Zn, Cd and Pb, but further baseline studies are needed for other elements, especially Al and Hg.

1 Introduction

The problems associated with heavy metal pollution are no doubt well known to most users of this booklet, since the deleterious effects of these metals to human health and the environment have long been recognized. Chemical techniques for analysis of heavy metals are now well advanced and concentrations of the order of about one part in a billion ($1\ \mu g\ l^{-1}$) can be analyzed routinely for many elements. There is however also increasing interest in biological monitoring methods for these metals, both to supplement chemical analysis of the water and for use when water analysis is not possible, as when a pollutant has already dispersed. This booklet summarizes the ways in which plants may be used for this purpose.

The term "heavy metal" itself has been criticized as ambiguous. Traditional definitions include all metals with a density greater than five (Passow *et al.*, 1961). The term has sometimes also been applied to lighter metals and metalloids, where there are similar connotations of toxicity, but these elements largely fall outside the scope of this booklet. Other terms which have been suggested include "potentially toxic element" (PTE) and a classification based on the affinities of metal ions for either oxygen or nitrogen and sulphur containing ligands (Nieboer & Richardson, 1980). Although these alternatives may be more logical, they seem unlikely to replace the term "heavy metal" for environmental monitoring.

There is extensive documentation on "safe" concentrations of heavy metals in water, such as the guidelines produced by the World Health Organisation and the European Economic Community. Traditionally, U.K. legislation has been based on ambient standards ("Environmental Quality Objectives", EQO), in contrast to the use of emission standards preferred by the EEC. As a result of negotiation a dual system was adopted and "directives" giving guidelines and mandatory concentrations issued. Detailed guidelines are available only for Cd and Hg (List I substances); these specify limit values and verification frequencies for emission standards and the maximum permissible concentrations in surface waters for ambient standards. These vary depending upon the hardness of the water and on whether it supports salmonid or cyprinid fish. The EQO also specifies that the metal concentration of sediment or a characteristic freshwater mollusc must not increase significantly with time. The present authors suggest that this could usefully be extended to include a characteristic freshwater plant.

This booklet gives detailed methods for the use of one technique (tissue analysis) for monitoring metal pollution, and describes three further techniques (bioassays, genetic tolerance, community structure) in more general terms, with references to the relevant literature. Features of the four techniques are compared with those of conventional chemical analysis in Table 1.1.

Definitions

Tissue analysis:

Analysis of total concentrations of metals in organisms or their parts. A preliminary "digestion" stage is usually required to release the metal into solution.

Bioassay:

"The use of living material to measure the concentration of a substance in water by determining its potency in producing some specific effect" (Standing Committee of Analysts, 1983). Toxicity tests are closely related, but largely fall outside the scope of this booklet. They are defined as "Use of living material to define the nature and degree of harmful effects produced by a single poison or a mixture of poisons" (Standing Committee of Analysts, 1983).

Table 1.1 Comparison of chemical and four biological techniques using photosynthetic organisms to monitor heavy metal pollution

	chemical analysis	tissue analysis	bioassays	genetic tolerance	community structure
taxonomic skill needed in field	none	recognize key species	none	recognize key species	preferably a biologist
laboratory facilities	standard in any water management body	mostly those for routine chemical analysis	relatively sophisticated facilities, though increasingly marketed as standard units	culture facilities	high quality microscope and wide range of floras
laboratory personnel	analytical chemist or skilled technician	analytical chemist or skilled technician	skilled technician	biologist	biologist, with specialist training in algae, including diatoms
minimum time period before results available	2 h	24 h	mostly 1–14 days, but more rapid methods becoming available	mostly c 3 days, but much more rapid methods being developed	3 h
ability to store sample	indefinite, provided care	indefinite as dried material	preferably used straight away: storage may introduce errors	1 month; otherwise open to doubt, unless ability to cryopreserve	indefinite as preserved material, but with preliminary inspection of live material
pollutant assessed	individual metals	individual metals	combined pollutants	one metal or mixture	combined pollutants
time period over which pollution assessed	point time	hours or days, depending on organism and environment	point time	days or weeks	days or weeks
general comments	simple, but no indication of ecological effects	flexible methodology adaptable for local conditions; not always easy in large rivers	suited where large number of routine samples permit semi-automation	can provide information not easily available otherwise e.g. short- v long-term response	aids assessment of pollutant interaction or likelihood of unrecognized pollutant

Genetic tolerance:

Measurement of the extent to which a population of a microorganism (chosen because of short generation times) from a site is adapted to heavy metals.

Community structure:

Based on either a diversity index (which counts all species of a particular group or groups) or a biotic index (which concentrates on a few taxonomic groups). In both cases the changes (spatial and/or temporal) in the distribution patterns of organisms are used to assess the effects of pollutants.

It is clear from Table 1.1 that tissue analysis compares favourably with the other techniques. Tissue analysis of animals may also be used, but animals have a number of disadvantages for routine use. Analysis of the heavy metal content of animals may be complicated by the time required for the clearance of gut contents, by the errant lifestyle of some species and, in the case of larger species, by the need for more detailed fractionation.

These methods are mostly intended to supplement chemical analysis of water and/or sediments, but there are situations where plants can provide information not easily obtained by other means. An example for rivers is the ability to detect pulses of metal pollution which have passed downstream some hours prior to the site being inspected. A more extreme example is the use of mosses from old herbarium sheets to comment on metal pollution at the time the mosses were collected. In general the methods described are of more value for monitoring flowing than standing waters, partly because of the greater tendency for environmental conditions to fluctuate in the former and partly because metal-rich inputs from mines or factory effluents are more likely to be released into flowing waters.

A further book is in preparation by the Standing Committee of Analysts on "Methods for the Determination of Metals in Plant Materials". This includes practical methods for plants with less easily digestible tissue than those discussed in the present book. It also differs from the present book in recommending that drying should be done at 80°C (rather than 105°C), as the lower temperature avoids caromelization in some agricultural crops. If the methodology in the present book is extended to larger aquatic plants such as *Typha*, it is recommended that dry weight measurements are made at both 80° and 105°C, in order to maximize the scope for comparisons with the literature.

2 Features of Tissue Analysis Methodology

2.1 Substances determined

Useful information can probably be obtained from plant tissues about all 'heavy metals' in their environment, but the method has been tested only for Cr, Ni, Cu, Zn, Cd, Pb and Hg; it is also suitable for monitoring Al, though more studies are needed before its reliability with this element can be assessed. It seems probable that the method is well suited for monitoring aqueous radionuclides, but few studies have been reported.

2.2 Type of sample

The method uses submerged, macroscopically recognizable photosynthetic algae, bryophytes and flowering plants. With bryophytes only younger tissue is used and with flowering plants only leaf and young stem tissue. For most analytical purposes, sufficient material should be sampled to produce 25 mg dry weight (usually about 250 mg wet weight), but a larger sample may be needed if it is necessary to reflect environmental heterogeneity. Most species of plant can probably provide useful information, but it is recommended that one or more of ten species (3.1) is chosen. A considerable body of data already exists for these species, making it easier to interpret the results.

2.3 Habitat sampled

The method is suited for most types of freshwater, but is of most use in small to medium-sized rivers. Suitable species are not always easy to find in large rivers, though this can be overcome by use of 'moss-bags' (4.3.3).

The majority of studies on which the methods have been developed have been carried out on waters of pH 6.0 or higher and only two of the species listed here (*Scapania undulata* and, rarely, *Nitella flexilis*) are likely to be found in waters with lower pH values. In addition, special care is needed if the methodology is required for waters with low, but fluctuating, pH values, in view of the fact that a drop in pH may lead to the loss of metals previously accumulated (Caines *et al.*, 1985).

2.4 Basis of method

In situ or transplanted material is sampled, digested and analysed. An indication of heavy metal contamination may be obtained by comparing various samples of a particular species, including some from unpolluted sites. A more quantitative approach may be adopted for some elements and some species, using datasets published in the literature which relate metal concentration in the plant to that in the water (Chapter 5). The method is unsuited for environments where there are marked pH fluctuations in the acidic range.

3 Choice of Material

3.1 Species

3.1.1 Selection of suitable species

Ten species are recommended for routine purposes. Use of a restricted number of species should encourage the accumulation of relevant background information and permit the comparison of results from different water management organizations. The species have been chosen according to several criteria. All are relatively easy to identify and are widespread not only in the U.K., but also in much of Europe; in most cases they are also widespread elsewhere in temperate regions. All occur in flowing waters and all but one (*Lemanea*) also in standing waters. Although several of the species are more frequent in standing than flowing waters, overall it is easier to find a population of a suitable species in the latter. The species are all easy to clean, or to obtain a young fraction free of a dense cover of epiphytes or crust of manganese and iron oxides (see 3.2). The various species differ in their physical and chemical requirements and some are much more seasonal than others.

3.1.2 Identification

The accompanying descriptions and illustrations should serve as pointers to the correct species and not as an exclusive aid to identification. The appropriate taxonomic literature should be used wherever possible. The following books may be helpful for identification. More detailed guides to the literature are given for algae by Whitton *et al.* (1978) and bryophytes and flowering plants by Holmes *et al.* (1978).

Algae:

Belcher & Swale (1976). Brief, well illustrated booklet which includes three of the algae recommended here.
Moore (1986). Pocket charophyte flora.
Pentecost (1984). General guide to British freshwater algae.
Prescott (1962). Although written for one specific area of the U.S.A. this serves as a good general flora for freshwater algae, but does not include *Enteromorpha*.

Bryophytes:

Smith (1978). Standard flora on U.K. mosses, detailed and technical.
Watson (1981). Includes all bryophytes in this booklet; easier to use than Smith (1978), but not exhaustive.

Flowering plants:

Clapham *et al.* (1987). Standard taxonomic work on U.K. flora. Also "Excursion Flora" (2nd edition, 1968).
Haslam *et al.* (1975). Low-cost guide to aquatic angiosperms, profusely illustrated.

General:

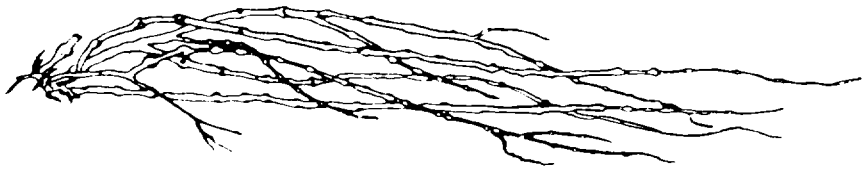
Standing Committee of Analysts (1987a). Methods for the Use of Aquatic Macrophytes for Assessing Water Quality.

Five of the species are illustrated in Fig. 3.1.

