GLOBAL ENVIRONMENT MONITORING SYSTEM

Concepts and Strategies for Biological Monitoring

by

D. V. Chapman

GEMS Monitoring and Assessment Research Centre
King's College London

Prepared by the

GEMS Monitoring and Assessment Research Centre

King's College London University of London
The Old Coach House Campden Hill London W8 7AD

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1. Introduction

Environmental contamination is a problem now facing all countries of the world. Preservation of human health and the natural environment has made it necessary to initiate management programmes to control the sources of this contamination. Such sources may be widespread or diffuse or they may arise from a single, identifiable location. For effective management of the environment some form of monitoring is usually necessary. The monitoring provides information of the extent of a pollution problem, its possible environmental impact and its deterioration or improvement over time. Monitoring can also be used to evaluate the effectiveness of a remedial action which has been taken to eliminate or reduce a source of contamination. It can also be used to check that any environmental guidelines or legislative standards are being met.

There are many definitions of monitoring some of which aim to separate it from the activities of survey and surveillance. Surveys can be considered to indicate the scale of pollution at a particular time, whereas surveillance enables a trend to be detected by a series of repeated measurements. Generally, however, the term monitoring is widely used to embrace all of these activities and can be defined as the systematic collection of data. In its strictest sense monitoring is carried out to test for compliance with standards and is action orientated, but for the purposes of this discussion it will be used to describe systematic collection of data on a spatial or temporal basis. The aim of a monitoring programme is to provide information which is useful for management and possibly research.

There are basically two types of monitoring, those methods which measure physical and chemical parameters in the environment and those methods which measure the responses of organisms to changes in their environment. The former physicochemical methods demonstrate that levels of contamination are changing but the latter biological methods are able to provide information on the response of organisms to the integrated effects of environmental conditions and contaminants. In practice monitoring programmes which use biological methods often include some physical and chemical measurements, or rely on a background of knowledge previously obtained by research, in order to explain biological changes or to relate biological accumulation of contaminants to environmental levels.

This handbook is the first of three which deal with the design of monitoring programmes using biological monitoring methods, many of which are simple and cheap. The methods discussed are based on the effects on organisms which are subjected to contamination or disturbance. These constitute the populations which are the focus of the monitoring programmes. With many environmental concerns man is the target, either directly such as in exposure to air pollutants or indirectly through food and other sources. Although biological monitoring can also be used to describe studies

investigating contaminants in man, this aspect is not dealt with in detail in this series. It must be borne in mind, however, that many management programmes are designed to protect man from any unnecessary health risks associated with environmental contaminants.

This series of documents aims to provide sufficient information for scientists and managers, without prior experience, to embark upon the design and implementation of a monitoring programme using biological monitoring methods. The first part describes basic principles and strategies which can be applied to the use of either plants or animals as the primary targets for monitoring. Methods applicable to these organisms will appear in parts two (plants) and three (animals) together with some examples of their successful use in monitoring programmes. More detailed reviews of programmes and studies from around the world, together with a presentation of some of the results obtained, are available in Burton (1986) and Samiullah (In Press).

2. Designing a monitoring programme

2.1 Setting objectives

Before embarking on the design of a monitoring programme it is important to have a clear impression of why such a programme is necessary. Reasons may include checking that environmental conditions meet regulations and standards, checking for potential health hazards arising from a pollution incident or measuring basic ecological changes that may arise due to some human impact such as industrialization of a rural area or increased urbanization. The subsequent planning of a monitoring programme must be clearly related to the reasons for the study and must aim to provide information which will be useful for management of the environment or incident. In practice the design of the monitoring programme will be constrained by the availability of resources such as manpower, finance or equipment. However, it is important that the final design of the scheme will be adequate to detect changes in the parameters of interest.

Firstly therefore, it is necessary to address the questions:

a) What is the purpose of the monitoring?
b) What is already known?
c) What is it necessary to find out?

The answers to these should indicate the possible targets of the monitoring programme, some background information which will enable the identification of gaps in current knowledge and the objectives for achievement by further monitoring. This further monitoring should provide information which is meaningful to managers and policy makers and some advice for achieving this is given in subsequent sections.

2.2 Programme design

Once all possible background information has been gathered the programme must be designed to meet the stated objectives (figure 1).

There are four basic steps to programme design:

1) Identifying the target organisms and the measurements that must be taken,
2) Selecting sampling sites and frequency of sampling,
3) Choosing the methods to be used
4) Designing a procedure for handling and presenting the data.

All these stages have many individual steps within them some of which will be covered in more detail in the following sections and the remainder in the accompanying two handbooks.

2.3 Identifying the problem

The requirement for a monitoring programme may have arisen as a result of newly introduced legislation requiring checking of levels of environmental contaminants in a defined environmental medium, or as a result of potential environmental degradation. Alternatively monitoring may be required to determine cause and effect relationships associated with particular environmental deterioration. In the first case, monitoring for compliance with standards, the nature of the problem can be clearly defined to measuring the specified contaminant(s) and a specific ecosystem or compartment of the environment. However, a requirement to monitor basic environmental change due to slow, progressive processes such as urbanization or increased agricultural development is a less well defined situation. In this case targets must be identified in the environment which are at risk and require preservation. In addition those factors which might cause deterioration, such as contaminants in the air or agricultural run-off, must be selected.

Where the impact of possible contamination on an ecosystem, or defined area, is of interest it is useful to construct an inventory of the possible sources of contamination to the area prior to embarking on the design of the monitoring programme. This will help identify specific contaminants which can be monitored using biological techniques and allow an assessment of existing knowledge to be made. The inventory, together with the aid of a suitable map of the area of interest, will help establish the areas which should be covered by the monitoring programme. Not all contaminants can, however, be related to a specific source as their origins may be widespread and diffuse. An example is the occurrence of agricultural pesticides in water bodies. Consideration of these problems will be dealt with in a later section on sampling strategies.

