4

Detection, Survival, and Removal of Pathogens in the Environment

THE COMMENSAL and pathogenic organisms found in human feces—and the environmental characteristics of the latter's transmission and control—have been examined in earlier chapters. The focus of this chapter is on the suitability of various excreted bacteria as diagnostic organisms to indicate environmental fecal pollution and on the relation of these bacteria to other bacterial and nonbacterial pathogens. In addition to bacterial indicators, generic "pathogen indicators" are proposed for assessing the safety of the products of excreta treatments. The survival times of indicators and pathogens in different environments (the reader is referred to Part Two in this regard) and several issues affecting the choice of excreta treatment technologies are analyzed in the remainder of the chapter.

Fecal Indicator Bacteria

Fecal indicator bacteria[1] are selected from among those commensal species that exclusively live in the intestinal tract of man and other warm-blooded animals without causing disease. Because they are always and naturally present in feces and are excreted in large numbers (up to 10^9 or 10^{10} cells per gram of feces), their presence in water indicates beyond doubt that the water has been contaminated with fecal material and possibly with excreted pathogens. If a water is shown to contain fecal indicator bacteria, it is considered unsafe for human consumption. This is the rationale for the bacteriological testing of public water supplies that was developed in Europe and North America at the turn of the century when the major concern of water supply engineers was to reduce the incidence of epidemics of strictly waterborne disease. It is still an epidemiologically valid testing technique for disinfected water supplies throughout the world, but it has certain limitations when applied indiscriminately in the examination of wastes and wastewaters, particularly in hot climates. (These limitations are discussed in the section "Relation of Fecal Indicator Bacteria to Excreted Pathogens," below.)

The ideal fecal indicator bacterium should be:

- A normal member of the intestinal flora of healthy people
- Exclusively intestinal in habitat, and hence exclusively fecal in origin when found in the environment
- Absent from nonhuman animals (a requirement not met by any of the indicator bacteria currently used)
- Present whenever fecal pathogens are present, and present only when fecal pathogens might reasonably be expected to be present
- Present in higher numbers than fecal pathogens
- Unable to grow outside the intestine, with a die-off rate slightly less than that of fecal pathogens
- Resistant to natural antagonistic factors and to water and waste treatment processes to a degree equal to or greater than that of fecal pathogens
- Easy to detect and count
- Nonpathogenic.

No one bacterial species or group completely fulfills all these requirements, but a few come close to doing so. Three main groups of bacteria are used as fecal indicators in conventional water bacteriology: the fecal coliforms, the fecal streptococci and the anaerobic bacterium *Clostridium perfringens*. Recently, some other members of the anaerobic intestinal flora,

1. Fecal indicator bacteria are discussed in greater detail in chapter 13; see also specific chapters in Part Two for notes on the taxonomic nomenclature of particular pathogens.

notably *Bifidobacterium* spp., have been proposed as additional indicator bacteria. *Pseudomonas aeruginosa* has also been proposed, but its status as an intestinal organism is in doubt. An analysis of these bacteria and their uses as indicators follows.

Coliform bacteria

There are two principal groups of coliform bacteria; the fecal coliforms (comprising mainly the bacterium *Escherichia coli*) and the total coliform group, that includes the fecal coliforms and comprises mainly species of the genera *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*. The former are exclusively fecal in origin, whereas the latter, although commonly found in feces also occur naturally in unpolluted soils and waters. Of the total coliform organisms found in fresh feces of warm-blooded animals, generally >90 percent are *E. coli*, the remainder being species of *Citrobacter*, *Enterobacter*, and *Klebsiella* (Dufour 1977).

Only the fecal coliforms (and especially *E. coli*) are definitive indicators of fecal pollution. In water bacteriology the total coliforms are regarded as "presumptive" indicators of pollution and should be absent from disinfected water supplies. In wastewater bacteriology, however, the total coliforms are of considerably less importance because many are nonfecal in origin and, especially in hot climates, they can multiply in the environment under suitable conditions, so that their presence or numbers may not necessarily relate to either the occurrence or degree of fecal pollution. In general, and despite the one report from India to the contrary (Raghavachari and Iyer 1939), only fecal coliforms (or better still, *E. coli*) should be used as indicators or tracers of fecal bacterial pathogens in wastes, wastewaters, and treatment and reuse processes.

Fecal and total coliforms are indistinguishable under the microscope: they are all Gram-negative rods measuring some 2–5 by 0.4 micrometers. In practice they are differentiated by the ability of fecal coliforms (mainly *E. coli* and thermotolerant *K. pneumoniae*) to ferment lactose with the production of acid and gas within 24 to 48 hours at a temeprature of 44°C. In addition, the most common fecal coliform, *E. coli*, can produce indole from the amino acid tryptophan at this temperature. In hot climates, however, some nonfecal coliforms can grow at 44°C and some can also produce indole at this temperature, thus mimicking the fecal coliforms (*E. coli* in particular). There are no satisfactory routine methods for differentiating between these organisms, and their simultaneous occurrence has prompted a search in recent years for alternative, more satisfactory indicator organisms for use in hot climates. A further disadvantage of fecal coliforms is that most standard enumeration procedures require an accurately controlled incubation period at 44° or 44.5°C, which is difficult to achieve in any small, or nonspecialized, laboratory.