3.1.3 Guide to the ten species

The seasonality shown for each species indicates the part of the year when there is a good chance of finding material suitable for analysis at sites where the organism is

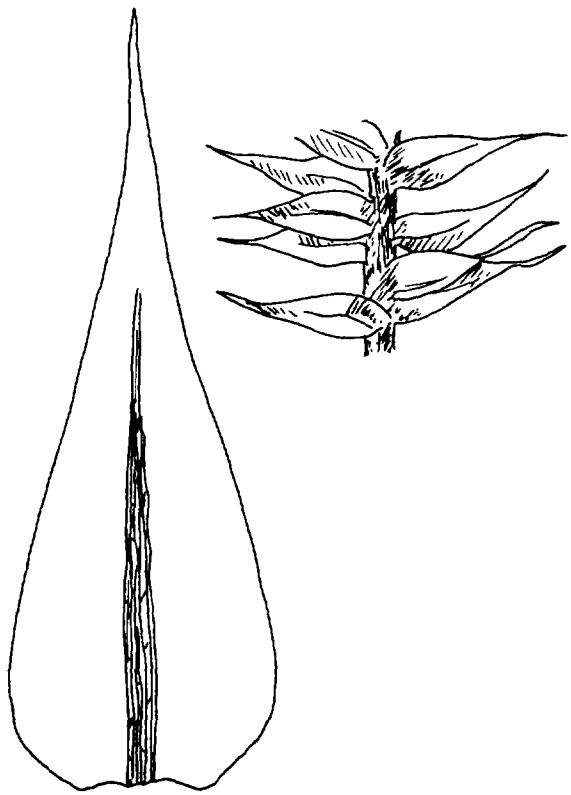
1. *Lemanea fluviatilis*



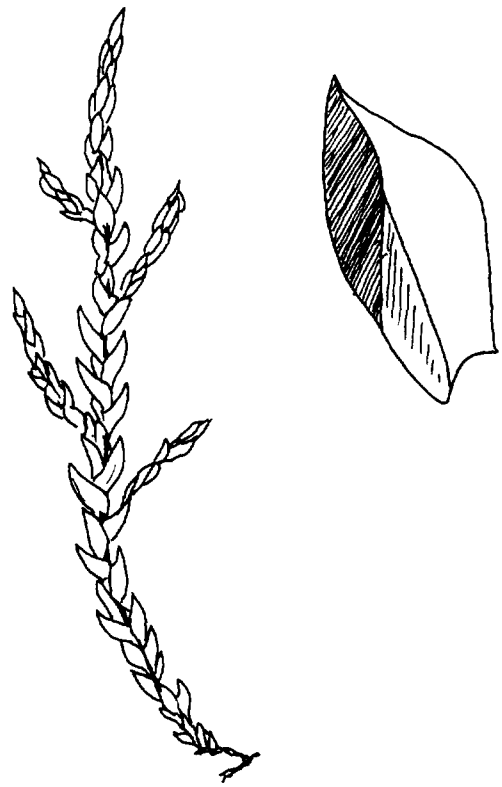
2. *Amblystegium riparium*

3. *Fontinalis antipyretica*

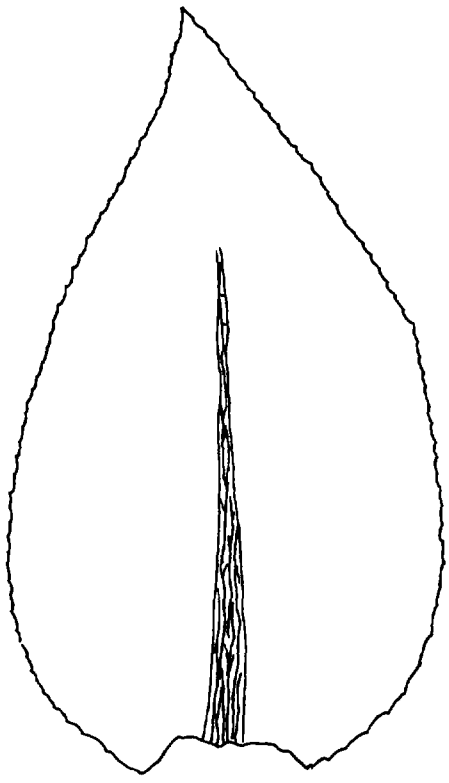
Fig. 3.1 Illustrations of *Lemanea fluviatilis* and the four bryophytes recommended.



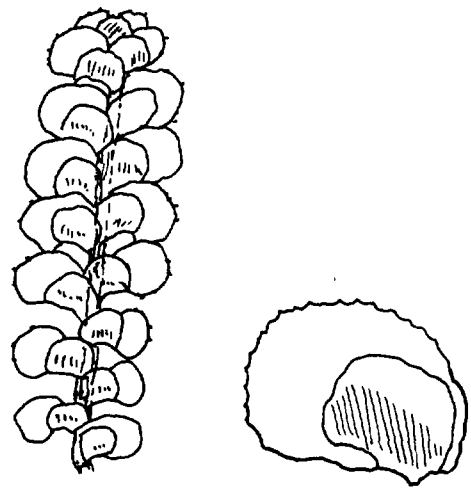
Amblystegium riparium leaf × 43
stem detail × 9



Fontinalis antipyretica stem detail × 2
leaf × 12



Rhynchostegium riparioides leaf × 42



Scapania undulata stem × 9
leaf × 19

Fig. 3.1 *continued*

known to occur; obviously this will vary slightly from year to year and according to the region under study.

Lemanea fluviatilis (L.) Agardh (Rhodophyta—red alga) (Figs 3.1, 3.2)

Reference: Harding & Whitton (1981)

It is unclear whether or not all forms of *Lemanea* in the U.K. belong to a single variable species, but it seems probable that all have similar properties with respect to metal accumulation.

Notes on identification:

Adult is a hollow thallus with the appearance of a rarely branched filament with a "knobbly" appearance. Colour mostly ranges from green to olive green, but is sometimes purple. Under certain conditions decaying or dead *Cladophora* and old, wiry bryophyte stems stripped of leaves may be confused with *Lemanea*. Closer examination should easily remove this ambiguity. The juvenile "chantransia" stage, which occurs in late autumn and early winter, consists of branched filaments one cell thick; little is known about its ability to accumulate heavy metals.

Distribution and ecology:

Widespread in temperate regions, including Europe, Asia and North America. It occurs attached to boulder in fast-flowing reaches of upper and middle stretches of rivers; absent from soft waters.

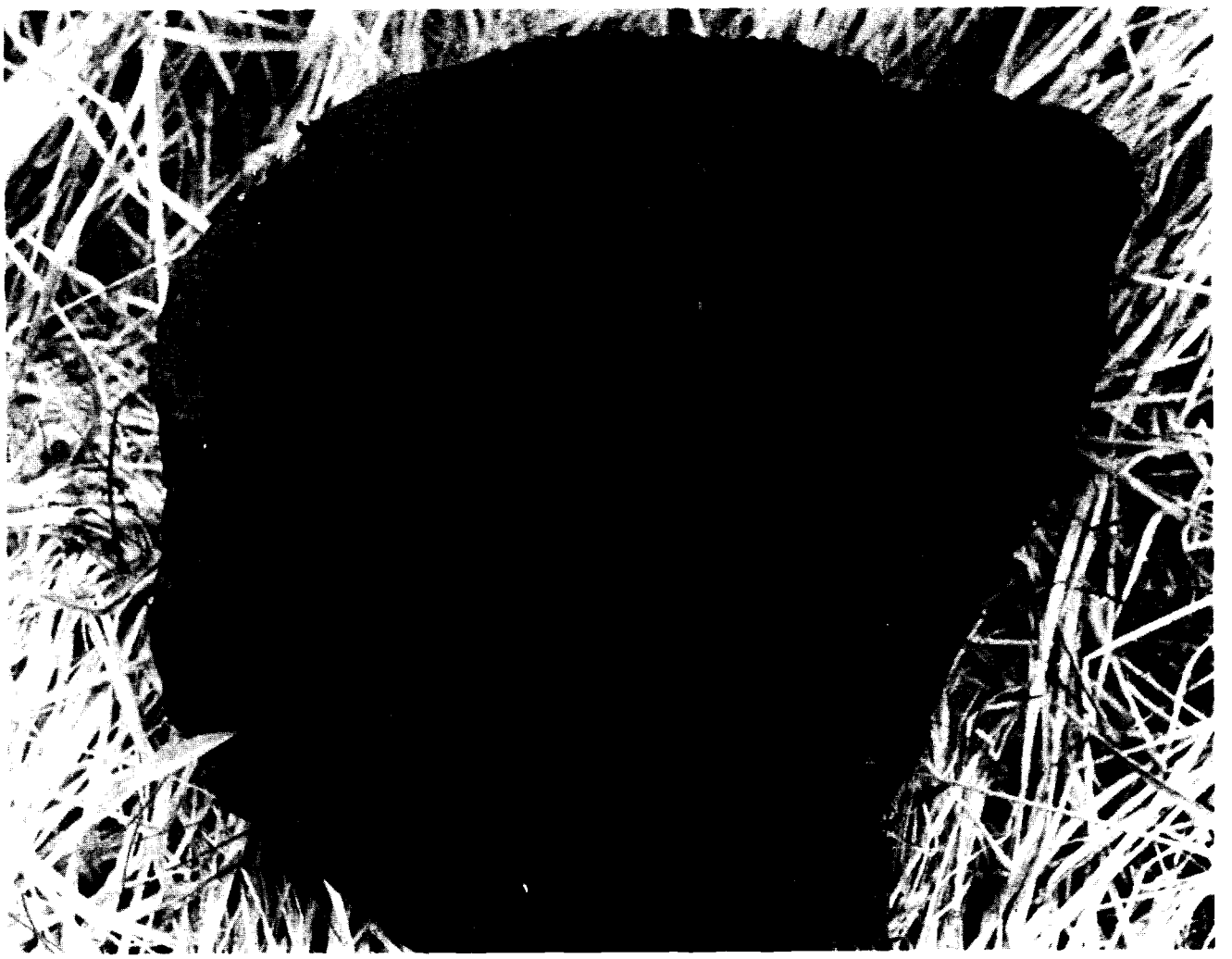


Fig. 3.2 *Lemanea* on boulder taken from River Wear in April; larger filaments are 15-20 cm long.

Seasonality for sampling purposes of adult plants >5 cm long:

J F M A M J J A S O N D

Fraction for analysis:

Only the adult stage is suitable; either 2-cm tips or whole plants are suitable for use, but the results are not directly comparable. Recommended: 2-cm tips.

Cladophora glomerata (L.) Kütz. (Chlorophyta—green alga)

Common name: blanket-weed

References: Whitton (1970); Whitton *et al.* (1989)

Notes on identification:

Coarse, green filaments, with branches usually clearly visible to the naked eye. Under favourable conditions it can form large masses up to 1.5 m in length. Other organisms which might be confused with it are:

Cladophora aegagropila (forms tufted carpet-like patches on bottom of fast-flowing shaded rivers);

Rhizoclonium (unbranched);

Vaucheria sessilis (wide filaments, lacking cross-walls; typically forms cushions of sub-erect filaments in silted situations, but sometimes forms large filamentous masses up to 1.5 m long on boulders in moderately fast-flowing rivers, when it is superficially somewhat like *Cladophora glomerata*).

It seems likely that *Rhizoclonium* will give quite similar results for metal accumulation to *Cladophora glomerata*, but this has not been investigated.

Distribution and ecology:

Worldwide; typically found in the U.K. in moderate to very hard eutrophic lowland rivers attached to boulders, pilings etc. or caught around other aquatic plants.

Seasonality for sampling purposes:

J F M A M J J A S O N D

Fraction for analysis:

Young bright green plants or terminal, approximately 2-cm lengths of larger plants; results using these two fractions are directly comparable.

Enteromorpha flexuosa (Wulfen ex Roth) J. Ag. (Chlorophyta—green alga)

The method adopted at University of Durham for the freshwater form of this species follows quite closely that described by Say *et al.* (1986) for processing marine *Enteromorpha* for metal analysis.