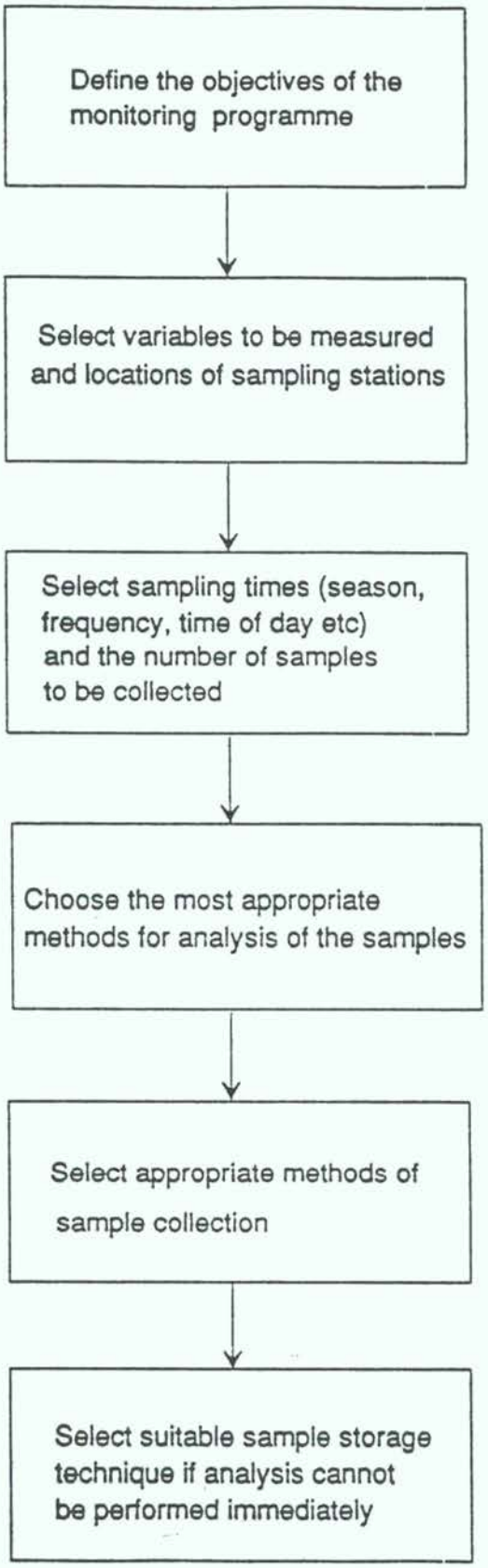


Figure 1 The sequential approach to designing a monitoring programme

2.4 Baselines and controls

With the exception of the situation where monitoring data are obtained to test for compliance with standards, any measurements made as part of a monitoring programme can only be fully interpreted if they can be related to baseline levels or measurements from a similar unperturbed environment. Alternatively they may be compared to a critical concentration level e.g. levels known to have determined toxic effects, either in the environment or specific organisms.

Having determined the targets of the monitoring programme and the possible contaminants or environmental area of concern, it is necessary, therefore, to obtain background information. In ideal circumstances this may consist of a set of data for the same variables obtained in an earlier monitoring programme. Such information provides a baseline for comparison with new data and a guideline for the expected levels. However, such baseline data is often not available and it may be necessary to go to published studies from elsewhere to obtain information on similar situations.

In practice baseline or control data may have to be obtained from field measurements made as part of the monitoring programme. An example is where measurements are made in a similar environmental area away from a source of contamination. This aspect is dealt with in more detail in the discussion of sampling strategies and site selection.

3. Sampling strategies

3.1 Basic principles

The choice of sampling strategy is specific to each monitoring programme and must be related to the nature of the problem to be investigated. In general equally-spaced, fixed sampling stations, monitored relatively infrequently, are useful for detecting trends over time, observing the effects of pollution, or monitoring recovery from pollution. Alternatively, cause and effect studies usually require multiple stations of high spatial density and high sample frequency.

Once the contaminants and the targets for monitoring have been selected there are three aspects to planning the sampling strategy: 1) site selection, 2) choosing the frequency and number of samples and 3) selecting the sampling technique to be used. Such strategies usually use either a random selection of samples from the target population (i.e. organisms or substrates) or systematic sampling at equal intervals in time or space, beginning at a point chosen at random.

Precise planning of sampling strategies may not be possible without first carrying out a preliminary survey. Such a survey may help establish the extent of the problem i.e. the area at risk and the levels of contaminant to be

expected. Alternatively the knowledge of an expert, with previous experience in the field, may define the locations or regions for choosing sample sites.

3.2 Site selection

Within a monitoring programme two types of sites may be selected, those which will give baseline information and those which will be related to some special interest e.g. source of contamination, geographical effect or human impact. Sites which are intended to give baseline information must be located away from unusual influences or disturbance and should all be similar in nature. It is helpful when interpreting effects on target organisms to have some basic physical measurements such as temperature, radiation, wind speed and direction or water velocity. Therefore, sites are often selected to coincide with existing programmes of physical and chemical measurements and are usually located where access is relatively easy. A site description should be kept for all sites.

3.2.1 Widespread contamination and monitoring networks

Where a monitoring programme is designed to determine the levels or effects of diffuse contaminants of uncertain origin, or of contaminants known to extend over wide regions such as in the case of global air pollutants, a network of sampling sites is necessary. In reality the number and location of these sites will probably be governed by the financial resources available to run the programme together with convenience of access. Nevertheless sites should be chosen which will give useful information, as time and effort may be wasted in gathering data which is irrelevant, or impossible to interpret.

If an intensive sampling programme is planned for a spatially distributed contaminant within a uniform substrate, an area of little physical variation, or an area for which no other criteria can be used to aid site-selection, sites must be chosen using a random sampling technique. This method is suitable if there are no major trends, cycles or pattens of contamination. The sites are selected using random number co-ordinates and must not be picked haphazardly. In order to ensure that samples are representative of the whole area a stratified random sampling procedure can be employed. The sampling area is divided up into several strata, such as different biotopes, which can be unequal in area if appropriate. This enables a better estimate of the mean or total contamination level for the whole area. The units for sampling within the strata are allocated in numbers proportional to the size of the strata and selected by simple random sampling.

In some situations it may be more appropriate to use a two stage sampling technique where the area is divided into primary units some of which are selected by simple random

