Table 2.1 Analysis of sewage effluents after primary and secondary treatment. Figures given are in $mg\,l^{-1}$, and are the range of values found at four different treatment works, as summarised by Bond and Straub (1974). Treatment plants operating under adverse conditions yield higher values than those shown here, but values at the upper end of the ranges shown would in many countries exceed modern effluent standards

Analysis	Range
Total solids	640–1167
Suspended solids	15–51
Biological oxygen demand	2–70
Chemical oxygen demand	31–155
Organic carbon	13–20
Anionic detergents	0.75–1.4
Ammonia	1.9–22
Nitrate	0.25–38
Nitrite	0.2–1.8
Chloride	69–300
Sulphate	61–270
Phosphate	6.2–9.6
Sodium	144–243
Potassium	20–26

be made. Many pollutants also enter water through fallout from the atmosphere. Historically, control and prevention of water pollution have concentrated on point sources as these are more obvious, easily identifiable and in theory easier to regulate at the point of origin. As awareness has increased of the significance of diffuse sources of pollution, control strategies have been under development but are based more on the application of good practices designed to reduce pollutant impact rather than on regulation of specific sources of input (see, for example, MAFF, 1991).

Most effluents are complex mixtures of a large number of different harmful agents. These include toxic substances of many kinds, extreme levels of suspended solids, and dissolved and particulate putrescible organic matter. In addition, many effluents are hot, of extreme pH value, and normally contain high levels of dissolved salts. Detailed compilations of data on the composition of sewage and industrial effluents of many kinds are given by Bond and Straub (1974), and by Sittig (1975). Some representative values for treated sewage effluent are given in Tables 2.1 and 2.2. Most effluents also vary in their strength and composition, on a seasonal, diurnal or even hourly basis. Most sewage treatment plants report regular diurnal peaks and troughs in their output according to patterns of water use. Sometimes storm-water drains are connected to the sewerage system, so the strength of the sewage effluent will vary with rainfall. Alterations in the

Table 2.2 Levels of heavy metals recorded in the final effluent of a typical sewage treatment plant operating under good conditions. Data from Bond and Straub (1974)

Metal	Concentration range ($\mu g\,l^{-1}$)	Typical % removal compared to raw sewage
Zn	85–190	65
Al	460–550	77.5
Fe	160–290	70.5
Cr	36–70	76
Cu	31–38	60.5
Pb	<20	
Ag	11–12	17.3
Cd	<20	
Ni	<10	
Sr	280–450	17.6
B	240–260	13.3

strength and composition of sewage also influence the efficiency of the sewage treatment process, so that dilution of the influent does not necessarily cause an improvement in the quality of the effluent. In industrial plants, variations in the quality of the raw materials, or changes in specification of the finished product, will require changes in the operating conditions of the plant and lead to changes in the composition of the effluent. Many industrial processes are 'batch' rather than continuous processes, so that some effluent discharges will be intermittent rather than continuous.

Nevertheless, it is often possible to generalise about the effects of different kinds of effluent on their receiving waters. Broadly speaking, the effects of sewage effluent, kraft pulp mill effluent, coal mine effluent and so on are consistent wherever they occur. However, a detailed understanding of the effects of individual components of an effluent, and of the consequences of variation in the composition of an effluent, is essential for pollution control. Waste-water treatment is expensive, and in order to devise cost-effective treatment processes it is necessary to identify those components of the effluent which cause the greatest damage to the environment. This is because it is usually impossible to devise an economically-feasible process which will be equally effective against all the components of a complex effluent. Frequently, treatment of an effluent to remove one component will exacerbate the problem of removing another; it is notoriously difficult, for example, to treat satisfactorily effluents which contain both cyanides and phenolic compounds, although many basic industrial processes produce just such an effluent. Therefore in order to devise the optimum pollution control strategy it is necessary to study, in the laboratory and in the field, the effects of effluents and of their individual components.

2.2 The Environmental Requirements of Aquatic Organisms

The effects of pollutants in aquatic ecosystems cannot be understood without some knowledge of the ecophysiology and basic biology of the aquatic biota. Those aspects which have most bearing on the interaction between water pollutants and aquatic organisms are briefly discussed here. There are many authoritative and readable texts on freshwater ecology which may be recommended to readers who require a fuller treatment. Among these are Hynes' (1970) classic text and more recent volumes such as Moss (1988), Jeffries and Mills (1990), Maitland (1990), Calow and Petts (1992, 1994) and Allan (1995).