Notes on identification:

Green tubular thallus, often with a rather shining appearance; mature thallus sometimes reaching 1 m in length and 1.5 cm in diameter; wall of thallus is one cell thick. Nothing else in freshwaters is likely to be confused with large plants of *E. flexuosa*, but partially exposed mats of *Cladophora* might perhaps be confused with *Enteromorpha* from a distance.

Distribution and ecology:

The marine form of this species has a wide distribution in Europe, but the geographical distribution of the freshwater form is not clear. It probably occurs throughout western Europe, but the situation is complicated by the fact that the species appears to have increased markedly in freshwaters in recent decades. There has also been an increasing number of reports of freshwater *Enteromorpha* from other parts of the world.

It is favoured by eutrophic hard water, typically occurring at the sides of rivers in ponded areas or entangled among submerged vascular plants. Most plants found in summer are unattached, but the species overwinters as small filaments attached to rocks. The unattached summer population may be washed away by floods. The species varies markedly in abundance from year to year.

Seasonality for sampling purposes:

J F M A M J J A S O N D

Fraction for analysis: Recommended: whole, bright green plants.

Nitella flexilis (L.) C. A. Agardh (Charophyta)

Reference: Harding & Whitton (1978)

Notes on identification:

This is best made by reference to Moore (1986). The thallus varies markedly in height; although typically 20–30 cm, it may reach 100 cm. It seems likely that all unencrusted charophytes may be equally suitable for monitoring purposes, but this has not been tested.

Distribution and ecology:

Widespread in northern hemisphere, attached to sediment of the shallower parts of oligotrophic and mesotrophic lakes and occasionally also slow-flowing rivers.

Seasonality for sampling purposes:

According to Moore (1986), it may be a winter or summer annual or live as a perennial in deeper water. The monthly distribution shown below gives the typical situation in northern England for populations growing in shallow water.

J F M A M J J A S O N D

Fraction for analysis:

The species is sometimes lightly encrusted with $CaCO_3$ in harder waters, but otherwise should present no difficulty. It is usually free of epiphytes and easy to wash free of loosely associated organisms. The thallus must be handled gently; breakage will lead to loss of the contents of one of the long internodal cells. Analytical studies have been made only on annual populations, but it might be necessary to remove the older parts of the thallus in longer-lived forms.

Recommended: whole, green plants.

Amblystegium riparium **(Hedw.) Br. Eur. (moss)** (Figs 3.1, 3.2)

Synonym *Leptodictyum riparium* (Hedw.) Br. Eur., 1853

Notes on identification:

Robust moss with soft texture and flattened appearance, widely spreading narrow, finely-pointed leaves (Fig. 3.1). Species that might be confused with it include *Rhynchostegium riparioides* (more rigid texture, less pointed leaves which are less widely spreading), *Drepanocladus* spp. (leaves often curved over at tips, generally less flattened appearance) and *Hygrohypnum ochraceum* (less frequent in lowland rivers).

Distribution and ecology:

It appears to be worldwide (Smith, 1978). It occurs attached to boulders, pilings and other firm substrata in eutrophic waters, both flowing and still; population often occur at sites with high organic pollution, such as filter beds of sewage treatment works; these might be useful for monitoring heavy metal concentrations in such situations.

Seasonality for sampling purposes: present all the year

Fractions for analysis:

Either 2-cm tips or whole plants are suitable, but the results are not directly comparable.

Recommended: 2-cm tips.

Fontinalis antipyretica **Hedw. (moss)** (Fig. 3.1)

Common name: willow moss.

Reference: Say & Whitton (1983)

Notes on identification:

Large, sparingly-branched shoots, often denuded of leaves below; leaves keeled, in three ranks; the var. *gracilis* is a more slender form. The only species likely to be confused with *F. antipyretica* is *F. squamosa*, which is also more slender than typical *F. antipyretica*, but has leaves without a keel. *F. squamosa* may be used instead of *F. antipyretica* for qualitative studies (4.1), but it will require further study to confirm whether or not data from the two species are comparable quantitatively.

Distribution and ecology:

Widespread in Europe and other parts of the northern hemisphere (Smith, 1978) and introduced in some parts of the southern hemisphere. Very widespread, found in both upland and lowland sites, although the type is more common in the latter, whereas

var. *gracilis* occurs in upland, soft and often peaty waters. In northern England, *F. squamosa* appears to be restricted to sites west of the Pennines.

Seasonality for sampling purposes: present all the year

Fraction for analysis:

Either whole plants or 2-cm tips are suitable for analysis, but the results are not directly comparable. *F. squamosa* is also suitable (Say *et al.*, 1981).

Recommended: 2-cm tips.

Rhynchostegium riparioides **(Hedw.) C. Jens. (moss)** (Fig. 3.1)

Synonyms:

Eurhynchium riparioides (Hedw.) Rich.;
Platyhypnidium riparioides (Hedw.) Dix.

References: Wehr & Whitton (1983a, b)

Notes on identification:

Robust moss, sparingly branched, often denuded of leaves below. Ovate to lanceolate leaves with denticulate margins. Species with which it might be confused are *Amblystegium riparium*, *Brachythecium rivulare* and *Hygrohypnum ochraceum*; the last two tend to be less robust and more confined to upland areas.

Distribution and ecology:

Widespread in northern hemisphere and reaching a few parts of the southern hemisphere (Smith, 1978; Crum & Anderson, 1981). It occurs attached to solid substrata in the faster flowing sections of both oligotrophic and eutrophic rivers and streams; it has also been found in sewage effluent channels (Wehr & Whitton, 1983), but is generally less tolerant of organic pollution than *Amblystegium riparium*.

Seasonality for sampling purposes: present all the year

Fraction for analysis:

Either whole plants or 2-cm tips are suitable for analysis, but the results are not directly comparable.

Recommended: 2-cm tips.

Scapania undulata **(L.) Dum. (liverwort)** (Fig. 3.1)

Reference: Whitton *et al.* (1982)

Notes on identification:

Leafy liverwort; leaves with underleaves, margin entire or denticulate; shoots vary greatly in size, but may reach 12-cm long. *Scapania* is a large and difficult genus and

some species are morphologically quite similar to *S. undulata*. Other leafy liverworts like *Chiloscyphus*, *Solenostoma* and *Nardia* might be confused with smaller forms of *S. undulata*.

Distribution and ecology:

Widespread species in north temperate region, typically occurring in stretches of upland streams and small rivers with moderate to fast current speeds. It forms especially luxuriant growths in peaty waters and near waterfalls; it is frequent in streams enriched by heavy metals as a result of mining activity, but is not tolerant of eutrophic or organically polluted conditions.

Seasonality for sampling purposes: present all the year

Fraction for analysis:

Although whole plants are sometimes suitable, the older parts frequently have an encrustation with manganese and iron oxides which is difficult to remove; in spite of the lower biomass, 1-cm tips are much easier to use. The results for whole plants and 1-cm tips are not directly comparable.

Recommended: 1-cm tips

Potamogeton pectinatus L. (flowering plant)

Common name: fennel pondweed; sago pondweed (U.S.A.)

Notes on identification:

No floating leaves; all leaves reduced, linear; stipule fused with leaf-base to form a single, basal leaf-sheath. Other common species of *Potamogeton* (*P. crispus*, *P. natans*, *P. perfoliatus*) all possess broader leaves, but two other genera may cause confusion. The most likely difficulty is with *Zannichellia palustris*, but the fine-leaved species of *Ranunculus* (*R. fluitans* and *R. penicillatus*) might also be confused. There should be little problem when plants are in flower or fruit, but anyone uncertain about identification should consult a flora. Useful diagnostic features of *Potamogeton pectinatus* are the stipular sheath, which is open, waved and with a white margin, and the two channels shown in a cross-section of a leaf.

Distribution and ecology:

This species is almost cosmopolitan (Clapham *et al.*, 1987), both in temperate regions and the tropics. It occurs rooted in fine sediments in lowland, eutrophic rivers with slow to medium current velocities, often downstream of major sewage treatment discharges.

Seasonality for sampling purposes:

J F M A M J J A S O N D

(In the mildest parts of the British Isles, healthy shoots may be present for much more of the year)

Fraction for analysis:

Recommended: young shoots

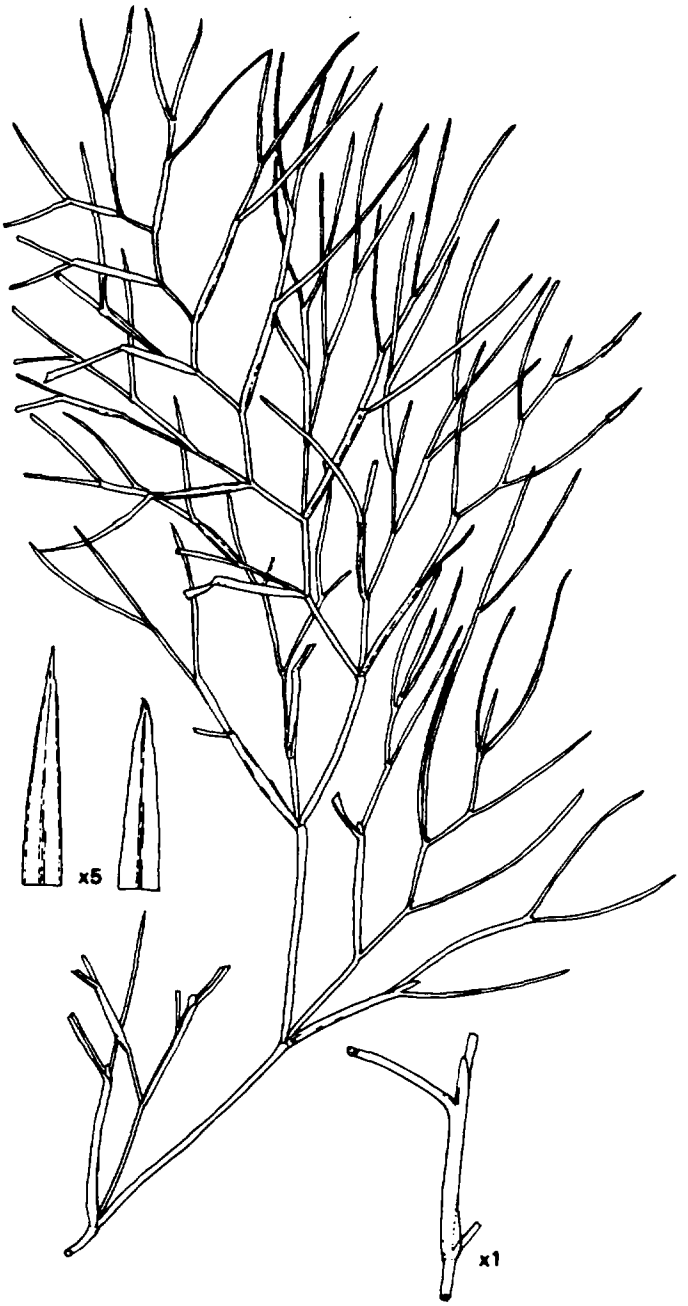


Fig. 3.3 Part of shoot of *Potamogeton pectinatus* with details of stipule, sheath and leaf (redrawn from Haslam *et al.*, 1975).

Elodea canadensis Michx.