Fecal streptococci

The fecal streptococci (or Group D streptococci) are a group of bacteria that are morphologically similar (Gram-positive cocci, measuring approximately 1 micrometer in diameter and occurring in short chains) and are mostly found in the intestines of man and other warm-blooded animals. The group includes species mainly associated with animals (*Streptococcus bovis* and *S. equinus*), other species with a wider distribution (for example, *S. faecalis* and *S. faecium*, which occur both in man and other animals), as well as two biotypes (*S. faecalis* var. *liquefaciens* and an atypical *S. faecalis* that hydrolyzes starch) that appear to be ubiquitous, occurring in both polluted and unpolluted environments. These last two strains, essentially nonfecal (although included in the group of fecal streptococci), are indistinguishable from the true fecal streptococci under routine detection or counting procedures. Because *S. faecalis* var. *liquefaciens* has been reported as the predominant biotype present at low densities (below about 100 fecal streptococci per 100 milliliters; Geldreich 1970), the usefulness of the fecal streptococci group as an indicator is open to question, especially in clean water bacteriology. Yet fecal streptococci may still have a place in wastewater bacteriology, although not as indicators of the bacteriological quality of wastewater-irrigated crops, on which the two nonfecal biotypes may both be present as natural flora unrelated to the degree of fecal pollution. There is no information, however, on the distribution of these two biotypes in tropical environments.

Aside from the possible problem of nonfecal strains of Group D streptococci, fecal streptococci have major advantages as fecal indicators. They are enumerated by a single-step membrane-filter procedure at 37°C, a temperature readily attained in small field laboratories. They are less prone to regrowth, and generally survive somewhat longer, than fecal coliforms and may thus be better indicators of excreted bacterial pathogens (that have little regrowth tendency) and excreted virus (that survive for longer than fecal coliforms in cool waters). These points are discussed in greater detail in chapter 13.

Fecal coliform to fecal streptococci ratio

It has been found that human feces in the USA contain at least 4 times as many fecal coliforms as fecal streptococci, but that animal feces contain at least 1.4 times as many fecal streptococci as fecal coliforms (Geldreich 1966). It was therefore suggested that American surface waters that have fecal coliform-streptococci ratios of >4 are likely to have received predominantly human pollution, whereas those with ratios of <0.7 mainly have been contaminated by the feces of wild and domestic animals (Geldreich 1966).

This method, however, is of no value in practice. The fecal coliform-streptococci ratios in fresh feces vary widely in different animal species and geographical locations. It is not true that humans the world over excrete a ratio of >4, and animals <0.7 (Wheater, Mara and Oragui 1979). Once the feces have been excreted, the ratios will change because of the differential death rates of the various bacteria. The enterococci (*S. faecalis*, *S. faecium*, and *S. durans*) typically survive longer than fecal coliforms which in turn survive longer than *S. bovis* and *S. equinus* (McFeters and others 1974). It was therefore suggested that for human pollution, in which enterococci are the dominant fecal streptococcal species, fecal coliform-streptococci ratios in water samples returned to the laboratory will fall; whereas for animal pollution, in which *S. bovis* or *S. equinus* may be more numerous, the ratios in stored samples may rise (Feachem 1975). But it now appears that, whereas enterococci are the dominant fecal streptococcal species in humans in developed countries (and therefore human pollution is associated with falling ratios), enterococci can also be the dominant fecal streptococcal species in some animals (for instance, cats, ducks, hens, mice, pigs, rabbits, rats, and seagulls in Scotland; Wheater, Mara and Oragui 1979). Furthermore, *S. equinus* and *S. bovis* are common in the feces of people in some countries (for intance, India and Uganda; Drasar and Hill 1974). It may be concluded, therefore, that neither the ratio at the time of sampling, nor the change in ratio in a stored sample, conveys useful information about the origins of fecal pollution. The development of a routine test to distinguish human from nonhuman fecal contamination is the highest current priority for research in santirary microbiology. [See note on page 66.]

Clostridium perfringens

The bacterium *Clostridium perfringens* (formerly *C. welchii*) is anaerobic, spore-forming, Gram-positive, and measures approximately 4–6 micrometers in length by 1–2 micrometers in width. It is exclusively fecal in origin and is also pathogenic, causing gas gangrene and food poisoning (Chakrabarty, Narayan and Chandiramani 1977). Because it is a spore-forming organism, it can persist for long periods outside the intestine, and therefore can be used as an indicator of occasional or intermittent pollution, or of previous pollution of waters in which the presence of neither fecal coliforms nor fecal streptococci can be demonstrated (Bisson and Cabelli 1980; Cabelli 1977). *C. perfringens* is also more resistant than both fecal coliforms and fecal streptococci to antagonistic substances such as chlorine.

In wastewater bacteriology, however, its long persistence is a disadvantage because residual, dormant populations of the bacterium in waters may not reflect the true degree of pathogenic contamination. Type A *C. perfringens* from human feces may also grow in the soil (in contrast to other types of *C. perfringens* of animal origin, which seem to die-out in soil).

Pseudomonas aeruginosa

The organism is an opportunistic human pathogen that causes infection in wounds (especially burns) and also ear and urinary tract infections, meningitis, respiratory infections and other conditions (Cross 1979). It is often associated with sepsis in otherwise debilitated patients in hospital wards. *P. aeruginosa* is being increasingly implicated as a cause of ear infection and skin rash following exposure in inadequately disinfected swimming pools and whirlpool baths (Jacobson, Hoadley and Farmer 1976; McCausland and Cox 1975; Seyfried and Fraser 1978; Washburn and others 1976).

P. aeruginosa is a Gram-negative, aerobic, nonsporulating rod measuring approximately 0.5 by 2 micrometers. It occurs, normally at low concentrations of about 50 organisms per gram, in the feces of a small proportion (about 3 to 15 percent) of healthy people. It probably does not grow in the intestine of healthy people, and *P. aeruginosa* isolated in feces may be survivors of ingested bacteria. Studies in which *P. aeruginosa* was fed to volunteers demonstrated that large numbers ($\geq 10^6$) must be ingested to produce fecal carriage, which did not persist for more than 6 days (Buck and Cooke 1969).