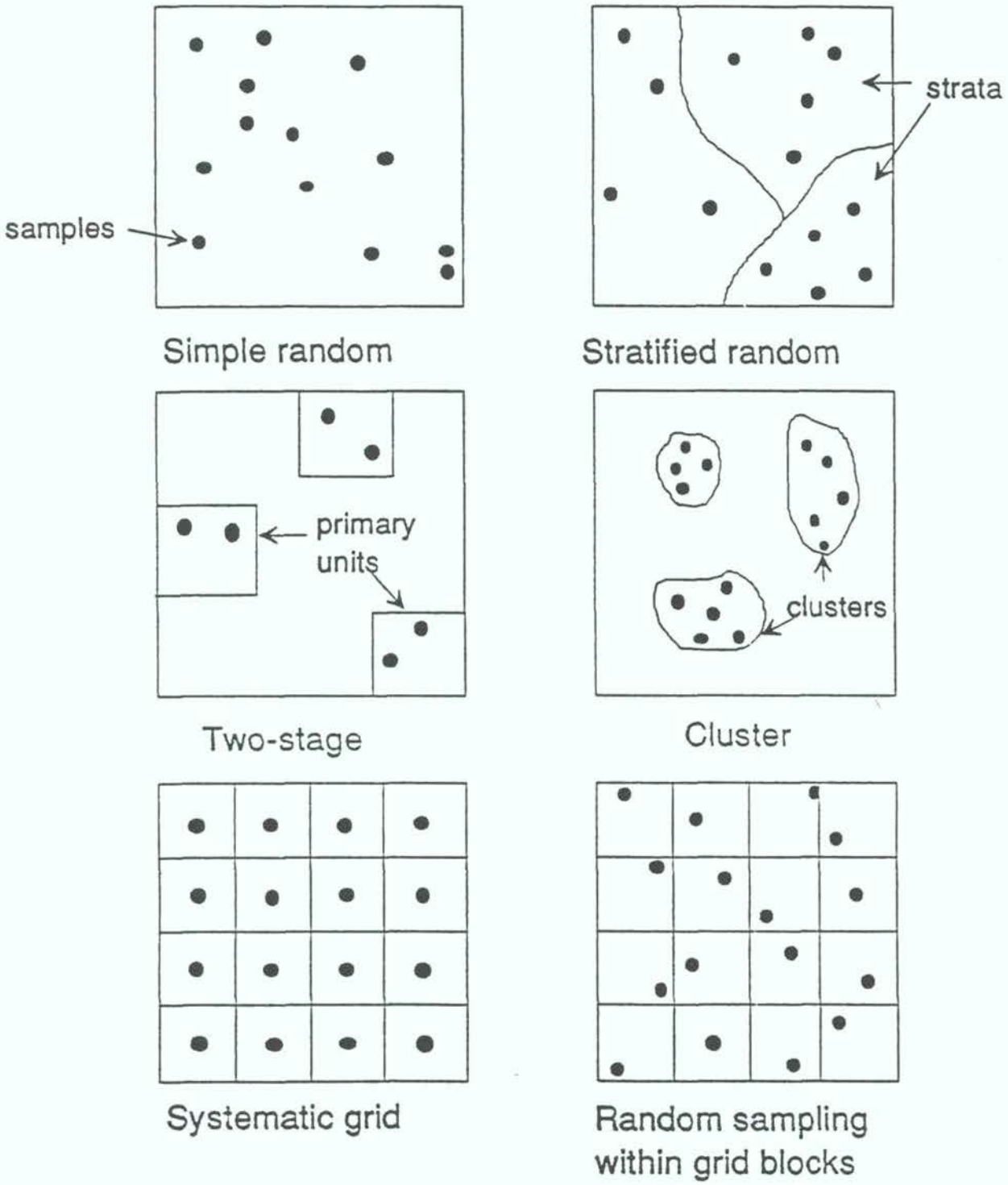


Figure 2 Sampling designs for two-dimensional spatial sampling.

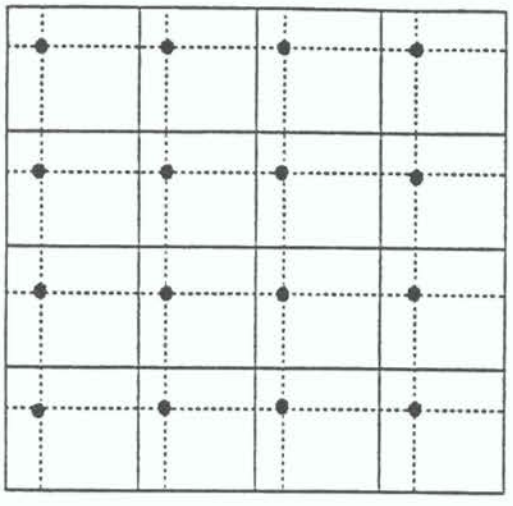
methods and each selected unit is then subsampled randomly (figure 2). An example would be random selection of soil samples and subsequent random sub-sampling. However, where it is difficult to obtain random samples of plants or other organisms, for example when access is awkward, cluster sampling is more suitable. The clusters of individuals are chosen at random and all individuals in the cluster sampled (figure 2).

Statistical studies indicate that systematic sampling methods are preferable to other methods for estimating means, totals or patterns of contamination. Systematic sampling consists of taking samples at locations and or times according to the spatial or temporal pattern e.g. on a grid pattern or equidistant along a transect. Such methods are also relatively easy to implement in the field but there is always the chance that the sampling pattern could correspond to the pattern of contamination in space or time. A more uniform coverage is obtained by taking samples from random locations within the blocks of a grid or a segment of a transect (figure 2). Figure 3a shows a simple central aligned square grid. An alternative is that of the triangular grid where a random point is chosen and the other points are fixed by the imposed triangular pattern (figure 3b). These methods are particularly suitable for estimating spatial distributions of contamination.

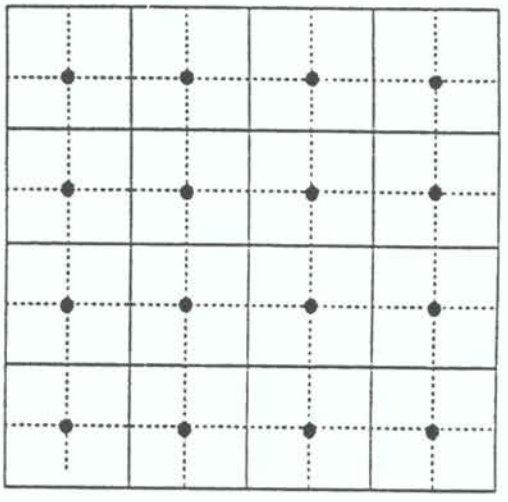
Ideally all units of a grid should be sampled on each occasion but the time and effort involved could be too great for frequent repetition during long-term monitoring programmes. Therefore, it is usual to select a sequence of sampling for the sites which allows for any one point to be repeated at least once in a prescribed time period e.g. once a year for long term monitoring. The determination of the order of sampling which ensures adequate repetition of sites is governed by sampling theory which is too complex to discuss in detail here. Further reading is suggested in Appendix A.

3.2.2 Point source emissions

If the monitoring programme is designed to determine the extent and effects of an emission from a point source, e.g. an incinerator chimney or an outfall pipe in a river, the sites chosen should extend away from the source in the predominant direction of travel of the likely plume of contamination. Transects can be chosen along which sampling is undertaken according to random or systematic methods as described above (figure 4). However, care must be taken to ensure that the sites are not influenced by other potential sources of contaminants that may have an effect on the target(s) being monitored. Additional sites should also provide background levels in uncontaminated areas of similar environmental type. In monitoring confined areas, such as narrow rivers, sample sites should also be kept away from the edges where additional natural factors may affect the type of biota found there. Such influences are often not well understood and cannot be allowed for in the interpretation of the resultant data.

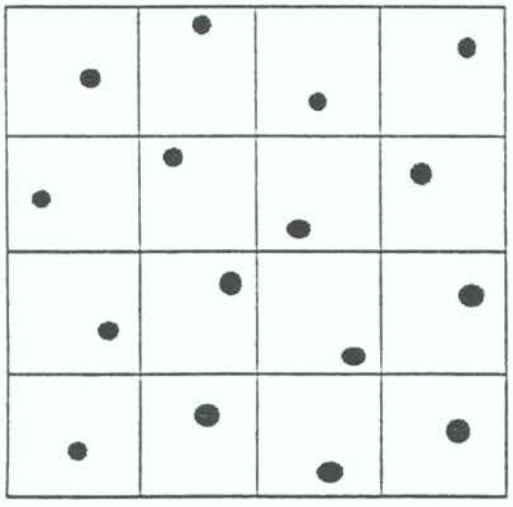


Aligned square grid

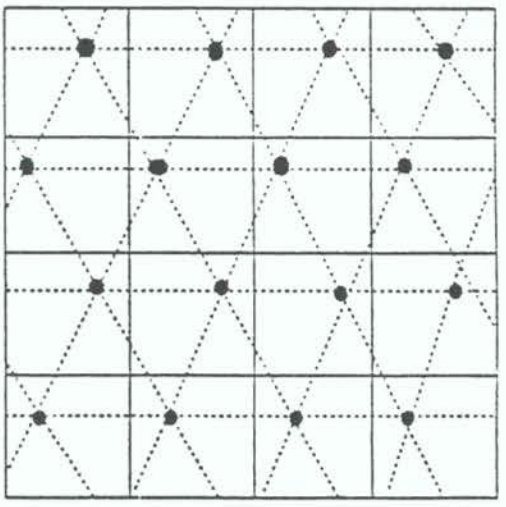


Central aligned square grid

A. The central aligned square grid.



Unaligned grid



Triangular grid

B. The triangular grid.

Figure 3 Two approaches to systematic sampling in space.