Synonym: *Anacharis canadensis* Planch.

common name: Canadian pondweed.

Notes on identification:

Stems sparsely branched, leaves (linear to oblong with central nerve) in whorls of 3–7. The rapidly increasing *E. nuttallii* is more slender, paler green and with narrower leaves. (The difference is usually obvious if the two species are placed side by side.) In practice the two species will probably provide similar analytical results, but this has not been tested critically. Two other species may sometimes look superficially like *Elodea canadensis* when viewed at a distance. *Groenlandia densa*, which may occur intermixed with *Elodea*, has opposite, serrate leaves. Late-season, epiphyte-covered plants of *Myriophyllum spicatum* can be distinguished by their finely-divided underwater leaves.

Distribution and ecology:

Native to North America, but now very abundant in the U.K. (Clapham *et al.*, 1987), the rest of Europe and has also spread to some other regions. It occurs rooted or unattached in lowland ponds and rivers. In rivers it is easily removed by high flows, so is most abundant in side-arms and backwaters.

Seasonality for sampling purposes:

Suitable material should be available at most times of year where it occurs in ponds. Populations in rivers are more likely to vary in size during the year; the seasonal distribution shown below indicates the times at which it is easiest to find the species in rivers.

J F M A M J J A S O N D

Fraction for analysis:

Although whole plants can be used, the older parts often have attached growths, so 2-cm tips are much more suited for analysis; the results for whole plants and 2-cm tips are probably not directly comparable, but critical tests have not been made.

Recommended: 2-cm tips.

Table 3.1 Typical habitats in which the ten species are found.

species	typical habitat
Cladophora glomerata	Attached to firm substrata in rivers and at edges of reservoirs and lakes, especially in nutrient-rich and harder waters
Enteromorpha flexuosa	Standing and flowing waters, including quite fast-flowing rivers, especially in nutrient-rich and harder waters; mostly free-floating, although loosely entangled with submerged flowering plants
Nitella flexilis	Ponds, shallow parts of lakes and slow-flowing stretches of rivers; apparently absent from nutrient-rich and hard waters
Lemanea fluviatilis	Attached to larger substrata in streams and rivers with high current speeds; absent from soft waters
Amblystegium riparium	Ponds, streams and rivers subject to high levels of organic pollution
Fontinalis antipyretica	Ponds, streams and rivers
Rhynchostegium riparioides	Streams and rivers; particularly abundant in harder and more eutrophic waters
Scapania undulata	Upland streams and small rivers
Elodea canadensis	Ponds, lakes and rivers
Potamogeton pectinatus	Eutrophic ponds and rivers with organic-rich silt

3.2. Plant fraction

The fraction to select has been considered for each species in 3.1. A few general comments may also be useful. The choice of whether to use whole plants or a particular fraction has a considerable effect on the final metal concentrations in all the species, except *Enteromorpha flexuosa*, though in some cases (especially *Cladophora glomerata*) very young plants may give results similar to apical parts of older plants.

The reasons for choosing a particular fraction are influenced by the objective of the investigation and various practical matters. The following comments apply to all the species except *Enteromorpha flexuosa* (where there is little distinction between different parts of the thallus), *Nitella flexilis* (which cannot be divided without damage) and *Potamogeton pectinatus* (which shows obvious differentiation into roots, stem and leaves):

i. Where there is continuous exposure to heavy metal pollution, whole plants contain higher concentrations of these metals than younger (apical) regions. These higher concentrations, especially in the case of bryophytes, often include a substantial contribution from the metals accumulated by the 'oxide' coatings on the older parts of the plant. This greatly increases the variability of analytical results.

ii. Young growths reflect recent events more efficiently than whole plants.

iii. Use of whole plants avoids waste of material if the plant is infrequent at the sample site and avoids the need to fractionate the material.

iv. Young growths are easier to wash clean and are less subject to differences in washing technique.

v. In the case of *Potamogeton pectinatus*, young shoots (including the leaves) reflect metal concentrations in the water much more closely than stems, roots or whole plants. In addition the size of a whole plant means that some sort of subsample must be taken for digestion purposes.

It is suggested that, in the case of bryophytes, whole plants are suited for broad surveys where the objective is to obtain a rough estimate of the extent of pollution. This approach is useful for general surveys of river catchments (Empain, 1976) and can be used to indicate the location of new factory discharges or where previous pollution problems have ceased. Many water management biologists in the U.K., who have used plant tissues to monitor heavy metals in freshwaters, have chosen whole moss plants (usually *Fontinalis antipyretica*).

The 2-cm fraction (or 1-cm fraction for *Scapania undulata*) is well suited for making detailed statistical comparisons of different samples, whether from different sites or times. It is important to use this fraction to monitor intermittent pollution events, such as when a body of polluted water is suspected of having passed downstream a few hours before sampling or for detecting an intermittent factory discharge. The comments for individual species in 3.2 are summarized in Table 3.2.

Table 3.2 Fractions of the ten species which it is recommended should be used (++) or which can be used (+).

species	whole plant	2-cm tip	other
Cladophora glomerata	+ (young)	++	
Enteromorpha flexuosa	++		
Nitella flexilis	++		
Lemanea fluviatilis	+	++	
Amblystegium riparium	+	++	
Fontinalis antipyretica	+	++	
Rhynchostegium riparioides	+	++	
Scapania undulata	+		++ 1-cm tip
Elodea canadensis	+	++	
Potamogeton pectinatus			++ young shoot

4 Procedures

4.1 Introductory note

The usual procedure is to remove plant material growing at the site under study, with at least some of the required washing steps being completed at the site, followed by any remaining ones in the laboratory. At sites without suitable plants, materials may be introduced for monitoring purposes. This may be achieved in a variety of ways, but essentially it either involves transplanting organisms with the minimum disturbance possible (e.g. Benson-Evans & Williams, 1976; Wehr *et al.*, 1987) or placing material in mesh bags suspended in the water (Kelly *et al.*, 1987). The latter is a development of the moss-bag methodology widely used in monitoring atmospheric pollution (Martin & Coughtrey, 1982).

Mouvet (1984) used a somewhat different approach to those recommended here (which he termed 'transplant'). This involved detached shoots of moss in plastic cylinders attached to portable concrete blocks; both ends of the cylinders were covered with plastic mesh and 4-mm diameter holes were drilled in the walls of the cylinder. The equipment is robust and could be used in many situations, but the results suggest that metal (Cr, Cu) accumulation under these conditions is slow. Sufficient information is given here for most practical purposes. However, a more critical account of the preparation and analytical methods for mosses is given by Wehr *et al.* (1983) and further general information on methodology by Say *et al.* (1986) and Kelly *et al.* (1987).

4.2 Collection of sample

4.2.1 Apparatus needed in field

The amount of equipment required depends on the extent to which washing is to be carried out at the site. A white enamel tray is useful for preliminary sorting, whether or not washing is done at the site. A cool box is not always essential, but it is advisable to use it wherever possible; it should always be used for *Cladophora* and *Nitella*. Care needs to be taken not to contaminate samples with any of the devices used for collecting or preparation (see note a at the end of 4.2.2). Metal items should be stainless steel, transported under clean conditions and rinsed at each site.

Standard requirements:

white enamel tray or approx. 20-cm diameter glass dish
stainless steel forceps
polythene bags and/or plastic jars (note a)
marker pen
cool box

Items needed if complete washing to be done in field:

stainless steel scissors
crystallization dishes (preferably) or plastic petri dishes
aspirator of deionized water
wash-bottle
tissue paper
qualitative grade filter paper or equivalent
adhesive tape

Optional requirements:

rake
grapnel

i. Site of collection should be defined by reference to one or more easily-recognised landmarks (note b).

ii. Wherever possible, plants should be taken from at least five different locations within the site. In some situations it is helpful to have a simple tool such as a rake or grapnel to help remove the samples from the water-body. In the case of flowing waters, several of the organisms (especially *Cladophora* and *Fontinalis*) are better collected from those parts of the site with the higher current speeds.

iii. All species should be given a preliminary wash in water from the site and, if possible, partial removal of associated materials. In the case of *Cladophora* much more satisfactory results are obtained if the full washing procedure is done at the site (Whitton *et al.*, 1989).

Cladophora glomerata

i. Sampling should be restricted to plants which are attached and submerged.

ii. Remove plants from stream and place in large glass or white enamel dish containing stream water.

iii. Unless young, bright green plants are available, remove 2-cm tips using stainless steel scissors. Tips on side branches may be used.

iv. Transfer tips to a smaller dish also containing stream water and clean off any debris, animals and other material associated with the alga.

v. Transfer to dish of deionized water, wash; repeat several times until alga appears clean.

vi. Remove tips from deionized water and place on tissue paper and blot to remove surplus water. Place tips on filter paper in a petri dish and secure lid.

vii. Place petri dish in polythene bag and place bag in cool box for return to the laboratory.

Enteromorpha flexuosa

i. As it will seldom be possible to find attached material, care needs to be taken in rivers to avoid selecting thalli that may have been washed downstream.

ii. Wash the plant in water from the site.

iii. Place moist plants in a polythene bag.

Nitella flexilis

i. Remove whole plant, including any rhizoids attaching it to the underlying silt. Handle this species with special care, as cytoplasmic contents are lost easily if damaged.

ii. Wash plant in water from the site.

iii. Place moist plants in plastic jar. (They require better protection than can be given by a bag.)

Lemanea, bryophytes and flowering plants

i. Collection should be restricted to plants which are attached and submerged (note c, d). Plants should be collected from at least five separate locations at the site.

ii. Plants should be rinsed and shaken several times in stream water to remove associated sediments, invertebrates and other plant material; excess water is removed from the plants with filter paper.

ii. Material is placed in a labelled polythene bag or jar and transferred to the laboratory in a cool box.

Notes

a. All apparatus manufactured from rigid plastics and glass should be acid washed in 4% HNO_3 for at least 30 min and rinsed 6–8 times with distilled or deionized water.

b. A 10-m stretch of river or stream, termed a reach, is recommended when sampling streams and smaller rivers (Holmes & Whitton, 1981). For rivers whose widths approach or exceed 10 m, it is recommended that a longer standard stretch is designated.

c. Although it is recommended that attached plants are collected wherever possible, under certain conditions (e.g. still water, dykes and ditches), where water movement is minimal and there is little chance of inoculation of plants from elsewhere, non-attached plants may be used instead.

d. A "fistful" of plant normally provides ample material for subsequent analysis.

4.3 Transplants and moss-bags

4.3.1 Additional apparatus

moss-bags (note a)
means of securing moss-bags (notes b, c, e)
twine
scissors
club or sledge hammer (note d)

4.3.2 Species

Transplants can be made with any of the species, but only those firmly attached to movable substrata are really of practical use for flowing waters; these are indicated in Table 4.1. *Elodea* and *Potamogeton pectinatus*, which might otherwise be of use, should not normally be transferred to a site where they are not already growing, because of the risk that they might spread to become a nuisance. The use of mesh bags is most likely to give useful results with mosses in flowing waters.

Table 4.1 List of species suitable for transplants or "moss-bags"

species	transplant	moss-bag
Lemanea	+	
Cladophora glomerata	+	
Amblystegium riparium	+	+
Fontinalis antipyretica	+	+
Rhynchostegium riparioides	+	+
Scapania undulata	+	

4.3.3 Methods

Transplants

i. At an unpolluted site, select five suitable boulders or other movable substrata with the required species attached; place them in bowls covered with a plastic sheet and transfer to the site to be monitored.

ii. Take one sample from each boulder for analysis at the time the boulders are placed at the experimental site.