In order to survive, a living organism must spend its life in an environment which meets its needs: a suitable physical habitat which provides space, shelter, and a sufficient supply of food, oxygen and other metabolic requirements; and which is not subject to extremes of temperature or other physical variables which lie outside the range which the organism can tolerate. Obviously, different habitats have very different physical characteristics, and organisms have evolved a fascinating array of adaptations which have enabled them to colonise every part of the Earth. To a greater or lesser extent, every living organism is adapted—in its morphology, physiology and behaviour—to the environment it normally inhabits. Some are remarkably specialised, that is they are adapted to specific places and/or modes of life in which they are very successful, but are excluded from living in most habitats. Others are more generalised in their adaptations, perhaps being nowhere particularly abundant but able to survive reasonably well in a wider range of habitats. Few organisms, however, are universally distributed.

Any of a living organism's many individual requirements may be a *limiting factor* preventing the establishment, survival or reproduction of a species in a particular habitat. Aquatic plants, for example, are commonly limited in their distribution and abundance by the availability of nutrients, such as phosphorus or nitrogen. An abundance of phosphorus is of no use to the plant if it has no nitrogen, and vice versa. Further, the nutrients must be present in a form which the plant can use. Photosynthetic plants, of course, also require light, an important limiting factor in most aquatic habitats. The non-photosynthetic flora (fungi, bacteria) are more likely to be influenced by the levels of dissolved or particulate organic material present. Animals, in turn, are greatly influenced by the quality and quantity of the aquatic flora, because many animals rely upon plants for food, shelter, as a repository for eggs, and so on. Animals are also influenced by the physical environment—current speed, nature of the substratum, temperature—but in addition are generally much more susceptible than plants and bacteria to the prevailing levels of oxygen.

Although some aquatic animals are air-breathers, the majority have to obtain their oxygen from water. Oxygen is not very soluble in water, and water is a rather dense and viscous medium. Moving through water requires a great deal of energy expenditure, and therefore a high oxygen consumption. To obtain from the water the meagre amount of oxygen dissolved therein requires that the respiratory surfaces be moved through the water, or the water be moved over the respiratory surfaces. Further, as the water temperature increases, the solubility of oxygen in water decreases, while the oxygen requirement of the animal actually increases. Thus the survival of animals in water is crucially dependent upon the extent to which their oxygen demand can be matched to the availability of oxygen from the environment.

Alexander (1970) describes elegantly and in detail the respiratory predicament of fishes. A fish at rest in well-oxygenated water ventilates its gills fairly slowly. Respiratory exchange is efficient, and the fish removes most of the oxygen from the water passing over its gills. If, however, the fish ventilates more quickly, as it must for example if it becomes physically active, the water passing over the gills has less time to equilibrate with the blood, and the efficiency of respiratory exchange drops. Thus, in order to double the rate of oxygen uptake, the fish must more than double the amount of water pumped over the gills. Measurements of the ventilatory mechanisms in fish suggest that a resting fish can remove 80% of the oxygen in the water passing over the gills, but this figure drops to about 30% in an actively swimming fish. Therefore, to increase its oxygen uptake by a factor of five, a fish must pump about 15 times as much water per unit of time. The density and viscosity of water are such that the muscles which pump the water over the gills themselves consume a significant proportion of the oxygen obtained from the water. This proportion increases dramatically as the fish's activity increases, or as the temperature rises, or as the dissolved oxygen level in the water falls. Alexander (1970) calculated that a resting fish in water containing 8 ml oxygen per litre might under typical conditions expend 0.025 ml oxygen to work the respiratory muscles, in order to obtain 6ml oxygen. However, if the oxygen concentration in the water fell to 1 ml per litre, the fish would expend 0.25 ml oxygen in order to obtain only 0.3 ml. At this point, the fish is clearly close to asphyxiation and has no ability to engage in physical activity.

