# 13

# Pathogenic and Nonpathogenic *Escherichia coli* and Other Bacterial Indicators of Fecal Pollution

THIS CHAPTER combines two distinct areas of knowledge. The first two sections ("Description of Pathogen and Disease" and "Control Measures") cover recent information on the role of certain types of *Escherichia coli* as major causes of acute diarrhea in many countries. Subsequent sections of the chapter briefly review the enormous compilation of literature on the fecal indicator bacteria, which have been used for 80 years as a measure of the degree of fecal contamination of the environment.

## Description of Pathogen and Disease

In the last 30 years, and especially in the last 10 years, it has become clear that various forms of *E. coli* are a major cause of diarrhea. This section briefly reviews *E. coli* diarrhea.

### *Identification*

Diarrhea produced by *E. coli* cannot be differentiated clinically from similar disease produced by other enteric pathogens. The spectrum of disease includes a cholera-like syndrome produced by enterotoxigenic organisms, a dysentery-like syndrome caused by enteroinvasive organisms, and many milder forms of diarrhea. Asymptomatic infection is very common. The severity of disease caused by the enterotoxigenic *E. coli* depends upon the degree of dehydration, and treatment is primarily by rehydration and electrolyte replacement—oral rehydration having proved very effective in most patients. Death rates of 5–10 percent may be experienced among untreated infants and children but are very low among those receiving rehydration therapy. Diagnosis of *E. coli* diarrhea is of limited clinical value and is, in any case, difficult because all patients are excreting large numbers of commensal *E. coli*, and the laboratory methods for identifying the suspected pathogens are complex and slow. The magnitude of the problem results from the fact that *E. coli* virulence factors are plasmid encoded and may be transmitted to many other Enterobacteriaceae.

### *Occurrence*

Gastroenteritis due to *E. coli* occurs in all parts of the world. Particular types of enterotoxigenic *E. coli* apparently cause infantile diarrhea in particular countries. It is thought that the acquisition of such infantile strains is one of the major causes of travelers' diarrhea. Enterotoxigenic *E. coli* appears to be a more important cause of diarrhea in developing countries than in developed countries. It may be that certain enteroinvasive strains, causing disease in adults, are also of restricted geographical distribution.

### *Infectious agents*

*E. coli* is a Gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae (figure 13-1). These organisms are usually thought of as lactose-fermenting saprophytes, in contrast with the non-lactose fermenting *Salmonella* spp. and *Shigella* spp. Lactose-fermenting *Salmonella* spp. and *Shigella* spp. do occur, however, and non-lactose-fermenting *Escherichia coli* may be common from some sources.

*E. coli* is a normal inhabitant of the intestinal tract of man and many other animal species. Conventional biochemical tests used in the identification of bacteria

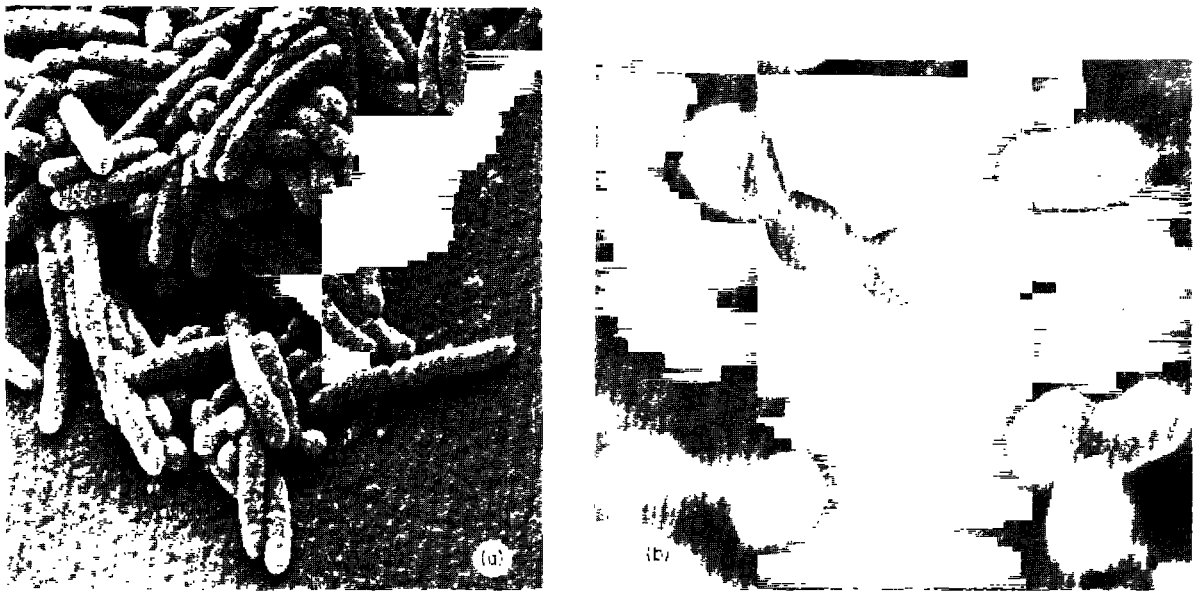

Figure 13-1. Escherichia coli *and* Streptococcus faecalis *under scanning electronmicroscopy.* (*a*) *E. coli.* Scale bar = 1 micrometer. Reproduced by permission of David Scharf 'Pile of *E. coli* cells', published in *Scientific American* Vol 237 No 1 July 1977 pp 22–23.) (*b*) *S. faecalis.* Scale bar = 1 micrometer. (Photo: N. J. Horan, Department of Civil Engineering, University of Leeds, Leeds, UK)

do not yield information that enables these saprophytic organisms to be differentiated from the pathogenic strains. Serological tests are more useful. Particular serological groups, distinguished by their somatic antigen (O antigen), are commonly associated with gastrointestinal disease. However, a particular strain cannot be assumed to be a pathogenic representative of a particular O group, unless a pathogenic mechanism (toxin production or invasiveness) can be demonstrated or epidemiological evidence links the strain to an outbreak.

It is valuable to distinguish between three different types of pathogen within the *E. coli* group, a description of which follows.

ENTEROTOXIGENIC *E. COLI* (ETEC). Enterotoxigenic strains of *E. coli* can be the cause of a cholera-like syndrome in infants, children, and adults. ETEC produce either a heat-labile enterotoxin (LT), serologically related to cholera enterotoxin, or a heat-stable enterotoxin (ST), which are structurally heterogeneous and may consist of LT complexed to endotoxin. Some strains produce both toxins. Action of LT is analogous to that of cholera toxin. Production of enterotoxin is controlled by extrachromosomal transferable DNA (plasmids). The ability to accept these plasmids may be enhanced by particular O group antigens but, although this may be important in nature, in the laboratory enterotoxin plasmids (ENT) can be transferred to nonpathogenic O groups. O groups particularly associated with toxigenicity are 6, 8, 15, 20, 25, 78, 115, 128, 148, 159.

The ability to cause disease depends not only on the production of enterotoxin but also upon the ability to colonize the intestine. Various colonization factors, or adhesins, have been described that enable the bacteria to attach to the small intestinal mucosa. These adhesins are plasmid controlled and are associated with hair-like protein structures on the bacterial cell, known as pili or fimbriae. There is now extensive evidence that the presence of one or more of three piliate bacterial antigens (K88, K99, and 987P) is required for successful colonization by ETEC of the small intestine of piglets and calves. More recent work has identified two pili, CFAI and CFAII, as the adhesins of functional importance in human infection. There is some degree of host specificity among adhesins: K88 is especially associated with piglet infections, and K99 is associated with calves and lambs.

ENTEROINVASIVE *E. COLI* (ETEC). Enteroinvasive *E. coli* produce disease by a mechanism similar to that of *Shigella* spp. These organisms invade the colonic mucosa and cause bloody diarrhea. The property seems to be restricted to a few O groups, O groups particularly implicated are 28, 112, 115, 124, 136, 143, 144, 147, 152, 164.

ENTEROPATHOGENIC *E. COLI* (EPEC). Organisms belonging to this group were first recognized as a result

of the serological examination of strains of *E. coli* isolated from outbreaks of diarrheal disease among infants. Though undoubtedly some enterotoxigenic and enteroinvasive strains have been included in this group, the pathogenic mechanism employed by most of these organisms is not known. These strains have been particularly associated with outbreaks of infantile gastroenteritis; they may, however, cause disease in adults. Experiments in adult volunteers have shown that nontoxigenic and noninvasive strains of *E. coli* isolated from epidemics are able to produce diarrhea O groups particularly implicated are 18, 20, 25, 26, 28, 44, 55, 86, 111, 112, 114, 119, 125, 126, 127, 128, 142.

*Reservoirs*

It seems likely that pathogenic *E. coli* is transmitted from man to man. Studies on infection in pigs and calves, when considered in the context of the problems that have been experienced in developing animal models of human infection, suggest a considerable degree of host specificity. Nonpathogenic or commensal *E. coli* are numerous in the gut of all warm-blooded animals and for this reason have been widely used as indicators of fecal pollution of the environment.

*Transmission*

In nursery outbreaks, the main route of transmission is by way of the hands of those nursing infected infants. It seems likely that fecal contamination of the environment, fomites, and hands constitute the primary means of transmission and infection both among children and adults. Water- and foodborne outbreaks have been described.

As with the other bacterial enteric pathogens (except *Shigella*), large numbers of ingested organisms are required to produce infection in healthy adults. Ferguson and June (1952) fed an EPEC serotype in milk to adult male prisoners in the USA. A dose of $6.5 \times 10^9$ produced moderate or severe diarrhea in seven of eleven volunteers, whereas a dose of $5.3 \times 10^8$ produced comparable symptoms in only one of twelve subjects. In subsequent experiments, in the same prison (June, Ferguson and Worfel 1953) with a different EPEC serotype, moderate or severe symptoms were produced by $1.6 \times 10^{10}$ organisms in three of eight volunteers, by $5.3 \times 10^9$ in one of eight, by $1.7 \times 10^9$ in one of seven, and by $1.4 \times 10^8$ in one of eight.

DuPont and others (1971) sought to infect adult male volunteers with ETEC and EIEC strains in milk. An ETEC strain associated with diarrhea in piglets failed to cause diarrhea in fourteen volunteers at doses of $10^6$–$10^{10}$. Two ETEC strains isolated from patients in Vietnam caused mild diarrhea (three watery stools in 24 hours) in three out of ten volunteers at a dose of $10^8$, and severe diarrhea (ten or more watery stools over 48 hours) in seven out of ten volunteers given a dose of $10^{10}$. Two EIEC strains at a dose of $10^8$ caused diarrhea (mild or severe) in eight of thirteen volunteers and dysentry in three of thirteen volunteers; at a dose of $10^6$ they caused diarrhea in one of fourteen volunteers; and at a dose of $10^4$ they caused no diarrhea in ten volunteers. However, when the $10^6$ dose of EIEC was preceded by 2 grams of sodium bicarbonate, severe diarrhea and dysentery were induced in two of three volunteers. These results suggest a median infective dose ($ID_{50}$) of around $10^9$ for the ETEC strains tested and an $ID_{50}$ of around $10^8$ for the EIEC strains tested. Those infected excreted $10^8$ EIEC per gram and $10^8$–$10^9$ ETEC per gram of feces.

*Incubation period*

Most reports suggest an incubation period of 6 to 72 hours.

*Period of communicability*

The organisms are excreted typically for 3–5 days, but sometimes for 2–3 weeks. Colonization of the intestine in which saprophytic *E. coli* is replaced by pathogenic *E. coli* can occur. Asymptomatic carriers of ETEC and EPEC have frequently been reported.

*Resistance*

The presence of the receptors for K88 antigen in the small intestine of the pig is genetically determined. Pigs lacking the receptors cannot be infected by ETEC. It seems likely that among human populations similarly unsusceptible individuals may occur. Over 50 percent of children have antibodies to common EPEC serotypes by the age of 1 year. These seem to confer resistance to infection. Adults are susceptible to strains they have not previously encountered. Neonates and infants are the most susceptible group, and breast feeding may confer some protection.

*Epidemiology*

The great importance of ETEC in childhood diarrhea in developing countries has only recently been recognized. Knowledge of which strains of *E. coli* cause diarrhea in which ways, and how these infections may

be diagnosed and categorized, is increasing rapidly. The newness and fluid state of the subject mean that understanding of *E. coli* epidemiology is limited and subject to continual revision. In addition, the laboratory techniques for declaring a particular organism to be ETEC (LT, ST, or LT + ST), EIEC, or EPEC are developing and are not entirely standardized among different laboratories. Much of the data produced by various surveys in the last five years are therefore not strictly comparable.

Several studies in different parts of the world have shown that *E. coli* is a major cause of diarrhea, especially among young children in poor communities. Sack and others (1975*a*) studied 59 Apache children, all under 5 years of age, hospitalized with acute watery diarrhea at Whiteriver (Arizona, USA). These patients had sixty-four episodes of diarrhea of which 9 percent were associated with ETEC, 11 percent with EPEC, 20 percent with *Shigella*, 3 percent with *Salmonella*, and 6 percent with ETEC plus another bacterial pathogen.

Guerrant and others (1975) studied forty infants and children (age 9 days to 10 years) admitted to the hospital in Florianópolis (Brazil) with diarrhea. A potential pathogen was isolated from thirty-one cases (78 percent). ETEC alone were isolated from twenty cases (50 percent), ETEC plus EIEC from five cases, ETEC plus *Salmonella* from two cases, EIEC alone from two cases, EIEC plus *Salmonella* from one case, and *Salmonella* alone from one case. Only one of twenty healthy controls was excreting ETEC.

Sebodo and others (1977) examined stool specimens from forty-one hospitalized children with acute diarrhea and sixteen healthy control children (all under 2 years old) in Central Java (Indonesia) in January and February. Among those with diarrhea, 15 percent excreted rotavirus, 12 percent excreted EPEC, 24 percent excreted ETEC, 2 percent excreted *Salmonella*, and 2 percent excreted *Shigella*. Among those without diarrhea, the equivalent percentages were 0, 19, 58, 0, and 0, respectively.

Freiji and others (1979) found that ETEC and EPEC accounted for around 6.6 percent of reported childhood diarrhea cases in the dry season (March–April), and around 14 percent in the wet season (May–June), in Addis Ababa (Ethiopia). The equivalent proportions for rotavirus were 11 and 34 percent in the dry and wet seasons, respectively.

Koornhof and others (1979) recorded in South Africa that 33 percent of 479 black and white children with diarrhea, mostly under 2 years of age, were excreting EPEC serotypes compared with only 15 percent of 498 age-matched healthy control children. Among those excreting EPEC, children with diarrhea excreted significantly greater numbers than healthy control children. ETEC were associated with only 10 percent of diarrhea cases, and no EIEC were recovered. EPEC appeared to be particularly prominent as a cause of diarrhea during the annual summer diarrhea peak.

In studies conducted prior to about 1970 it was common to fail to identify a known pathogen in the stools of approximately 70 percent of diarrhea cases (see, for instance, Gordon 1964). The enormous progress in diarrheal etiology is illustrated by studies in Bangladesh. One study investigated forty-eight patients with diarrhea admitted to Matlab hospital who did not have *V. cholerae*, *Salmonella*, or *Shigella* in their

Table 13-1. *Etiology of diarrhea reported to Matlab Hospital, Bangladesh, during 1977*

| | *All patients* | | *Patients under 5 years* |
|---|---|---|---|
| *Pathogen* | *Annual incidence per 1,000* | *Percentage with stated infection* | *Percentage with stated infection* |
| Enterotoxigenic *E. coli* | 8.1 | 25 | 25 |
| Rotavirus | 7.5 | 23 | 40 |
| *Vibrio cholerae* | 3.7 | 12 | 5 |
| Other vibrios | 3.1 | 9 | 5 |
| *Shigella* | 1.5 | 5 | 5 |
| *Salmonella* | | <1 | <1 |
| *Giardia lamblia* | | 2 | <1 |
| *Entamoeba histolytica* | | 3 | <1 |
| Mixed | | 6 | 10 |
| Unknown | | 15 | 10 |
| All diarrhea-causing | 30.3 | 100 | 100 |

*Note:* *Campylobacter* was not included as a possible cause of diarrhea.
*Source:* Adapted from Black and others (1979).

stools (Ryder and others 1976). Twenty-three percent had ETEC infection, and they were all over 2 years old; 29 percent had rotavirus infection, and they were all under 2 years old. ETEC was isolated from 2 percent (10 of 575) of healthy individuals in the community and from one out of thirty-nine contaminated water sources.

Black and others (1979) investigated 4,498 diarrhea cases reporting to Matlab hospital and were able to identify a pathogen in the stools of 85 percent of all cases, and in the stools of 90 percent of cases under 5 years old. Their results are reproduced in table 13-1. ETEC and rotavirus alone were associated with 48 percent of all reported diarrhea and 65 percent of diarrhea in children under 5 years. Children under 5 years constituted 57 percent of all diarrhea cases reporting to Matlab hospital. Table 13-1 also shows that the estimated incidence of reported ETEC diarrhea was over twice that of reported cholera, even though during 1977 the cholera incidence was nearly double the yearly average for that area. The incidence of reported ETEC diarrhea was around 8 times higher in children under 2 years than in all age groups over 4 years. ETEC showed a marked peak in August, compared with a cholera peak that year in September–October. Dehydration was moderate-to-severe in 33 percent of ETEC reported diarrhea cases and in 70 percent of reported cholera cases.