Fig. 4.1 Moss-Bag before attaching to a submerged part of a post or similar fixed object.

i. Select suitable whole plants of (preferably) *Fontinalis* or *Rhynchostegium* from an unpolluted site.

ii. Wash thoroughly in water and place approximately 15 – 20 g (wet weight) of moss in each bag (Fig. 4.1).

iii. Instal bags in the main flow of the river to be monitored, taking care to avoid "backwaters" unless information on these areas is specifically required. The method of installation of the bags depends on the situation being monitored. Placement of bags should take into account problems of vandalism, interference from other water users, changes in water level and fouling with extraneous material.

iv. An exposure period of at least 24 h is recommended. If metal contamination is only slight or intermittent, and there is no urgency in obtaining results, it is recommended that transplants and moss-bags are left for one week. (Under circumstances where the concentration of contaminant metal remains the same, the length of period for which the moss must be exposed to the water in order to obtain something approaching an equilibrium between the concentrations of metal in the moss and metal in the water varies according to metal and environmental conditions. This probably reflects differences in the mechanisms of accumulation.)

v. At the end of the period of exposure, moss is collected from bags and washed as described in 4.4.2.

Notes

a. Recommended sizes for moss-bags are 20–30 cm long by 15–20 cm wide, open at one end and between 0.6 and 1.0 meshes cm^{-1}, although experimental studies have shown the mesh size not to be critical, provided that it is more than 0.5 cm^{-1} (Kelly *et al.*, 1987). Suitable meshes are available from: Henry Simon Ltd, Cheadle Heath, Stockport, Cheshire (061-428-3621). Other meshes (e.g. used to protect horticultural crops) may also be suitable. These may be sewn together with nylon monofilament fishing line to form a bag.

b. Types of *in situ* structure which may be used include fences across a stream, bridges, pilings and piers; when used in rivers they must be located in the main flow and not subject to excessive turbulence.

c. In some rivers the only feasible method of attachment is to use stakes hammered into the bed. However care needs to be taken in selecting sites to avoid any risk to other river users and the stakes should always be removed after use. It may be necessary to place a warning notice on the bank. Stakes can be made from ca. 8–9 mm mild steel rod ca. 1 m in length and are driven into the river bed inclined upstream so that river flow will not dislodge them. In water of greater depths, longer stakes will be required.

d. Hammer is required only if bags are to be secured with a stake.

e. Housebricks are recommended for use as weights in deeper water. In water with a current velocity less than 1.0 m s^{-1} a single housebrick should be sufficient. Where the velocity is more, then a greater weight should be employed. Nylon cord (4-mm diameter) is tied around the weight and the bag attached leaving 1 m between the brick and the bag. A second length of cord is tied around the brick and attached to the bank. The point of attachment to the bank should be selected with regard to possible changes in the water level, vandalism and to safety of other water users. In deeper waters a float may also be required.

f. When moss-bags are used in lakes it may be necessary to secure the bags at specified depths in the water column (Everard & Denny, 1985) and to tease material out to form a flat "disc".

4.4 Treatment

4.4.1 Storage

4.4.1.1 Short-term storage

Ideally, material should be processed immediately. If this is impossible, fresh material of all species except *Cladophora glomerata* can be stored for up to four days at 4°C, and probably longer for the bryophytes.

4.4.1.2 Long-term storage

Material processed as in 4.4.2 and 4.4.3 below may be stored either as dried whole plants or as a dried powder for at least 12 months without loss of metals. (Tests have not been made to check whether this applies in the case of Hg.) It seems likely that storage of dried materials can be indefinite, but tests over periods longer than one year have been made only for some metals and some species.

Samples of the bryophytes may also be stored before drying in sealed containers at −20°C, but for at least some of the other organisms (*Enteromorpha*, *Nitella flexilis*) this procedure is unsuitable.

4.4.2 Washing and fractionation

4.4.2.1 Apparatus

stainless steel or nylon forceps
scalpel or razor blade
nylon sieve
petri dishes (note a)

crystallization dishes (note a)
laboratory paper towel
glass vials (note a)
deionized water

Note

a. All apparatus manufactured from rigid plastics and glass should be acid washed in 4% HNO_3 for at least 30 min and rinsed 6–8 times with distilled or deionized water.

4.4.2.2 Methods

In most circumstances *Cladophora* should be prepared for drying at the field site (3.13). The other species should be treated as follows, apart from samples to be analyzed for Hg (see below).

i. Sample is held in a nylon sieve and washed thoroughly in a stream of water to remove obvious attached sediment. As far as possible, this should be done entirely with deionized water.

ii. Washed material is transferred to a petri dish containing deionized water.

iii. The relevant fractions are removed, using a scalpel and forceps where necessary, and given three rinses in crystallizing dishes containing deionized water.

iv. Sufficient material to give a final dry weight of about 40 mg is taken, if a single sample is to be analyzed, or 200 mg (divided into five sub-samples), if replicates are required for statistical purposes. Typically this requires about 25 tips per replicate for the mosses and about 40 tips per replicate for *Lemanea* and *Scapania*. The sample is blotted dry on laboratory paper towel and placed in a glass vial.

Analysis for Hg typically requires more material, in view of the need to monitor this metal at very low concentrations. For this reason it is suggested that 5–10 times as much plant material should be collected as for the other metals.

4.4.3 Drying

The following procedure applies to samples for analysis for all metals except Hg. On return to the laboratory, *Cladophora* should be air-dried for 24 h on a petri dish with the lid slightly open, and then placed in a glass vial prior to drying at 105°C. Tests have shown that *Cladophora* treated with the procedures given in 3.2 and above loses less than 1% of any metal to the filter paper. Without the pretreatment, the filaments stick to the surface of the vial. Other species can be dried straight away at 105°C, without the preliminary air-drying. Dried material should be cooled in a desiccator and weighed prior to digestion.

If Hg is to be monitored, drying must be done at 40°C. The length of period required for drying to constant weight should be extended to 48 h for the bryophytes; the time needed for the other species needs checking.

4.5 Analysis

4.5.1 Introduction

There are three approaches to the decomposition of plant material: digestion, dry ashing and fusion. Few studies have used the last, but the other two techniques have been used widely. Digestion involves treatment of the sample usually with strong acid(s), but sometimes other oxidizing agents. Dry ashing involves removal of organic material, either using high temperature, typically in a muffle furnace, or lower temperatures under reduced pressure in an oxygen plasma discharge. In either case the "ash" is subsequently dissolved in a moderately dilute acid such as 2 M HNO_3. For routine monitoring, digestion has the advantage of the relatively higher speed with which large batches of samples can be processed and the lower chance of losses of volatile elements. The acid used for digestion should not be stronger than needed. All the species recommended here can be digested with 4 M HNO_3 and the algae and bryophytes can be digested with 2 M HNO_3. However, if plants like *Typha* with lignified tissue are brought into the

methodology for monitoring metals, then stronger digestion procedures may be needed (see Standing Committee of Analysts, 1987b). Studies with the species recommended here have shown that in most cases more metal is brought into solution by 4 M HNO_3 than concentrated HNO_3. Use of the more dilute acid also makes atomic absorption spectroscopy easier and reduces the level of element contamination introduced with the acid.

A variety of vessels and methods of heating may be employed; the methodology below employs equipment likely to be available in most analytical laboratories. A possible improvement that should be considered by a laboratory that wishes to analyse biological samples routinely is the use of PTFE vessels within a stainless steel pressure 'bomb' (Uhrberg, 1982), which can be placed in an oven at the desired temperature. Another possibility is the use of PTFE containers in a microwave digestion system (Kingston & Jassie, 1986; Nakashima *et al.*, 1988). A booklet in this series on the preparation of plant and related material for analysis is in preparation.

4.5.2 Digestion

4.5.2.1 Apparatus

(see also 4.4 note a)

stainless steel forceps
boiling tubes, internal diameter = ca. 15 mm
calibrated pipette, 5-ml capacity
heating block with temperature control
centrifuge
centrifuge tubes
25-ml volumetric flasks

4.5.2.2. Reagents

2 M HNO_3 (algae and bryophytes) or 4 M HNO_3 (flowering plants) – atomic absorption grade diluted with deionized water
(reagents for samples to be analysed for Hg are given in 4.5.2.3.)

4.5.2.3. Method

All metals except Mercury:

i. Material is transferred to a boiling tube and 5 ml HNO_3 (2 M or 4 M, according to material) added.

ii. A FUME CUPBOARD MUST BE USED FOR THE NEXT STAGE OF THE PROCEDURE. The sample is digested in boiling acid on the heating block set at ca. 105°C for 45 min, removed and allowed to cool for a further 20–30 min. Note that during the early stages of digestion material may be carried up the walls of the tube. This should be gently shaken down or, if this is not successful, a small quantity of deionized water should be trickled down the edge of the tube to dislodge it.

iii. The digested sample is centrifuged to separate any solid material remaining and the supernatant is poured into a 25-ml volumetric flask. The pellet is resuspended in deionized water, recentrifuged and the supernatant added to the volumetric flask.

iv. The solution is made up to 25 ml with deionized water.

v. The resulting solution may then be stored in the original glass vial at 4°C.

Mercury:

The above procedure needs to be modified according to the increased biomass of material being digested. The method of digestion may also need to be modified for routine studies on Hg in tissues. The use of the same relatively dilute acid as for samples to be analysed for other metals leads to problems during measurement

of Hg by atomic absorption spectroscopy (see below) due to effervescence in the reaction vessel of the atomic vapour accessory. This can be overcome by using a digest mixture of 18 ml of concentrated HNO_3 and 2 ml of 60% $HClO_4$. (SAFETY PRECAUTIONS MUST BE CHECKED BEFORE USE OF PERCHLORIC ACID.)

4.5.3. Atomic absorption spectroscopy

The digest solution can be analysed by atomic absorption spectroscopy. Full details of the relevant techniques can be found in the relevant manuals in this series. A graphite furnace may be necessary for low concentrations of some elements (e.g. Cd) and an atomic vapour accessory for Hg (see 4.5.2.).

4.6 Presentation of results

The results for each site should be presented as a mean value for five replicates plus the 95% confidence interval. Comparison between sites may be made using the Student t-test (see Chapter 5).

A study should generally include confirmation of the reliability of the analytical data by inclusion of analyses using standard reference material. Reference material of *Rhynchostegium riparioides* has been prepared by the Commission of the European Communities (as *Platyhypnidium riparioides*: Colinet *et al.*, 1982; = CRM 061, Community Bureau of Reference, 1990); reference material of *Lagarosiphon major*, a submerged flowering plant not native to the U.K., though naturalized at some sites, has also been prepared by the Commission of the European Communities (Colinet *et al.*, 1982; CRM 060, Community Bureau of Reference, 1990).

5 Use of Data

5.1 Introductory comments

A variety of approaches can be used to interpret the analytical results, but essentially they fall into two categories. The simpler (qualitative) approach is where analytical data are used to show whether or not temporal or spatial differences occur between sets of samples; statistical tests may be applied to the results (5.2.2). The other (quantitative) approach is where the results of analysis for a particular plant sample are used to comment on the concentration of a metal in its environment. Useful background information on uptake and accumulation of heavy metals is given in the book by Kelly (1988), which includes tables of metal concentrations in aquatic plants.