P. aeruginosa is common in sink traps and flower water. It has been reported in fairly high concentrations ($>10^3$ per 100 milliliters) in urban stormwater runoff in Canada (Qureshi and Dutka 1979), and in higher concentration in sewage ($>10^5$ per 100

milliliters) and hospital sewage ($>10^6$ per 100 milliliters) in Scotland (Wheater and others 1980). It has been suggested (Cabelli, Kennedy and Levin 1976) that a consideration of *P. aeruginosa-E. coli* ratios in fecally contaminated waters can provide evidence on the possible origins of the pollution, with counts of >1,000 fecal coliforms and <1 *P. aeruginosa* per 100 milliliters being associated putatively with animal, rather than human, pollution. But *P. aeruginosa* occurs widely (albeit in highly variable numbers) in nature as a free-living organism (Green and others 1975; Parker 1971); it can therefore have little usefulness in studies of fecal contamination.

Bifidobacterium and other anaerobic bacteria

Bifidobacteria (previously known as anaerobic lactobacilli) are nonsporulating, anaerobic organisms that occur in the intestines of man and other animals; they are Gram-positive V- or Y-shaped cells, with each branch measuring about 0.8 by 3 to 4 micrometers. The most common species in man are *Bifidobacterium adolescentis* and *B. longum*. Bifidobacteria have recently been proposed as indicator organisms for use in tropical waters because the lactose-fermenting species are exclusively fecal in origin (Cabelli 1978; Levin 1977). They therefore overcome the principal disadvantage of fecal coliform counts on tropical samples—that such samples may contain a significant proportion of strains that can ferment lactose and produce indole at 44°C but do not derive from feces. An additional advantage of bifidobacteria is that, because they are strict anaerobes and grow poorly below 30°C, they have very low multiplication potential in extraintestinal environments. Work on bifidobacteria has only commenced relatively recently, and there is little information on their survival in extraintestinal environments other than in riverwater (Evison and Morgan 1978).

The bacterial flora of feces is predominantly composed of anaerobic bacteria (table 1-6). Bifidobacteria have been described, but feces contain large numbers of other nonsporulating anaerobes, such as *Bacteroides* spp. (commonly *B. fragilis*), the anaerobic Gram-positive cocci (*Peptoccus* spp. and *Peptostreptococcus* spp.) and *Eubacterium* spp. Current research is investigating the usefulness of these organisms as fecal indicators, but at present there are insufficient data on their extraintestinal ecology to know whether or not use of all or some of them as indicators will be practicable. Moreover, current techniques for their detection and enumeration are rather complex for routine use.

Fecal concentrations, detection, and enumeration of bacterial indicators

Approximate numbers of indicator bacteria commonly found in human feces are given in table 4-1. The cell counts in the table are average figures only and are mainly derived from American literature. Some communities, because of dietary differences (see table 1-6), may display considerably different numbers for one or more of the listed indicators (see also chapter 13).

Methods suitable for the detection and enumeration of coliform bacteria, fecal streptococci, and *C. perfringens* are described in the 15th edition of *Standard Methods for the Examination of Waters and Wastewaters* (American Public Health Association 1980) and in the 4th edition of *The Bacteriological Examination of Water Supplies* (Department of Health and Society Security 1969). A membrane-filter technique for enumerating *C. perfringens* is also described by Bisson and Cabelli (1979). *P. aeruginosa* populations can be counted by membrane filtration using the medium of Levin and Cabelli (1972) supplemented with 0.1 percent cetrimide (Wheater and others 1980; see also Brodsky and Ciebin 1978; Dutka and Kwan 1977; Hoadley 1977). The membrane filtration method and medium for *Bifidobacterium* spp. are described by Evison and Morgan (1978), but no satisfactory routine procedure for enumerating these bacteria has yet been developed. Reference may also be made to Mara (1974). [See note on page 66.]

Relation of Fecal Indicator Bacteria to Excreted Pathogens

Fecal indicator bacteria were originally identified to assess the bacteriological quality of potable waters at a time when only the transmission of bacterial

Table 4-1. *Number of indicator bacteria commonly found in human feces*

Indicator	*Cells per gram of feces (wet weight)*
Bacteroides spp.	10^7–10^{11}
Bifidobacterium spp.	10^7–10^{11}
Clostridium perfringens	10^3–10^{10}
Coliforms	
Fecal	10^6–10^9
Nonfecal	10^7–10^9
Fecal streptococci	10^5–10^8

enteropathogens (such as salmonellae, shigellae, and cholera vibrios) was considered the major public health risk from drinking water. Historically (and to some extent, even now), attention has therefore focused on the relation of the fecal indicators to bacterial pathogens. Recent literature, continuing this emphasis, contains many reports on the persistence of fecal coliforms and salmonellae in the extraintestinal environment, but only a few reports on the comparative survival of the fecal indicators and nonbacterial fecal pathogens (such as viruses, protozoal cysts, and helminth eggs). This has partly been a result of the difficulty of routinely analyzing samples for these other pathogens (especially viruses), but an uncritical acceptance of the historical direction of research in the field has also contributed to the neglect. Thus, for example, there has been no report on the relation of the indicator bacterium *C. perfringens* and the eggs of the fecal helminth *Ascaris lumbricoides* (both persist for longer periods in the extraintestinal environment than do other organisms of their respective kind). Knowledge of such a relation would be of little value in assessing the safety of urban water supplies (in which *Ascaris* eggs are not a public health hazard) but it might be of assistance in assessing the quality of sewage sludges, composted feces, and some wastewater effluents.

This one example illustrates the longstanding preoccupation of sanitary bacteriologists with urban water supplies to the near exclusion of appropriate consideration of wastes and wastewaters, and of the comparative removal and persistence of fecal pathogens and indicator bacteria in treatment processes and reuse products. There are many data (mainly from North America and admittedly of variable quality) on the relation between the survival of bacterial pathogens and indicators in sewage treatment processes in temperate climates, but very little data from tropical countries. Predicting with confidence the likely density of salmonellae in a tropical sewage effluent, even when the number of fecal coliforms present is known, is extremely difficult; in contrast, reasonable estimates are possible with a temperate climate effluent. This neglect makes the establishment of a fecal coliform standard for most tropical sewage effluents a highly unscientific process. Because engineers design, for example, maturation pond systems on the basis of fecal coliform removal to the desired standard, this state of scientific uncertainty can lead to either overdesign (with a consequent unnecessary increase in cost) or underdesign (with a consequent increased risk, and perhaps actual damage, to public health).