Further studies in Bangladesh (Black and others 1979) investigated family contacts of 82 index cases of diarrhea associated with ST- or ST + LT-producing ETEC of four serogroups (O6, O8, O78 and O115). Out of 446 family contacts, 53 (12 percent) became infected with the same serogroup and toxin type of ETEC as the index case within 10 days. Of the 53 infected contacts, 20 (38 percent) developed diarrhea. Among children under five years old, the proportion of contacts infected was 25 percent (21 of 84) and the proportion of infected individuals with diarrhea was 67 percent (14 of 21). Water sources used by households having index cases were investigated, and 10 percent (15 of 152) yielded ETEC of the same serogroup and toxin type as that infecting the index case, whereas only 0.7 percent (1 of 144) of sources used by control households were contaminated by ETEC. Ditch, tank, and canal waters were more often positive for ETEC than tubewell or river water, and 7 of the 15 ETEC-positive sources were used for drinking, whereas the remaining 8 were used for bathing and cooking. The proportions of other environmental and livestock samples containing ETEC, in case and control households respectively, were 0.8 percent (2 of 244) and 0.4 percent (1 of 242) of stored drinking water, 1.4 percent (4 of 287) and 0 percent (0 of 220) of food, 1.3 percent (3 of 237) and 0 percent (0 of 222) of heathy cows, and 0 percent (0 of 127) and 0 percent (0 of 100) of healthy goats. Contacts of index cases in houses having ETEC-positive drinking water or cooked food had a risk of infection 4.5 times that of contacts living in houses with no ETEC-positive environmental or animal specimens.

Some studies, however, have shown either that *E. coli* were not associated with a major proportion of diarrhea cases, or that the prevalence of pathogenic *E. coli* excretion among diarrhea patients was the same as that among healthy controls. Echeverria and others (1977) studied eighty infants and children (age 3 days to 4 years) with diarrhea seen at three hospitals in Taipei (Taiwan) during the summer. Fifty-six percent of cases were associated with rotavirus. The proportion of children excreting ETEC was low (7 percent) and was not significantly different from the proportion of healthy controls excreting ETEC. Three out of six sick children with bacteriological or serological evidence of LT-producing ETEC infection, and four out of five children with ST-producing ETEC, also had rotavirus infection.

Echeverria and others (1978*a*) studied eighty-two children (age 6 months to 13 years) with diarrhea in a hospital in Manila (Philippines). ETEC were isolated from 11 percent of these children and from 8 percent (4 of 49) of healthy controls. Rotavirus infection was associated with 17 percent of the diarrhea cases, *Salmonella* with 6 percent, *Shigella* with 1 percent, *Giardia* with 5 percent and *Entamoeba histolytica* with 2 percent. To determine the source of these infections, human, animal, and environmental samples were collected in the town of San Jose and examined for ETEC (Echeverria and others 1978*b*). Five out of 1,086 human fecal samples (individuals without diarrhea, ages not stated) were positive for ETEC (all LT-positive, ST-negative). ETEC were also isolated from two of twenty-eight pigs, one of ten water buffalo, zero of twenty-six pieces of beef, zero of twenty-five pieces of pork, zero of fifty-two vegetable samples and zero of forty-seven polluted water samples. The meat, vegetable, and water samples were contaminated with *E. coli*. None of the ETEC serotypes from livestock were the same as those from humans.

Spencer and others (1980) studied 156 cases of diarrhea occurring over a 1-year period among 2,400 people living in a rural, coastal area of El Salvador. Stool specimens were collected from all diarrhea cases and were compared with stools from healthy age- and sex-matched controls living nearby. ETEC were isolated as frequently from controls (13 percent) as from cases (12 percent). ETEC-producing LT were isolated more

frequently in children under 5 years among both cases and controls. ETEC isolations were more common among cases during April–June—the beginning and middle of the rainy season and the time of the annual diarrhea peak. Among controls, there was no clear seasonality of ETEC isolation. EPEC serotypes were isolated from 8 percent of cases and controls, and no EIEC were isolated. This study in a rural non-hospitalized population failed to show an association between ETEC or EPEC infections and diarrhea.

Although ETEC is a major cause of diarrhea in Bangladesh (table 13-1), Gilman and others (1980) reported that EIEC was not a major cause of dysentery. Of 132 nonamebic dysentery cases in Dacca, 81 percent excreted *Shigella*, 1 percent excreted *Vibrio parahaemolyticus*, and 1 percent excreted EIEC.

Although information is scanty, ETEC does not appear to be a major cause of diarrhea in many developed countries. Gangarosa (1978) concluded that ETEC was not readily transmitted in highly sanitized environments and had not become a significant problem in the USA despite multiple introductions by infected travelers returning from abroad (see below).

Transmission of ETEC and EPEC among small children and infants is clearly vigorous where hygiene is less than optimal. This is suggested by the very high age-specific incidences of infection and disease in children under 2 years old compared with older age groups and by serological surveys showing that a high proportion of children have antibodies to particular forms of *E. coli* or their toxins. Studies among the Apache at Whiteriver (Arizona, USA) showed widespread exposure to LT among small children (Sack and others 1975*b*).

Travelers' diarrhea has attracted increased attention recently, and it is now clear that a substantial proportion of cases are caused by ETEC. An adult visitor to a country, especially an individual from a developed country visiting a developing country, may be immunologically unprepared for the strains of *E. coli* that will be encountered. Kudoh and others (1979) studied 320 Japanese travelers returning to Tokyo with acute diarrhea. The following pathogens were associated with various percentages of the diarrhea cases: ETEC 30 percent, EIEC 1 percent, EPEC 1 percent, *Shigella* 6 percent, *Salmonella* 12 percent, *V. parahaemolyticus* 7 percent, and non-O1 *V. cholerae* 2 percent. Of the 95 individuals with ETEC infection, 20 also had other bacterial infections, most commonly salmonellosis. Echeverria and others (1979) reported that ETEC was the most common identifiable enteric pathogen among Americans with diarrhea at Clark Air Force Base in the Philippines.

Sack and others (1978) studied thirty-nine Peace Corps volunteers during the first few weeks of their work in Kenya. Eighteen of the volunteers were given a daily dose of 100 milligrams of doxycycline (a derivative of tetracycline) as a prophylactic, and only one (6 percent) of this group developed diarrhea—caused by an antibiotic-resistant strain of *Shigella sonnei*. The remaining twenty-one volunteers took a placebo, and 62 percent of them developed diarrhea during their first 4 weeks in Kenya. No salmonellae or vibrios were recovered from the placebo group, but ETEC was isolated from 57 percent (8 of 14) of individuals with diarrhea and from 57 percent (4 of 7) of those without. There were no ETEC infections among the doxycycline group.

Merson and others (1976) conducted a prospective study of 73 physicians and 48 family members (94 percent of whom were from the USA) attending the Fifth World Congress of Gastroenterology in Mexico City (Mexico) in October 1974. Of the 121 participants, 59 (49 percent) had travelers' diarrhea (51 percent of physicians and 45 percent of spouses). All pre-Mexico stool specimens were negative for bacterial pathogens. Subsequently, a pathogen was isolated from 63 percent of those with diarrhea and 21 percent of those without. ETEC was isolated from 45 percent of those with diarrhea and 7 percent of those without. In 81 percent of those with ETEC and diarrhea, ETEC was the only pathogen found. Other potential pathogens isolated from participants with diarrhea were: EIEC 4 percent, *Salmonella* 16 percent, *Shigella* 4 percent, *V. parahaemolyticus* 2 percent, *Giardia* 2 percent, and rotavirus 4 percent (there were several multiple infections). Occurrence of illness in one spouse did not increase the risk of illness in the other, and only one ill couple had the same serotype of ETEC. Illness was not associated with the consumption of water or iced beverages, but ETEC infection was associated with the consumption of salads containing raw vegetables. Nineteen percent of those with diarrhea were confined to bed. The authors point out that if these figures apply to the 3 million annual visitors from USA to Mexico (a conservative assumption, since many of the 3 million visitors are not physicians and do not stay at major hotels), then there may be 1.5 million cases of travelers' diarrhea per year among these visitors, of which 300,000 may be bedridden.

Despite dramatic figures of this kind, the problem of travelers' diarrhea is trivial compared with that of *E. coli* diarrhea among infants and children in developing countries, and deaths from travelers' diarrhea are extremely rare. Some of the recent comments (for instance, Lee and Kean 1978) concerning the

relationship between travelers' diarrhea and the economics of tourism—and the value to the general population of improving the hygiene of the hotel, restaurant, and food and beverage industries—are somewhat naïve and have led to excessive emphasis being given to travelers' diarrhea by some international development agencies.

Food is a likely route for the transmission of pathogenic *E. coli*. Barrell and Rowland (1979*a*) found that *E. coli* could be isolated from 35 percent of freshly prepared food samples in the Gambian wet season (June–October) and from 7 percent in the dry season. When food that had been stored for 8 hours or more was sampled, the percentages were 85 in the wet season and 59 in the dry season. No serotyping or toxin assays were reported. Sack and others (1977) examined 240 strains of *E. coli*, of non-EPEC serotypes, isolated from food samples collected throughout the USA and not known to be associated with diarrheal disease. Five percent of isolates produced LT alone, 6 percent produced ST alone, and 3 percent produced LT and ST—a total ETEC proportion of 14 percent (see also Mehlman and others 1976).

Polluted water is also a likely vehicle for *E. coli* transmission. Sack and others (1975*a*) examined eighteen water sources used by Apaches at Whiteriver (Arizona, USA). The water sources contained 200–300 coliforms per 100 milliliters; out of forty-seven *E. coli* isolates, three strains (6 percent) were toxin producing. Freij and others (1979) tested river water and wellwater around Addis Ababa (Ethiopia) and found ETEC in 55 percent and 14 percent of samples from the two respective sources, EPEC in 70 percent and 53 percent of samples, and EIEC in 10 percent and 20 percent of samples.

Rosenberg and others (1977) investigated an outbreak of diarrhea at Crater Lake National Park (Oregon, USA) in June 1975. Illness (defined as diarrhea or vomiting) was reported by 90 percent (288 of 320) of resident park staff, by 64 percent (68 of 107) of visitors contacted by phone, and by 44 percent (2,310 of 5,273) of visitors contacted by mail. Illness was significantly associated with drinking the park water supply, and the duration of illness increased as reported daily water consumption increased. The park water supply came from a shallow spring and was chlorinated before distribution. There was no systematic monitoring of chlorine in the supply, and dye tests showed the spring to be contaminated from an overflowing sewer above it. A single ETEC serotype (O6:H16, LT- and ST-positive) was isolated from 43 percent (17 of 40) of active diarrhea cases and from no (0 of 71) individuals who had not had diarrhea during the previous 4 days. No other viral, bacterial, or parasitic pathogen was recovered from ill or well persons. Of 396 *E. coli* isolates from the water supply, 14 (3.5 percent) were ETEC, and all of these were the same serotype as found in the diarrheal stools.

## Control Measures

Awareness that *E. coli* is a major cause of diarrhea is so recent, and understanding of *E. coli* epidemiology so partial, that few definitive statements about control can be made. Most of what can be said is based on the assumptions that, first, *E. coli* transmission is broadly similar to that of other anthroponotic, endemic bacterial agents of diarrhea (such as *Shigella*), and, second, that the information concerning *E. coli* in the environment reviewed below applies to ETEC, EPEC and EIEC as well as to nonpathogenic *E. coli*.

### *Individual*

Chemoprophylaxis with antibiotics or intestinal antiseptics is not to be encouraged. Antibiotic-resistance plasmids that may also code for virulence factors are present in the *E. coli* population in man and animals, and use of antibiotics encourages their spread. Merson and others (1976), studying travelers' diarrhea in Mexico, recorded that 33 percent of ETEC isolates showed multiple resistance to antibiotics. Kudoh and others (1979), studying travelers' diarrhea among Japanese, recorded that 38 percent of ETEC isolates had resistance to 1 or more antibiotics. Resistance to streptomycin and tetracycline was particularly common in both studies. However, there is some evidence that the prevalence of antibiotic resistance among ETEC strains is lower than among the *E. coli* population at large (Sack and others 1978).

Considerable success has been achieved in protecting suckling piglets and calves against fatal diarrhea by vaccinating their mothers with products containing K88, K99, and 987P adhesins. There is currently considerable research activity directed toward identifying the most appropriate preventive antigens for ETEC in man, and subsequently developing an oral vaccine. Prospects are reasonably good for an ETEC vaccine but are remote for a vaccine for EPEC and EIEC.

Scrupulous personal hygiene, and caution over choice and use of water and food, are probably the most effective protective methods for adults. Breast feeding appears to give some protection to infants.

### *Environmental*

Human feces, most probably from children and infected with EPEC, ETEC, or EIEC, are the major source

of infection. Control in the community therefore rests upon good water quality, adequate personal and domestic cleanliness (which will often require improved water availability), and hygienic excreta disposal. The hygiene of infants and young children—and those who handle, clean, and feed them—are of paramount importance.

## Fecal Indicator Bacteria

The previous sections of this chapter have dealt with that small subgroup of all *E. coli* which are able to cause diarrhea. The remainder of the chapter deals with the occurrence and survival of the fecal indicator bacteria in the environment. These fecal indicator bacteria include the coliforms, the fecal coliforms, *E. coli*, fecal streptococci, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Bifidobacterium*, *Bacteroides*, and other bacteria that are excreted in large numbers by healthy warm-blooded animals and that are not normally enteric pathogens. These indicator bacteria, and the concepts underlying their use as measures of the fecal contamination of the environment, are discussed in chapter 4 of Part One. In the remainder of this chapter, the most emphasis is given to coliforms, fecal coliforms, *E. coli*, and fecal streptococci because these are the excreted bacteria for which most environmental data exist and the ones which are most commonly used today as fecal indicators.

Very little information yet exists on the occurrence of pathogenic strains of *E. coli* in the environment, and little is known of their abilities to survive or multiply in extraintestinal settings. A few reports of ETEC isolations from water and food have been reviewed in the section above on epidemiology. For the time being, therefore, it must be assumed that the behavior of ETEC, EPEC, and EIEC in the environment is identical to that of the general *E. coli* population. Research currently under way, or being planned, will reveal the degree to which this assumption is valid.

## Occurrence and Survival in the Environment

Because the fecal coliforms and fecal streptococci are excreted by all warm-blooded animals, they are widespread in the environment wherever animal life is present. In addition, because the coliforms have been the main indicator of fecal pollution for over 60 years, there is more accumulated information on their presence and behavior in the environment than for any other excreted organism discussed in this book. Space only permits that a brief summary of the massive literature on coliforms in the environment, dating back to the end of the last century, can be given.

It must be stressed that the source of fecal coliforms and fecal streptococci in the environment is both humans and other animals. In an area populated mainly by humans (for instance, many urban areas), most enteric bacteria present may derive from man. However, in many rural areas the dominant source of indicator bacteria is domestic animals. An adult person may excrete only 150 grams of feces per day, but a pig may excrete 1.2 kilograms, and a cow may excrete 15–20 kilograms of feces. A hen may excrete almost the same weight of feces per day as a man. Feces from all these sources may contain comparable numbers of indicator bacteria per gram.

### *In surface waters*

Rain and snow generally contain no fecal bacteria. Geldreich (1978) collected rainwater in Cincinnati (Ohio, USA) and found no fecal coliforms or fecal streptococci in forty-six of forty-nine samples. Fallen precipitation, however, becomes stormwater or overland flow and may pick up a considerable load of fecal bacteria before it reaches a stream or soaks into the ground (Geldreich and others 1968). In urban areas, stormwater becomes contaminated by human and animal feces, and in some towns also by sewage, which may collect in drains and pools. Geldreich (1978) cited estimates that 70,000 kilograms of dog feces are deposited in the streets and parks of New York City (USA) every day. He also reported (1978) up to $10^5$ fecal coliforms and fecal streptococci per 100 milliliters of stormwater in suburban Cincinnati. Olivieri, Kawata and Krusė (1978) found that stormwater in Baltimore (Maryland, USA) contained indicator concentrations similar to weak raw sewage, with geometric mean values for fecal coliforms and fecal streptococci being generally over $10^5$ per 100 milliliters.

Stormwater run-off in rural areas may also collect large concentrations of indicator bacteria from animal feces and septic tank discharges (Dudley and Karr 1979). Typically, concentrations of fecal coliforms and fecal streptococci in stormwater are somewhat lower in rural catchments than in urban areas (Davis 1979), but this is not the case where manure, sewage effluent, or sludge is being applied to fields (Dunigan and Dick

1980; Evans and Owens 1972) or where the catchment supports intensive livestock farming (Geldreich 1976). For example, in the highlands of Papua New Guinea the large herds of domestic pigs (Feachem 1973), and the tendency of these pigs to pass the day foraging near streams, caused elevated fecal coliform and fecal streptococci concentrations in streams and rivers used for domestic purposes (table 13-2; Feachem 1974).

Major rivers flowing through populated areas always contain a considerable concentration of fecal indicator bacteria. Berg and Metcalf (1978) reported that the Seine (France) contained up to $5 \times 10^4$, the Missouri (USA) $4 \times 10^4$, and the Mississippi (USA) $3 \times 10^5$ fecal coliforms per 100 milliliters. Poynter and Stevens (1975) reported that water supply intake points on the Thames (UK) contained up to $3.5 \times 10^4$ *E. coli* per 100 milliliters. Evison and James (1973) found up to $10^5$ fecal coliforms and up to $10^4$ fecal streptococci per 100 milliliters of Nairobi River (Kenya) water, and Petr (1980) recorded over $10^4$ fecal coliforms per 100 milliliters in some sections of the Purari River (Papua New Guinea). Saleh (1980*a*, 1980*b*) found up to $10^3$ fecal streptococci per 100 milliliters in the Nile at Cairo (Egypt).

There is a growing tendency in Europe and North America to set indicator bacterial standards for body-contact recreational waters. A commonly adopted standard in the United States is less than 200 fecal coliforms per 100 milliliters (Cabelli 1978; Train 1979), and the European Economic Community has proposed a guideline of less than 100 fecal coliforms or fecal streptococci per 100 milliliters and a mandatory limit of 2,000 fecal coliforms per 100 milliliters. These standards for bathing water remain a controversial subject, and there is no clear consensus of opinion among regulatory authorities or individual experts (Evison 1979). Very few developing countries have standards of this kind, and most governments take the very reasonable view that, with the exception of schistosomiasis (see chapter 32), the risk of disease related to recreational bathing comes very low on the list of national health priorities.