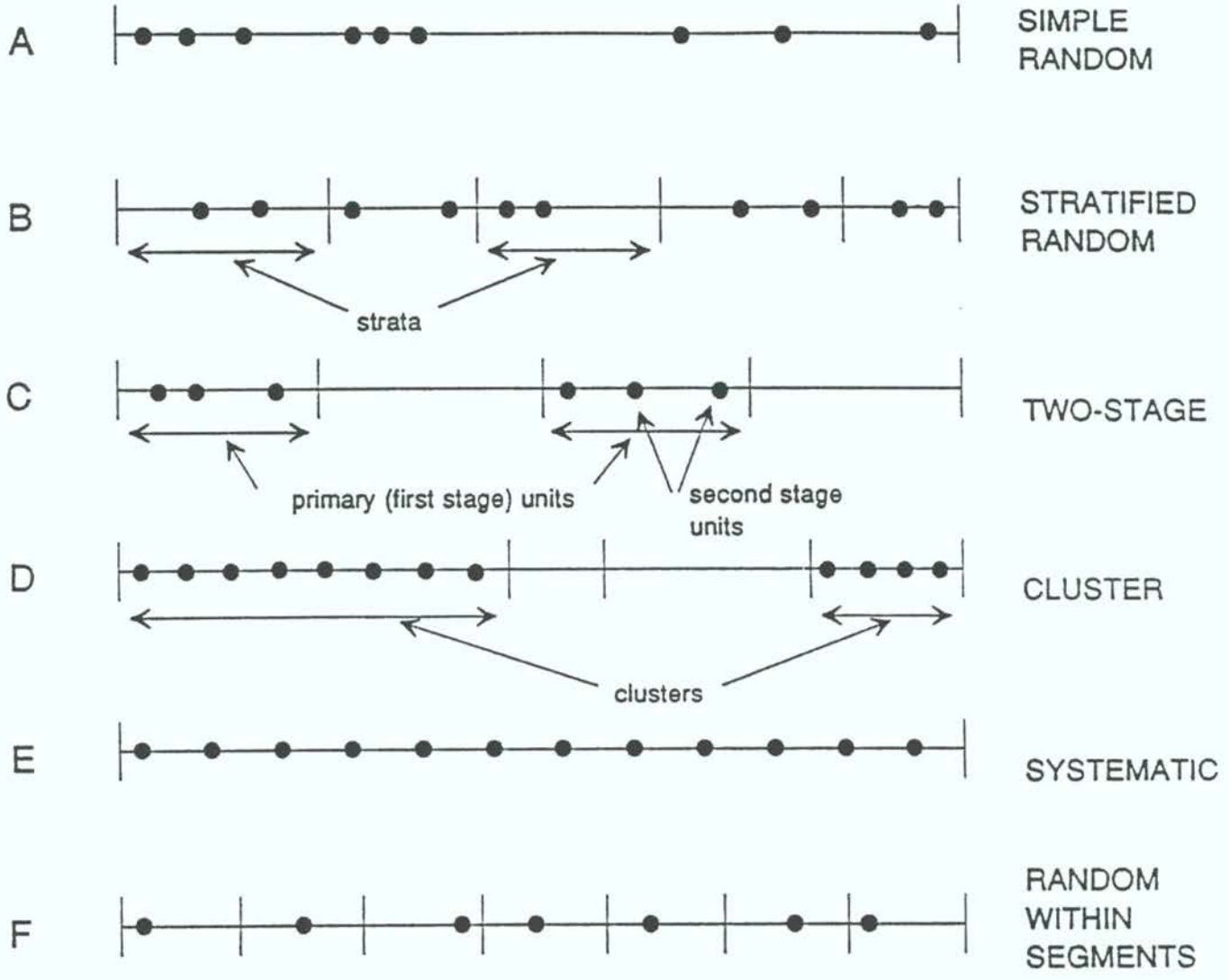


Figure 4 Sampling designs for sampling along a transect or over time

A Samples chosen at random over whole line.
B Samples chosen at random within strata. Strata are usually different in size and based on existing information about variation and the site.
C Primary units chosen at random. Samples chosen at random within selected primary units.
D All possible samples taken within randomly selected clusters.
E Samples evenly spaced after a random start position A is chosen.
F One or more samples chosen at random within each segment. Segments may be different sizes, not based on existing information about variation or the site.

Appropriate use of transect sampling is illustrated by a programme designed to determine the extent of effects of emissions from an incinerator chimney. Sites are selected along one or more transects radiating from the chimney taking into account any prevailing wind direction where the effects may be greatest. Sites should also radiate from the chimney in other directions to determine the total extent of the effects and concentrations of contaminants with distance from the source. Control or baseline levels may be obtained well away from the source but must be of similar environmental character e.g. same vegetation, rainfall, substrate etc.

In the case of an outfall in a river, baseline samples may be obtained by sampling upstream of the source of contamination. The effects of the contaminate input can be monitored with a series of sampling points at intervals downstream. It is important, however, to ensure that the physical environment, for example the sediment type and rate of water flow, is similar at both upstream and downstream sites.

A similar approach to sample selection can be used when sampling over a long time span to detect temporal change. The time span can be considered as a transect in much the same way as a transect is used on the ground to look at changes with distance from a point source (figure 4).

3.3 Sampling frequency

The frequency of collecting samples will be determined by the nature of the monitoring programme, its purpose and the variability of the data. When using biological monitoring methods the season of the year may also be an important consideration (see below). Without knowledge of the way in which organisms respond to contaminants at different times of the year it may be necessary to sample at least once each season.

When collecting data for long-term trend analysis, annual or seasonal sampling may be adequate. However, if annual sampling is used it is important to collect samples always at the same time of the year. When monitoring in relation to a potential public health risk it may be necessary to relate the frequency of sampling to the anticipated period of risk to the human population from the source of contamination. If sampling in response to an isolated contamination incident, rather than general environmental pollution, the frequency which will indicate the subsequent recovery of the environment may only be determined by a trial period of intense sampling. Such sampling may be at hourly, daily or weekly intervals and the number of samples taken becomes important in order to obtain meaningful statistical analysis.

3.4 Sample numbers

When planning a monitoring programme consideration must be given to the final statistical treatment of the data obtained. It is far better to select sample frequency and numbers with the final data analysis in mind rather than attempt to make an analysis at the end of the programme with inappropriately collected data. It may be advisable to consult a statistician when designing the sampling regime, as the number of samples required is usually determinable from the pattern of site selection e.g. random or cluster sampling.

If possible any available data should be examined to obtain an estimate of the ranges of expected levels of a contaminant or the expected variability for specific populations or groups. If this information is not available then a preliminary study, such as the collection of a series of sub-samples, may have to be carried out to determine the range of expected results in order to plan the best statistical approach to the final monitoring programme. A typical set of sub-samples should ideally number 50 to 100 for a homogeneous population. In practice, in order to give a high reliance to the mean of the results, a total of 18 to 24 samples are required for statistical analysis but the greater the number of samples the more the standard error of the mean value can be reduced. The improvement in confidence in estimates of the mean value with the number of samples collected is illustrated in figure 5. The improvement in the standard error will, however, be small for any increase in the number of samples above the optimum suggested.