When the hazards from nonbacterial excreted pathogens are considered, the bacterial fecal indicators are of limited usefulness. They are of some use in assessing the quality and resulting risks to health of irrigation waters, but even here the gaps in knowledge are considerable. Much of the existing information on the relation of fecal indicators and excreted pathogens comes from relatively wealthy communities (for example North America, South Africa, and Israel), and these data cannot be applied with much confidence to other communities in which climate, diet, disease patterns, agricultural practice and cultural attitudes to excreta reuse products are all different. This does not mean that information on, say, fecal coliform survival in Israel cannot be used to predict fecal coliform survival in, say, rural India but it does mean that the information may not be all that relevant to conditions in rural India, where the ability to make statements about fecal coliform survival may not help assess the degree of fecal pathogen contamination of crops irrigated with sewage effluent or fertilized with treated excreta. Caution must therefore be exercised in applying data on fecal indicator survival in environments other than those from which the information was obtained.

In summary, little is known about the relative concentrations of indicator bacteria and bacterial pathogens in effluents and fecal products in warm climates and practically no information exists on the relative concentrations of indicator bacteria and nonbacterial pathogens. In addition, it must be noted that the stability of the ratio between the concentration of an indicator bacteria and the concentration of a particular pathogen decreases as the size of the contributing population decreases. Thus, for systems serving small communities, or for individual systems such as aquaprivies or composting toilets, the ratios will vary enormously from place to place and from time to time, and no organism will act as a good indicator of another organism.

Pathogen Indicators

Fecal indicator bacteria only demonstrate fecal contamination. This fact is useful in assessing the safety of drinking water supplies, but when the health aspects of sanitation systems, excreta and sewage treatments and reuse processes are considered, what is needed is not a fecal indicator bacterium (for feces are obviously present) but, rather, a pathogen indicator organism. A reliable measure of the total pathogen content of the end product of a treatment process is needed, so that the health risks associated with any reuse of the end

product, or with its discharge into the environment, can be gauged as accurately as possible. If these risks can be judged, responsible and informed decisions can be made—for instance, on whether the benefits from end-product reuse outweigh the possible health costs and whether further treatment is necessary to protect the health of those involved (either as producers or consumers) in the reuse process or, in the case of the end products being discharged into the environment, of the users of the environment.

It would be unrealistic to expect the same pathogen indicator organism to be useful in assessing the pathogen content of different kinds of fecal products—for example, composted feces and the effluent from waste stabilization ponds. In the former case the concern is primarily the viability of the persistent helminth eggs (notably *Ascaris lumbricoides* eggs) whereas in the latter case it is known that, if the total retention time in a stabilization pond system is more than 20 days, the effluent will be free of both helminth eggs and larvae but may contain excreted viruses and bacteria. Because of these variations, it is convenient to divide fecal products into two groups, effluents and noneffluents, and to examine which organisms are suitable pathogen indicators for each.

Pathogen indicators for pond effluents

The effluents from waste stabilization ponds and other sewage treatment processes are best considered separately because the vastly different retention times involved (weeks in ponds, hours or days in other processes) produce effluents with markedly different pathogen contents. If a pond system has a retention time of more than 20 days its effluent will be free from both pathogenic protozoa and helminth eggs and larvae, but it may still contain viral and bacterial pathogens. Because a routine analysis of pond effluents for pathogenic viruses and bacteria is not yet feasible (nor likely to become so in the immediate future), the choice of a suitable pathogen indicator is exceedingly difficult. Bacteriophages—and more specifically, coliphages—may provide a solution in the future but the laboratory techniques are not yet widely known. Fecal coliforms or fecal streptococci would seem an obvious choice, but there is little data on the usefulness of either as viral indicators, and the literature on their respective survival compared to pathogenic bacteria is only slightly less scant (especially for tropical pond effluents). There is no information available on the usefulness of bifidobacteria and the other non-sporulating anaerobes.

Although they are less than ideal for the purpose, fecal coliforms and fecal streptococci are perhaps the best pathogen indicators. It is difficult to determine what density of fecal coliforms or fecal streptococci—as an indication of the presence of endemic pathogens—should be permissible. The rather unhelpful answer is that the densities should be as low as possible, which in practice means at least below 1,000 per 100 milliliters of effluent (and preferably below 100 per 100 milliliters). Effluents reused for the irrigation of crops that may be consumed raw must have fecal coliform and fecal streptococci counts that are both below 100 per 100 milliliters. Viral and bacterial pathogens may or may not be absent at these indicator organism densities, but in general health risks will be so minimal that further treatment will not normally be economic.

Pathogen indicators for effluents from other sewage treatments

The effluents produced by sewage treatment processes other than waste stabilization ponds are likely to contain a full range of fecal pathogens—viruses, bacteria, protozoal cysts, and helminth eggs. There is no suitable fecal indicator organism in these circumstances; it is not possible to have a single organism indicate the presence of so diverse a group of pathogens. Fecal coliforms have been used, but only for historical reasons (they are totally inappropriate indicators for the helminth eggs for instance). This subsection can conclude with the generalization that if a sound economic argument can be put forward for the use of treatment processes other than ponds, then the effluent from such treatments should undergo tertiary treatment or be heavily disinfected or discharged well out to sea because, in the tropics, the health risks from the effluent may be similar to those from raw sewage. It should be noted that heavy disinfection is often ineffective (especially against viruses and helminth eggs) and has undesirable environmental consequences.[2]

Pathogen indicators for noneffluents

Noneffluents are taken here to include night soil, the contents of pit latrines and composting toilets and the sludges from aquaprivies, septic tanks and conventional sewage treatment works. It is reasonable to assume that, if ascariasis is endemic and there are no viable *Ascaris* eggs present in the wastes analyzed, then

2. Effluent disinfection is discussed further in chapters 6, 9, 13, and 23.

other pathogens are absent as well, since *Ascaris* eggs are so resistant. Thus, and in the current absence of any data on the comparative survival of the bacterium *C. perfringens*, the viable eggs of *Ascaris lumbricoides* would appear to be the best pathogen indicator currently available for noneffluents. This indicator has been accepted in China, where standards of 95 percent *Ascaris* egg mortality have been adopted for the agricultural reuse of excreta (McGarry and Stainforth 1978).