Early studies on coliform die-off in water (see the appendixes to Feachem and others 1980) reported the time required for the bacterial population to become undetectable. These data are of limited value because a small proportion of organisms will be especially hardy and because the time over which residual numbers can be detected is very dependent upon the isolation technique being employed. Therefore, most recent work has concentrated on the rate of die-off that may be expressed as the time required for a given level of reduction; for instance, the time required for a 90 percent reduction ($t_{90}$) or the time required for a 50 percent reduction (half life, or $t_{50}$).[1]

Chamberlin and Mitchell (1978) and Mitchell and Chamberlin (1978) reviewed the literature on coliform decay rates in streams and found that $t_{90}$ values ranged from 20 to 115 hours, with a median value of about 60 hours. Chamberlin and Mitchell (1978) went on to analyze physical, chemical, and biological influences on coliform death rates and concluded that solar radiation is the dominant lethal factor (see also Chojnowski, Mancini and Jeris 1979).

Zanoni and others (1978) found a $t_{90}$ value for coliforms in Lake Michigan (USA) in July (water temperature 15°C) of only 6 hours. Dutka and Kwan (1980) reported $t_{90}$ values of 23–720 hours for *E. coli*, 19–360 hours for *Streptococcus faecalis*, and 19–180 hours for *Salmonella thompson* in Lake Ontario (Canada) at 17–19°C. Poynter and Stevens (1975) recorded $t_{90}$ values of 88 hours for *E. coli* and 96 hours for fecal streptococci in reservoir water stored at 15°C. McFeters and others (1974) found that the $t_{50}$ times for coliforms and fecal streptococci in well water at 9–12°C were around 20 hours, which was similar to times for shigellae and some salmonellae, but considerably

1. It has been often found experimentally, and it is usually assumed, that bacterial survival in water follows an exponential curve; that is, that the probability of a bacterium dying in a given time interval is independent of its age. In other words, the reduction in bacterial concentration in water follows a first-order equation of the form:

$$\frac{dC}{dt} = -kC,$$

where $C$ = concentration of bacteria per volume of water (say, organisms per 100 milliliters) at time $t$
$t$ = time (say, hours)
$k$ = first order decay or die-off rate constant (expressed as reciprocal of units of $t$; say per hour).

Integrating:

$$C = C_0 e^{-kt}$$

where $C_0$ = concentration of bacteria at $t = 0$.

Changing to base 10 and rearranging:

$$k = \frac{2.3}{t} \log_{10} \frac{C_0}{C}$$

If $C = 0.1C_0$, then:

$$k = \frac{2.3}{t_{90}}$$

In much of the literature, death rates are expressed as $k$, in hourly or daily units, rather than as $t_{90}$ values. The significance of $t_{90}$ is that it is the time for reduction to a fraction of 1/10 of the starting population, or a 1 log reduction. Since large changes in bacterial populations are best handled in logarithmic terms, it is of particular convenience. Times required for greater reductions may be readily calculated: $t_{99} = 2 \times t_{90}$, $t_{99.9} = 3 \times t_{90}$, and so forth.

Table 13-2. *Some reported concentrations of fecal bacteria in untreated domestic water sources in developing countries*

| *Country* | *Water source* | *Fecal coliforms per 100 milliliters* | *Fecal streptococci per 100 milliliters*[a] | *Source* |
|---|---|---|---|---|
| Gambia | Open hand-dug wells, 15–18 meters deep | Up to 100,000 | ND | Barrell and Rowland (1979*b*) |
| Indonesia | Canals in central Jakarta | 3,100–3,100,000 | ND | Gracey and others (1979) |
| Kenya | Springs | 0 | 0 | Evison and James (1973) |
| | Dam | 0–2 | 0–14 | |
| | Waterhole | 11–350 | 50–90 | |
| | Large river | 10–100,000 | 10–10,000 | |
| Lesotho | Unprotected springs | 900 | 1,700 | Feachem and others (1978) |
| | Waterholes | 860 | 1,610 | |
| | Small dams | 260 | 360 | |
| | Streams | 5,000 | 4,100 | |
| | Protected springs | 200 | 250 | |
| | Tap water from springs | 9 | 29 | |
| | Tap water from bored holes | 1 | 10 | |
| Nigeria | Ponds | 1,300–1,900 | 1,300–3,900 | Essien and Osuhor (1979) |
| | Open hand-dug wells | 200–580 | 180–630 | |
| | Tap water from bored hole | Up to 35 | Up to 6 | |
| | Ponds | 4,000,000[b] | ND | Tomkins and others (1978) |
| | Open hand-dug wells, 6–12 meters deep | 50,000[b] | ND | |
| | Stored in home | 100[b] | ND | |
| Papua New Guinea | Streams | 0–10,000 | 0–4,000 | Feachem (1974) |
| Tanzania | Rainwater | 3 | 13 | Brokunsult and Ross Institute (1978) |
| | Waterholes | 61 | 974 | |
| | Ponds | 163 | 590 | |
| | Streams | 128 | 293 | |
| | Unprotected springs | 20 | 58 | |
| | Protected springs | 15 | 40 | |
| | Open wells | 343 | 1,761 | |
| | Protected wells | 7 | 33 | |
| | Bored holes | 1 | 11 | |
| | Treated tap water | 3 | 13 | |
| Uganda | Rivers | 500–8,000 | ND | White, Bradley and White (1972) |
| | Streams | 2–1,000 | ND | |
| | Unprotected springs | 0–2,000 | ND | |
| | Protected springs | 0–200 | ND | |
| | Hand-dug wells | 8–200 | ND | |
| | Bored holes | 0–60 | ND | |

ND No data.

*Note:* These figures are not necessarily typical of the domestic water quality in the countries concerned. They are measurements taken from selected sources during specific investigations. It is generally true, however, that people in developing countries who must use surface sources or open wells are often drinking water with $>10^3$ fecal coliforms per 100 milliliters.

a. When only a single value is given, it is a geometric mean.

b. Total coliforms rather than fecal coliforms.

longer than those for *Vibrio cholerae* and other salmonellae.

Waters with little or no microbial life will sustain indicator bacteria for considerably longer than similar waters with an active flora and fauna. Geldreich (1976) reported that the $t_{90}$ for fecal coliforms in filtered stormwater was 40 hours at 20°C and 230 hours at 10°C. Gallagher and Spino (1968) found that the $t_{90}$ for fecal coliforms in filtered streamwater was 168 hours at 20°C.

As with all the microbial survival data discussed in this book, temperature is a crucial factor. Mancini (1978) used published data to compute a relationship between decay rate and temperature in fresh water. The results showed a $t_{90}$ of about 120 hours at 0°C, falling to about 15 hours at 30°C. Evison and James (1973) found that indicator bacteria in sewage effluent were reduced by 96.5 percent within 5 miles following discharge into the Nairobi River (Kenya; temperature 18.5°C), whereas only a 56 percent reduction took place over an equivalent distance in the River Tees (UK; temperature 2°C). Davenport, Sparrow and Gordon (1976) found fecal coliform and fecal streptococci reductions of only 84 and 67 percent, respectively, after 170 hours of travel under the ice of the frozen Tanana River (Alaska, USA), whereas a similar travel time in a tropical river might cause a reduction of over six log units assuming that there were no additional inputs of fecal pollution.

Many investigators have found that total coliform and fecal coliform decay rates are similar, whereas fecal streptococci often persist for longer (for instance, Cohen and Shuval 1973; Poynter and Stevens 1975).[2] *Str. bovis*, and to a lesser extent *Str. equinus*, however, die-off considerably faster than fecal coliforms and other species of fecal streptococci (Geldreich 1976; Geldreich and Kenner 1969; Guy and Small 1977; McFeters and others 1974). Therefore, because *Str. bovis* and *Str. equinus* are the dominant streptococcal species in some animal feces but never in human feces, it is sometimes the case that in stored samples containing mainly human fecal pollution the fecal coliform to fecal streptococci ratio falls over time, whereas when nonhuman pollution predominates the ratio may rise (Feachem 1975; see chapter 4).

As a general rule indicator bacteria die in fresh water, and the warmer the temperature the higher the death rate. Death rates are also higher in natural waters with an active biological population, than in sterilized, filtered, or other "dead" waters (Poynter and Stevens 1975). Under certain special conditions, however, growth of indicator bacteria may occur. The growth phase is usually of limited duration and is especially likely where nutrient levels are high, temperatures are warm, but overall microbial and zoological activity is low. Thus chlorinated effluents sometimes provide suitable growth environments for indicator bacteria (see the section below on effluent chlorination). Hendricks (1972) found that *E. coli* would grow at 30°C in autoclaved river water collected downstream of a sewage outfall, but not at 20°C or 5°C and not in autoclaved water collected upstream of the outfall (see also Gorden and Fliermans 1978; Hendricks 1971; McFeters, Stuart and Olson 1978).

Coliform growth is more likely than fecal coliform growth, which is more likely than fecal streptococcal growth (see, for instance, Allen, Pasley and Pierce 1952). The growth of indicator bacteria is more likely than the growth of pathogenic bacteria. This latter fact seriously reduces the value of the indicator bacteria as indicators of pathogenic microbes in situations where growth is possible or suspected; also, the human excreted viruses (chapters 9–11) can *never* increase in numbers in the aquatic environment.

### *In groundwater*

The microbiological quality of groundwater is becoming an increasing cause for concern worldwide as greater use is made of limited groundwater resources and as the practice of disposing of fecal wastes in on-site sanitation systems or by land application becomes more common. The potential for groundwater contamination depends upon a complex of factors including the rainfall, the rate of groundwater abstraction, groundwater depth and flow patterns, the method of waste disposal, and the type, texture, and depth of the overlying soil or rock. Marzouk, Goyal and Gerba (1980) reported on the quality of ninety-nine groundwater samples in Israel. Measures of fecal coliforms per 100 milliliters were range $0–2 \times 10^4$, mean $10^3$, and median 0. Measures of fecal streptococci per 100 milliliters were range $0–10^4$, mean 300, and median 0. Tjostem and others (1977) found low levels of coliform contamination in groundwater pumped from a limestone aquifer in northeastern Iowa (USA). Below the limestone, and separated from it by a shale band, was a sandstone aquifer. Water pumped from the sandstone in uncased wells was also contaminated, but

2. A recent report, however, suggested that *Str. faecalis* survived for a shorter period in Canadian lake water than *E. coli* (Dutka and Kwan 1980). There is probably considerable inter- and intra-species variation in survival ability, and studies on mixed populations of fecal streptococci and *E. coli* cannot be compared directly with studies on the survival of single laboratory-maintained strains.

wells that were fully cased and grouted through the limestone into the sandstone produced relatively unpolluted water. This illustrates an important general principle—that unpolluted groundwater can often be obtained from below polluted shallow aquifers if carefully designed abstraction technologies are employed.

Over the past 60 years, studies have been conducted to determine the risks of shallow groundwater pollution from pit latrines and septic tank soakage systems. Stiles and Crohurst (1923) recorded the movement of *E. coli* for 20 meters horizontally through fine sand in the direction of groundwater flow. In an early study in Singapore (Yeager 1929) to investigate the required separation of bored hole latrines from shallow unprotected wells, it was found that coliforms traveled for more than 23 meters but less than 31 meters through very permeable soils. Similar studies in West Bengal (India) showed that wells located 1.6 meters from a bored hole latrine (dug in alluvial sandy loam with a percolation rate of about 56 meters per day) became heavily contaminated with fecal coliforms, whereas most wells located 3.3 meters distant remained uncontaminated (Dyer, Bhaskaran and Sekar 1945). Further information on the ability of excreta disposal systems, especially septic tank drainfields, to pollute groundwater is reviewed below in the sections on septic tanks and land treatment.

Groundwater pollution by fecal coliforms and fecal streptococci may also be due to the deliberate recharge of sewage effluents to groundwater. Vaughn and others (1978) studied three sewage recharge installations on Long Island (New York, USA). At site 1, the chlorinated effluent contained up to $2.4 \times 10^6$ fecal coliforms per 100 milliliters, and the groundwater (9 meters below the recharge basins) contained up to 150 fecal coliforms per 100 milliliters. At site 2, the chlorinated effluent contained up to $4.3 \times 10^5$ fecal coliforms per 100 milliliters, and the groundwater (24 meters below the recharge basins) contained up to 930 fecal coliforms per 100 milliliters. At site 3, the tertiary effluent contained up to $9.3 \times 10^5$ fecal coliforms per 100 milliliters, and the groundwater (5.5 meters below the recharge basins) contained up to 150 fecal coliforms per 100 milliliters at a point 46 meters horizontally downslope from the recharge site. Slade and Edworthy (1981) isolated up to $1.2 \times 10^5$ *E. coli* and fecal streptococci per 100 milliliters of groundwater from a chalk aquifer directly below groundwater recharge lagoons receiving raw comminuted sewage. Bacteriological aspects of groundwater recharge in Israel are summarized by Goldshmid (1974). Groundwater contamination may also result from seepage through the base of waste stabilization ponds (see, for instance, Ciravolo and others 1979).

There are few data available on the survival of indicator bacteria in groundwater. It may be anticipated that survival will be for longer than in must surface waters because of the absence of sunlight, cool temperatures, and a low level of microbial and biological activity. Kudryavtseva (1972) reported that coliforms introduced into saturated alluvial sands in the USSR during the summer survived for up to 3.5 months. A pathogenic serotype of *E. coli* similarly inoculated into the groundwater survived for 3 months. In groundwater samples returned to the laboratory and stored in darkness, coliforms survived for up to 5.5 months, and pathogenic *E. coli* survived for up to 4 months.

These and other data (Allen 1979) show that whether or not on-site soakage or land application cause bacterial pollution of the groundwater depends on numerous site-specific factors. Where soils are of fine or medium texture, unsaturated, and more than 1 meter deep, little or no bacterial contamination of the underlying aquifer may occur. Where wastewater can drain down through "macropores" (such as root channels, structural voids, rodent burrows, solution channels or fissures) the groundwater may become significantly polluted with fecal coliforms and fecal streptococci. Even in this latter case, however, the enteric bacterial concentrations in groundwater are likely to be far less than in surface waters in the same location and will be readily eliminated by any water treatment process including chlorination. Even where untreated waters are being used for domestic purposes, contaminated groundwater will usually pose a lesser health risk than available surface water.

### *In drinking water*

Treated and chlorinated drinking water should contain no fecal indicator bacteria. Most people in developing countries, however, drink unchlorinated and untreated water. In cases where this water derives from protected groundwater, or upland surface water sources, it may be of moderately good quality (say $< 100$ fecal coliforms per 100 milliliters). In other cases the water used may be highly polluted and, on occasions, has an indicator bacteria concentration similar to that of a weak raw sewage. In table 13-2, some information on the pollution of drinking water sources in developing countries is summarized.

This problem of fecally polluted drinking water is by no means restricted to the developing countries. Sandhu, Warren and Nelson (1979) reported that

around 60 percent of people in three counties in South Carolina (USA) were served by wells, springs, and other private water sources. In one county, private water sources had a mean *E. coli* count of $1.4 \times 10^6$ per 100 milliliters and a mean fecal streptococci count of $2.6 \times 10^6$ per 100 milliliters. It was concluded that defective septic tank systems were responsible for most of the fecal contamination.

The question of bacteriological standards for drinking water remains a subject of considerable debate. In countries where all, or nearly all, the population drink treated piped water it is reasonable and correct to stipulate that no coliforms or other indicator bacteria should be detected in tap water. Failure to meet this standard indicates a malfunction of the treatment plant (especially of the chlorination unit) or an inflow of pollution through a damaged section of the distribution system, which should be immediately investigated and rectified. In developing countries, however, the great majority of the population drink water that is untreated, either from improved but untreated supplies (such as handpumps) or from unimproved supplies (such as ponds). This water, as indicated in table 13-2, may be grossly polluted, and it is pointless for the government of such a country to require that all water supplies contain no fecal coliforms. At the best such a ruling will simply be ignored and thus bring similar regulations into disrepute; at the worst it may force people to abandon improved but lightly contaminated supplies in favor of the only alternative, which may be unimproved and heavily polluted supplies. For example, there have been cases where overzealous health officials have closed down contaminated shallow tubewells in a village because the wells were found to contain 50 fecal coliforms per 100 milliliters and have thus forced the villagers to use polluted irrigation canals containing $10^4$ fecal coliforms per 100 milliliters.

The World Health Organization (WHO) has generally advocated standards or guidelines for small untreated water supplies that stipulate less than 10 coliforms and zero fecal coliforms per 100 milliliters (WHO 1971). These recommendations have been questioned by those primarily concerned with water supplies in developing countries on the grounds that they are too stringent (for instance, see Feachem 1977). Even if great attention is paid to selecting the purest available water source and distributing the water through a well-designed and well-maintained system, it will not in general be possible to meet a zero fecal coliform standard without incorporating chlorination. Well-designed untreated spring supplies, for instance, will typically contain up to about 25 fecal coliforms per 100 milliliters, and this level of contamination can only be removed by adding a chlorination unit. Therefore, to set a zero fecal coliform standard is equivalent to requiring that all water supplies be chlorinated (at least). Many developing countries, however, have decided to adopt a flexible policy toward water treatment and are installing numerous spring, well, or upland stream supplies that have no treatment processes. The advantage of designing a supply without treatment is that it is somewhat cheaper, and much easier to operate and maintain, than a similar supply with treatment (for instance, slow sand filtration and chlorination). For many developing countries, therefore, a zero fecal coliform standard is inappropriate. A preferable approach is to set flexible quality goals that can be changed as the water supply sector progresses. To install an improved supply providing water with up to 50 fecal coliforms per 100 milliliters, for instance, is a great advance when many people in the same country may be drinking water containing over $10^3$ fecal coliforms per 100 milliliters.

The effect collecting, carrying, and storing water have on bacteriological water quality has attracted increasing concern in recent years. Clearly, there is less purpose in supplying good quality water at a public tap if it is to become subsequently polluted prior to use. If water is collected in clean vessels and stored in such a way that polluting material cannot enter, water quality is likely to improve—as suggested by the data from Malumfashi (Nigeria) presented by Tomkins and others (1978) and summarized in table 13-2. It may be more usual, however, for water quality to deteriorate between collection and use because the water collection vessels are contaminated and the water is stored in the home in such a way that it can be further contaminated by children and animals. Studies in Lesotho (Feachem and others 1978) showed that clean water collected from a handpump (0–6 fecal coliforms and 0–1 fecal streptococci per 100 milliliters) could become considerably contaminated before use (maximum of 1,340 fecal coliforms and 4,280 fecal streptococci per 100 milliliters). Similarly, Oluwande (1980) reported that public tap water in Western State (Nigeria) contained 0–3 coliforms and 0 fecal coliforms per 100 milliliters, whereas stored water in homes contained 0–1,800 coliforms and 0–10 fecal coliforms per 100 milliliters. The epidemiological significance of water pollution occurring after collection is different from that of pollution of the water source. The first type of pollution promotes intrafamilial disease transmission, whereas the second allows the spread of infection throughout a community using a common source.