Clearly, oxygen is an important limiting factor to fishes. Equally clearly, however, limiting factors interact with one another. An oxygen concentration which is acceptable at 10°C is limiting, even lethal, at 20°C, because as the temperature rises the metabolic rate of the fish also rises; thus most aquatic animals need more oxygen at higher temperatures, but the solubility of oxygen in water decreases with temperature, as does the efficiency of respiratory exchange. Varley (1967) described very simply the interactions of temperature and oxygen and their influence on the distribution patterns of freshwater fishes in Britain. Similar principles are involved among the invertebrates. Nymphs of stoneflies (Plecoptera), for example, are particularly fastidious in their requirements for oxygen, and are absent from waters whose oxygen concentration drops much below saturation value for appreciable periods of time. Ephemeroptera (mayfly) nymphs are a little more tolerant, but are generally more sensitive to oxygen depletion than Trichoptera (caddis fly) larvae. The amphipod crustacean *Gammarus pulex* and the isopod *Asellus aquaticus* are remarkably similar in their habits and environmental requirements, but above a critical oxygen concentration *Gammarus* predominates, while below that level it tends to be replaced by *Asellus*. The distribution of individual species is often correlated with dissolved oxygen levels in the water. Indeed, the response of invertebrates to the prevailing levels of dissolved oxygen is so well known that their distribution patterns can be used as indicators of the prevailing environmental conditions (see Chapter 3).

Other correlates with distribution patterns can be found. An excellent example is Maitland's (1980) study of mayfly (Ephemeroptera) species in the British Isles. There are 47 species of Ephemeroptera recorded in the British Isles, a rather smaller number of species than is found in continental Europe. This illustrates an important general biogeographical principle; the British Isles, like other islands situated offshore from the continental land mass, has a relatively poor fauna and flora. Second, there is a distinct pattern of distribution in relation to altitude, each species having its own upper and lower limit of distribution, some being confined to upland areas and others to lowland waters. Altitude, of course, will tend itself to be correlated with other factors, such as temperature, and a variety of physical and chemical characteristics of the water, so in fact it would be expected that several correlations may be found between physico-chemical parameters and the presence or absence of species. At its simplest level, Maitland's (1980) study identified four specific habitat types which would *not* be expected to sustain mayfly nymphs: small water bodies liable to desiccation; water bodies susceptible to saline intrusion; nutrient-poor upland waters in exposed areas; and lowland waters subject to eutrophication or pollution.

The patterns of distribution and abundance of organisms cannot, of course, be explained solely in ecophysiological terms. A habitat may be perfectly adequate, physically and chemically, for a herbivorous animal, but if the habitat is not suitable for the animal's food plants the herbivore will be absent. Where a habitat is marginally acceptable to a species, the organism may nevertheless be absent because another species, with similar requirements, is better adapted to those conditions and competes more successfully for the available resources. Some organisms are absent from particular habitats not because the habitats are unsuitable, but simply because the species concerned have so far failed to overcome a geographical barrier which prevents their spread. In other words, the observed patterns of distribution and abundance of species are the net result of a complex interaction of physical, chemical and biological factors. Patterns of distribution in relation to these factors are, however, discernible and with an adequate database and suitable methods of analysis can be formed into very useful tools for assessing the impact of pollution on a habitat. The type of study represented by Maitland (1980) was developed into

RIVPACS (River Invertebrate Prediction and Classification System), which is now widely used in pollution monitoring. RIVPACS is described more fully in Chapter 3. Even with simple methods of analysis, within a habitat it is frequently possible to observe a zonation pattern in the distribution of species in response to directional changes in the physical environment. The zones may be sharply delineated, as on a steeply-sloping seashore, or they may merge gradually one into another, as is more typically found along the length of a river (Hawkes, 1975; Hynes, 1970). Pollution may directly influence one or more components of the dynamic equilibrium of physical, chemical and biological phenomena which give an ecological community its characteristics. The influence of the pollution will extend even to organisms which are not directly susceptible, as the system accommodates itself to the new conditions. Indeed, it is frequently possible to observe consistent zonation patterns in the biological community which occur in response to sources of pollution. The response to organic pollution in rivers, for example, is particularly well known.

2.3 Organic Pollution

The discharge of excessive quantities of organic matter is undoubtedly the oldest, and even today the most widespread form of water pollution. Its significance to human health is discussed in Chapter 5; the present discussion is concerned with its effect on the receiving waters.