5.2 Qualitative comparison of samples

5.2.1 Practical uses

The use of this approach is apparently quite widespread, but seldom reported in the literature. In most cases the sampling strategy and interpretation of results have been developed locally to solve a particular problem. A helpful guide to sampling and experimental design for anyone planning such work is given by Standing Committee of Analysts (1980).

Examples which have been carried out in the U.K. include:

i. Tracing the source of an unmapped drain carrying Zn-rich effluent (Say *et al.*, 1981).

ii. Supplementing routine chemical monitoring at sites where it is suspected that metal pollution in an effluent is partially or entirely intermittent (Holland & Harding, 1984).

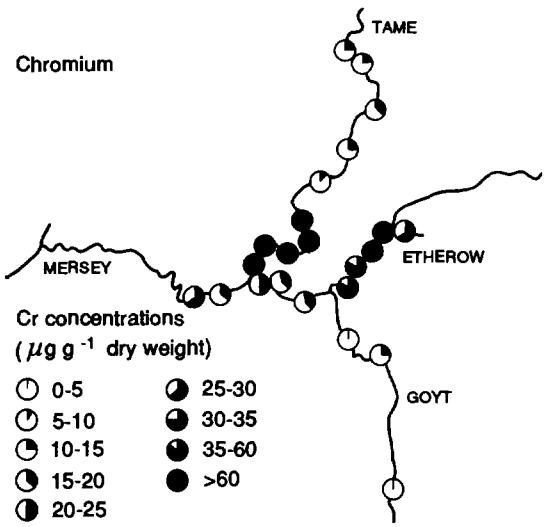
iii. Establishing how long it takes for metal released accidently to disappear from the biota.

iv. Preliminary survey to establish which parts of a catchment are most influenced by Hg pollution originating from pesticide application.

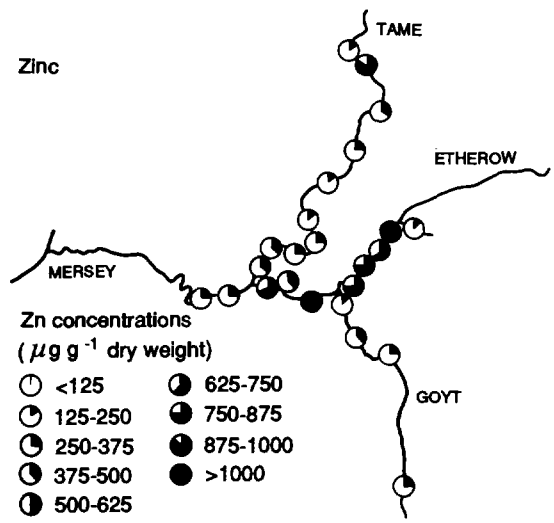
The use of many samples from one catchment to map pollution problems (Fig. 5.1) has been extended to a large area of Belgium (Descy *et al.*, 1981). Such maps can supplement or replace maps based on water chemistry, and provide a useful visual impact. Another potentially important use of plant material is to provide baseline data for future comparison. Material can be prepared and stored indefinitely as dried fragments; only a small mass (perhaps 100 mg) need to be stored, hence requiring only a very small volume. A sample could be analysed at any future time to demonstrate whether or not there had been an environmental change in the concentration of a particular metal.

5.2.2 Use of statistics to compare samples

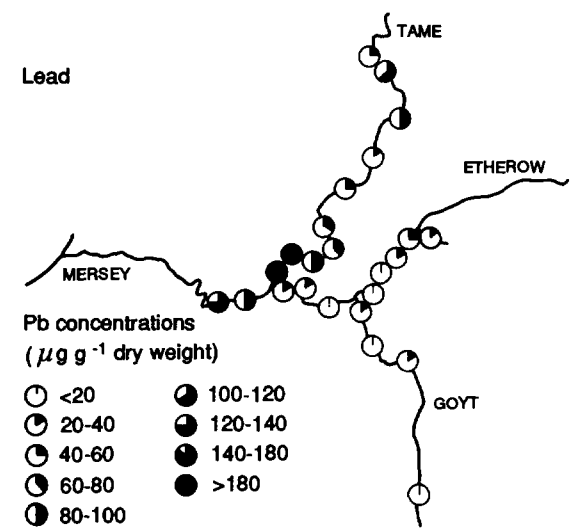
Detailed comments on statistics are outside the scope of this manual, but a few comments may be helpful. Statistics texts (e.g. Green, 1979; Parker, 1979; Mead & Curnow, 1983) should be consulted for details of the methods. If only a limited number of comparisons is required, the "Student" t-tests may be used. If repeated use of "Student" t-tests is considered, the user should be aware of the danger of making a "Type I" error by interpreting as significant, differences that are in fact non-significant. A more general method for comparison of sites is to use Analysis of Variance and, if the results are significant, to compare the sample means by the methods of "Least Significant Difference" or a "Multiple Range Test". Prior to the use of parametric statistical procedures the conditions of the normal distribution must be met. A $\log_{10}$-transformation is usually adequate to normalize the data; however, for certain purposes, Taylor's Power Law (Elliott, 1977) may be used to determine which transformation should be used.



A. *Chromium.* Concentrations increase markedly downstream of the effluent from Dukinfield S.T.W., which treats tannery waste. High levels were found also in the Etherow, providing evidence for short-term releases from industrial premises.



B. *Zinc.* The high concentrations in the Etherow were due to continuous input of metal from an industrial source reaching the river via old culverts.



C. *Lead.* A marked increase occurred on the Tame downstream of the effluent from Denton S.T.W. The high concentrations probably result from battery manufacture.

Fig. 5.1 Cr, Zn and Pb in 2-cm tips of shoots of *Fontinalis antipyretica* in the R. Mersey system. (Samples collected October 1979–January 1980, see Holland & Harding, 1984.)

5.3 Quantitative approach

In order to make quantitative estimates of aqueous metal concentrations from known concentrations in the plant samples, it is necessary to establish a dataset based on populations where both water and plant material have been sampled simultaneously and then analysed. Where datasets have been assembled relating concentrations of heavy metals and the fractions of algal or bryophyte species recommenced in Section 3, there has in all cases been a significant positive correlation between $\log_{10}$ concentration in the plant and $\log_{10}$ concentration in the water (Kelly & Whitton, 1989). (Full datasets are not available for *Nitella* and the two flowering plants in Section 3.)

In order to make the most effective use of such datasets, several of their features need to be considered. The datasets are based on spot samples and so are most reliable when aqueous metal concentrations have not varied greatly over time. However the need to use plants is most likely to occur in situations where pollution is intermittent or even the results of a single event, where the pulse of metal has passed downstream and is no longer detectable in the water. The only way to tackle this problem is to combine information from the relevant dataset with information on rates of uptake or loss of the particular metal; species transplant experiments are valuable here; such experiments have been carried out for *Lemanea* (Harding & Whitton, 1981) and *Rhynchostegium riparioides* (Wehr *et al.*, 1987).

The simplest method for using the datasets is to consider the bivariate relationship between ($\log_{10}$) metal in plant and ($\log_{10}$) metal in water. Examples of bivariate equations are given below for *Rhynchostegium riparioides* apical tips:
($_{aq}$ = metal concentration passing through 0.2 μm Nuclepore filter)

$$\log Zn_{aq} = 1.301 \log Zn_{pl} - 4.614 \quad r^2 = 0.64$$
$$\log Cd_{aq} = 0.481 \log Cd_{pl} - 3.744 \quad r^2 = 0.17$$
$$\log Pb_{aq} = 0.672 \log Pb_{pl} - 3.328 \quad r^2 = 0.36$$

However, the precision and accuracy of an estimate from a dataset can be increased markedly by including other variables in the dataset. These variables can relate to

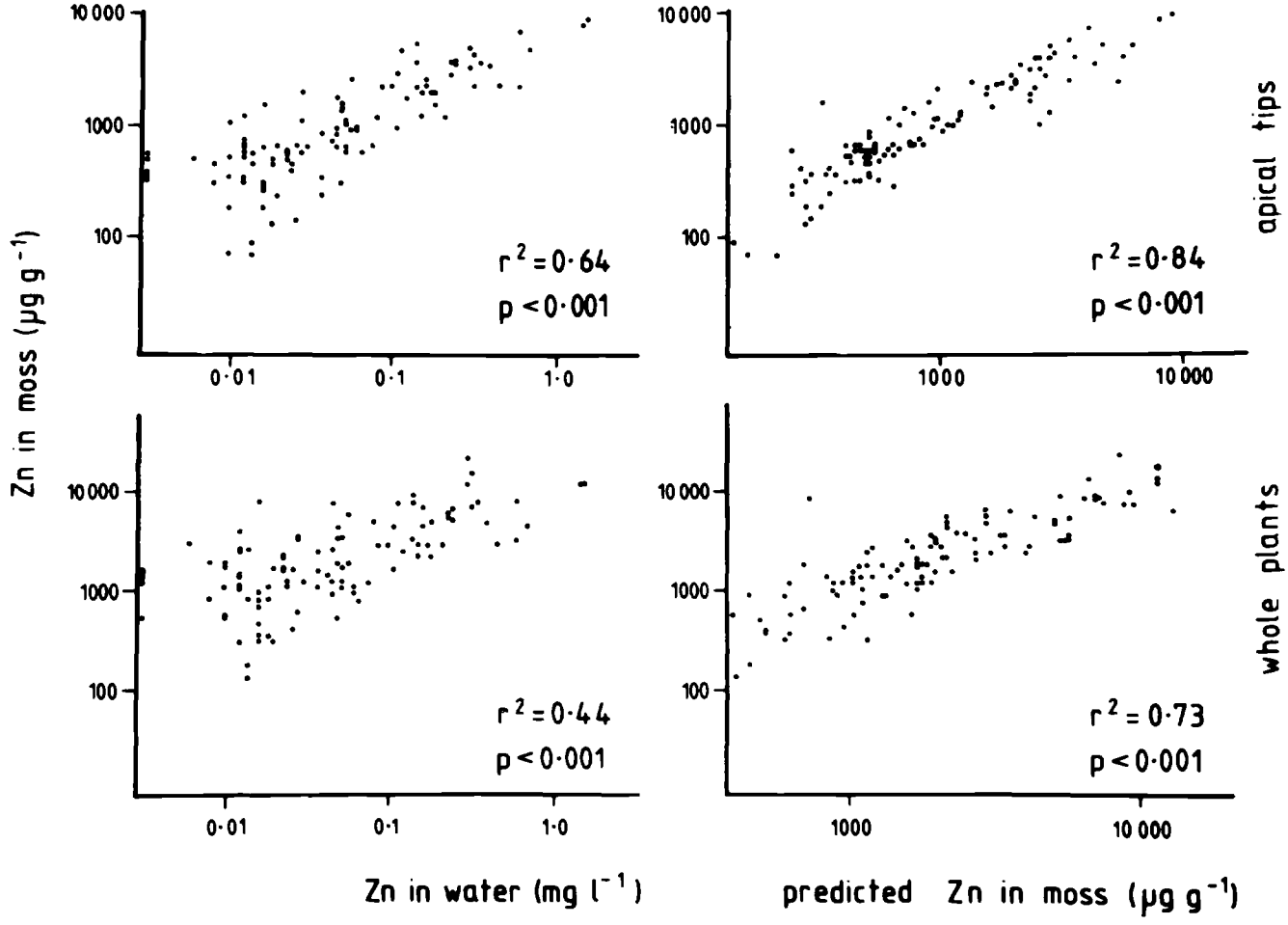


Fig. 5.2 Bivariate and predicted regressions for accumulation of Zn by apical tips and whole plants of *Rhynchostegium riparioides*. For details of the other variables used to obtain the predicted regressions, see Wehr *et al.* (1983).

the composition of both plant and water. Consider *Rhynchostegium riparioides* as an example (Wehr & Whitton, 1983a). A prediction of aqueous Zn concentrations based on accumulated Zn only has a predictive value (as coefficient of multiple determination, r^2) of 0.64. Inclusion of eight other variables into the equation via multiple stepwise regression (Draper & Smith, 1981; Berry & Feldman, 1985) improves the predictive ability to 0.84 (Fig. 5.2). It is, however, appreciated that under most conditions the range of additional determinands available will be limited and that the easiest ones to measure will be additional accumulated metal concentrations, which can be measured on the digest solutions prepared for the original analysis.