In concluding this section on indicator bacteria in drinking water, a note of caution must be sounded about the validity of the standard tests for fecal coliforms, which were developed in Europe and North America, when they are applied to tropical waters. Some workers studying water pollution in upland tropical areas, where surface water temperatures are not greatly higher than in temperature zones, have obtained satisfactory results using standard methods for enumerating fecal coliforms [for instance, Feachem (1974) in the highlands of Papua New Guinea and White, Bradley and White (1972) in Uganda]. Other studies in the tropics [for instance, Banerjea and Sen (1940) and Raghavachari and Iyer (1940) in India; Boizot (1941) in Singapore; Evison and James (1973) in Kenya; Katugampola and Assim (1958) in Sri Lanka; and Moussa (1965) in Egypt] have detected a considerable proportion of coliforms of probable nonfecal origin that have the ability to ferment lactose at 44°C. In this respect they mimic the truly fecal coliforms and are thus able to give false positive reactions on standard fecal coliform tests. Recent water testing in the Gambia (Barrell and Rowland 1979*b*) and Tanzania (Brokunsult and Ross Institute 1978) has shown a high prevalence (up to 55 percent) of false positive results presumably caused by nonfecal coliforms that reside in warm tropical waters and have the ability to ferment lactose at 44°C. There is an urgent need for the development of a test for fecal indicator bacteria in tropical waters that will reliably and simply distinguish between organisms of enteric origin and others that are free-living and adapted to warm, aqueous habitats. [See note on page 66.]

*In seawater*

The great majority of coastal towns and cities that have a sewerage system discharge their sewage into the sea following little or no treatment. This is true throughout the world. The design of these marine outfalls has attracted considerable interest over the past two decades and involves complex decisions about the degree of treatment and the design of the outfall and complex tradeoffs between costs and environmental hazards. The principal health-related fecal hazards are the risks to swimmers and the contamination of fish and shellfish.

To design outfalls in such a way that fecal bacteria and viruses do not pollute beaches or seafood requires a detailed knowledge of the dispersion, sedimentation, and death of fecal microorganisms discharged into coastal waters. The information on viruses is briefly reviewed in chapter 9, and the available knowledge of fecal bacteria, especially coliforms, is considerably more extensive.

Numerous studies have documented high levels of indicator bacteria (up to $10^3$–$10^5$ per 100 milliliters) in ocean or estuarine waters near sewage outfalls. Recent examples from the USA include studies at Miami Beach (Florida; Edmond, Schaiberger and Gerba 1978), Honolulu (Hawaii; Loh, Fujioka and Lau 1979), the Texas Gulf Coast (Gerba and others 1977; Goyal, Gerba and Melnick 1977, 1978, 1979), Long Island, (New York; Vaughn and others 1979), and the New York Bight (Berg and Metcalf 1978). Studies from other countries include those at Tel Aviv (Israel; Shuval 1978), Alexandria (Egypt; Hakim 1978), Kerala (India; Raveendran, Gore and Unnithan 1978), Naples (Italy; Evison and Tosti 1980), Tuscany (Italy; Petrilli and others 1979), Whitely Bay (UK; Evison and Tosti, 1980), Liverpool (UK; Karthegisan and Pugh Thomas 1980), Belgium (Yde and de Maeyer-Cleempoel 1980), and New South Wales and Tasmania (Australia; Roper and Marsall 1979). In several of these studies (for instance, those in Texas) fecal indicator bacteria were isolated at higher concentrations (10–1,000 times higher) from bottom sediments than from the overlying waters. Roper and Marshall (1979) showed that *E. coli* in saline sediments were protected against attack by viruses, bacteria, and amoebae, and growth of coliforms in marine sediments has been demonstrated (Gerba and McLeod 1976).

Numerous estimates of coliform death rates have been made. Chamberlin and Mitchell (1978) and Mitchell and Chamberlin (1978) reviewed eighty-seven of these estimates and concluded that the times of 90 percent reduction ($t_{90}$) lay between 0.6 and 8 hours, with a geometric mean of about 2 hours (corresponding $k$ values are 0.3–4 per hour with a mean of 1.15 per hour—see footnote 1, above). These values reveal considerably faster death of coliforms in seawater than in fresh water (where $t_{90}$ values are between 20 and 115 hours, with a median of about 60 hours). Death rates of coliforms in seawater are also considerably faster than the death rates of viruses in seawater ($t_{90}$ values in the range of 15–70 hours—see chapter 9). There is now widespread agreement that, owing to the greater persistence of enteric viruses, fecal coliforms are an inadequate index of saline water quality, especially in shellfish-growing areas (Berg and Metcalf 1978). Fecal streptococci survive longer in marine environments than fecal coliforms (Baross, Hanus and Morita 1975; Hanes and Fragala 1967; Petrilli and others 1979; Vasconcelos and Swartz 1976), but not sufficiently long for them to act as an adequate indicator of the enteroviruses. Pichot and Barbette (1978) found a $t_{90}$

of 3.7 hours for fecal coliforms and 5.7 hours for fecal streptococci under the same experimental conditions.

The reasons for the rapid death of coliforms in seawater have been the subject of many investigations (Mitchell 1968). Faust, Aotaky and Hargadon (1975) found temperature, dissolved oxygen, and salinity to be the major determinants of the rate of death, and Enzinger and Cooper (1976), McCambridge and McMeekin (1979) and Mitchell and Yankofsky (1969) drew attention to the important role of protozoan predators. Gerasimenko (1977) found that oil pollution did not affect coliform death rates.

An increasingly convincing case has been built for the importance of light-induced cell damage in determining coliform death rates in sea water (Chamberlin and Mitchell 1978; Chojnowski, Mancini and Jeris 1979; Gameson and Gould 1975; Gameson and Saxon 1967; Mitchell and Chamberlin 1975, 1978). Experiments on fecal coliforms in Sydney harbor (Australia) showed a minimum daytime $t_{90}$ of 1.9 hours and a night time $t_{90}$ of 40 hours (Bellair, Parr-Smith and Wallis 1977). Fecal streptococci appear to be substantially less sensitive to light than coliforms (Chamberlin and Mitchell 1978).

Little information is available on the survival of indicator bacteria in tropical seawater. In extrapolating results from temperate areas, temperature is the variable of most importance. Even relatively small temperature differences can substantially affect the death rate. Jamieson, Madri and Claus (1976) reported that in sterilized saline waters a pathogenic serotype of *E. coli* had a $t_{90}$ of about 40 hours at 4°C and about 8 hours at 37°C. Vasconcelos and Swartz (1976) reported that *E. coli* concentrations in seawater declined by less than 2 log units at 8.9°C, but by 7 log units at 14.5°C, after 6 days. Burdyl and Post (1979) studied *E. coli* survival in the Great Salt Lake (USA) and estimated a $t_{90}$ of about 110 hours at 9°C and about 21 hours at 19°C. Faust, Aotaky and Hargadon (1975) reported $t_{50}$ values for *E. coli* in estuarine water of 39 hours at 0°C and 14 hours at 30°C. Mancini (1978) reviewed reported death rates and temperatures and computed typical $t_{90}$ values of 60 hours at 0°C and 7 hours at 30°C. Clearly, coliforms discharged into tropical seawater will rapidly decline in numbers, as will other excreted bacteria, although not necessarily at the same rate. Excreted virus concentrations will decline considerably more slowly (see chapter 9).

An active debate continues about the magnitude of the health risk associated with swimming in fecally polluted seawater and the correct approach to water quality standards and legislation (Cabelli 1979; Evison and Tosti 1980; Moore, Perin and Maiden 1979). Recent evidence from Egypt and the USA (Cabelli 1979; Cabelli and others 1979) revealed a small but measurable difference in the incidence of gastrointestinal illness between swimmers and nonswimmers at polluted beaches. The recorded risks of swimming in seawater containing $10^2$–$10^3$ fecal coliforms per 100 milliliters were an additional attack rate of 1–2 cases of gastrointestinal illness per 100 people in the 8–10 days following the visit to the beach. It must be kept in mind, however, that especially in developing countries the infections that may be transmitted to swimmers at polluted beaches will usually be highly endemic in the community at large (the community producing the wastes which are polluting the sea), and swimming may constitute a negligible additional risk. Set against this is the possibility that swimmers from high socioeconomic strata (who experience a low risk at home due to adequate water supply, sanitation, and hygiene) may be exposed to a substantially increased risk of infection when they bathe in seawater polluted by the wastes of all socioeconomic strata. The same level of additional risk may apply to tourists—who are usually either local residents from upper socioeconomic groups or foreign visitors.

### *In feces and night soil*

The fecal indicator bacteria are excreted by almost all people, and by almost all warm-blooded animals, nearly all of the time. They are therefore ubiquitous and numerous in all materials containing fresh human or animal feces. The fecal indicator bacteria are a part of the vast total intestinal microflora. A healthy individual may commonly excrete $10^{11}$–$10^{12}$ bacterial cells per wet gram of feces, and these cells may constitute about 9 percent of the total fecal wet weight or 25 percent of the fecal dry weight (Geldreich 1978). The composition of this total bacterial population is set out in table 1-6. It is usual for the anaerobes, especially *Bifidobacterium* and *Bacteroides*, to be a more numerous and more stable component of the fecal flora than the fecal coliforms or fecal streptococci (see, for instance, Mata, Carrillo and Villatoro 1969; Tomkins and others 1981; Zubrzycki and Spaulding 1962).

The numbers and the serotypes or species of fecal coliforms and fecal streptococci excreted by humans vary considerably between individuals according to age (Gorbach and others 1967), diet (Bettleheim and others 1977), state of health, and chemical and microbiological properties of the intestine. Wheater, Mara and Oragui (1979) studied twelve adults in Dundee (Scotland) and found between $8 \times 10^4$ and

$8 \times 10^7$ fecal coliforms, and between $3 \times 10^2$ and $2 \times 10^7$ fecal streptococci, per gram of wet feces. However, community averages are less variable and are in the ranges of $10^6$–$10^9$ fecal coliforms per gram and $10^5$–$10^8$ fecal streptococci per gram (table 1-6). Fecal coliform concentrations are usually higher than those for fecal streptococci by a factor of 4 or more, at least in the developed countries whence most such data come.

The numbers and serotypes of fecal coliforms excreted by a single individual vary through time due to the influence of many factors. In particular, the proliferation of a pathogenic bacterium may modify the commensal flora in the intestine. Dale and Mata (1968) found that eight children in Guatemala with shigellosis excreted between $10^4$ and $10^8$ coliforms per gram and that coliform excretion had a roughly inverse relation to *Shigella* excretion. Streptococci were excreted by the same children in concentrations of $10^8$–$10^9$ per gram.

Most nonhuman animals excrete $10^5$ or more fecal coliforms and fecal streptococci per gram of feces. However, some animals excrete $<10^5$ per gram, as reported for horses and rabbits, respectively, by Geldreich (1978) in the USA and by Wheater, Mara and Oragui (1979) in Scotland. It has often been claimed in the literature from the USA (for instance, Geldreich 1976) that fecal streptococci concentrations generally exceed fecal coliform concentrations in animal feces and that the reverse is true for human feces. However, Wheater, Mara and Oragui (1979) showed that this was not the case for sheep, pigs, cats, dogs, hens, ducks, pigeons, and seagulls in Scotland (see also Williams Smith and Crabb 1961). This variability in fecal coliform to fecal streptococci ratios is one of the reasons for the current rejection of the ratio as a method for distinguishing between human and nonhuman fecal pollution (see chapter 4).

Jordan (1926) studied the survival of *E. coli* in stored feces. At room temperature, numbers increased to $10^8$–$10^{11}$ per gram after 2–5 days and subsequently decreased to undetectable levels in 6–12 weeks. At 37°C, numbers initially increased markedly but then declined to zero within 1–3 weeks. At 10°C, a slower increase in numbers occurred, to a maximum of $10^8$–$10^{10}$ per gram after 20 days, and $10^4$ per gram could still be detected after 23 weeks.

### *In sewage*

Because the fecal indicator bacteria are ubiquitous and numerous in feces, they are also ubiquitous and numerous in raw sewage and in most treated sewage effluents. Concentrations of indicator bacteria in sewage vary through the day, but this variability is considerably less in the sewage derived from large communities than in that derived from small communities. Communities with a high water use per capita produce a sewage with a lower concentration of indicator bacteria than communities with lower water usage. Thus, indicator bacteria concentrations in sewage in developing countries are generally higher than those reported from industrialized countries.

Raw sewage typically contains between $10^5$ and $10^8$ fecal coliforms and fecal streptococci per 100 milliliters. Berg and Metcalf (1978) reported between $3.8 \times 10^4$ and $4.6 \times 10^6$ fecal coliforms per 100 milliliters of sewage in the USA. Geldreich (1978) reported that twenty-one towns in the USA had between $3.4 \times 10^5$ and $4.9 \times 10^7$ fecal coliforms and that seven towns had between $6.4 \times 10^4$ and $4.5 \times 10^6$ fecal streptococci per 100 milliliters of sewage. Davis (1979) found that raw sewage in Houston (Texas, USA) contained $3 \times 10^6$ to $3 \times 10^7$ fecal coliforms and $5 \times 10^5$ to $2 \times 10^6$ fecal streptococci per 100 milliliters. In the Dundee area (Scotland), raw sewage contained $5.8 \times 10^6$ to $1.5 \times 10^7$ *E. coli* per 100 milliliters (Wheater and others 1980).

Evison and James (1973) reported that raw sewage in Nairobi (Kenya) contained up to $1.6 \times 10^8$ *E. coli*, and up to $3.5 \times 10^7$ fecal streptococci, per 100 milliliters. In contrast, and presumably because of higher levels of water use, raw sewage in Pietermaritzburg (South Africa) contained only $1.5 \times 10^4$ *E. coli* per 100 milliliters (Grabow and Nupen 1972). In Brazil, Mara and Silva (1979) reported a mean of $5 \times 10^7$ fecal coliforms and $7 \times 10^6$ fecal streptococci per 100 milliliters of raw sewage.

Concentrations of indicator bacteria in sewage may be affected by the presence of industrial wastes that often contain chemicals antagonistic to enteric bacteria. Data on raw sewages from different areas of Birmingham (UK) showed *E. coli* concentrations of $1.7$–$3.7 \times 10^8$ per 100 milliliters where sewage was principally of domestic origin, compared with only $9 \times 10^5$ per 100 millimeters where sewage flow was 60 percent of industrial origin and contained 20–30 milligrams per liter of phenols (Pike and Carrington 1979).

Studies of sewage produced by small rural communities in the hills of Yorkshire (UK) have shown the fecal coliform concentrations to vary between $10^5$ and $10^8$ per 100 milliliters, whereas fecal streptococci vary between $10^4$ and $10^7$ per 100 milliliters (Feachem, unpublished data). Sanitation technologies that use little water, for instance the pour-flush designs, will

produce a sewage with an exceptionally high concentration of indicator bacteria. Daniel and Lloyd (1980) reported a geometric mean from twelve samples of $8.4 \times 10^8$ coliforms per 100 milliliters of sewage flowing into an Oxfam sanitation unit installed in a refugee camp near Dacca (Bangladesh).

Some studies on the survival of coliforms and streptococci in sewage are listed in the appendixes of Feachem and others (1980). Survival is greatly prolonged at cool temperatures, when dissolved oxygen is low (Hanes, Sarles and Rohlich 1964), or when the overall microflora have been reduced by chlorination or some other means. In warm climates, with sewage temperatures around 25–30°C, a >99 percent reduction in indicator bacteria concentrations may be expected in about 10–15 days, depending on the level of oxygenation of the sewage. It is generally reported that fecal streptococci survive for a little longer than fecal coliforms in sewage (see, for instance, Berg and Metcalf 1978; Cohen and Shuval 1973).

One study recorded a far more rapid rate of death for fecal indicator bacteria in sewage under natural conditions (Dor, Schechter and Shuval 1976). The undiluted raw sewage of Jerusalem (Israel) entered the Nahal Soreq wadi at a rate of about 21,000 cubic meters per day. After a flow of 45 kilometers (which took about 44 hours), at a temperature of around 20°C (March), the fecal coliform and fecal streptococci concentrations were reduced by 99.9 and 99 percent, respectively. The combination of warm temperatures, highly turbulent flow in some sections of the wadi, and a rich algal community contributing photosynthetic oxygen produced a warm and well-aerated environment that caused rapid death of excreted bacteria.

### *In sludge*

Fecal indicator bacteria are always present in high concentrations in fresh sewage works sludge. Concentrations of $10^6$–$10^8$ total coliforms, $10^5$–$10^7$ fecal coliforms, and $10^4$–$10^6$ fecal streptococci per gram are normal. Dudley and others (1980) investigated an anaerobically digested sludge and two primary sludges at San Antonio (Texas, USA) and found $5 \times 10^5$–$5 \times 10^6$ fecal coliforms and $7 \times 10^4$–$5 \times 10^5$ fecal streptococci per gram of suspended solids.

As with coliforms in feces, night soil, and soil, coliforms in sludge may survive for several months in cool, moist conditions. Growth may also occur, and this will be more rapid at warmer temperatures. Edmonds (1976) reported that fecal coliforms in sludge applied to forest soil in Washington State (USA) survived for longer in summer than in winter. At warmer temperatures (say >25°C), however, it is likely that a vigorous growth period would be followed by a rapid decline. Overall survival times of indicator bacteria in sludge in the tropics will typically be shorter than in temperate climates.