The major sources of organic pollution are sewage and domestic wastes; agriculture (especially runoff from inadequately stored animal wastes and silage); various forms of food processing and manufacture; and numerous industries involving the processing of natural materials such as textile and paper manufacture. Most organic waste waters contain a high proportion of suspended matter, and in part their effects on the receiving water are similar to those of other forms of suspended solid (see Section 2.8). However, the most important consequences of organic pollution can be traced to its effect on the dissolved oxygen concentration in the water and sediments. In an unpolluted water, the relatively small amount of dead organic matter is readily assimilated by the fauna and flora. Some is consumed by detritivorous animals and incorporated into their biomass. The remainder is decomposed by bacteria and fungi, which are themselves consumed by organisms at higher trophic levels. The activity of microorganisms results in the breakdown of complex organic molecules to simple, inorganic substances, such as phosphate and nitrate, carbon dioxide and water. During these metabolic processes, oxygen is consumed. However, where the organic load is light, the oxygen removed from the water is readily replaced by photosynthesis and by re-aeration from the atmosphere.

Where the input of organic material exceeds the capacity of the system to assimilate it, a number of changes take place. How far the sequence of

Table 2.3 Approximate analysis of the organic component of sewage (after Higgins and Burns, 1975)

Lipids	30%
Amino acids, starch, glycoproteins	8%
Hemicellulose	3%
Cellulose	4%
Lignin	6%
Protein	25%
Alcohol-soluble fraction	3%
Ash	21%

changes proceeds depends upon the severity of the organic load and the physical characteristics of the receiving water. Initially, the enhanced level of organic matter will stimulate increased activity of the aerobic decomposer organisms. When their rate of oxygen consumption exceeds the rate of re-aeration of the water, the dissolved oxygen concentration in the water will fall. This alone may be sufficient, as argued earlier, to eliminate some species, which may or may not be replaced by others with less rigorous demands for oxygen. If the drop in oxygen concentration is very severe, the aerobic decomposers themselves will no longer be able to function, and anaerobic organisms will become predominant.

The biochemical reactions involved in the breakdown of organic matter, and the microorganisms involved, are described in general terms by Dugan (1972) and by Higgins and Burns (1975). The composition of organic waste varies according to its source, and in particular according to the relative abundance of material of plant, animal or microbial origin. Typically, however, the composition of organic waste is approximately as shown in Table 2.3. Most effluents, of course, also contain other materials, in particular toxic matter (see Tables 2.1 and 2.2), derived from various sources. To illustrate the effects of the breakdown of organic matter on the receiving water, proteins may be used as an example. The first stage of decomposition of proteins is usually their breakdown by hydrolysis to their constituent amino acids. A typical amino acid is alanine, and its breakdown under aerobic conditions may be summarised:

$$\underset{\text{Alanine}}{CH_3.C\text{—}\underset{\displaystyle H}{\overset{\displaystyle NH_2}{\overset{|}{\underset{|}{C}}}}OOH} + \tfrac{1}{2}O_2 \rightarrow \underset{\text{Pyruvic acid}}{CH_3.\overset{\displaystyle O}{\overset{\|}{C}}\text{—}COOH} + \underset{\text{Ammonia}}{NH_3}$$

Pyruvic acid is an important substance in the metabolism of most living organisms. It is produced in normal metabolism during the glycolytic (anaerobic) phase of the breakdown of carbohydrates and, as in this case, from the breakdown of excess amino acids. In aerobic organisms, the pyruvate enters the citric acid cycle, the primary means by which compounds are broken down to release energy, carbon dioxide and water. Under aerobic conditions, therefore, proteins will be broken down ultimately to these relatively innocuous compounds, while providing a source of metabolic energy for the organisms responsible for the catabolism. Ammonia is also a common end product of the metabolism of nitrogenous compounds (such as amino acids) and in aquatic organisms is generally excreted as such. Normally, the ammonia diffuses rapidly into the environment, but where the level of organic enrichment is high, it can create difficulties for living organisms as it is very toxic (see Section 2.7.2). Typically, therefore, organic wastes contain high levels of ammonia; the eventual fate of the ammonia is very relevant to the effects of water pollution and is discussed below.

Under anaerobic conditions, the breakdown of amino acids takes place through different metabolic pathways. Some amino acids, such as cysteine, contain sulphur as well as nitrogen, and its breakdown is used as an example in the following sequence of reactions which are catalysed by acid-producing and methanogenic bacteria:

$$4CH_3H_7O_2NS + 8H_2O \rightarrow 4CH_3COOH + 4CO_2 + 4NH_3 + 4H_2S + 8H$$
$$4CH_3COOH + 8H \rightarrow 5CH_4 + 3CO_2 + 2H_2O.$$

In this case, the products of decomposition include (in addition to ammonia, carbon dioxide and water) acetic acid, hydrogen sulphide and methane. These compounds are very toxic to most forms of aquatic life and, in addition, they are aesthetically undesirable by virtue of their unpleasant odours.