Satisfactory use of the quantitative approach, and in particular the use of many variables combined with multiple stepwise regression, is only feasible with access to the datasets on a computer file. (These are at present held at Durham University, but it would be helpful if they were more widely available.)

6 Bioassays and Toxicity Tests

Unicellular algae have been used widely for bioassay purposes, but their use in relation to heavy metals has been fragmentary. No one technique has been tested sufficiently that it can be put forward as a standard method.

At the present time EQOs (Environmental Quality Objectives) for heavy metals are defined in terms of chemical and zoological parameters and consequently most bioassay and toxicity testing procedures are designed to fulfil these criteria. The publication in this series, "Acute Toxicity Testing with Aquatic Organisms" (Standing Committee of Analysts, 1983), for example, includes tests for five animals. Under what circumstances, then, would bioassays using algae and other aquatic plants be of relevance to a water manager? In situations where information on all levels of the ecosystem is required (e.g. when setting consent values) it would clearly be of value to have standard methods available for plants as well as for animals. Inclusion of plants in a toxicity testing scheme extends coverage to the primary producers and, therefore, to the base of the "ecological pyramid".

The volume edited by Shubert (1984) provides a useful introduction to algal bioassays, with sections on the use of unicellular cultures (Trainor), mixed populations (Schelske) and heavy metals (Whitton). More recent literature is reviewed by Stratton (1987) and Kelly (1988). Most algal methods rely on strains which have been isolated and maintained in the laboratory, but there are a few which use natural populations or assemblages. The final choice depends on the aims of the study. Addition of a standard laboratory strain to a filtered sample of the water provides results that are more readily compared with other studies. On the other hand, use of field material allows the effect of a discharge on indigenous organisms to be monitored and also to predict or interpret changes in species composition and dominance (likely to be) caused by the input.

The reliable assay system for nutrients using *Selenastrum capricornutum* developed by US Environmental Protection Agency (Miller *et al.*, 1978) has been modified for use in metal toxicity tests, such as a study of the influence of Cd on specific growth rate by Parrish and Burks (1977). However application of this assay system for toxicity tests, especially ones for heavy metals, has been much less extensive than for the determination of nutrient limitation. Because the method requires 2–3 weeks to obtain results, a variety of short term assays have also been developed. These mostly depend on the impact of a toxicant on oxygen evolution by algal cultures. Effects have usually been measured over a 24-h period, but the time-scale can be made much shorter by incorporating sensitive methods of oxygen measurement. When the two methods were compared for a range of heavy metals and herbicides (Turbak *et al.*, 1986), the long-term Bottle Test proved to be the more sensitive assay system, but the responses of the two bioassays correlated well. The impact of Cu and Cd on oxygen evolution has also been tested for *Fontinalis antipyretica* (Sommer & Winkler, 1982).

There have also been a range of studies on the use of aquatic vascular plants in toxicity tests for heavy metals and other toxicants. Most have used species which are not native to the U.K., but Wang (1986) summarizes results from studies with the common duckweed *Lemna minor*. The results for 96-h toxicity tests for seven metals on duckweed and a fish indicated that the two test organisms compared very well.

7 Genetic Tolerance

A further approach to quantifying the impact of current heavy metal contamination at a site is to assess genetic differences between organisms at the site and organisms of the same species from an uncontaminated site. No technique has been developed sufficiently to recommend it as a standard method for freshwaters, but it is likely that this approach will eventually prove important if rapid screening techniques can be developed.

The metal-tolerance of populations of various green algae has been shown to be related to the environmental concentration of the particular metal, and the evidence suggests that much of the tolerance is genetic (Whitton, 1984). For instance, this has been shown for zinc and species in the filamentous genera *Klebsormidium*, *Microthamnion*, *Mougeotia* and *Stigeoclonium*. Laboratory assays of tolerance of populations from contaminated sites can therefore be used to assess the impact of a metal at these sites (Harding & Whitton, 1976; Kelly & Whitton, 1989). Similar studies have been made with protonema from terrestrial moss populations at copper and zinc contaminated sites (Shaw, 1987; Shaw *et al.*, 1987) and it would be easy to develop the methodology for aquatic bryophytes.

A more elaborate, but one of the most successful, algal test system has been developed at the University of Göteborg, Sweden, for quantifying the influence of toxic materials. Practical assessment of the impact of contaminant metal has been made so far only for the marine environment (Blanck & Wängberg, 1988; Blanck *et al.*, 1988), but the method has been tested for other toxicants on stream algal populations (Blanck, 1985). Periphytic communities are allowed to develop on glass discs placed at the study site. The discs are then returned to the laboratory and used for toxicity determinations, with the impact measured as percentage decrease in O_2 evolution and/or $^{14}CO_2$ fixation. The method can be used as a straighforward bioassay to help predict the impact of a toxic effluent on a mixed community developed at an uncontaminated site (as in Chapter 6) or to assess the extent to which a community from a particular site has become tolerant to a potentially toxic metal (Blanck *et al.*, 1988).

It seems likely that methods for assessing genetic tolerance based on molecular biological techniques will eventually become important in monitoring the impact of heavy metals. Such methods might include rapid screening for the presence of particular metallothionein genes or demonstration of their products by fluorescent antibodies.

8 Species and Community Structure

There are many accounts in the literature of the impact of one or more heavy metals on the floristic composition at freshwater sites (see Whitton, 1984, for examples concerning the algal flora). Similar studies have been reported for aquatic bryophytes and flowering plants (e.g. Besch & Roberts-Pichette, 1970), though apparently no detailed studies in the U.K. These provide some guidance for anyone needing to predict the short- and long-term effects of heavy metal contamination at particular sites.

At least in rivers, macroinvertebrates have been used much more widely than plants to assess overall water quality (Hellawell, 1978; Metcalfe, 1989). Nevertheless a number of authors have also tested the use of species diversity indices or measures of community structure based on plants to monitor river water quality. The majority of these studies are based on benthic algae, either the whole periphyton community or just the diatoms. The methods have mostly been applied to assessing organic contamination and nutrient enrichment, although they have sometimes also been applied to sites influenced by heavy metal contamination. Insufficient data have been obtained relating results for diversity indices or community structure to analyses of the water chemistry of sites with heavy metal contamination for this approach to be used as a standard method for monitoring heavy metals. Several of the techniques might however prove a useful complement to macroinvertebrate surveys if this database were assembled.

A major text on periphyton in the Mitteilungen series of the Society for Theoretical and Applied Limnology is planned to include chapters on monitoring, but no publication date has been announced yet. The Saprobien system (Sládeček, 1973), which is still used in parts of eastern Europe, has sometimes been modified to incorporate information about the effects of metal contamination. A number of recent papers dealing specifically with diatoms also include summaries of the literature and theoretical approaches concerning the use of benthic algae for monitoring purposes (e.g. Descy, 1979; Stevenson, 1984; Mouthon & Coste, 1984; Leclercq & Maquet, 1987; Leclercq & Depiereux, 1987).

References

Belcher J.H. & Swale E.M.F. (1976). *A Beginner's Guide to Freshwater Algae.* 48 pp. Institute of Terrestrial Ecology/Natural Environment Research Council. H.M.S.O., London.

Benson-Evans K. (1976). Transplanting aquatic bryophytes to assess river pollution. *J. Bryol.* 9: 81–91.

Berry W.D. & Feldman S. (1985). *Multiple Regression in Practice.* 95 pp. *Sage University Paper Series on Quantitative Application in the Social Sciences No. 50.* Beverly Hills and London.

Besch K.W. & Roberts-Pichette P. (1970). *Effects of mining pollution on vascular plants in the Northwest Miramichi River System. Can. J. Bot.* 48: 1647–1656.

Blanck H. (1985). *A simple, community level, ecotoxicological test system using samples of periphyton. Hydrobiologia* 124: 251–261.

Blanck H. & Wängberg S-A. (1988). *Induced community tolerance in marine periphyton established under arsenate stress. Can. J. Fish. aquat. Sci.* 45: 1816–1819.

Blanck H., Wängberg S-A. & Molander S. (1988). *Pollution-induced community tolerance – a new ecotoxicological tool.* In: Cairns J., Jr, & Pratt J.R. (Eds) *Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals.* ASTM 988.

Caines L.A., Watt A.W. & Wells D.E. (1985). *The uptake and release of some trace metals by aquatic bryophytes in acidified waters in Scotland. Environ. Pollut.* B10: 1–18.

Clapham A.R., Tutin T.G. & Moore D.M. (1987). *Flora of the British Isles. 3rd Edn.* 688 pp. Cambridge University Press, Cambridge.

Community Bureau of Reference (1990). *BCR Reference Materials.* 60 pp. Commission of the European Community, Brussels.

Crum H.A. & Anderson L.E. (1981). *Mosses of Eastern North America* 2. pp. 665-1328. Columbia U.P., N.Y.

De Boer J.L.M. & Maessen F.J.M.J. (1983). *A comparative examination of sample treatment procedures for ICAP-AES analysis of biological tissue. Spectrochimica Acta* 38B, 739–746.

Descy J-P., Empain A. & Lambinon J. (1981). *La Qualité des Eaux Courantes en Wallonie – Bassin de la Meuse.* 18 pp. Secretariat d'Etat a l'Environnement, à l'Amenagement du Territoire et à l'Eau pour l'Wallonie.

Draper N.R. & Smith H. (1981). *Applied Regression Analysis.* 2nd Edn. 709 pp. Wiley, N.Y.

Elliott J.M. (1977). *Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. 2nd Edn.* 160 pp. Sci. Publs Freshwat. Biol. Ass. U.K. 25.

Empain A. (1976) *Les bryophytes aquatiques utilisés comme traceurs de la contamination en métaux lourds des eaux douces. Mem. Soc. r. bot. Belg.* 7: 141–156.

Empain A., Lambinon J., Mouvet C. & Kirchmann R. (1980). *Utilisation des bryophytes aquatiques et subaquatiques comme indicateurs biologiques de la qualité des eaux courantes.* In: Pesson P. (Ed.) *La Pollution des Eaux Continentales, 2nd Edn.* pp. 195–226. Gauthier-Villars, Paris.

Everard M. & Denny P. (1985). *Flux of lead in submerged plants and its relevance to a freshwater system. Aquatic Botany* 21: 181–193.