### *In soil*

Fecal indicator bacteria occur in only very low concentrations (typically less than 2 per gram) in most uncontaminated soils (Geldreich and others 1962). In contrast, they are found in high concentrations in soil wherever effluent, night soil, sludge, or manure are being used for irrigation or fertilization or where livestock are grazing. The literature on the survival of enteric bacteria in soil is extensive and dates back to the early 1920s (see the appendixes of Feachem and others 1980). This literature has been periodically reviewed (see, for instance, Elliott and Ellis 1977; Gerba, Wallis and Melnick 1975*a*; Rudolfs, Falk and Ragotzkie 1950).

Reported survival times vary widely. The work of Van Donsel, Geldreich and Clarke (1967) clearly demonstrated the importance of sunlight and temperature in determining the death rate of fecal bacteria in soil. Times for 90 percent reduction ($t_{90}$) varied from a minimum of about 3 days in summer in exposed sites to maxima of about 14 days for *E. coli* and 20 days for *Str. faecalis* in autumn and winter at shaded sites. In spring and winter *Str. faecalis* survived for approximately twice as long as *E. coli*, whereas in summer and autumn the two survival times were similar. This may have been because some *E. coli* growth was occurring in summer and autumn.

In Alberta (Canada) studies were conducted on the weekly application of 45 millimeters of unchlorinated waste stabilization pond effluent to plots of canary grass (Bell and Bole 1978). The effluent contained between $2.3 \times 10^4$ and $1.7 \times 10^5$ fecal coliforms per 100 milliliters, and most coliforms were retained in the upper 80 millimeters of the loamy sand. Fecal coliform dieoff occurred in two phases. Over the first 2 days after effluent application a 90 percent reduction occurred. Subsequently, the reduction was about 33 percent per day at 15°C and about 25 percent per day at 10°C. In spring and summer, little or no fecal coliform contamination could be detected 2 weeks after the cessation of irrigation.

Chandler and Craven (1978*a*) studied the disposal to land of piggery waste containing $10^5$–$10^8$ *E. coli* per 100 milliliters in Victoria (Australia). *E. coli* concentrations in the soil declined by 99 to 99.99 percent

after 3–6 weeks, and rapid downward movement of *E. coli* was recorded in late summer when the soil was dry and cracked. In other experiments, Chandler and Craven (1978*b*) recorded a 99 percent reduction of *E. coli* in 1 day in dry soil, whereas in saturated soil the reduction was less than 90 percent after 3 weeks. Similarly, Chandler and Craven (1980) recorded $t_{90}$ values for *E. coli* at 20°C of 18 days in soil with 30 percent moisture and 2.5 days in soil with 10 percent moisture.

Kibbey, Hagedorn and McCoy (1978) found that the survival of *Str. faecalis* in various loams was mostly dependent upon temperature and moisture levels. The time for 95 percent reduction ($t_{95}$) in saturated soils was 94 days at 4°C, 80 days at 10°C, 53 days at 25°C, and 29 days at 37°C. In air-dried soil the $t_{95}$ values were 23 days at 4°C, 18 days at 10°C, 9 days at 25°C, and 5 days at 37°C.

Under certain soil conditions coliform and fecal coliform concentrations will increase, and this increase will be more rapid at warmer temperatures (see, for instance, Guy and Small 1977). Survival times and the potential for growth are influenced by the availability of nutrients in the soil. Thus, survival is prolonged in soil that is regularly receiving effluent or night soil applications. Dazzo, Smith and Hubbell (1973) recorded $t_{90}$ values for *E. coli* of 4 days in soil receiving no manure and 8.5 days in soil receiving 50 millimeters of cow manure slurry per week.

Survival of indicator bacteria in soil is influenced by moisture content, temperature, shade, soil organic content and the overall biological activity present in the soil. These conditions are so variable that reported survival times and $t_{90}$ values cover a wide range. From an overview of the literature (see the appendixes of Feachem and others 1980), it appears that fecal coliforms generally survive for less than 10 weeks, with a 90 percent reduction taking place within 10 days. Under cool, moist conditions a hardy residual fraction of fecal coliforms may survive for many months. Where conditions are hot and arid very limited survival can be expected, and it is probable that almost complete elimination of fecal indicator bacteria will occur within 2 weeks.

### *On crops*

Crops irrigated or fertilized with effluent, night soil, sludge, or manure may be heavily contaminated by fecal indicator bacteria, whereas untreated plants and crops are not (Geldreich, Kenner and Kabler 1964).

Studies in Victoria (Australia) demonstrated the very limited survival times of excreted bacteria on plants compared with their survival on the underlying soil (Chandler and Craven 1978*a*, 1978*b*, 1980). Under conditions in which *E. coli* survived on soil for 8 weeks, no *E. coli* were recovered from grass after 16 days. Persistence of *E. coli* on the grass stems was greatest near the soil surface.

Experiments in Alberta (Canada) on the spray irrigation of fodder crops with waste stabilization pond effluent (containing $10^2$–$10^6$ fecal coliforms per 100 milliliters) have demonstrated the importance of climatic conditions and the anatomy of the plant in determining the survival of excreted bacteria (Bell 1976; Bell and Bole 1976). Fecal coliforms on alfafa (*Medicago sativa*) declined by over 99 percent in 1 day when temperatures were warm (12–23°C), relative humidity was low (20–65 percent) and there were about 9 hours per day of bright sunshine. When temperatures were cooler (9–18°C), relative humidities higher (48–95 percent), and the sky was overcast, a 99 percent reduction required 4 days. In contrast, fecal coliforms on reed canary grass (*Phalaris arundinacea*), bromegrass (*Bromus inermus*) and orchard grass (*Dactylis glomerata*)—grasses that, unlike alfafa, possess leaf sheaths—only underwent a 99 percent reduction in 5 days even in bright weather conditions. The authors argued that sunlight is an important determinant of bacterial death rates and that plant anatomy controls the degree to which effluent droplets on plant surfaces are exposed to sunlight. Greenhouse studies (Brown, Jones and Donnelly 1980) showed that 25 millimeters of simulated rainfall, falling in 1 hour, reduced *E. coli* on grass by 90–99.9 percent. In the absence of rainfall, the same level of reduction under the same conditions took 10–25 days.

Sadovski and others (1978) studied the survival of a mutant *E. coli* inoculated into waste stabilization pond effluents that were applied to drip irrigation to cucumber plots on two farms in Israel. At one site (air temperature 13–30°C, soil temperature at noon 22–30°C, sunlight 9.5 hours per day, relative humidity 27–55 percent), a single irrigation was performed with inoculated effluent containing $10^7$ mutant *E. coli* per 100 milliliters. Mutant *E. coli* were still detectable in the irrigation water, at a concentration of $>10^3$ per 100 milliliters, 8 days after the flow of inoculated effluent. The soil contamination immediately after irrigation with inoculated effluent was $10^7$ mutant *E. coli* per 100 grams of dry soil and persisted at a level of $>10^6$ per 100 grams for at least 8 days. Cucumbers grown in exposed soil were contaminated by $10^4$ mutant *E. coli* per 100 grams immediately following the inoculated irrigation, and this contamination fell to 65 per 100 grams after 8 days. When the soil and drip lines were

covered with polyethylene sheets to reduce evaporation and raise temperature, no mutant *E. coli* could be detected on cucumbers 1 day following the inoculated irrigation. At the second site (air temperature 23–28°C, soil temperature at noon 40–43°C, sunlight 11.8 hours per day, relative humidity 62–70 percent), three irrigations were performed with inoculated effluent containing $4.4 \times 10^{11}$ mutant *E. coli* per 100 milliliters. After the third inoculated irrigation the soil contained $1.3 \times 10^6$ mutant *E. coli* per 100 grams (dry weight), and this contamination fell to 130 per 100 grams after 9 days and maintained this level for a further 11 days. Unlike at the first site, where irrigation with uninoculated effluent had continued throughout the study, in this case the irrigation terminated 5 days after the third inoculated irrigation, and consequently the soil moisture content fell from 15 percent to 3 percent. Bacterial contamination on cucumbers grown in exposed soil rose to $1.7 \times 10^3$ mutant *E. coli* per 100 grams but rapidly declined following the last inoculated irrigation. When the soil and drip lines were covered with polyethylene sheets, no mutant *E. coli* could be detected on the cucumbers. Earlier studies at the first site by the same workers (Sadovski, Fattal and Goldberg 1978) showed that harvested cucumbers and eggplants, irrigated with sewage effluent containing $10^6$ fecal coliforms per 100 milliliters, were contaminated by, on average, 389 fecal coliforms per 100 grams when drip-irrigated throughout the growing season on exposed soil, but by only 30 per 100 grams when drip-irrigated on soil covered by polyethylene sheets. Irrigating with sewage effluent only during the early stage of growth (up to flowering) produced a final contamination level on harvested vegetables similar to that found when irrigation was with fresh water (around 2.5 fecal coliforms per 100 grams).

Other reports of coliform survival on crops are listed in the appendixes of Feachem and others (1980) and have been reviewed elsewhere (for instance, Elliott and Ellis 1977; Geldreich and Bordner 1971; Rudolfs, Falk and Ragotzkie 1950). Fecal bacteria in soil may be in relatively sheltered and supportive microhabitats where moisture, shade, and nutrient availability permit survival for many weeks. In contrast, bacteria on crop surfaces will in general be exposed to desiccation and sunlight, and their survival is very much shorter than in soil. Rapid death is promoted by high air temperatures, bright sunlight, low relative humidity, and a plant anatomy that does not offer many sheltered sites. Under most conditions, fecal indicator bacteria are unlikely to survive for more than 4 weeks on crop surfaces with at least a 99 percent reduction taking place within 1 week. In arid regions with low cloud cover, complete elimination is likely to take place within 1 week.

The reduction of fecal contamination of harvested crops depends not only on the microbial death rates on plant surfaces but also on the technology employed for irrigation or fertilization. The use only of treated effluent, night soil or sludge; the application of the fecal material to the soil (for instance, by drip irrigation) rather than over the crops (for instance, by spray irrigation); covering the soil with plastic sheets to reduce evaporation; and the cessation of irrigation about two weeks prior to harvesting will all greatly reduce crop contamination.

### *In fish and shellfish*

Fish and shellfish that live in water contaminated by fecal discharges are frequently found to contain fecal indicator bacteria. Several studies have shown that these bacteria are not part of the normal flora of the intestines of freshwater or saltwater fish (Geldreich and Clarke 1966; Guélin 1962). Fish intestines may contain fecal coliforms and fecal streptococci only when the fish have been living in fecally contaminated water, and these bacteria may survive, and perhaps multiply, for periods of up to 14 days (Glantz and Krantz 1965) in the fish intestines.

Most investigations have concentrated on the bacterial contamination of shellfish rather than fish. This is because the method of filter feeding of bivalve molluscs concentrates bacteria in the same way as it concentrates viruses (see chapter 9; Metcalf 1978; Wood 1979) and because molluscs are often eaten raw or only lightly cooked. Goyal, Gerba and Melnick (1979) investigated oyster beds in Galveston Bay (Texas, USA) and found fecal coliform concentrations per 100 milliliters of up to 2,400, 46,000, and 46,000, respectively, in water, sediment, and oysters. Similar results were obtained by Slanetz, Bartley and Stanley (1968). Munger, Heyward and Dutton (1979) recorded that fecal coliform concentrations in clams in the Seattle area (Washington, USA) were up to 59 times higher than in the surrounding water.

Mitchell and others (1966) studied the uptake and elimination of *E. coli* by the Eastern oyster (*Crassostrea virginica*) in sterilized seawater at 20°C. When the seawater was inoculated with $10^3$ *E. coli* per milliliter, the oysters accumulated over $10^4$ *E. coli* per gram within 4 hours. When the seawater contained about 10 *E. coli* per milliliter, the oysters accumulated over 100 per gram within 4 hours. When the contaminated oysters were rinsed in clean water and

placed in sterilized seawater, the *E. coli* concentrations declined by 99 percent in 10 hours, and fell below the level of detection after 50 hours. In similar experiments Hoff and Becker (1969) reported that Olympia oysters (*Ostrea lurida*), in sterilized seawater containing 10 *E. coli* per milliliter, accumulated 110–320 *E. coli* per gram after 24 hours at 6–11°C. When the oysters were rinsed and replaced in sterilized seawater, *E. coli* levels fell to about 1 per gram after 48 hours.

Jegathesan and others (1976) purchased three species of commonly eaten shellfish (cockles, *Anadura granosa*, and two species of mussels, *Modiolus senhaussi* and *M. metcalfi*) from markets in Malaysia and examined them for bacterial enteric pathogens. Of twenty cockles examined, nine contained coliforms (mean concentration of $8.9 \times 10^4$ per gram), and seven isolations of pathogenic serotypes of *E. coli* were made. Of eighteen mussels examined, six contained coliforms (mean concentration of $1.2 \times 10^6$ per gram), and twelve isolations of pathogenic serotypes of *E. coli* were made. The authors noted that these shellfish are normally eaten partially cooked in Malaysia.

Although most attention in the developed countries has turned to the risks of contaminated shellfish transmitting excreted virus (especially hepatitis A virus, rotavirus, and Norwalk agent), the risks of bacterial infections due to pathogenic *E. coli*, shigellae, salmonellae, and *Vibrio cholerae* being spread by shellfish harvested from polluted waters are very real (Hughes, Merson and Gangarosa 1977; Janssen 1974).

Considerable debate surrounds the use of quality standards for shellfish growing waters based on permissible concentrations of fecal indicator bacteria (Cabelli 1978; Evison 1979; Metcalf 1978). The USA sets limits of 70 coliforms per 100 milliliters (median value) and 14 fecal coliforms per 100 milliliters (median value) for waters used for shellfish production. It is widely accepted, however, that these bacteriological measures are poor indicators of the risk of viral contamination, and most countries have yet to legislate for shellfish water quality or to decide whether a standard based on indicator bacteria alone is adequate.

### *In the air*

Airborne droplets of water and wastewater may contain excreted bacteria, and these may cause respiratory or enteric infection if inhaled in sufficient numbers by a susceptible host. The mechanisms by which aerosolized bacteria may be produced are similar to those that generate viral aerosols (see chapter 9). Baylor, Peters and Baylor (1977) bubbled air through a column of liquid containing *E. coli* and produced droplets that contained an average concentration of *E. coli* 30 times greater than that in the column. In similar experiments Blanchard and Syzdek (1970) produced droplets that contained up to 1,000 times more *Serratia marcescens* per milliliter than the water column from which the droplets were produced. The concentration of bacteria in the droplet depended on the size of the droplet, with highest concentrations occurring in droplets 60–80 micrometers in diameter.

The aerosolized excreted bacteria most encountered by people in developed countries are those produced by the flush toilets in their houses. Darlow and Bale (1959) inoculated wash-down toilet bowls with $10^{11}$–$10^{12}$ *Serratia marcescens* and investigated the production of airborne organisms when the low-level, 9-liter cistern was flushed. At the level of the seat, near the bowl, $7 \times 10^4$ *Serratia* per cubic meter of air were isolated, and 1.2 meters above the seat the concentration was $7 \times 10^2$ per cubic meter. About 10 minutes after flushing there remained 70 bacteria per cubic meter widely distributed about the toilet room. A tenfold reduction in the inoculum to the bowl reduced the aerosol concentrations by about one-quarter. A second flush, 15 minutes later and without reinoculation of bacteria, still produced $2.8 \times 10^4$ bacteria per cubic meter at seat level. Swabs taken from surfaces throughout the room yielded *Serratia*. Adding sheets of toilet paper to the bowl before flushing did not affect aerosol production by the first flush, but increased aerosol production by the second flush by retaining a greater number of organisms in the bowl during the first flush. Even with the lid closed, the first flush produced $3.1 \times 10^4$ bacteria per cubic meter of air at seat level. Aerosol production was unaffected by whether the seat was covered by its lid or by a cardboard replica of the human buttocks.

Bound and Atkinson (1966) compared aerosol production by wash-down and syphonic types of flush toilets, each flushed by a low-level, 9-liter cistern. The bowls were inoculated with a suspension of *E. coli*, and air samples were taken 0.4 meters from the bowl at seat level. Wash-down toilets produced on average 13 *E. coli* per cubic meter of air, whereas syphonic bowls produced only 0.9 per cubic meter. Closing the lid before flushing did not affect aerosol production.

Newson (1972) studied hospital toilets in England. He sampled water from the bowls of toilets in normal use and found no *E. coli* in 62 percent of samples (109 of 176); only 5 percent of samples (9 of 176) contained more than $2.5 \times 10^5$ per 100 milliliters. The seats, lids, flush handles, and door handles in the same toilets were swabbed, and 6 percent of specimens (19 of 293) were positive for *E. coli*. When $10^{11}$ *E. coli* were added to a

bowl, an aerosol of up to 186 *E. coli* per cubic meter of air was produced. The same procedure also produced 70–80 visible droplet splashes, of which 39–75 contained *E. coli*. More droplet splashes were produced by high-level cistern flushes and, surprisingly, syphonic bowls generated more splashes than wash-down bowls. The first flush reduced the concentration of inoculated *E. coli* in the bowl water from $10^8$ to $10^6$ per 100 milliliters; the second flush took the concentration down to $10^3$ per 100 milliliters; and the third flush left no *E. coli* detectable in bowl water.

Gerba, Wallis and Melnick (1975*b*) seeded $10^{11}$ *E. coli* into a flush toilet bowl with a 14-liter cistern flush. Total numbers of *E. coli* in the bowl water were reduced to $10^7$ after one flush, $10^4$ after two flushes, and remained at around $10^4$ for at least seven flushes. This was because *E. coli* were adsorbed to the porcelain inner bowl surface and were gradually eluted by successive flushes. Public toilet bowls in normal use were sampled and found to contain between $10^2$–$10^8$ coliforms per total bowl volume. One flush ejected 27–104 visible droplets, and up to $6.6 \times 10^4$ *E. coli*, when $10^{11}$ organisms were seeded into the bowl. A reduction of 5 log units in the number of *E. coli* in the bowl reduced the number ejected by a flush by under 3 log units. The numbers of *E. coli* ejected were not appreciably affected by whether the bowl contained similar original numbers in culture, homogenized stool, or fecal pellets. An estimated 800–1000 bacteria fell out on bathroom surfaces after a flush ($10^{11}$ seeded *E. coli* in the bowl), and 75–80 percent of these fell out in the first 2 hours after the flush. Coliforms were detected on surfaces of all of twenty private and public toilets in normal use. Walls, floors, toilet seats, and bowl rims were the most contaminated surfaces.