Survival of Indicators and Pathogens

From the time of excretion, the concentration of all pathogens usually declines from the death or loss of infectivity of a proportion of the organisms. Viruses and protozoa will always decrease in numbers following excretion, but bacteria may multiply if they find themselves in a suitably nutrient-rich environment with a minimum of competition from other microorganisms. This can occur when salmonellae, for instance, contaminate certain foods, or when *E. coli* multiply in a chlorinated sewage effluent from which many other bacteria have been eliminated. Multiplication of bacterial pathogens is generally rare, however, and is unlikely to continue for very long. Intestinal helminths—except the trematodes, which have a multiplication phase in their molluscan intermediate hosts—will decrease in numbers following excretion. The multiplication possibilities for the excreted pathogens were summarized in table 2-3.

The ability of an excreted organism to survive is defined as its persistence (discussed in chapter 2). The natural death of organisms when exposed to a hostile environment is of the utmost importance because it reduces the infectivity of excreta independently of any treatment process. In fact, some treatment processes have little effect on excreted pathogens and simply allow the necessary time for natural die-off to occur. The effect of conventional sewage treatment on protozoal cysts is of this kind (see chapter 20). Certain treatment processes, however, create conditions that are particularly hostile to excreted pathogens and that promote their rapid death. The effects of activated sludge on fecal bacteria, or of thermophilic digestion on all organisms, are of this kind. The essential environmental factors in limiting pathogen persistence are time and temperature. The success of a given treatment process in reducing the pathogenicity of an effluent or sludge thus depends, in general, upon its retention time and its creation of an environment especially hostile to particular organisms. The sole environmental condition likely found in a night soil or sewage treatment system that is highly fatal to all pathogens in a reasonably short time (a few hours) is raised temperature (in the range 55–65°C). The only other low-cost process that causes 100 percent removal or destruction of most pathogens is the waste stabilization pond system with its long retention times, exposure to sunlight, and good sedimentation properties.

The elapse of time is a feature common to all treatment, disposal and reuse technologies; in many cases, it is the feature that most determines the pathogen removal achieved. The rate of loss of infectivity of an organism also depends very much on temperature; most organisms survive well at low temperatures ($\approx$5°C) and rapidly die at high temperatures (>40°C). Except in sludge or night soil digestion processes, temperatures approximate environmental temperatures—in most developing countries, generally in the range of 15–35°C and commonly of 20–30°C. It is therefore useful to know the persistence of pathogens at ambient temperatures in different environments so that the likely pathogen content of various fecal products can be predicted. In this section pathogen survival at ambient temperatures is reviewed with the following considered in turn: survival in feces, night soil and sludge; survival in water and sewage; survival in soil; and survival on crops. Under each heading that follows, the available knowledge is summarized as succinctly as possible. A greal deal of additional information is given in Part Two and some of the data are further tabulated in Feachem and others (1980).

The shape of the curve describing pathogen survival over time should determine the way in which survival is reported. Many bacterial populations decline exponentially, so that 90 or 99 percent of the bacteria are lost relatively quickly with a few organisms persisting for longer periods. Such a situation is best described by the probability of survival for a given time or by half life, the time required for half the population to die. For instance, 50 percent of fecal coliforms may die in 20 hours in water, whereas a few may persist for up to 50 days, and the results obtained will depend heavily on sampling procedures. Most of the literature gives data on the persistence of the small proportion of long-term survivors and only a few authors have reported the shape of the death curve or given the 50 to 90 percent destruction times. The discussion below will therefore mainly concern the overall persistence of a few organisms. This focus is epidemiologically appropriate for organisms that can replenish their numbers if they find themselves on food or other suitable substrates

(for example shigellae, salmonellae and pathogenic *E. coli*) or for organisms whose infective dose is believed to be low (the excreted viruses, for example). It is less appropriate for cases in which regrowth is unlikely and infective doses may be high (for example, *Vibrio cholerae*); in these cases it is the rapid decline of the bacteria to a level that no longer presents a major public health hazard that is important. In organisms having several developmental stages outside the human host (such as hookworms and schistosomes), each stage will have its own separate survival pattern. When a developmental stage is actively moving yet dependent on an unreplenished energy source (for example, the schistosome miracidium seeking its snail host) the length of life may be precisely defined.

In feces, night soil, and sludge

There is less literature on the survival of pathogens in these media than in the aqueous environments discussed in the following subsection. Some sources refer to survival of pathogens in sewage works' sludges, but survival in feces and night soil may be assumed to be broadly similar. Research on pathogen survival in these media may be summarized as shown in table 4-2.[3]