3.5 Sampling technique

Practical sampling techniques are related to the final monitoring methods chosen and will be discussed in more detail in the appropriate handbooks dealing more specifically in plant and animal methods. The final choice of method will depend on the equipment available and the nature of the sample sites. There are many specialized texts which discuss the currently available methods for collecting samples in different environments and this aspect will not be dealt with in detail here. However, there are some general guidelines which may be useful for certain types of monitoring depending on the subsequent use of the samples e.g. chemical analysis or population estimates. Once a suitable regime for sample handling and preservation has been designed it should be recorded so that all subsequent sample handling follows the same procedure. In this way greater consistency and comparability in results can be obtained.

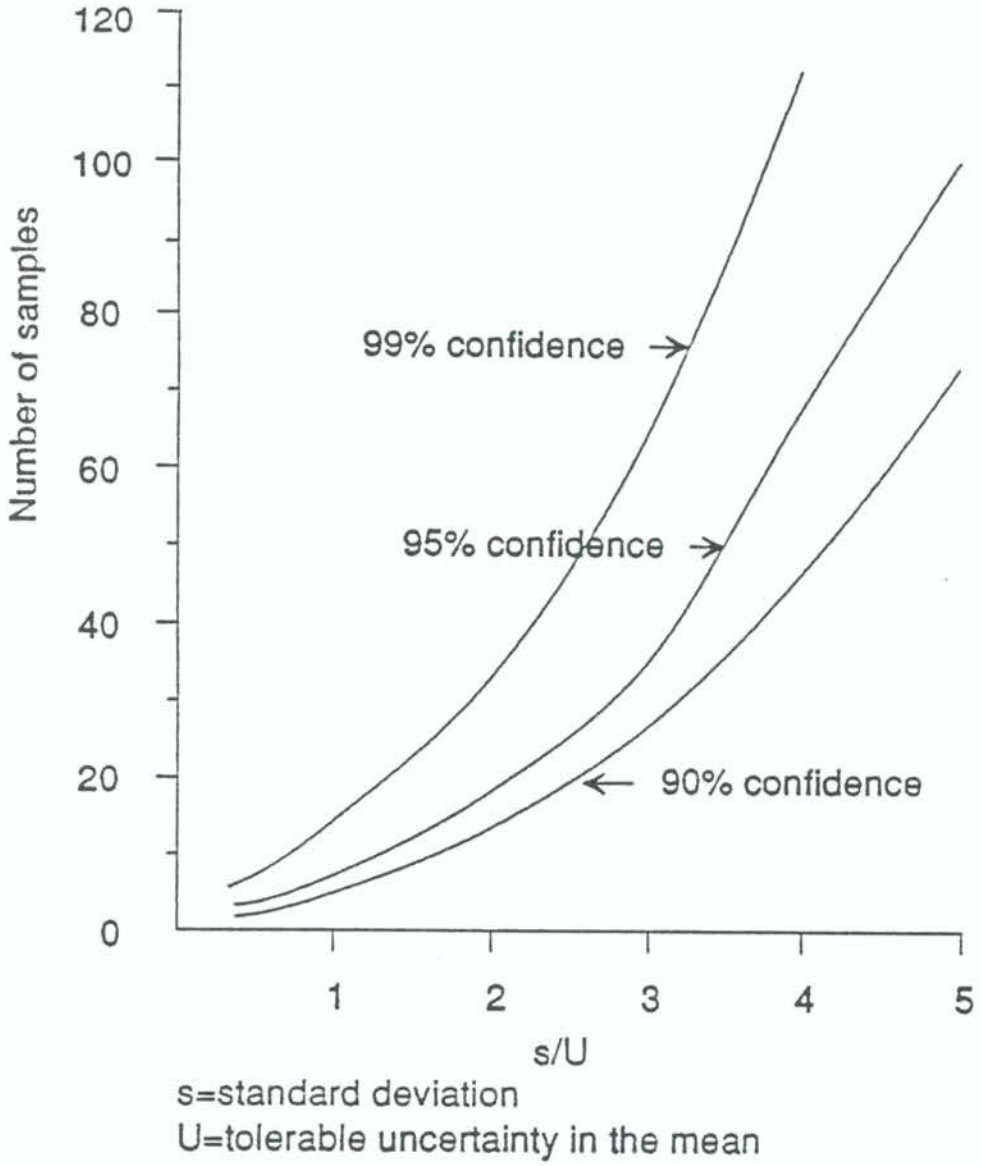


Figure 5 The approximate number of samples required to estimate a mean concentration with different levels of confidence

3.5.1 Sample handling

It is important to avoid disturbing samples as much as possible and to prevent any change in the condition or nature of the samples which may affect their subsequent use. If the monitoring method involves determining the levels of a contaminant within parts or whole organisms it may be necessary to wash off contaminants which may be attached to the organisms surface e.g. organic debris. Such sources of extraneous contamination can lead to misleading results.

If samples are being collected for later analysis by chemical techniques any further contamination during handling or storage must be prevented. If, for example, the monitoring programme includes metal concentrations within organisms the samples must be handled with plastic instruments and stored in plastic containers, all of which have been cleaned in dilute nitric or hydrochloric acid and washed off in metal-free distilled water to remove any traces of metals. Samples required for analysis of organic compounds may be most appropriately stored in glass or stainless steel containers.

3.5.2 Sample preservation

If samples cannot be analyzed for some time after collection it is necessary to store them in such a way that the distribution of the contaminant of interest within the sample tissue does not change. In most instances this can be achieved by freezing the samples to -20°C as soon as possible after collection. Samples can then be thawed slowly at room temperature whenever further treatment can be continued.

If populations of organisms are being collected for identification and enumeration rather than analysis, a preservation technique which is adequate for long-term storage, and will prevent decay at room temperature, is preferable. It should also cause minimal physical disruption to the organisms in the sample, possibly by the use of suitably buffered preservation solutions such as formaldehyde or other less toxic alternatives.

Further reading on appropriate sample handling and preservation techniques is provided in Appendix A.

4. General concepts of biological monitoring

Biological monitoring methods may be used to measure or determine the effects of contaminants on the environment, or they may be used to monitor actual contaminant levels within the environment. A detailed review of the many methods, and uses, of these techniques has been carried out in *Biological Monitoring of Environmental Contaminants (Plants)* and *Biological Monitoring of Environmental Contaminants (Animals)*, MARC Reports Nos 32 and 37. Guidelines for the use of some of these techniques in monitoring programmes are covered in two separate handbooks in the MARC series. However, some general precautions applicable to both plants and animals are dealt with in the following sections.

The advantage of biological methods for monitoring the effects of contamination is that they use organisms to indicate directly the responses of the environment to the physical and/or chemical changes that may have arisen. Such methods provide more information on the resultant stress on ecosystems than physical and chemical measurements alone. They illustrate the integrated effects of contamination and natural environmental conditions. Many of the techniques

involve the study of populations and communities or the measurement of physiological or biochemical changes in individuals and populations. Simple community analyses can be carried out with the minimum of equipment and at very low cost, although they require trained personnel.

Both plants and animals, as individuals, can be useful for monitoring environmental quality in three main ways: 1) as indirect (biological) indicators of the levels of contaminants within the environment, 2) the presence or absence of species can be indicative of environmental disturbance and 3) selected species may show behavioural or physiological response, including cellular damage or even death, due to exposure to certain levels of pollution.

The first method uses individuals of a single species which accumulate the contaminant within their bodies. In order to determine the concentrations of the contaminants some analytical capability is necessary and therefore, these methods are more expensive than other methods. However, the levels of contaminants in the organisms may be sufficiently high to enable less sophisticated apparatus to be used more effectively than for chemical analysis of trace levels in other media such as water.