Sewage treatment plants, especially those that involve the pumping of air or oxygen into sewage or the mechanical aeration of a sewage tank, produce aerosol droplets that contain excreted bacteria as well as excreted viruses (see chapter 9). Bitton and Smith-Holmes (1978) sampled air above the aeration tank of a package sewage treatment plant, above an activated sludge plant, and on the roof of a building in Florida (USA). In a cubic meter of air above the package plant there were 154 coliforms, 22 fecal coliforms, and 2 fecal streptococci; above the activated sludge plant there were 165 coliforms, 27 fecal coliforms, and 5 fecal streptococci; and on the roof there were 16 coliforms, 2 fecal coliforms, and 1 fecal streptococcus.

Fannin and others (1977) investigated airborne coliform bacteria near an activated sludge and a trickling filter sewage treatment plant in Michigan (USA). The sewage contained $2 \times 10^{10}$ coliforms per 100 milliliters. Within 5 meters of the source, the air at the activated sludge plant contained 130–390 coliforms per cubic meter, whereas at the trickling filter plant the air contained 21–79 coliforms per cubic meter. Wind speed and air temperature were significantly correlated with the concentration of airborne coliforms. Katzenelson, Teltch and Shuval (1977) isolated up to 452 coliforms per cubic meter of air 30 meters downwind of an aerated lagoon in Israel.

Goff and others (1973) isolated up to 965 coliforms per cubic meter of air 100 meters downwind from trickling filter plants in the USA. Coliform aerosol detection increased at high relative humidities and low solar radiation. Cronholm (1980) detected aerosolized enteric bacteria up to 930 meters downwind from small activated sludge plants in Kentucky (USA). Foliage downwind of the plants was contaminated by 1–830 enteric bacteria per square millimeter of leaf surface. Six mice forced to inhale air at a sewage treatment plant and observed for 2 weeks exhibited no symptoms, and cultures of their respiratory organs were negative for enteric bacteria.

Fedorak and Westlake (1980) sampled total airborne bacteria at an activated sludge plant at Edmonton (Canada) and found up to $1.8 \times 10^3$ per cubic meter of air, compared to a background level of 27 per cubic meter. Similar bacterial concentrations (up to $2.6 \times 10^3$ per cubic meter) were found indoors near taps used to sample sewage and sludge from the treatment plant. Increased airborne bacterial concentrations occurred in the laboratory when sludge was being dispensed for analysis and when the floor was being mopped.

Randall and Ledbetter (1966) studied the aerosols produced by an activated sludge plant in Texas (USA). The opportunistic respiratory pathogens *Klebsiella*, *Aerobacter*, and *Proteus* constituted 11 percent of all isolates, 19 percent of isolates of enteric origin, and 56 percent of Enterobacteriaceae. The *Klebsiella-Aerobacter* group survived for longer in the air than other enteric bacteria and made up an increasingly high proportion of isolates further from the activated sludge tanks. The authors suggested that *Klebsiella* should be used as an indicator of bacterial air pollution from sewage treatment plants.

Crawford and Jones (1978) studied the emissions of airborne bacteria from aerated grit removal tanks at a sewage treatment plant in Toronto (Canada). The rate of bacterial emission from the tanks was strongly inversely correlated to relative humidity but not to air temperature, pressure, or sewage flow rate. The rate of decay of downwind airborne bacterial concentrations

was correlated only to wind speed. When the decay rates of various indicator bacteria were compared it was found that fecal coliforms decayed faster than total coliforms, which decayed considerably faster than fecal streptococci. By the use of a mathematical model, the authors predict a maximum downwind travel distance of 505 meters for fecal coliforms and 757 meters for *Str. faecalis*.

The source of excreted bacterial aerosols that is receiving the most scientific attention at present is the spray irrigation of sewage effluents. Sorber and others (1976) investigated airborne bacteria produced by the spray irrigation of secondary sewage effluent at a golf course in Arizona (USA). The effluent contained a mean of $3.7 \times 10^5$ coliforms and 170 fecal streptococci per 100 milliliters, and the average background level of coliforms in the air was 2.4 per cubic meter. At 47 meters downwind of the sprinklers up to 330 coliforms per cubic meter were recovered, and at 152 meters only 30 coliforms per cubic meter were found. Coliform levels significantly higher than the background were detected up to 198 meters downwind. The aerosolization efficiency (the proportion of effluent that was divided into droplets sufficiently small to remain airborne) was estimated as 0.32 percent. The decay rate of airborne bacterial concentrations was markedly reduced at night. In subsequent experiments at the same site, effluent contained $2.8 \times 10^5$ coliforms, $2.3 \times 10^4$ fecal coliforms, and $1.3 \times 10^3$ fecal streptococci per 100 milliliters (Bausum, Schaub and Kenyon 1978). The concentrations of aerobic bacteria-bearing particles per cubic meter of air, downwind of the sprinklers, were up to 10,500 at 46 meters, up to 4,700 at 76 meters, up to 3,200 at 100 meters, up to 500 at 150 meters, and up to 13 at 560 meters. (All these concentrations are expressed as values in excess of the background levels of 15–198 colony-forming particles per cubic meter.) When the effluent was heavily chlorinated prior to spraying, colony-forming particles fell to between 0 and 57 per cubic meter at 46 meters downwind. The proportion of Enterobacteriaceae in the total aerobic bacterial flora was 2 percent in the effluent but 26 percent in the aerosol isolates.

Parker and others (1977) investigated a spray irrigation system that utilized effluent from a potato-processing plant. The sprayed effluent contained $1.6 \times 10^6$ coliforms per 100 milliliters. At 15 meters downwind there were up to 1,100 coliform-bearing particles per cubic meter, and at 1–1.5 kilometers downwind there were up to 30 coliform-bearing particles per cubic meter.

A series of experiments were conducted in Israel to investigate the production of airborne bacteria at spray irrigation sites utilizing sewage effluents (Katzenelson and Teltch 1976; Katzenelson, Teltch and Shuval 1977; Teltch and Katzenelson 1978). Coliform bacteria were found in the air up to 350 meters downwind from the irrigation site. The concentration of bacteria in the air was directly related to the concentration in the effluent, and coliforms could only be detected in the air when their concentration in effluent exceeded $10^5$ per 100 milliliters. Coliforms survived for longer in aerosols when relative humidity was high, when solar radiation was low, and when the effluent contained a higher concentration of organic matter. Up to 10 times more aerosolized bacteria were detected at night than during the day. Earlier studies in Israel (Katzenelson, Buium and Shuval 1976) had suggested that residents on kibbutzim that practised spray irrigation with sewage effluent experienced a higher incidence of some bacterial excreted infections (shigellosis, salmonellosis, and typhoid) than members of kibbutzim that practiced no form of wastewater irrigation. These findings have been doubted and appear to be contradicted by subsequent work (Feliciano 1979).

Airborne excreted bacteria can be produced by many situations other than toilet flushing, sewage treatment, or spray irrigation. Edmonds and Littke (1978) sampled airborne coliforms 80 millimeters above anaerobically digested, dewatered (20–40 percent solids) sludge applied to land. Coliforms in the sludge over a 7-month sampling period were between $5 \times 10^5$ and $3.5 \times 10^7$ per gram, while coliforms in the air ranged from 0 to $1.5 \times 10^4$ per cubic meter. Maximum coliform emission was associated with no rainfall and high air temperature, wind speed, and solar radiation. Adams and others (1980) have reported on bacterial aerosol emissions from cooling towers using disinfected sewage effluents or polluted river water.

The risks to health associated with inhaling aerosolized bacteria depend on factors such as the dose inhaled, the dose required to cause an infection, and the aerosol size. Katzenelson, Teltch and Shuval (1977) estimated that an individual working 100 meters from an effluent sprinkler in Israel would inhale about 36 coliforms every 10 minutes. Considering the very high ratio of coliforms to pathogens, this rate of inhalation appears low. Infective doses for some pathogens may be lower, however, by the respiratory route than by the alimentary route. Crozier and Woodward (1962) reported that typhoid fever was established in chimpanzees by the respiratory route with doses of *S. typhi* a thousandfold less than those needed for infection by oral challenge.

The infective dose of inhaled bacteria depends in part upon the ability of the aerosols to penetrate deep

into the lungs. Lung penetration is especially important in the establishment of respiratory infections. Aerosols that penetrate best are those which are less than 5 or 6 micrometers in diameter (Druett, Henderson and Peacock 1956; Druett and others 1953; Harper and Morton 1953; May and Druett 1953). Reported aerosol sizes from sewage sources vary considerably but, in general, smaller aerosols predominate as distance from the source increases because larger particles have settled. Although only small aerosols are likely to penetrate to the lower respiratory tract, it is probable that bacteria (and viruses) in larger aerosols caught in the upper respiratory tract may subsequently be swallowed.

Baylor, Peters and Baylor (1977) found a significant negative correlation between droplet size and the concentration of *E. coli* within a droplet. Droplets produced by surf and blown into the beach had a mean size of 35 micrometers at the water's edge and 21 micrometers 10 meters up the beach (Baylor and others 1977). Sorber and others (1976) found that 50 percent of airborne bacteria at a spray irrigation site were associated with particles in the range of 1–5 micrometers. Darlow and Bale (1959) found that a toilet flush produced aerosols with a mean diameter of 2.3 micrometers, 87 percent of them being less than 4 micrometers. Goff and others (1973) recorded that the majority of aerosols emitted by trickling filter plants were less than 5 micrometers in diameter. Randall and Ledbetter (1966) found that 40 percent of viable airborne bacteria in the immediate vicinity of activated sludge units were associated with particles of less than 5 micrometers in diameter, but that this proportion rose to 70 percent at a downwind distance of 6 meters. Teltch and Katzenelson (1978) found that the median aerosol size produced by effluent sprinklers in Israel was greater than 7 micrometers but that 50 percent of bacteria sampled were associated with aerosols of less than 7 micrometers in diameter.

The studies reported above, and the laboratory investigations of Poon (1968), clearly show that, although bacterial aerosol production may be increased by low relative humidity, high temperature, high solar radiation, and high wind speed, these same conditions also promote rapid death and dispersion of bacteria in the air. Thus the dissemination of aerosolized bacteria from treatment plants and spray irrigation sites in hot arid climates should be appreciably less than that reported from temperate climates. In addition there is no convincing epidemiological evidence that those exceptionally exposed to sources of bacterial aerosols (for instance, workers in fields receiving sprayed effluent or sewage plant operators) have higher infection or disease rates than others (Feliciano 1979). It may be that, in most situations, the inhalation of excreted bacteria is insignificant compared with the ingestion of the same pathogens and that the numbers of bacteria inhaled are usually well below the required infective doses.

## Inactivation by Sewage Treatment Processes

The fate of bacterial indicators of pollution in sewage treatment processes has been the subject of a number of studies over the past 50 years. The literature is not, however, as extensive as might be expected, partly because it is only very recently that the importance of *E. coli* as an enteric pathogen has been recognized and partly because the traditional focus of attention has been the ability of sewage treatment processes to improve the physicochemical quality, rather than the microbiological quality, of sewage. Indeed, interest in the performance of sewage treatment plants in removing enteric bacteria has been so low that many engineers are unaware of the poor bacterial quality of secondary effluents from conventional treatment plants.

The interest in enteric bacteria in sewage treatment processes is now increasing for two reasons. First, more stringent effluent quality legislation is being introduced in the developed countries, and this has promoted research into disinfection and tertiary treatment as methods for improving the quality of unsatisfactory secondary discharges. Second, there is growing awareness in developing countries of the dangers of discharging highly pathogenic secondary effluents into streams that downstream communities may use for domestic purposes.

### *By primary and secondary sedimentation*

Primary sedimentation tanks, with retention times of 2–6 hours, produce quite variable results in removal of indicator bacteria (see the appendixes to Feachem and others 1980). Usually a reduction of fecal coliforms and fecal streptococci is reported in the range 0–60 percent. For instance, at two sewage treatment plants in Scotland, primary sedimentaton removed on average 32 and 50 percent of *E. coli* and 57 and 53 percent of *Pseudomonas aeruginosa* (Wheater and others 1980). Removal is primarily caused by settlement of bacteria, which are adsorbed to, or entrapped within, solid particles in the influent sewage. The data on bacterial dieoff in sewage reported above

suggest that only a very small reduction (< 10 percent) would be expected due to natural death in the short period during which the sewage resides in the sedimentation tank. Growth of indicator bacteria, especially of total coliforms, is sometimes recorded in sedimentation tanks.

*By storage*

Storage will be an especially effective method of reducing enteric bacteria in sewage at warm tropical and subtropical temperatures (say, >25°C). Although little specific information is available, it may be anticipated that fecal bacteria in warm sewage would have a $t_{90}$ of 120 hours or less.

*By septic tanks*

The removal of fecal indicator bacteria by septic tanks has attracted increased interest recently because of concern about the pollution of groundwater by septic tank effluents. A septic tank is simply a settling chamber, or chambers, usually having retention time of 3 days or less. In poorly designed tanks, or those requiring desludging, there is very considerable solids carryover into the effluent. Enteric bacteria are removed both by death in the anaerobic liquor and by association with solids that settle to the sludge layer. The short retention times, and poor sedimentation performance that is often the result of insufficiently frequent desludging, are the reasons why high concentrations of fecal indicator bacteria are found in septic tank effluents.

Viraraghavan (1978) reported that a septic tank in Canada produced an effluent containing geometric mean values of $2.3 \times 10^6$ coliforms, $1.6 \times 10^5$ fecal coliforms, and $1.1 \times 10^5$ fecal streptococci per 100 milliliters. Brandes (1978*a*) reported that approximately 2.5 million residents of Ontario (Canada) use septic tank systems. He studied three septic tanks with retention times of 2–10 days and recorded the following ranges of concentrations of fecal coliforms per 100 milliliters: $4 \times 10^5$–$2 \times 10^6$ in first compartment supernatant, $1 \times 10^5$–$1 \times 10^6$ in second compartment supernatant, $9 \times 10^5$–$8 \times 10^6$ in first compartment sludge, $6 \times 10^4$–$6 \times 10^5$ in second compartment sludge, and $5 \times 10^5$–$4 \times 10^6$ in the effluents (see also Brandes 1978*b*). McCoy and Ziebell (1976) sampled effluents from five septic tanks in the USA with retention times of 2–13 days and found geometric mean values of $4.2 \times 10^5$ fecal coliforms and $3.8 \times 10^3$ fecal streptococci per 100 milliliters.

McGarry and Stainforth (1978) have described a three-compartment septic tank used in Kiangsu Province, China (see also figure 6-1 in Part One). The first two compartments have retention times of 10 days each, and the third has a retention time of 30 days. Tests on these tanks showed that, in winter at temperatures of 3–7°C, *E. coli* reductions of 4 log units were achieved (suggesting a $t_{90}$ of 300 hours).

The data (see the appendixes of Feachem and others 1980) suggest that in a well-designed and well-maintained septic tank with 3 days' retention in a warm climate (>25°C), a reduction of fecal indicator bacteria of 50–95 percent may be achieved. In poorly designed and poorly maintained tanks (the most usual kind), little or no reduction can be expected. It must be stressed, however, that the distinction between a 90 percent removal (say, influent = $10^6$ per 100 milliliters; effluent = $10^5$ per 100 milliliters) and a 10 percent removal (say, influent = $10^6$ per 100 milliliters; effluent = $9 \times 10^5$ per 100 milliliters) is trivial. In either case the effluent is heavily contaminated with enteric bacteria, and the ultimate fate of these bacteria depends on the method of disposal of the effluent and the sludge.

Effluents are normally discharged to soakaway pits or drainfields where bacteria may be retained in the soil and eventually die. Under certain conditions, however, fecal bacteria may travel from the drainfield to pollute shallow groundwater aquifers or nearby wells. Kudryavtseva (1972) recorded that coliforms inoculated into saturated alluvial sands (percolation rate 13 meters per day) in the USSR traveled for a horizontal distance of not more than 3 meters in the direction of groundwater flow. However, the same author cited other data from the USSR indicating a horizontal travel of indicator bacteria of 850 meters through pebble deposits and 1 kilometer through weathered limestone.

Several studies have shown that the travel of bacterial indicators through soil from pit latrines or septic tank drainfields decreases over time as the soil becomes increasingly clogged with fecal solids and biological slime. Caldwell and Parr (1937) found that, after two months of operation, fecal coliforms and *Clostridium perfringens* could be recovered at 8 meters, and occasionally at 11 meters, from a bored hole latrine. After 7 months, however, bacterial travel was less than 1.5 meters. McCoy and Ziebell (1976) applied septic tank effluent ($5.1 \times 10^6$ fecal coliforms and $7.3 \times 10^6$ fecal streptococci per 100 milliliters) to 0.6 meter deep columns of loamy sand at 25°C. At an application rate of 0.1 cubic meters per square meter per day, 92 percent of fecal coliforms were removed over the first 100 days of application. After this time a

clogging zone developed at the top of the column, and removal of fecal coliforms improved to 99.999 percent. Fecal streptococci removal rates were around 3 log units during the first period and over 6 log units after 100 days. In field experiments it was found that an effluent contained $1.9 \times 10^6$ fecal coliforms per 100 milliliters; the soil in the clogging zone at the base of the drainfield trench contained $4 \times 10^6$ per 100 grams; and the soil 0.3 meters below the clogging zone contained less than 200 fecal coliforms per 100 grams. The authors noted, however, that these are optimal removals under ideal conditions in nonaggregated soils.

Brown and others (1979) studied the movement of septic tank effluent containing $10^6$ fecal coliforms per 100 milliliters through a sandy loam, a sandy clay, and a clay, with percolation rates of 6, 0.9, and 0.06 meters per day, respectively. Fecal coliform concentrations decreased greatly with increasing distance from the septic line, and a 90–99 percent reduction in 50 millimeters was common. In most tests fecal coliforms were not present more than 0.3 meters below the septic line, and only very occasionally were they detected in leachate drawn from 1.2 meters below the septic line. The tendency for nearly all fecal coliforms to be concentrated at the interface between the gravel packing around the septic line and the soil was especially marked in soils of low permeability and increased with time of effluent application for all soils. Fecal coliform concentrations in the soil decreased by about 99 percent in 2 weeks following the termination of effluent application.