The fate of the ammonia largely depends upon the level of oxygen present. Under aerobic conditions, nitrifying bacteria predominate and the ammonia is converted to nitrite (e.g. by bacteria of the genus *Nitrosomonas*) and subsequently to nitrate (e.g. by *Nitrobacter* spp.). Thus the toxic ammonia is oxidised to the less toxic nitrite and to the relatively innocuous nitrate. Since, however, both ammonia and nitrate are important plant nutrients, problems related to eutrophication can arise as a consequence of organic inputs to water (see Section 2.4). Under anaerobic conditions, denitrification of the nitrate typically takes place under the influence of other bacteria such as *Thiobacillus denitrificans* and some *Pseudomonas* species. These cause the reduction of nitrate to elemental nitrogen, which readily displaces the less-soluble oxygen from solution and contributes still further to the deoxygenation of the water. The processes are most easily understood by reference to Figure 2.1 which summarises the nitrogen cycle in the aquatic environment. Note that the gaseous phases of the cycle are usually of limited significance in the aquatic environment.

These chemical changes, combined with the blanketing effect of fine organic particles on the substratum, lead to the deoxygenation of the water

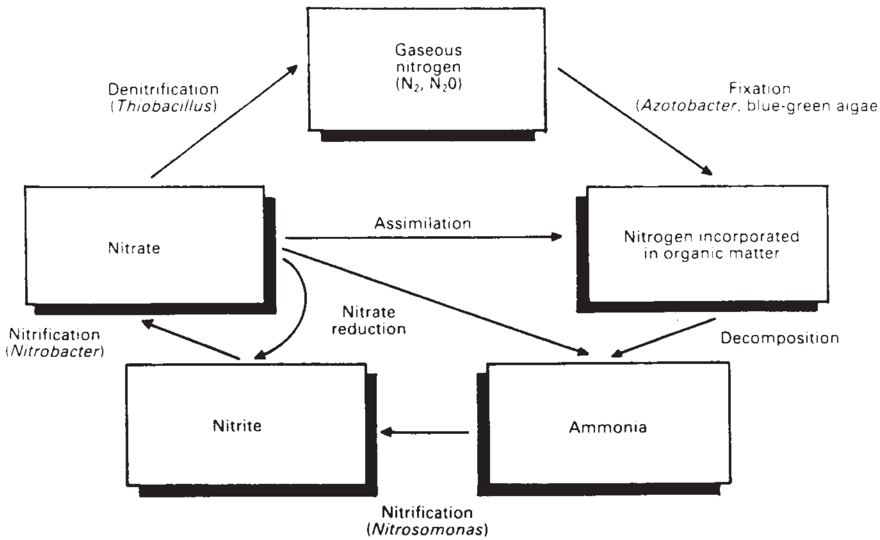

Figure 2.1 Summary of the nitrogen cycle in fresh-water ecosystems

and substratum, and readily bring about profound changes in the fauna and flora of the receiving water. In severe cases, animal life may be completely eliminated. Hynes (1960) summarised in diagrammatic form the changes which typically occur in a river below a discharge of organic effluent (Figure 2.2).

The strength of an organic effluent is frequently expressed in terms of its biological oxygen demand (BOD). This is defined as the quantity of oxygen utilised, expressed in mg l^{-1}, by the effluent during the microbial degradation of its organic content. BOD is typically measured by taking a sample of water or effluent and aerating it until it is saturated with oxygen. The dissolved oxygen concentration in part of the aerated sample is then determined by one of the well-known standard procedures. Another part of the aerated sample is incubated, in a sealed bottle of known volume for a period of (typically) five days, at a controlled temperature which is usually 15°C or 20°C. Usually the incubation is carried out in the dark, to eliminate oxygen production by any photosynthetic organisms which may be present. At the end of the incubation period, the concentration of dissolved oxygen remaining in the sample is determined. The difference between the initial and final dissolved oxygen concentrations is used to determine the BOD of the sample, which is expressed as milligrams of oxygen consumed per litre of sample. The basic assumption of the method is that oxygen is mainly consumed by aerobic microorganisms during the metabolism of organic matter. This is not necessarily true, since many effluents contain chemically-reduced compounds which undergo oxidation by purely chemical reactions.