In water and sewage

Many studies on the survival of excreted organisms in water and sewage have been conducted.[4] The data are summarized in table 4-3. For all organisms survival is highly dependent on temperature, with greatly increased persistence at lower temperatures. Survival of bacteria is also highly dependent on the presence of other microorganisms in the water that might provide competition or predation. Bacteria often survive longer in clean water than in dirty water and the longest survival times are obtained by inoculating a single bacterial species into sterilized polluted water. There is some evidence that virus survival is enhanced in polluted waters, presumably a result of some protective effect that the viruses may receive when they are adsorbed onto solid particles in dirty water (see chapter 9). Coliforms, in particular *E. coli*, have attracted the most interest because of their established role as indicator bacteria. Substantial regrowth of coliforms is possible in organically polluted waters, but this growth phase will give way to a progressive die-off. Survival in excess of 50 days is most unlikely and, at 20–30°C, 20 days is a more likely maximum survival time. Mixed fecal streptococci have a similar (perhaps a little longer) survival but, if the streptococci are predominantly *S. bovis* or *S. equinus*, the survival times are substantially shorter (see chapter 13). *Salmonella* survival has also been widely reported. Survival of over 2 months has been recorded, but 1 month is a more common upper limit (see chapter 15). *Shigella* spp. and

Table 4-2. *Survival times of excreted pathogens in feces, night soil, and sludge at 20–30°C*

Pathogen	*Survival time* (days)
Viruses	
Enteroviruses[a]	<100 but usually <20
Bacteria	
Fecal coliforms	<90 but usually <50
Salmonella spp.	<60 but usually <30
Shigella spp.	<30 but usually <10
Vibrio cholerae	<30 but usually <5
Protozoa	
Entamoeba histolytica cysts	<30 but usually <15
Helminths	
Ascaris lumbricoides eggs	Many months

a. Includes polio-, echo-, and coxsackieviruses.

3. A compilation of original sources and findings on survival in feces, night soil, and sludge can be found in the appropriate sections of Part Two, chapters 9 through 35.

Table 4-3. *Survival times of excreted pathogens in fresh water and sewage at 20–30°C*

Pathogen	*Survival time* (days)
Viruses[a]	
Enteroviruses[b]	<120 but usually <50
Bacteria	
Fecal coliforms[a]	<60 but usually <30
Salmonella spp.[a]	<60 but usually <30
Shigella spp.[a]	<30 but usually <10
Vibrio cholerae[c]	<30 but usually <10
Protozoa	
Entamoeba histolytica cysts	<30 but usually <15
Helminths	
Ascaris lumbricoides eggs	Many months

a. In seawater, viral survival is less, and bacterial survival is very much less, than in fresh water.

b. Includes polio-, echo-, and coxsackieviruses.

c. *V. cholerae* survival in aqueous environments is a subject of current uncertainty—see chapter 17.

4. These studies are reviewed in the appropriate sections of Part Two, chapters 9 through 35.

Vibrio cholerae are less persistent, and survival of these bacteria for more than 20 days is seldom reported (see chapters 16 and 17).

The development of viral detection techniques in the 1950s led to the demonstration of the presence of excreted viruses in the environment. The enteroviruses (polio-, coxsackie-, and echoviruses) have been frequently isolated from water and wastewater (chapter 9) and the literature on this subject is growing rapidly at the present time. Viral survival may be longer than bacterial survival and it is greatly increased at lower temperatures. In the 20–30°C range, 2 months seems a typical survival time, whereas at around 10°C, 9 months is a more realistic figure.

Protozoal cysts are poor survivors in any environment. A likely maximum for *Entamoeba histolytica* in sewage or polluted water is about 20 days (see chapter 20). Helminth eggs vary from the very fragile to the very persistent. The most persistent of all are *Ascaris* eggs, which may survive for a year or more (see chapter 23).

In soil

Survival times in soil are relevant in all situations where effluent, sludge, compost, or other fecal products are being applied to the land as fertilizers or soil conditioners.[5] Several factors, shown in table 4-4, affect the survival time of enteric bacteria in soil (Gerba, Wallis and Melnick 1975). Fecal coliforms can survive for many months under optimal conditions. In warm climates, especially when arid, survival is limited to 2–3 months at most. Fecal streptococcal survival is likely to be longer if human enterococcal species are dominant (see chapter 13). Survival of salmonellae may be up to 1 year if the soil is cool, moist and rich in organics (for example, if it is fertilized), but strain variation is considerable and 50 days would be a more typical maximum (see chapter 15). Data on *Shigella* or *Vibrio cholerae* survival in soil are limited (see chapters 16 and 17).

The information available on viruses suggests that virus particles adsorb to soil particles and become protected from environmental factors. Viral survival is greater at low temperatures: survivals of up to around 3 months have been reported in warm weather, increasing to around 5 months in European winter conditions (see chapter 9). Protozoal cysts in soil are most unlikely to survive for more than 10 days (see chapter 20). Helminth egg survival varies enormously,

5. A comprehensive review of the persistence of excreted pathogens in soil is contained in the appropriate sections of Part Two, chapters 9 through 35.

Table 4-4. *Factors affecting survival time of enteric bacteria in soil*

Soil factor	*Effect on bacterial survival*
Antagonism from soil microflora	Increased survival time in sterile soil
Moisture content	Greater survival time in moist soils and during times of high rainfall
Moisture-holding capacity	Survival time is less in sandy soils than in soils with greater water-holding capacity
Organic matter	Increased survival and possible regrowth when sufficient amounts of organic matter are present
pH	Shorter survival time in acid soils (pH 3–5) than in alkaline soils
Sunlight	Shorter survival time at soil surface
Temperature	Longer survival at low temperatures; longer survival in winter than in summer

Source: Adapted from Gerba, Wallis and Melnick (1975).

but *Ascaris* eggs can survive for several years (see chapter 23). The situation is summarized in table 4-5.