Studies on septic tank drainfields have clearly shown that the risks of groundwater pollution are very much greater if the drainfield is located in, or only a little above, the saturated zone. Viraraghavan (1978) studied the movement of indicator bacteria horizontally through sandy clay and clay (percolation rates 0.02–1.0 meters per day) from a septic tile discharging effluent containing about $10^5$ fecal coliforms and fecal streptococci per 100 milliliters. The septic line was 0.6 meters below ground level, and the water table was only 0.15 meters or less below the line. Fifteen meters downslope from the septic line the groundwater contained about $10^2$ fecal coliforms and $10^2$–$10^3$ fecal streptococci per 100 milliliters.

Hagedorn, Hansen and Simonson (1978) seeded antibiotic-resistant *E. coli* and *Str. faecalis* into two pits, 0.3 and 0.6 meters deep, dug into saturated silty loam and clay loam (percolation rates 0.01–0.2 meters per day) in Oregon (USA). The bacteria were detected in wells 15 meters from the pits within 8–16 days of inoculation, and they survived in appreciable numbers in the saturated soils throughout a 32-day sampling period. Reneau and others (1977) recorded the travel of fecal coliforms for a horizontal distance of at least 28 meters from a septic tank drainfield through saturated sandy loam in Virginia (USA). At one site, fecal coliform concentrations were up to $4.6 \times 10^4$ per 100 milliliters adjacent to a drainfield but were never more than 430 per 100 milliliters at a distance of 28 meters (see also Reneau and Pettry 1975). Further important work on bacterial movement through saturated soil was reported by McCoy and Hagedorn (1979) and Rahe, Hagedorn and McCoy (1979).

Other literature on the retention and survival of bacteria in soil is reviewed above in the sections on groundwater and soil and below in the sections on filtration and land treatment. A detailed review of the fate of enteric bacteria in septic tank and drainfield systems has been published (Small Scale Waste Management Project 1978).

Septic tank sludge is rich in excreted bacteria (Brandes 1978*a*, 1978*b*) and requires treatment by digestion, drying, or composting prior to application to agricultural land. The destruction of fecal indicator bacteria in tanks, in drainfields, and in sludges will be more rapid at warmer temperatures; therefore, given correct design and good maintenance, performance in developing countries may often be better than that reported from temperate areas.

### *By trickling filters*

Little information is available on the performance of trickling filters as a unit process. Most studies report the removal of fecal indicator bacteria across a complete trickling filter plant (pretreatment–primary sedimentation–trickling filters–secondary sedimentation).

Literature on fecal indicator bacteria removal in trickling filters is listed in the appendixes of Feachem and others (1980). In a well-operated plant the trickling filter itself may remove 20–80 percent of influent enteric bacteria, whereas the total treatment plant will remove 70–97 percent. Poor maintenance or overloading will result in considerably lower removal rates. Fecal coliform and fecal streptococci removal rates are generally similar.

In Britain it is common for the removal of fecal coliforms across complete trickling filter plants to be 90–95 percent. This poor removal performance results in an effluent containing $10^4$–$10^7$ fecal coliforms per 100 milliliters. Wheater and others (1980) recorded that two trickling filter plants in Scotland achieved average overall *E. coli* reductions of 87 and 90 percent

and produced effluents containing up to $6.9 \times 10^6$ and $1.4 \times 10^6$ *E. coli* per 100 milliliters.

*By activated sludge*

The removal of fecal indicator bacteria by activated sludge processes is poor, although removal is somewhat better than by trickling filters. The mean retention time in the aeration tanks (6–12 hours) is such that only a less than 50 percent reduction by natural die-off would be expected, even at warm temperatures and assuming that all the liquor were held for the mean retention time. Most reduction of indicator bacteria is in practice achieved by adsorption to flocs, which are subsequently removed in secondary sedimentation tanks, and by the grazing of ciliated protozoa (Van der Drift and others 1977).

Studies on removal of fecal indicator bacteria by activated sludge are listed in the appendixes of Feachem and others (1980). Reductions of influent fecal bacteria are between 0 and 99.9 percent, with some experimenters reporting increasing numbers during aeration. Most experience suggests that reductions will be 80–99 percent across a complete activated sludge plant that is well-operated and well-maintained but that does not include effluent disinfection or tertiary treatment. Overloaded or poorly maintained plants will achieve very much lower removal rates. These low levels of removal (always less than 2 log units) mean that activated sludge plant secondary effluents contain high concentrations of enteric bacteria. Berg and Metcalf (1978) reported $1.1 \times 10^5$–$4.9 \times 10^6$ fecal coliforms per 100 milliliters of activated sludge effluent. The settled sludge from the secondary sedimentation tanks will also contain a high concentration of fecal indicator bacteria that have adsorbed to flocs in the aeration tanks.

*By oxidation ditch*

Practically no information has been published on removal of fecal indicator bacteria by oxidation ditches, although a certain amount is known about removal of *Salmonella* (see chapter 15). The process is essentially similar to that of activated sludge, but the longer hydraulic retention times (1–3 days), and the higher proportion of sludge recycling giving a solids retention time of 10–30 days, are features that should produce improved bacterial removal. Laboratory studies in the USSR indicated that coliforms declined by 6 log units after 3 days in an oxidation ditch, and that an EPEC strain (O111) survived for 3–7 days when seeded at a concentration of $10^5$ per 100 milliliters and for 15–18 days when seeded at $10^7$ per 100 milliliters (Goncharuk and others 1970).

*By waste stabilization ponds*

The removal of *E. coli* and other fecal indicator bacteria in waste stabilization ponds has been studied by many investigators throughout the world during the last 30 years (see the appendixes of Feachem and others 1980). It is now well established that ponds, if properly designed, can achieve substantially higher removal rates of fecal bacteria (and indeed of other excreted pathogens) than other forms of sewage treatment. For example, Mara and Silva (1979) report the reduction of fecal coliform bacteria in a series of five ponds in northeast Brazil, with a total retention time of 29 days and an average temperature of 26°C, from $5 \times 10^7$ per 100 milliliters in raw sewage to 17 per 100 milliliters in the final effluent; this represents a very high overall reduction of 99.99996 percent.

MECHANISMS OF *E. COLI* REMOVAL IN PONDS. There is a variety of environmental factors that are considered to be responsible, or at least partially so, for the removal of *E. coli* and other fecal indicator bacteria in ponds. These factors include time and temperature, ultraviolet radiation, the antibacterial effect of extracellular algal toxins, low concentrations of dissolved carbon dioxide, high pH, high (especially supersaturated) concentrations of dissolved oxygen, and predation by the microinvertebrate fauna. These factors are reviewed briefly below, but it should be emphasized that their relative importance, apart from perhaps that of time and temperature, is largely unknown.

Compared with other forms of sewage treatment, ponds are characterized by long mean hydraulic retention times, ranging from a few weeks in hot climates to several months in cold climates. Thus, ponds provide a considerably greater opportunity for fecal bacterial removal than other treatment processes. It is now well established (Mara 1976), both theoretically and from field observation, that removal of fecal bacteria is greater in a series of ponds than in a single pond providing the same overall hydraulic retention time, and that this efficiency increases with the number of ponds in the series. The microbial flora and fauna vary considerably from pond to pond in a series of ponds, and it is therefore likely that the relative effect on fecal bacterial removal of the environmental factors discussed below changes in a similar manner.

Moeller and Calkins (1980) investigated the effect of the ultraviolet component of solar radiation (wavelength range: 280–320 nanometers) on the removal of fecal coliforms in a series of four maturation ponds treating the effluent from a conventional activated sludge plant in Kentucky (USA). The overall hydraulic

retention time in the pond series was 5–7 days, and the pond temperatures varied from below 10°C to above 25°C. No relationship between fecal coliform removal and either temperature or algal density was found, but a significant correlation between fecal coliform removal and the received dosage of ultraviolet radiation was apparent, with the bacterial removal rate being directly proportional to the dose received.

Several investigators have studied the direct and indirect effects of pond algae on the removal of fecal bacteria. Many common pond microalgae produce antibacterial substances that have been shown to be inhibitory to *E. coli* (Davis and Gloyna 1972), although *in vitro* toxicity tests have shown that the degree of inhibition effected by different algae not only varies from alga to alga, but that there appears to be a marked synergistic effect when two or more algae are present, with the synergism increasing with the number of algal species present. Moreover, removal of fecal bacteria in samples of pond water is higher than in laboratory mixtures of large numbers of different algal species (Jackson 1979).

Algal demand for carbon dioxide is often greater than its supply as an end-product of pond bacterial metabolism, with the result that bicarbonate ions reverse to carbon dioxide and hydroxyl ions, leaving an excess of the latter that raises the pH of the pond. High pH values (above 9.5) are known to be detrimental to the survival of *E. coli* in ponds (Parhad and Rao 1974), although Gray (1975) suggests that, since carbon dioxide is an essential growth factor for *E. coli*, its unavailability to *E. coli* as a result of its rapid utilization by photosynthesizing algae is an important factor in determining the removal of *E. coli* in natural environments.

The concentration of dissolved oxygen in ponds is controlled by the pond algae. In facultative ponds there is a diurnal variation in dissolved oxygen concentration at any depth above the oxypause and also in the position of the oxypause itself. The survival of *E. coli* is enhanced under anaerobic conditions (Klock 1971; Marais 1974). In thermally stratified facultative ponds in northeast Brazil, *E. coli* forms a reasonably stable layer some 10–20 centimeters below the oxygen-supersaturated algal zone, presumably to provide protection against the detrimental effect of very high dissolved oxygen concentrations (Pearson and Mara, unpublished data). However, Davis and Gloyna (1972) have shown that in Texas (USA) pretreatment in anaerobic ponds is advantageous in that the overall removal in a series of ponds which includes an anaerobic pond is greater than in one which does not. From the details given by these authors it is not possible to determine whether this is due merely to the anaerobic pond functioning as an additional unit in the series, or whether solids removal in anaerobic ponds permits an enhanced efficiency of whatever factors are responsible for fecal bacterial removal in facultative ponds. The results obtained by Mara and Silva (1979) in northeast Brazil suggest, however, that the latter explanation is not valid under all climatic conditions.

Loedolff (1965) examined the removal of *E. coli* through predation by microinvertebrates in experimental ponds in Pretoria (South Africa). Cladocera were found to be the numerically greatest group of microinvertebrates, with *Moina dubia* and *Daphnia magna*, respectively, predominating in facultative and maturation ponds. *In vitro* studies showed that *M. dubia* remove 93 cells of *E. coli* per individual per hour and *D. magna* 55, the difference being ascribed to the difference in coarseness of the filtering setae of the two species. At these rates of predation it was concluded that Cladocera do not contribute significantly to bacterial removal in ponds because, in practice, their numbers never rise to the level required for them to have a major effect on bacterial numbers.

KINETICS OF *E. COLI* REMOVAL IN PONDS. Removal kinetics have been studied in detail by only a few investigators. The most favored approach (for example, Marais 1974) is the assumption that removal of fecal bacteria follows first-order kinetics and that the pond is a completely mixed reactor.[3] Although this assumption undoubtedly represents a gross

3. The resulting kinetic equation is thus:

$$N_e = N_i/(1 + K_b t^*),$$

where $N_e$ and $N_i$ are the numbers of a fecal indicator bacterium (or bacterial pathogen) per 100 milliliters of pond effluent and influent, respectively; $K_b$ is the first-order rate constant for the removal of the bacterium in reciprocal days; and $t^*$ is the mean hydraulic retention time in the pond in days ( = the pond volume in cubic meters divided by the influent flow rate in cubic meters per day). A formal derivation of the equation is given, for example, by Mara (1976). A more rigorous approach to pond kinetics would be to use the Wehner and Wilhelm (1956) equation for first-order removal in dispersed flow reactors; such an approach is not normally possible, however, as pond dispersion numbers are usually unknown. For a fuller discussion of this point, see Mara (1976).

The value of $K_b$ is strongly temperature dependent and is usually described by an Arrhenius equation of the form:

$$K_{b(T)} = K_{b(20)}\theta^{T-20},$$

where $K_{b(T)}$ is the value of $K_b$ at $T$°C, $K_{b(20)}$ its value at 20°C, and $\theta$ the dimensionless Arrhenius constant. Marais (1974), using the results reported by Slanetz and others (1970), calculated values of 2.6 reciprocal days and 1.19 for $K_{b(20)}$ and $\theta$, respectively, for facultative and maturation ponds in the temperature range 5–20°C.

simplification of the environmental factors involved in fecal bacterial removal in ponds and also of the hydraulic regime therein, it is nonetheless empirically justified and in the past has served as a reasonable basis for design (Marais 1974; Mara 1976). However, a more recent and more rigorous analysis of removal of fecal bacteria in ponds is given by Dissanayake (1980), who studied the removal of fecal coliforms in laboratory-scale, pilot-scale, and full-scale ponds in Bangkok (Thailand). He found that the first-order rate constant for fecal coliform removal ($K_b$, in reciprocal days) was best described by the following multiple linear regression equation:

$$\exp(K_b) = 0.7716(1.0281)^T(1.0016)^{C_s}(0.9990)^{\lambda},$$

where $T$ is the temperature in degrees Celsius, $C_s$ the average concentration of algae in the pond in milligrams per liter,[4] and $\lambda$ the organic loading on the pond in kilograms of chemical oxygen demand per hectare per day. The intensity of ultraviolet radiation was shown to be an unimportant factor in influencing the value of $K_b$, and no account was taken of predation by microinvertebrates (which, as noted above, is insignificant). When used with the Wehner and Wilhelm (1956) model for first-order removal of fecal coliforms in dispersed flow reactors, this equation was found to be very satisfactory in predicting fecal coliform removal in full-scale ponds. Dissanayake (1980) also gives regression equations for predicting the value of $C_s$, so that his model for fecal coliform removal can be used by design engineers. Application of Dissanayake's model has of course been limited because of its recentness, and further work is required to determine the global applicability of its regression constants. Nonetheless, the model at least gives some idea of the relative importance of the principal environmental factors involved in removal of fecal bacteria in ponds.

Much of the large volume of literature in removal of fecal bacteria in ponds does not contain all the information required for a kinetic analysis of the results given therein; for example, many publications do not contain details of the retention time, and almost none gives information on the dispersion number or algal biomass of the ponds studied. The complexity of the removal of fecal bacteria in ponds is shown in table 13-3, which presents values of the first-order rate constant for the removal of various fecal bacteria in four series of ponds located in Australia, Brazil, and South Africa.[5] These series of ponds were selected because they are representative of well-operated ponds, with sufficient information given about their performance to permit the kinetic constants to be calculated. It is apparent from table 13-3 that there is considerable variation in the values of the kinetic constants for each bacterial group—even for ponds in the same series at the same temperature—and that little can be concluded about the relative removal rates of the different groups. Insufficient data were reported for this series of ponds to permit the validity of Dissanayake's (1980) model to be ascertained.

At present it appears, therefore, that design engineers have no alternative but to follow the design procedure based on the work of Marais (1974) and Mara (1976) for the removal of fecal bacteria in a series of ponds, even though its only environmental parameter is temperature. It is clear, however, that in the future pond design will have to include the effect of other variables such as algal biomass and organic loading. The pioneering approach shown by Dissanayake (1980) requires that it be followed by further work to determine its validity as a design tool.

### *By aerated lagoons*

The survival of fecal indicator bacteria in aerated lagoons has scarcely been studied. Menon and Bedford (1973) reported that coliform and fecal coliform removal rates were 38 and 63 percent, respectively, in an aerated lagoon treating wood pulp processing effluents at Fort Frances (Canada). The study was conducted during summer when the lagoon temperature was 28°C, but other process details were not given. There was evidence of coliform and fecal coliform growth on some occasions in the aerated lagoon, and laboratory studies showed that in sterilized aerated lagoon liquor total coliforms multiplied at 15°C and 28°C, that fecal coliforms multiplied at 28°C but not at 15°C, and that fecal streptococci died at both temperatures. This particular study was not well designed or reported, and it may be that the results are not typical. From a theoretical standpoint, an aerated lagoon (retention time 2–6 days) may be expected to have bacteria removal properties similar to, or a little better than, an oxidation ditch. If the effluent is treated

4. Rather than using algal dry weight in milligrams per liter, it is preferable to express algal biomass concentrations in ponds in terms of photosynthetic pigment; for example, micrograms of chlorophyll a per liter.

5. Since the dispersion numbers for these ponds were not given in the literature cited, it is only possible to analyze the results therein on the assumption of either complete mixing or plug flow. For the purpose of table 13-3, the former was used, although the latter would have served the argument equally well. The kinetic equation for a completely mixed pond is given in footnote 3, above.