Whether individuals or populations are used for monitoring there are some general precautions for regular sampling which must be observed in order to obtain comparability between samples collected. Principally, the sampling method used must be the same, and the sample sites must be similar in nature, throughout the monitoring programme (see above). For long term programmes samples should be collected at the same time each year, e.g. in the spring, to eliminate as far as possible any seasonal changes in the physiology, behaviour or life cycles of the organisms.

Where organisms are used over long time periods the possibility of species acclimatization to either the contaminant or the disturbance is possible. Such acclimatization may be due to genetic changes in local populations of the organisms and may be very difficult to detect. Where this occurs it may make meaningful comparisons between samples from different areas impossible.

5. Monitoring with individuals and single species

5.1 The use of individuals as indicator species

If the correlation between the level of a chemical in the tissues of a certain species and the surrounding environmental concentrations is good, those organisms can be used directly for monitoring. At present there are few species that can be found all around the world which are known to be good biological indicator organisms. Therefore, it may be necessary to carry out studies on local species to determine those organisms which are appropriate. A good indicator

organism must fulfil the following criteria before it can be used in a biological monitoring programme:

a) it should be easy to sample and survive for long enough in the laboratory to enable studies of contaminant uptake to be performed
b) it should accumulate the contaminant at the levels present in the environment without being killed
c) it should be abundant in the desired area and representative of that area
d) it should be large enough to provide sufficient tissue for analysis
e) preferably it should bioaccumulate the contaminant to a level high enough to enable direct analysis of the tissues
f) there should be a simple correlation between the content of the contaminant in the organism and the average contaminant concentration in the surrounding environment
g) all the organisms in a monitoring programme must show the same correlation between their contaminant levels and those in the surrounding environment at all locations studied under all conditions

Suitable organisms are typically immobile, or at least unlikely to travel far from one place. Both plants and animals have been found suitable in many studies (Burton, 1986; Samiullah, In Press). Examples range from seaweeds and mosses to pine tree needles and from bivalve molluscs and worms to land and sea mammals.

Unless comparative experimental observations are made on the toxicity of the chemicals to the indicator organisms they cannot be used to assign any environmental significance to the measured levels although they may be used to indicate trends. However, they can also provide useful information on the availability, mobility and fate of contaminants. Such organisms, collected from their natural habitat also provide a time-integration of ambient concentrations of a contaminant, averaging out temporal fluctuations.

5.2 Factors affecting contaminant levels in individuals

It is preferable when using organisms as indicators that as much information as possible is gathered about the species used. The way in which the organism accumulates contaminants, e.g. from solution directly through the body surface or by ingesting contaminated food, will suggest whether it will be accurately reflecting the presence of a contaminant or the possible risk of human effects from that chemical. Organisms which are high up a food chain may acquire levels of contaminants in excess of the environmental levels because they consume large numbers of organisms from lower trophic levels which in turn have also accumulated the contaminants. This may lead to possible risks to humans who may consume the same, or similar, organisms as a food source. It is therefore important to ascertain the manner in which the organisms are

accumulating the pollutants. This may have to be done by laboratory experimentation if there is insufficient information already published.

Some metals are required for normal metabolic functions, for example as constituents of enzymes, and therefore some organisms may have an expected body concentration of those metals even though they may not be present in the environment as contaminants. Such information may be already available in the published literature and can provide guidelines for the expected baseline or uncontaminated levels within the indicator species.

Before embarking on the use of organisms for biological monitoring additional information should be sought, or experimental analyses done, to determine whether the species of interest accumulate contaminants in certain tissues of the body more than others. For example, some metals such as Cd, Hg and Pb may be accumulated mainly in the liver and kidney of certain mammals and in the hepatopancreas or exoskeleton of crustacean species. In plants metals may be immobilized, to a large extent, in cell walls. Therefore, for a monitoring programme it may be more "time and cost" effective to collect and analyze certain tissues only.

Some organisms regulate, e.g. by excretion, particular elements when they are exposed to levels above a certain tissue or environmental concentration. This is particularly true for those elements which are essential for metabolic processes. As a result, any correlation between tissue concentrations and environmental levels which may have previously existed breaks down, and the organism ceases to be useful as an indicator. This can be tested within the laboratory before embarking on a monitoring programme.

In addition to the above there are many natural variations in species which affect the metabolism and associated levels of accumulated contaminants. The stage of growth i.e. the age and the related size and weight of the individual may affect the concentration of an accumulated contaminant. Some organisms may accumulate continuously or throughout their growth period and therefore, older organisms may be expected to have higher levels of non-regulated contaminants. In this situation all organisms collected for monitoring should be of a comparable size or a similar age. In some mammals there have been reported differences in metal accumulation between males and females and in some invertebrates the gonads may form a substantial proportion of total body weight. This proportion may vary between males and females and therefore sex should be taken into consideration when measuring whole body contaminant levels.

The physiological condition of an organism may also affect accumulation, regulation and mobilisation of contaminants within the body or tissues. In preparation for

reproduction, or as a result of factors like starvation, body reserves, e.g. fats or starches, may be redistributed or used up. Contaminants which are rendered harmless to the organism by immobilization or storage in fat tissues then become re-mobilised into other tissues where they may exhibit toxic properties. Thus the fitness and reproductive cycles of an organism, which are in turn related to the season of the year, must be taken into account when using it for biological monitoring, particularly as an indicator organism. If monitoring continues throughout the year the relationship between reproductive state and contaminant levels must be determined before programme results are analyzed.

In a complete monitoring programme biological methods would normally be combined with some physical and chemical measurements. These environmental factors may also affect the way in which an organism accumulates or regulates contaminants. Examples include changes in pH in soil or water, changes in temperature, irradiance or rainfall and the possible interference between two different contaminants. In the latter case the sudden appearance of a different contaminant could affect the way in which the indicator organism accumulated the contaminant of interest. If the indicator organism is not well known such information will not be available in the literature and will have to be determined experimentally, particularly if implicated by unusual results from a monitoring programme.

5.3 Practical considerations in using indicator organisms

More detailed advice for different types of indicator organisms used successfully in biological monitoring programmes is given in the appropriate handbooks of this series. General advice on collection and storage of samples has already been discussed in earlier sections.

The use of indicator organisms to monitor contaminant levels requires some analytical capability for metals or organic compounds. The required sensitivity of the instrumentation for measuring the metal or chemical will depend on the degree to which the organism accumulates it. If no rough guidelines are already available for the species to be used then some preliminary analyses will be necessary. If no measurable levels are detectable in the whole organisms (i.e. for some invertebrates and plants) or in dissected tissues or sub-samples, several samples can be bulked together to increase the total contaminant level. The mixing of the samples must be thorough and then, if sufficient material is available, several sub-samples can be taken from the bulked sample and a mean value calculated from the analyses. Figure 6 illustrates a typical procedure for bulking randomly collected field samples and taking sub-samples.