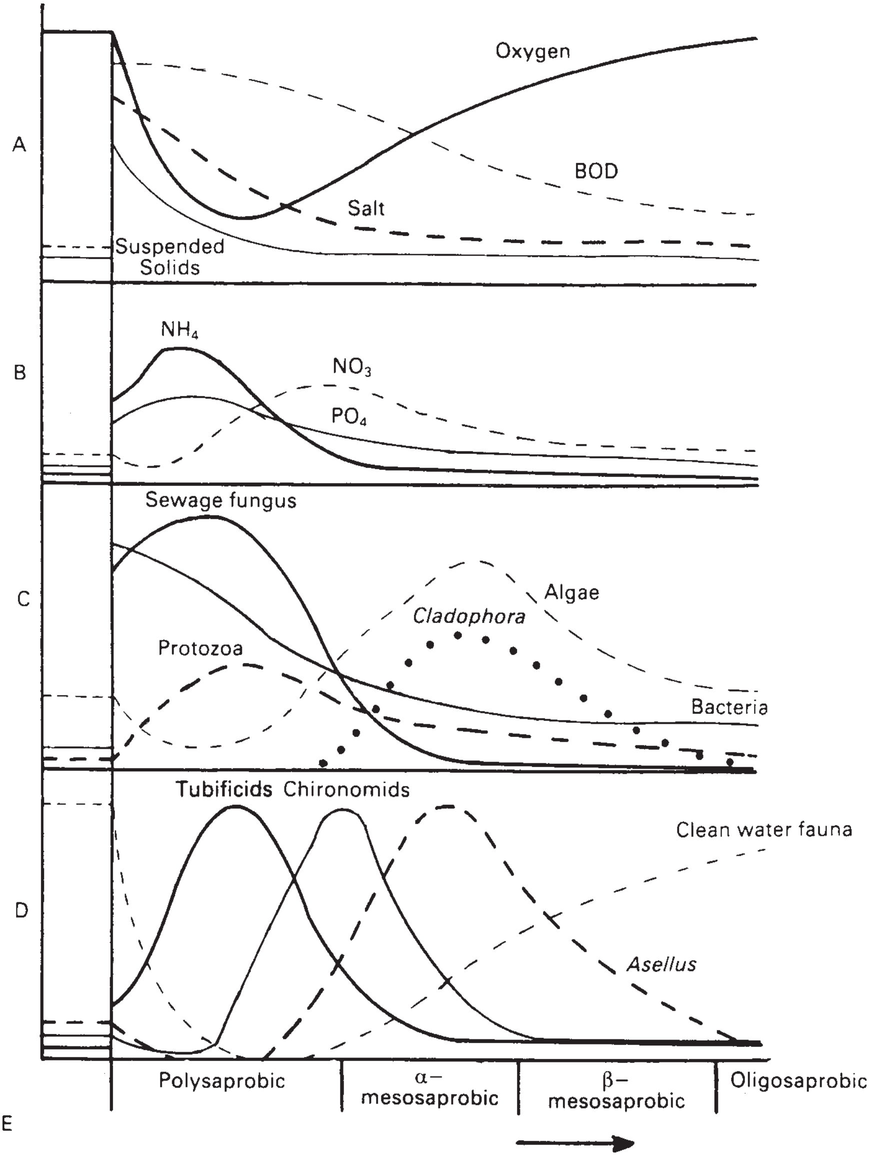

Figure 2.2 Changes in water quality and abundance of selected organisms typically found in a river below the discharge of an organic effluent, after Hynes (1960). See text for explanation. (E) shows the zones described by Kolkwitz and Marsson (1909). Other descriptive systems have been used (see Hellawell (1986) for a summary) but are not significantly different

For this reason, it is often necessary to carry out other determinations (for example, of chemical oxygen demand, or of permanganate value) in addition to BOD in order to characterise precisely the likely effects of an effluent or correctly interpret the significance of a BOD value. Within these limitations, BOD values are generally useful as indicators of the organic loading of water. They typically range from one or two milligrams per litre in unpolluted water, to 10000 milligrams per litre or more in raw wastes, untreated effluents or severely polluted receiving waters. The wide range of expected values means that the precise details of the method of determination have frequently to be modified according to the circumstances; raw samples, for example, often need to be diluted before the determination is carried out. The incubation period of five days (rather than some shorter period) in practice compensates for the fact that some samples will have, initially, very few microorganisms present, while others will contain a large inoculum. Detailed descriptions of the procedures which may be employed for BOD and other relevant determinations, and for the interpretation of their results, are available in several handbooks, for example APHA (1995). Note that in recent years, the term *biochemical oxygen demand* has become a popular synonym for *biological oxygen demand*. The present author prefers the term *biological* oxygen demand as it has historic precedence and avoids confusion with the *chemical oxygen demand,* which refers to the oxygen demand imposed by the *chemical* oxidation of reduced substances in the water.