Foster P.L. (1982). *Species associations and metal contents of algae from rivers polluted by heavy metals. Freshwat. Biol.* 12: 17–39.

Green R.H. (1979). *Sampling Design and Statistical Methods for Environmental Biologists.* 257 pp. Wiley Interscience, N.Y.

Harding J.P.C. & Whitton B.A. (1976). *Resistance to zinc of* Stigeoclonium tenue *in the field and the laboratory. Br. phycol. J.* 11: 417–426.

Harding J.P.C. & Whitton B.A. (1978). *Zinc, cadmium and lead in the water, sediments and submerged plants of the Derwent Reservoir. Water Res.* 12: 307–316.

Harding J.P.C. & Whitton B.A. (1981). *Accumulation of zinc, cadmium and lead by field populations of Lemanea. Water Res.* 15: 301–319.

Haslam S.M., Sinker C.S. & Wolseley P.A. (1975). *British Water Plants.* Field Studies Council, London.

Hellawell J.M. (1978). *Biological Surveillance of Rivers.* Water Research Centre, Medmenham, Bucks.

Holland D.G. & Harding J.P.C. (1984) *Mersey.* In: Whitton B.A. (Ed.) *Ecology of European Rivers.* pp. 113–144. Blackwell, Oxford.

Holmes N.T.H., Whitton B.A. & Hargreaves J.W.G. (1978). *A Coded List of Freshwater Macrophytes of the British Isles. Water Archive Manual Series No. 4.* 201 pp. Department of the Environment, Water Data Unit, Reading.

Kelly M.G. (1988). *Mining and the Freshwater Environment.* Elsevier Applied Science. 231 pp.

Kelly M.G., Girton C. & Whitton B.A. (1987). *Use of moss-bags for monitoring heavy metals in rivers. Water Res.* 21: 1429–1435.

Kelly M.G. & Whitton B.A. (1989). *Relationship between accumulation and toxicity of zinc in* Stigeoclonium *(Chaetophorales, Chlorophyta). Phycologia* 28: 512–517.

Kingston H.M. & Jassie L.B. (1986). *Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Analytica Chemistry* 58: 2534–2541.

Leclercq L. & Depiereux E. (1987). *Typologie des riviers oligotrophes du massif Ardennais (Belgique) par l'analyse multivariée de relevés de diatomées benthiques. Hydrobiologia* 153: 175–192.

Martin M.H. & Coughtrey P.J. (1982). *Biological Monitoring of Heavy Metal Pollution.* 475 pp. Applied Science Publishers, London & N.Y.

Mead R. & Curnow R.N. (1983). *Statistical Methods In Agriculture and Experimental Biology.* 335 pp. Chapman & Hall, London.

Metcalfe J.L. (1989). *Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe Environ. Pollut.* B60: 101–139.

Miller W.E., Green J.C. & Shiroyama T. (1978). *The Selenastrum capricornutum Printz Algal Assay Bottle Test.* Corvallis Environmental Research Laboratory office of Research and Development. 126 pp. US EPA, Corvallis.

Moore J.A. (1986). *Charophytes of Great Britain and Ireland. B.S.B.I. Handbook No.* 5. 140 pp. Botanical Society of the British Isles, London.

Mouthion J. & Coste M. (1984). *Qualité hydrobiologique de la Loire. Etat de référence du site de Villerest.* Etude du CEMAGREF–Lyon. 110 pp.
Mouvet C. (1984). *Accumulation of chromium and copper by the aquatic moss* Fontinalis antipyretica *L. ex Hedw. transplanted in a metal-contaminated river. Environ. Technol. Lett.* 5: 541–548.
Mouvet C. (1985). *The use of aquatic bryophytes to monitor heavy metals pollution of freshwaters as illustrated by case studies. Verh. int. Verein. Limnol.* 22: 2420–2425.
Nakashima S., Sturgeon R.E., Willie S.N. & Bernab, S.S. (1988). *Acid digestion of marine samples for trace element analysis using microwave digestion. Analyst* 113: 159–163.
Nieboer E. & Richardson D.H.S. (1980). *The replacement of the nondescript term 'heavy metal' by a biologically and chemically significant classification of metal ions. Environ. Pollut. (Ser.B)* 1: 3–26.
Parker R.E. (1979). *Introductory Statistics for Biology. 2nd Edn.* 122 pp. Edward Arnold, London.
Parrish D. & Burks S.L. (1977). *A Comparison of the Effects of Chelated and Non-chelated Cadmium on the Specific Growth Rate of* Selenastrum capricornutum. *US Dept. of Commerce National Technical Information Series* PB-275 775.
Passow H., Rothstein A. & Clarkson T.W. (1961). *The general pharmacology of heavy metals. Pharmac. Rev.* 13: 185–224.
Pentecost A. *Keys, Descriptions, and Line Drawings of the Commoner British Freshwater Algae.* 247 pp. +48 pls. Richmond Publishing Co.
Prescott G. W. (1962) *Algae of the Western Great Lakes.* W.C. Brown & Co., Iowa.
Say P.J., Burrows I. G. & Whitton B.A. (1986). *Enteromorpha as a Monitor of Heavy Metals in Estuarine and Coastal Intertidal Waters.* 25 pp. *Occasional Publication No. 1,* Northern Environmental Consultants Ltd, Consett.
Say P.J., Harding J.P.C. & Whitton B.A. (1981). *Aquatic mosses as monitors of heavy metal pollution in the River Etherow, Great Britain. Environ. Pollut.* B2: 295–307.
Say P.J. & Whitton B.A. (1983). *Accumulation of heavy metals by aquatic mosses. 1.* Fontinalis antipyretica. *Hydrobiologia* 100: 245–260.
Schelske C. L. (1984). *In situ and natural phytoplankton assemblage bioassays.* pp. 15–47. In: Shubert L.E. (Ed.) *Algae as Ecological Indicators.* Academic Press, London.
Shaw J. (1987). *Effect of environmental pretreatment on tolerance to copper and zinc in the moss* Funaria hygrometrica. *Am. J. Bot.* 74: 1466–1475.
Shaw J., Antonovics J. & Anderson L.E. (1987). *Inter- and intraspecific variation of mosses in tolerance to copper and zinc. Evolution* 41: 1312–1325.
Shubert L.E. (Ed.) (1984). *Algae as Ecological Indicators.* 434 pp. Academic Press, London.
Sládeček V. (1973). *System of water quality from the biological point of view. Arch. Hydrobiol., Ergebn. Limnol.* 7: 1–218.
Smith A.J.E. (1978). *The Moss Flora of Britain and Ireland.* 706 pp. Cambridge University Press, London.
Sommer C. & Winkler S. (1982). *Reaktionen im Gaswechsel von* Fontinalis antipyretica *HEDW. nach experimentellen Belastungen mit Schwermetallverbindungen. Arch. Hydrobiol.* 93: 503–524.
Standing Committee of Analysts (1980). *General Principles of Sampling and Accuracy of Results 1980.* 57 pp. Methods for the Examination of Waters and Associated Materials, H.M.S.O., London. (see also SCA 1991 ref. below).
Standing Committee of Analysts (1983). *Acute Toxicity Testing with Aquatic Organisms.* H.M.S.O., London.
Standing Committee of Analysts (1984). *Sampling of Non-Planktonic Algae (Benthic Algae or Periphyton) 1982.* (Eds Marker A.F.H. & Bolas P.M.) 27 pp. H.M.S.O., London.
Standing Committee of Analysts (1986). *The Sampling and Initial Preparation of Sewage and Waterworks Sludges, Soils, Sediments, Plants and Contaminated Wild Life (2nd Edn). 1986.* H.M.S.O., London
Standing Committee of Analysts (1987a). *Methods for the Use of Aquatic Macrophytes for Assessing Water Quality 1985–86.* 176 pp. H.M.S.O., London.
Standing Committee of Analysts (1987b). *Methods for the Determination of Metals in Soils, Sediments and Sewage Sludge and Plants by Hydrochloric–Nitric Acid Digestion, with a note on the Determination of the Insoluble Metal Contents 1983.* (Methods for the Examination of Waters and Associated Materials) 36 pp. H.M.S.O., London.
Standing Committee of Analysts (1991). *General Principals of Sampling (2nd Edn) with Supplements 1990.* H.M.S.O., London.
Stevenson R.J. (1984). *Epilithic and epipelic diatoms in the Sandusky River, with emphasis on species diversity and water pollution. Hydrobiologia* 114: 161–175.
Stratton G.W. (1987). *The effects of pesticides and heavy metals towards phototrophic microorganisms. Reviews in Environmental Toxicology* 3: 71–147.
Trainor F.R. (1984). *Indicator algal assays: laboratory and field approaches.* pp. 3–14. In: Shubert L.E. (Ed.) *Algae as Ecological Indicators.* Academic Press, London.
Turbak S.C., Olson S.B. & McFeters G.A. (1986). *Comparison of algal assay systems for detecting waterborne herbicides and metals. Wat. Res.* 20: 91–96.
Uhrberg R. (1982). *Acid digestion bomb for biological samples. Anal. Chem.* 54: 1906–1908.
Wang, W. (1986). *Toxicity tests of aquatic pollutants by using common duckweed. Environ. Pollut. B* 11: 1–14.
Watson E.V. (1981). *British Mosses and Liverworts. 3rd Edn.* Cambridge U.P.
Wehr J.D., Empain A., Mouvet C., Say P.J. & Whitton B.A. (1983). *Methods for processing aquatic mosses used as monitors of heavy metals. Wat. Res.* 17: 985–992.
Wehr J.D., Kelly M.G. & Whitton B.A. (1987). *Factors affecting accumulation and loss of zinc by the aquatic moss Rhynchostegium riparioides. Aquatic Botany* 29: 261–274.
Wehr J.D. & Whitton B.A. (1983a). *Accumulation of heavy metals by aquatic mosses. 2.* Rhynchostegium riparioides. *Hydrobiologia* 100: 261–284.
Wehr J.D. & Whitton B.A. (1983b). *Accumulation of heavy metals by aquatic mosses 3. Seasonal changes. Hydrobiologia* 100: 285–291.
Whitton B.A. (1970). *Biology of Cladophora in freshwaters. Water Res.* 4: 87–114.
Whitton B.A. (1984). *Algae as monitors of heavy metals.* pp. 257–280. In: Shubert L.E. (Ed.) *Algae as Ecological Indicators.* Academic Press, London.
Whitton B.A., Burrows I.G. & Kelly M.G. (1989). *Use of* Cladophora glomerata *to monitor heavy metals in rivers. J. appl. Phycol.* 1: 293–299.
Whitton B.A., Holmes N.T.H. & Sinclair C. (1978). *A Coded List of 1000 Freshwater Algae of the British Isles.* 335 pp. Department of the Environment, Water Data Unit, Reading.
Whitton B.A., Say P.J. & Jupp B.P. (1982). *Accumulation of zinc, cadmium and lead by the aquatic liverwort Scapania. Environ. Pollut.* B3: 299–316.
Whitton B.A., Say P.J. & Wehr J.D. (1981). *Use of plants to monitor heavy metals in rivers.* In: Say P.J. & Whitton B.A. (Eds) *Heavy Metals in Northern England: Environmental and Biological Aspects.* 198 pp., pp. 135–145. Department of Botany, University of Durham.

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