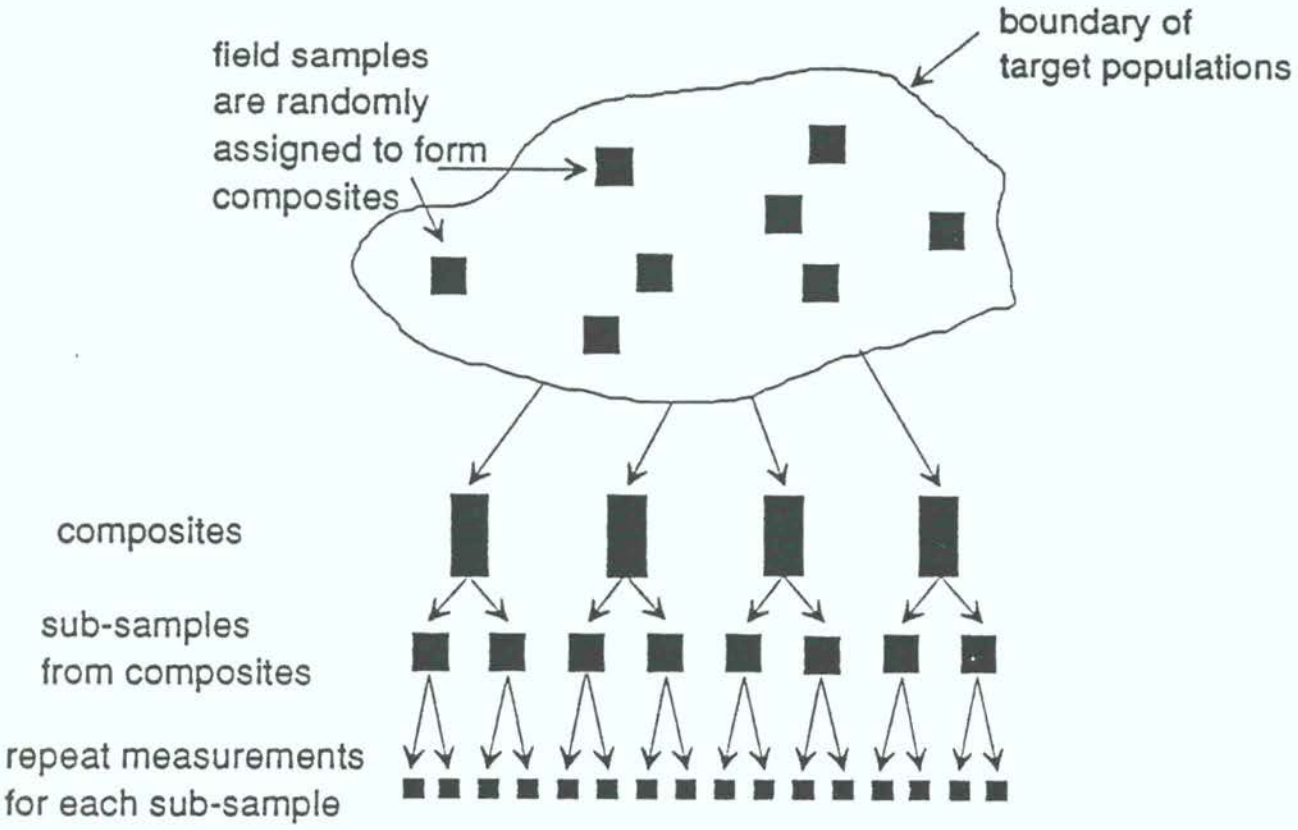


Figure 6 Obtaining a mean value by bulking samples and analyzing sub-samples.

If after bulking samples no contaminant measurement is obtained and the analytical equipment is functioning properly it is possible that the levels present in the organisms are below detection level using that technique. More sensitive, and probably more expensive, equipment will be required. Further information on detection limits and errors is usually available in the instruction manuals for the equipment. It is worth noting that when measuring contaminants close to the detection limit of the equipment, the errors involved in the analysis could be so large that the results may be virtually meaningless. The time and effort involved in using the chosen indicator organism as part of a monitoring programme may, in that situation, not be justified. Suggested methods for checking the accuracy of analysis for contaminants are discussed below.

5.4 Other uses of individuals for monitoring

Individuals or single species can be sensitive to certain types of pollution and exhibit external signs of stress which can be attributed to a particular contaminant. Sub-lethal effects may therefore be used to indicate the presence of contamination but not generally the levels of individual contaminants in the environment. Examples include damage to plant leaves caused by atmospheric pollution, reduced capacity to grow in invertebrates, i.e. reduced fitness which may be measurable in the laboratory, impaired reproductive capacity and physical deformities in animals such as shellfish. The best known methods within this category are bioassay techniques and standard toxicity tests. Specific examples are discussed in the appropriate handbooks.

When using effects of contamination as a biological monitoring tool a subjective assessment of severity may be possible. When combined with data on the occurrence in samples collected from the monitoring network the method can indicate spatial distribution of contamination, and probably monitor recovery, but not actual levels of contamination present.

6. Monitoring with populations of organisms

6.1 General considerations

Organisms have a preferred environment in which they will live and thrive. Many factors contribute to the suitability of the environment and variation in one or more of them can lead to stress on individuals and possible reduction of the total numbers that are present. In an extreme situation of environmental change, perhaps due to contamination, certain species will be unable to tolerate the changes in their environment and will disappear completely from the area concerned, either as a result of death or migration. Thus the presence and absence of certain species, or the total species number and abundance has been exploited as a means of measuring environmental degradation. Two main approaches have been used: measures based on community structure and measures based on indicator organisms. In this case an indicator organism is a test species picked for its physiological response i.e. sensitivity or tolerance (more frequently sensitivity) to various sorts of pollution such as organic or heavy metal pollution. The specificity of such indicators means that any biotic index derived from them must also be specific in its use, both with respect to the type of pollution and environment to which it is applicable.

However, when interpreting results from monitoring programmes incorporating the use of community structure and indices it is important to realise that the absence of a species does not always indicate contamination. It could also be due to unfavourable changes in the environment which occur naturally. For example severe storms can lead to changes in the substrate of rivers carrying flood waters or changes in

the vegetation of an area. These in turn will affect the species inhabiting such areas.

6.2 The use of animal and plant communities

The community structure approach looks at the numerical abundance of each species in a community in one of two ways. One method orders the species according to the overall similarities and looks at the major environmental factors such as pollution. The other method uses an index of community structure, either a diversity index or a similarity index. A diversity index attempts to combine the data on species abundance in a community into a single number. A similarity index is obtained by comparing two samples, one of which is often a control. These methods have the advantage that a knowledge of biology or ecology is not required in order to interpret whether a situation is getting better or worse, since this can be interpreted from the scale of the index.

The theory behind the designing of such indices is well known and beyond the scope of this handbook. Many books and reviews are available on the subject. The most common diversity indices in use are those based on information theory, such as the Shannon-Weiner Index (H'). They are applicable to a wide variety of environmental conditions but have not been thoroughly investigated with respect to their biological relevance. This index can, however, be used until other indices have been adequately field tested or developed.

6.3 The use of indicator species

Biotic indices are specific for one or two particular types of pollution since indicator organisms are unlikely to be equally sensitive to all types of pollution. Such indices are frequently based on macroinvertebrate populations and not universally applicable since taxonomy varies widely in different world regions. The basic principles can, however, be applied to developing regionally suitable indices. At present such indices have been most widely applied to aquatic pollution, particularly from excess organic matter.