Figure 2.2(A) shows the immediate effect of the effluent on the BOD of the receiving water. Correspondingly, the dissolved oxygen (DO) level drops, gradually recovering as the BOD falls with increasing distance downstream. The oxygen sag curve varies in its dimensions according to the strength of the effluent and the physical characteristics of the river. (Knowing the BOD of the effluent and certain physical characteristics of the river, oxygen sag curves can be predicted according to mathematical models. This can be useful in planning, for example by predicting the likely effect of a proposed new outfall on a receiving water.) Figure 2.2(A) also shows the typical changes in the suspended solids and dissolved salt concentrations of a receiving water downstream of an organic effluent. Figure 2.2(B) shows how ammonia, nitrates and phosphates typically behave. Ammonia in the receiving water reaches peak levels at the point of greatest deoxygenation, but declines as aerobic conditions are re-established and reduced nitrogen compounds are oxidised to nitrates.

Figures 2.2(C) and (D) show the responses of the plant and animal communities to these physical and chemical changes. 'Sewage fungus' is a characteristic and conspicuous feature of waters which are heavily polluted with organic matter. It is not, in fact, a fungus at all, although its dense, blanketing growths of matted, greyish-brown filaments suggest this idea to many people. It is an aggregation, of varying composition, of bacteria, algae, fungi and protozoa, frequently dominated by the slime-forming bacterium *Sphaerotilus natans* (Curtis and Curds, 1971). Algae are generally reduced initially (partly because the high levels of suspended solids prevent photosynthesis), but increase rapidly in abundance downstream as light penetration improves and levels of nitrate and phosphate remain high. *Cladophora* is a particularly conspicuous attached filamentous alga, widely associated with mild organic pollution or with the early stages of recovery from more severe pollution, and appears to be especially responsive to elevated phosphate levels (Pitcairn and Hawkes, 1973; Whitton, 1970).

The animal community (Figure 2.2(D)) also shows a clear pattern of response. The 'clean-water fauna' initially declines, and may be entirely eliminated, in the region immediately below the outfall. Tubificid worms, being typically tolerant of low dissolved oxygen levels and silty substrata, usually dominate the fauna in the more seriously affected areas. The larvae of the midge *Chironomus* (Diptera) spp. typically become established further downstream, followed by the isopod crustacean *Asellus,* and the gradual re-establishment of the 'clean-water fauna' as the river returns to its normal physical and chemical status. In any particular situation, the total number of species involved can be very large. The general pattern, subject to many variations in detail, is of a zonation, with increasing distance from the source of pollution, every bit as obvious as that along the length of an unpolluted river, or along a transect on a steeply-sloping seashore. The distribution of many individual species in response to organic pollution has been studied in detail in many different parts of the world. Hellawell (1986) gives a detailed summary of these observations. Several early investigators attempted to provide systematic descriptions of the zonation pattern of plants and animals observed in response to organic pollution with increasing distance from the pollution source, and one such description is shown in Figure 2.2(E). These descriptive systems eventually gave rise to the idea of indicator organisms, and to the development of numerical indices (pollution indices and biotic indices), which are widely used in the biological surveillance of water quality. These topics are further discussed in Chapter 3.

2.4 Nutrient Pollution

Plant growth in water may be limited by any of several factors, including light and the physical characteristics of the habitat. In many cases, however, the limiting factor is the availability of inorganic nutrients, particularly phosphate (Moss, 1988). Increased input of nutrients can therefore trigger increased plant growth which, if excessive, leads to changes in the biological characteristics of the receiving water. The discharge of organic matter to water is an important source of plant nutrients, since the aerobic decomposition of organic matter results in the release of phosphate, nitrate and other nutrients. Domestic sewage typically contains high levels of phosphate because detergent washing powder formulations normally contain high levels of phosphate. For example, the level of phosphate typically found in treated