On crops

Excreted viruses and bacteria cannot penetrate undamaged vegetable skins. However, there are many reports in the literature on the isolation of all kinds of excreted pathogens from the surface of vegetables that have been irrigated or fertilized with fecal products.[6] Root vegetables are more prone to contamination than others. Weather conditions have an important

Table 4-5. *Survival times of excreted pathogens in soil at 20–30°C*

Pathogen	*Survival time* (days)
Viruses	
Enteroviruses[a]	< 100 but usually < 20
Bacteria	
Fecal coliforms	< 70 but usually < 20
Salmonella spp.	< 70 but usually < 20
Vibrio cholerae	< 20 but usually < 10
Protozoa	
Entamoeba histolytica cysts	< 20 but usually < 10
Helminths	
Ascaris lumbricoides eggs	Many months

a. Includes polio-, echo-, and coxsackieviruses.

influence on the survival of pathogens on plants; warmth, sunshine, and low air humidity greatly promote pathogen death. The survival characteristics of various excreted organisms on crops may be summarized as shown in table 4-6. As indicated in the table, pathogen survival times on vegetables are short compared to survivals in other environments. Protozoal cysts are rapidly killed. Viruses, bacteria, and worm eggs survive for longer, but little survival of any species is to be expected after 2 months.

Pathogen Survival versus Removal in Waste Treatment

Pathogen survival, rather than pathogen removal, is purposely referred to in this book. This is because health hazards are posed by the pathogens that survive a treatment process, not by those that are removed by treatment. Figures such as 99 percent or 99.9 percent removal appear highly impressive but they represent 1 or 0.1 percent survival, respectively, and this degree of survival may be highly significant wherever incoming concentrations are great. If an influent to a sewage works contains, say, 10^5 pathogenic bacteria per liter, then 99 percent removal will produce an effluent with 10^3 pathogenic bacteria per liter. In areas where the effluent is to be reused, or where it is to be discharged to a stream that populations downstream use as a source of drinking water, such effluent quality may be inadequate.

Table 4-6. *Survival times of excreted pathogens on crops at 20–30°C*

Pathogen	*Survival time* (days)
Viruses	
Enteroviruses[a]	<60 but usually <15
Bacteria	
Fecal coliforms	<30 but usually <15
Salmonella spp.	<30 but usually <15
Shigella spp.	<10 but usually <5
Vibrio cholerae	<5 but usually <2
Protozoa	
Entamoeba histolytica cysts	<10 but usually <2
Helminths	
Ascaris lumbricoides eggs	<60 but usually <30

a. Includes polio-, echo-, and coxsackieviruses.

6. These reports are reviewed in the appropriate sections of Part Two, chapters 9 through 35.

The emphasis in the literature on the exact proportions of pathogens removed by various treatment processes is thus misleading. For instance, most conventional treatment plants remove 90 to 99 percent of enteric bacteria.[7] This is a very poor removal; whether trickling filters remove a little less (say 95 percent) than activated sludge plants (say 99 percent), they are both technologies with poor pathogen removal characteristics (but they were never designed to have them—see the next section). A removal ability of less than 99 percent means always more than 1 percent survival, or always less than a log unit reduction of 2. In developing countries, where incoming wastes have high concentrations of pathogens (especially viruses, bacteria, and protozoal cysts—see table 1-10), a survival of more than 1 percent is usually inadequate.

In considering treatment technologies by their ability to remove pathogens, it is necessary not to dwell on trivial differences (for instance, 92.3 percent versus 97.8 percent removal), but to translate removal efficiencies into orders of magnitude. Conventional treatment works remove between 1 and 2 log units of enteric bacteria and should be contrasted with technologies, such as waste stabilization ponds, which remove 5 log units. In considering stabilization ponds or thermophilic digesters, which have high removal performances, it is also misleading to talk in terms of percentage removal (use of this convention disguises, for instance, the important difference between 99.99 and 99.999 percent removal).

The removal characteristics of treatment technologies should be related to the incoming concentrations of particular pathogens, to the intended reuse or disposal arrangements, and to the associated health risks. Different pathogens occur in varying concentrations and are affected in different ways by a given treatment technology. For instance, protozoal cysts will be found in raw sludge in relatively low numbers and will not survive sludge treatment. In contrast, *Ascaris* eggs may be found in sludge in high concentrations and will survive most sludge treatment processes.

Objectives of Night Soil and Sewage Treatments

The primary objective in the treatment of night soil or sewage from communities in which excreted infections are endemic is the destruction of excreted

7. The processes that conventional sewage treatment comprises, and their ability to remove various excreted pathogens, are discussed in chapter 6 and reviewed at length in the chapters of Part Two.

pathogens. This is principally achieved by a combination of time and temperature, although other conditions of the extraintestinal environment are also important (for example, sunlight and oxygen availability). From the extensive literature review in part 2, it appears that no excreted pathogen—with the exception of spore-forming bacteria (for example *C. perfringens*) and possibly hepatitis A virus—can survive a temperature of more than 65°C for a few minutes. As the temperature falls survival increases; thus, at 10°C, for instance, *Ascaris* eggs may survive for several years, enteroviruses for 12 months, and shigellae for 2–3 months.

The degree to which night soil and sewage are treated is largely influenced by what is to be done with the sludge, compost or sewage effluent.[8] It is thus accepted engineering practice to discharge untreated sewage into the sea, provided that the outfall is designed to ensure that no pollution of beaches or shellfish-growing areas will occur; but if reuse of an effluent for the irrigation of edible crops is intended, the designer's goal should be the absence of excreted pathogens on the surface of crops, and he should accordingly design the treatment works for a very low degree of pathogen survival.

Excreta and night soil treatment

The effectiveness of treatment methods for excreta and night soil depends greatly upon their time-temperature characteristics. The effective processes are those that retain the excreta for a long time (>1 year), or make it warm (>55°C), or effectively combine adequate retention time and high temperature.

Pit latrines (see the section of that title in the next chapter) have a useful life of a few years; when one becomes full, a second is dug, and the contents of the first are left undisturbed while the second is in use. Because of the time interval there are no health hazards associated with digging out the contents of previously filled and covered pit latrines. Provided the squatting plate is regularly cleaned, pit latrines pose no greater risks to health than do flush toilets (though insect breeding can be a serious problem—see chapters 36 and 37—and odor can be a nuisance).