One such index used in pollution monitoring in rivers which is well known is the Trent Biotic Index. This index is based on the number of defined taxa of benthic invertebrates in relation to the presence of six key organisms found in the fauna. Depending on the number of groups present and the key organisms found in the fauna the index ranges from 10 for clean water to 0 for polluted water (Table 1). The index has been found to be rather insensitive and has been largely replaced by further developments of the basic principle. One such, widely used in the UK, is the Chandler's Biotic Index. To derive the index for a particular river station the fauna (collected according to a standard procedure) is identified and counted. Each group is given a score according to its abundance and the total score represents the index. The higher the score the cleaner the water. With knowledge and

Table 1 Classification of biological samples using the Trent biotic index

			Total number of groups present				
Clean			0–1	2–5	6–10	11–15	16
					Biotic index		
Organisms in order of tendency to disappear as degree of pollution increases	Plecoptera nymphs present	More than one species	—	VII	VIII	IX	X
		One species only	—	VI	VII	VIII	VX
	Ephemeroptera nymphs present	More than one species*	—	VI	VII	VIII	IX
		One species only*	—	V	VI	VII	VIII
	Trichoptera larvae present	More than one species†	—	V	VI	VII	VIII
		One species only†	IV	IV	V	VI	VII
	Gammarus present	All above species absent	III	IV	V	VI	VII
	Asellus present	All above species absent	II	III	IV	V	VI
	Tubificid worms and/or red chironomid larvae present	All above species absent	I	II	III	IV	—
	All above types absent	Some organisms such as *Eristalis tenax* not requiring dissolved oxygen may be present	0	I	II	—	—
Polluted							

**Baetis rhodani* (Ephem.) is counted in this section for the purpose of classification.

†*Baetis rhodani* excluded.

Groups: The term "Group" here denotes the limit of identifiation which can be reached without resorting to lengthy techniques. Thus the Groups are as follows: Each known species of Platyhelminthes (flatworms). Annelida (worms) excluding genus *Nais*. Genus *Nais* (worms). Each known species of Hirudinae (leeches). Each known species of Mollusca (snails). Each known species of Crustacea (log-louse, shrimps). Each known species of Plecoptera (stone-fly). Each known genus of Ephemeroptera (may-fly) excluding *Baetis rhodani*. *Baetis rhodani* (may-fly). Each family of Trichoptera (caddis-fly). Each species of Neuroptera larvae (alder-fly). Family Chironomidae (midge larvae) except *Chironomus Ch. thummi*. *Chironomus Ch. thummi* (blood worms). Family Simulidae (black-fly larvae). Each known species of other fly larvae. Each known species of Coleoptera (beetles and beetle larvae). Each known species of *Hydracarina* (water-mites).

experience of aquatic invertebrates in a particular country or region such indices could be devised for other situations, such as tropical waters.

7. Data collection and presentation

The data obtained from a monitoring programme must be collected in an orderly manner that makes it readily available for analysis and assessment. The results of such analyses must then be presented such that managers and policy makers can understand and act upon the information. It is important, therefore to consider for whom the data are being gathered and how it will be used before embarking on data storage or assessment procedures. In order to act upon the results of a monitoring programme managers and policy makers must know the quality and reliability of the data, i.e. its precision and accuracy.

The quality of the data obtained, particularly analytic data, such as for specific contaminant concentrations in biota, can be ascertained by carrying out parallel analyses on recognized standard reference materials which contain the contaminant in question. The degree of accuracy in the method being applied can then be determined and applied to the results of the monitoring programme.

Where many laboratories are participating in a monitoring programme which requires comparison of data obtained from the different laboratories it is necessary to carry out an analytical quality control exercise. For this purpose all participating laboratories are required to perform the same tests on samples of a reference material which are sent to them all. Such analyses are usually performed without prior knowledge of the expected results. From such an exercise it is possible to determine which monitoring data from within the network is reliable and comparable. It can also be used as a means of monitoring laboratory performance.

Within a monitoring programme it is important to define the method of recording results. This is frequently achieved with data reporting sheets, often one sheet per station, and the use of these can help avoid incorrectly recorded or accidently omitted data. The data recording mechanism should always be kept as simple as possible to allow for use and interpretation by different levels of expertise. Such data sheets typically contain information on 1) the site location, such as with grid location and relevant geographical information, 2) the time and date of the sampling, and 3) the types of samples collected and/or the variables to be measured, together with the units in which the results should be expressed and a space for recording the final results and any other relevant information. The latter helps to ensure that for the whole programme the data are reported on a consistently comparable basis e.g. $\mu g\ g^{-1}$ dry weight. If any of the techniques are changed during a monitoring programme it is essential to have a period of overlap when both techniques

are used so that any differences in sensitivity can be established.

Computers are now widely used for data storage and handling. The variety of techniques and databases available for this is enormous and beyond the scope of this handbook. Once experience has been gained in computer data handling considerable time can be saved in data assessment and presentation of results. Only for very automated situations can data be logged directly onto a computer and therefore there is often still a need for a data logbook. These should be properly maintained as they provide a valuable backup in the event of any problems with electronically stored data and can be used to record any personal observations that may not be easily entered onto a numerical database.

There are many existing manuals on the best methods of analysing and presenting data and these will not be discussed in detail here. Some basic principles are, however, important. The precision and accuracy of the data should always be stated. This can be in terms of the likely degree of error or the confidence limits. It is helpful to relate data obtained to any other relevant information such as guideline values, control measurements or background values. Where available results can be related to any defined standards.

There are two main ways of presenting the data, in tabular form or graphically. The former requires careful study in order to glean the necessary facts whereas the latter offers an instant visual impression, particularly useful when presenting data to non-specialists. The relationship of the data to guidelines, background values etc. can be incorporated into such presentations as illustrated in figure 7.

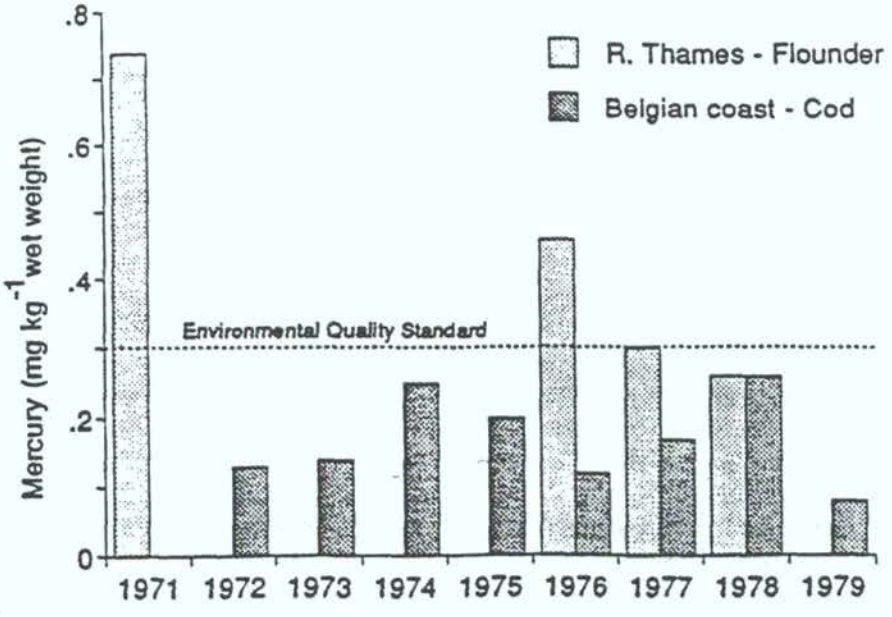


Figure 7 Mean concentration of mercury in fish from European coastal waters.

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