Composting toilets (see the section of that title in the next chapter) are of two types: batch and continuous. If the composting period is over 1 year, only a few *Ascaris* eggs will be present in the product. With composting periods of under 1 year, varying numbers of other excreted pathogens will be present (see table 5-1). Composting toilets thus have definite health risks that, although slight, should be recognized by the designers and users of these systems. In strictly economic terms the value of the compost must be greater than the possible cost to health from its use.

The health hazards associated with the collection of night soil from bucket and vault latrines are described in the section "Cartage Systems" in the next chapter. If urine is collected as well as feces, the night soil is a fecal suspension similar to primary sewage sludge and may be treated by mesophilic or thermophilic digestion. It also may be treated in a pond system which can be designed to produce little effluent so that very long retention times are possible (>1 year) and, consequently, no survival of excreted pathogens. If the urine is not collected, or is allowed to drain away, the night soil (now principally feces) may be disposed of, treated, and reused in a number of ways (see also the section "Composting" in chapter 5). Night soil cartage and treatment systems will tend to have higher health risks than many other systems, although risks can be greatly reduced by the use of modern methods (such as those found in Japan). In high-density urban settings, where the only technological alternative may be a sewerage system, cartage systems will often be economically attractive despite their health hazards. In other settings, where a greater range of technologies is feasible, cartage may be less attractive.

Sewage treatment

Those whose job is to select and design appropriate systems for the collection and treatment of sewage in developing countries must bear in mind that European and North American practices do not represent the zenith of scientific achievement, nor are they the product of a logical and rational design process. Rather, treatment practices in the developed countries are the product of history, a history that started about 100 years ago when little was known about the fundamental physics and chemistry of the subject and when practically no applicable microbiology had been discovered. Only since 1970 have the tools to do serious work in water and wastewater virology been developed, and only since 1975 have the roles of rotavirus, *Campylobacter*, and *E. coli*[9] in the etiology of diarrheas been demonstrated.

8. Treatment strategies for different reuse and disposal practices are discussed in chapter 7.

9. The epidemiology of infections with rotavirus, *Campylobacter*, and pathogenic *E. coli* are reviewed in chapters 11, 12, and 13, respectively.

The historical development of European and North American sewerage systems can be roughly summarized as follows:

- A growing awareness of squalor in the large cities and the consequent risks to health led to the construction of sewers that discharged raw wastes into rivers (in the mid-nineteenth century in London, for instance).
- This discharge of raw wastes yielded massive pollution and oxygen depletion in the rivers, which often became foul, open sewers.
- Various treatment technologies were developed to reduce the suspended load and the oxygen demand of the discharged wastes (for example, the UK Royal Commission on Sewage Disposal, 1899–1915, proposed effluent standards of <30 milligrams per liter for suspended solids and <20 milligrams per liter for biochemical oxygen demands, or BOD).
- In the 1950s and 1960s, a growing awareness of environmental problems, coupled with a now greatly increased population, led to tertiary treatment processes being introduced to protect receiving waters from further oxygen depletion, toxic substances, and eutrophication.
- At the same time it became clear that these sophisticated treatment technologies were not efficient at removing pathogenic microorganisms. Thus, in countries where environmental concern was acute (for example, the USA), or where effluents were commonly reused (for example, Israel), effluent chlorination was borrowed from the water treatment industry as a way of killing bacteria (and possibly viruses) in effluents. This technology, however, brought with it new and different environmental concerns.[10]

This highly simplified account illustrates the historical and conservative nature of the development of current waste treatment practices in industrialized countries. These practices are not especially clever, nor logical, nor completely effective—and it is not necessarily what would be done today if these same countries had the chance to start again.

Fluid retention times in conventional sewage works, oxidation ditches, and aerated lagoons treating domestic sewage are commonly less than 1, 3, and 6 days, respectively. Septic tanks typically have retentions of 1–3 days. These short retention times, in conjunction with temperatures that rarely exceed 35°C, allow high pathogen survivals, and the full range of excreted pathogens present in the raw sewage appears in the effluent. The sludges produced in conventional sewage works and oxidation ditches also contain the full range of excreted pathogens and require some form of treatment before disposal or reuse.

Conventional sewage works were originally developed in order to prevent gross organic pollution in European and North American rivers; they were never intended to achieve high removal of excreted pathogens. Their use in tropical countries in which excreted infections are endemic is only justifiable in special circumstances, for there is an alternative treatment process much superior in obtaining low survivals of excreted pathogens—the waste stabilization pond system.[11] Retention times commonly encountered in properly designed pond systems are >25 days, and this feature, in conjunction with such environmental factors as sunlight, high oxygen content and the presence of algal toxins, is responsible for the ability of pond systems to reduce greatly the survival of excreted pathogens. Indeed, protozoal cysts and helminth eggs and larvae can be completely eliminated from pond effluents. Pond systems have several more advantages over other treatment methods: they are the cheapest form of treatment, both to construct and operate, with minimal requirements for foreign exchange; their maintenance is very simple, requiring only unskilled labor; they are easily designed to achieve any required degree of treatment; and the algae produced in the ponds are a potentially valuable source of protein.[12]

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10. See chapter 6, the section "Effluent Chlorination."

11. Waste stabilization ponds are examined in more detail in the section of that title in chapter 6.

12. See the section "Reuse in Aquaculture" in chapter 7.

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Note added in proof

Recent work in Yorkshire, England (Oragui 1982) has led to the development of bacteriological methods for distinguishing between human and animal fecal pollution of waters. *Streptococcus bovis*, which can be enumerated in water samples by the method of Oragui and Mara (1981), appears to be excreted exclusively by animals, whereas sorbitol-fermenting strains of *Bifidobacterium adolescentis* and *B. breve* are only excreted by man. Enumeration media for both sorbitol-fermenting and total bifidobacteria are described by Oragui (1982). These methods for distinguishing between human and animal pollution are currently being evaluated in Mexico, Nigeria and Zimbabwe.

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