Table 13-3. *Fecal bacteria removal rate constants in series of waste stabilization ponds*

| *Country reference* | *Temperature* (°C) | *Pond number* | *Retention time (days)* | *First-order rate constant (reciprocal days, base e) for removal of:* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *Fecal coliforms* | *Fecal streptococci* | Clostridium perfringens | *Salmonellae* | Pseudomonas aeruginosa |
| Australia (Parker 1962)[a] | 21 | 1 | 3.8 | 0.13 | 0.21 | | | |
| | | 2 | 8.0 | 1.13 | 1.30 | | | |
| | | 3 | 13.0 | 0.07 | 0.13 | | | |
| | | 4 | 18.0 | 0.12 | 0.61 | | | |
| | | 5 | 23.0 | 1.11 | 0 | | | |
| | | 6 | 28.5 | 0.55 | 0.91 | | | |
| | | 7 | 33.5 | 0.38 | 0.99 | | | |
| | | 8 | 38.5 | 0.19 | 0.23 | | | |
| Australia (Parker 1962)[a] | 9 | 1 | 4.1 | 0.11 | 0.16 | | | |
| | | 2 | 8.6 | 0.44 | 0.21 | | | |
| | | 3 | 14.0 | 0.23 | 0.17 | | | |
| | | 4 | 19.2 | 0.28 | 0.04 | | | |
| | | 5 | 24.6 | 0.14 | 1.01 | | | |
| | | 6 | 30.5 | 0.28 | 3.17 | | | |
| Brazil (Mara and Silva 1979)[b] | 26 | 1 | 6.75 | 2.17 | 3.69 | | | |
| | | 2 | 5.46 | 2.26 | 2.17 | | | |
| | | 3 | 5.46 | 2.40 | 2.38 | | | |
| | | 4 | 5.46 | 15.00 | 5.79 | | | |
| | | 5 | 5.79 | 1.91 | 0.12 | | | |
| South Africa (Coetzee and Fourie 1965)[c] | ND | 1 | 20.0 | 1.96 | | 2.44 | 0.55 | 4.19 |
| | | 2 | 15.0 | 8.47 | | 1.40 | 1.27 | 0.26 |
| South Africa (Coetzee and Fourie 1965)[d] | ND | 1 | 2.5 | 1.71 | | | 0.17 | 3.73 |
| | | 2 | 2.5 | 0.43 | | | 0.19 | 0.24 |
| | | 3 | 2.5 | 0.35 | | | 0.62 | 2.36 |
| | | 4 | 2.5 | 1.11 | | | 0.15 | 3.60 |

ND No data.

a. Data for full-scale ponds receiving raw sewage (anaerobic, facultative and six or four maturation ponds).

b. Data for pilot-scale ponds receiving raw sewage (anaerobic, facultative and three maturation ponds).

c. Data for full-scale ponds receiving raw sewage (facultative and maturation ponds).

d. Data for full-scale ponds receiving humus tank effluent (four maturation ponds).

in maturation ponds, removal as in waste stabilization ponds is anticipated. The sludge drawn off from secondary sedimentation tanks or settling ponds will be rich in excreted bacteria.

*By tertiary treatment*

The growing environmental concern in developed countries in recent years, coupled with some awareness that the effluents from conventional secondary processes (trickling filter and activated sludge) are heavily contaminated with excreted viruses and bacteria, has led to an increasing use of tertiary treatment. This section discusses the effect on fecal indicator bacteria of some of the tertiary processes that are being used to upgrade the chemical or microbiological quality of secondary effluents prior to discharge or agricultural reuse.

This section does not consider the advanced wastewater reclamation plants designed to transform sewage into drinking water. Such plants incorporate a complex combination of biological, physicochemical, and disinfection processes and can eliminate excreted bacteria completely. Grabow and Isaacson (1978) reported that the water produced by the sewage reclamation plants in Windhoek (Namibia) and Pretoria (South Africa) contained no fecal coliforms in 94 percent of samples, no fecal streptococci in 100 percent of samples, no *Pseudomonas aeruginosa* in 96 percent of samples, no *Staphylococcus aureus* in 91 percent of samples, and no *Clostridium perfringens* in 92 percent of samples. The Windhoek and Pretoria plants receive secondary effluents and treat them by lime treatment, primary coagulation and sedimentation, ammonia stripping, primary chlorination, recarbonation, secondary coagulation and sedimentation, pH adjustment, sand filtration, secondary chlorination, activated carbon, and final chlorination (Grabow, Bateman and Burger 1978). A treatment train of this complexity, although highly effective, has no application in most countries (whether developed or developing) because of its high cost and excessive operation and maintenance problems.

LAGOONING. If retention times are several days, lagooning can be highly effective in removing excreted bacteria. Removal mechanisms and rates are as reported above for waste stabilization ponds. With short retention times, lagooning will not achieve worthwhile reductions in concentrations of excreted bacteria. Grabow, Middendorff and Basson (1978) report a removal of only 31 percent of coliforms and 18 percent of enterococci from lime-treated tertiary effluent (pH 9.6) held in an aerated pond for a mean period of 10 hours in Pretoria (South Africa).

COAGULATION. Coagulation or flocculation of a secondary effluent, followed by solids removal in sedimentation or flotation chambers, will remove those bacteria which are bound up within, or adsorbed to, the flocs. This bacterial removal mechanism is analogous to that of activated sludge, although the retention times in the sedimentation tanks are considerably shorter and poor removal percentages may be expected. Coagulation is commonly promoted by the addition of alum [$Al_2(SO_4)_3$], iron salts (for example, $FeCl_3$), or polyelectrolytes.

High removal rates can be achieved by lime treatment followed by sedimentation. The lime treatment raises the pH to a level that is extremely hostile to many bacteria and viruses, although some Gram-positive bacteria (for example, fecal streptococci) and spore-forming bacteria (for example, *Clostridium* spp.) are comparatively resistant to high pH. Grabow, Middendorff and Basson (1978) studied the effect of lime treatment, plus sedimentation with added $FeCl_3$ (1.5–2.5 milligrams per liter as Fe) and polyelectrolyte (0.5 milligrams per liter), on enteric bacteria in activated sludge effluent. With a final pH of 9.6, 62 percent of coliforms and 68 percent of enterococci were removed. When the lime dose was raised to give a final pH of 11.1, the removals were 99.98 and 97 percent, respectively, for coliforms and enterococci.

FILTRATION. The data on bacterial retention and death in soil, reviewed elsewhere in this chapter (see above, the sections on groundwater, soil, and septic tanks and below, the section on land treatment), show that a well-designed sand filter, with a sufficiently deep bed and a sufficiently low filtration rate, will remove a considerable proportion of fecal indicator bacteria in the effluent. In particular, a slow sand filter, receiving 2–5 cubic meters per square meter per day of effluent, should remove around 99 percent of enteric bacteria. Rapid processes, however, may be relatively ineffective. A combination of ammonia stripping, recarbonation, secondary clarification (with added $FeCl_3$ and polyelectrolyte), and sand filtration reduced the average concentration of enterococci by only 98 percent (Grabow, Middendorff and Basson 1978). Similarly, a tertiary effluent from a gravity sand filter on Long Island (New York, USA) contained up to $9.3 \times 10^5$ fecal coliforms per 100 milliliters (Vaughn and others 1978). Removal rates will be enhanced at warm temperatures.

DISINFECTION. The realization that secondary effluents from conventional sewage treatment plants (trickling filters or activated sludge) contain high concentrations of excreted viruses and bacteria has caused a growing interest in effluent disinfection as a means of achieving a major improvement in microbiological quality prior to discharge. The technique most practiced and most studied is effluent chlorination.

As with water chlorination, the level of bacterial kill achieved in effluent chlorination increases as chlorine dose, temperature, and contact time increase and as pH decreases. The additional factor of critical importance is the chemical quality of the effluent being chlorinated. When chlorine is added to an effluent, free chlorine ($HOCl + OCl^-$) disappears almost immediately, and the chlorine rapidly combines with ammonia and organic compounds. This combined chlorine is very considerably less bactericidal than free chlorine—although, as with free chlorine, it will be more effective as contact times and temperatures increase.

When a chlorinated effluent is discharged into a river, it is possible that the fecal indicator bacteria in the river will also be reduced in the stretch of river immediately downstream of the outfall (Snow 1977). The chlorine may also have a negative impact on the receiving water by killing certain species of flora and fauna and thus upsetting the aquatic ecology (Silvey, Abshire and Nunez 1974). In addition, the discharge of chlorinated effluents will add to the load of chlorinated organic compounds in the water and in the food chain, and some of these compounds are known or suspected carcinogens.

Properly controlled effluent chlorination can produce a very dramatic reduction in the concentrations of excreted bacteria. The better the quality of the effluent, the greater will be the bacterial reduction achieved by a given dose of applied chlorine. Berg and others (1978) added sodium hypochlorite (NaOCl) to primary effluents to achieve final combined chlorine residuals of 11 to 23 milligrams after 15 minutes at pH 8.2–9.2 and 22°C. This treatment reduced enterovirus (initial concentrations 109–427 per liter) by 85–99 percent, fecal coliforms (initial concentrations $10^4$–$10^7$ per 100 milliliters) by 99.95–>99.99998 percent, and fecal streptococci (initial concentration $10^5$ per 100 milliliters) by 99.997–>99.9998 percent (see also Berg and Metcalf 1978).

Kott and others (1974) reported that the addition of 8 milligrams per liter of chlorine to waste stabilization pond effluent (1 hour contact time at 20°C) reduced the concentration of coliforms from $10^5$–$10^7$ per 100 milliliters down to less than 2 per 100 milliliters in 50 percent of tests. Stenquist and others (1977) found that treating a secondary effluent ($BOD_5$ = 19 milligrams per liter) with 39 milligrams per liter of chlorine reduced coliform concentrations from $10^6$ to <2 per 100 milliliters after a 1-hour contact time.

Although effluent chlorination can produce very low concentrations of indicator bacteria at the point of discharge, it does not always do so. Vaughn and others (1978) reported that chlorinated secondary effluents on Lond Island (New York, USA) contained between 0 and $2.4 \times 10^6$ fecal coliforms per 100 milliliters. Kampelmacher, Fonds and van Noorle Jansen (1977) found that the chlorination of three secondary effluents in the Netherlands (2–6 milligrams per liter of chlorine added) reduced *E. coli* by between 24 and 99.999 percent and fecal streptococci by 0 to 99.99 percent. This great variability of bacteria removal is characteristic of effluent chlorination systems and in part is a result of the variable chlorine demand of the effluent.

The environment produced by effluent chlorination, namely one of high nutrients but low microbiological activity, is ideal for the growth of some excreted bacteria. Several studies have reported massive regrowth of fecal indicator bacteria, especially coliforms, in chlorinated sewage effluents. Shuval, Cohen and Kolodney (1973) studied trickling filter effluent in Jerusalem (Israel) that was treated with 10 milligrams per liter of chlorine and then stored for 3 days prior to agricultural use. The geometric mean concentrations of coliforms per 100 milliliters were $5 \times 10^6$ in the secondary effluent, 120 in the chlorinated effluent, and 800 in the stored effluent. Laboratory experiments with 5 milligrams per liter of chlorine added to secondary effluent stored at 20°C showed that coliform concentrations fell to 0.001 percent of their initial value after chlorination but 4 days later had regained their initial level, whereas fecal coliform concentrations fell to 0.0001 percent of initial levels and had climbed back to 0.1 percent after 5 days.

Kinney, Drummond and Hanes (1978) compared bacterial death rates in chlorinated secondary effluent mixed with stream water and in unchlorinated tertiary effluent mixed with stream water (three parts effluent to one part of stream water). The effluent had a $BOD_5$ value of 7–11 milligrams per liter. The applied chlorine dose was 2.3–3.5 milligrams per liter, which produced a total residual of around 1 milligram per liter after 15 minutes. The mixtures were held in the dark at 20°C. The chlorinated mixture contained 6 *E. coli* per 100 milliliters, which rose to $10^2$ per 100 milliliters after 5 days. The unchlorinated mixture contained $1.9 \times 10^3$–$2.2 \times 10^4$ *E. coli* per 100 milliliters, which fell to $10^2$ per 100 milliliters after 5 days. Similar

experiments were conducted with total coliforms, *Klebsiella*, *Enterobacter*, and *Citrobacter*. In all cases there was no statistically significant difference in bacterial concentrations between the chlorinated and unchlorinated mixtures after 4 days of storage.

Irving (1980) reported great variability in the bacterial reductions obtained when chlorine was added to raw sewage. Average results showed that, after a contact time of 30 minutes, the coliform reductions were 3 log units at 5 milligrams per liter of applied chlorine, 4 log units at 10 milligrams per liter of applied chlorine, and $>5$ log units at 15 milligrams per liter of applied chlorine. To investigate regrowth, samples of chlorinated sewage were added to seawater and stored in the dark at 15°C. When the effluent to seawater mix was 1:10, coliform concentrations increased in the chlorinated mixture to more than the initial (pre-chlorination) level after 160 hours' storage, whereas the unchlorinated control had concentrations of only 10 percent of initial values. When the effluent to seawater mix was 1:100, no regrowth occurred and the chlorinated mixture had coliform concentrations of 0.001 percent of initial levels after 160 hours' storage, whereas the unchlorinated control had concentrations of 1 percent of initial values. The ratio of effluent to seawater, and thus the concentration of bacterial nutrients, was found to influence strongly the coliform regrowth potential. When actual marine outfall conditions were simulated (ten-fold dilution of sewage initially rising to a hundredfold dilution after 3.5 hours), no regrowth of coliforms or fecal coliforms was recorded in the chlorinated effluent and seawater mixture.

Fecal coliforms are more susceptible to chlorination, and undergo lesser regrowth in chlorinated effluents, than coliforms as a whole. Fecal streptococci may be slightly less susceptible to chlorination of effluents than fecal coliforms. Major regrowth of fecal indicator bacteria in chlorinated effluents and their receiving waters is more likely in fresh water than in seawater, where dilution is low (less than ten-fold) and where temperatures are warm. Thus, the discharge of chlorinated effluents into tropical streams, which have little or no flow during the dry season, is a situation almost certainly accompanied by major regrowth of coliforms, fecal coliforms, and possibly other excreted bacteria.

Chlorine dioxide ($ClO_2$) has attracted interest recently as a disinfectant of water and wastewater, although its mode of action is not elucidated (Roller, Olivieri and Kawata 1980). Longley, Moore and Sorber (1980) compared chlorine and chlorine dioxide applied to secondary effluent at doses of 5 milligrams per liter (as $Cl_2$) with a contact time of 3 minutes at 22°C. Reduction of fecal coliforms averaged over 5 log units with chlorine dioxide and over 3 log units with chlorine. This and other studies (for instance, Cronier, Scarpino and Zink 1978) have shown that chlorine dioxide is often a more powerful bactericide than chlorine and has other advantages such as being less affected by pH and less prone to generate carcinogenic trihalogenated methanes. Similarly, Keswick and others (1980) found that bromine chloride (BrCl) had several advantages over chlorine as a wastewater disinfectant.

Various other disinfecting methods have been tried, at least on an experimental basis. Ozone is able to eliminate fecal solids-associated coliforms after 20 seconds with an applied dose of less than 0.1 milligram per liter (Foster and others 1980; see also Wyatt and Wilson 1980). Ultraviolet and gamma radiation have also been shown to be effective in killing bacteria in effluent (Myhrstad 1979; Woodbridge and Cooper 1979). These techniques are very much at the experimental stage, and their economical and technical appropriateness are doubtful. The same comments apply to disinfection by photodynamic oxidation (Gerba, Wallis and Melnick 1977).

LAND TREATMENT. The treatment of a primary or secondary effluent by application to land, with subsequent flow through the soil to underdrains or to groundwater, can be an effective method of removing high concentrations of excreted viruses (see chapter 9) and bacteria. Lance, Rice and Gilbert (1980) reported a 5 log removal of fecal coliforms from primary effluent (settled sewage) as it passed through 2.5 meters of loamy sand at the rate of 0.2 cubic meters per square meter per day and at a temperature of 24°C. Under similar conditions, but with double the infiltration rate, there was a 3 log removal of fecal coliforms from secondary effluents. A theoretical approach to computing the degree of bacteria removal by land treatment systems has been proposed by Hendricks, Post and Khairnar (1979).

Gilbert and others (1976) recorded that the fecal coliforms and fecal streptococci in a secondary effluent were reduced by 99.9 percent after flow through 9 meters of soil (fine loamy sand underlain by coarse sand and gravel) under groundwater recharge basins at Phoenix (Arizona, USA). The application rates average 0.25 cubic meters per square meter per day, and the mean daily air temperatures ranged between 10°C in January and 32°C in July. Fecal coliforms were not detected in water from a well 90 meters distant from the recharge basins (Bouwer and others 1980). Schaub

and Sorber (1977) studied a rapid infiltration site (1.4 cubic meters per square meter per day) in Massachusetts (USA) where primary effluent had been applied to unconsolidated silty sand and gravel for over 30 years. Nearly all indicator bacteria were removed in the top 1 meter of soil, but, on occasion, up to $10^4$ fecal streptococci per 100 milliliters were detected in the groundwater, as compared with $10^5$–$10^6$ per 100 milliliters of effluent.

Land treatment will in general be more effective in warm climates than in temperate ones. Maintenance and management of these installations must be highly efficient, otherwise, the treatment site will degenerate into an unsanitary bog.

OTHER PROCESSES. A variety of other processes for the treatment of sewage are being tried on a laboratory or pilot scale. Many of these research and development projects focus on the need to produce a final effluent almost completely free of excreted organisms and attempt to reclaim sewage for agricultural or other productive purposes. Some of the technologies being developed have high performance in removing fecal indicator bacteria—for instance, solar distillation (Qasim 1978)—but are unlikely to be economically and technically appropriate for application in developing countries. Technologies that are simple, relatively low cost, and highly effective in removing excreted bacteria are already known (for instance, waste stabilization ponds or land treatment of secondary effluents), and the priority in most developing countries is the successful and widespread application of these established technologies.

## Inactivation by Night Soil and Sludge Treatment Processes

Since fresh feces commonly contain $10^5$–$10^9$ fecal coliforms and fecal streptococci per gram, the fecal products from dry sanitation systems contain high concentrations of fecal indicator bacteria. It is this very concentration of excreted organisms that makes the dry systems attractive compared with the wet systems, which mix the fecal material with large volumes of water that are then difficult to purify and contain. From a microbiological viewpoint the essential principle of the dry sanitation systems is that they should concentrate and contain the excreted organisms in the smallest possible volume where they may then be killed. The sludge from a sewage treatment plant contains those bacteria which were adsorbed to, or trapped within, settleable solids, and high concentrations of fecal indicator bacteria are typically found ($10^5$–$10^8$ per gram). This section describes methods of killing bacteria contained in night soil or sludge and emphasizes the important role of time and temperature in creating conditions lethal to bacteria.

### *By pit latrines*

Pit latrines are an effective method of containing and storing excreted bacteria. Death rates will be very much more rapid at warm temperatures than at cold temperatures and may be more rapid in dry pits than in flooded pits. A pit in use will always contain fresh layers of pathogenic material. The contents of a sealed pit in a warm climate, however, should contain a very low concentration of fecal indicator bacteria after 3 months' storage.

Stiles and Crohurst (1923) buried human feces in pits in an area with a high water table and covered them with sawdust. Three years and 2 months later, the feces were both macroscopically, and, microscopically recognizable but had developed a "musty" odor. Three out of five samples contained viable *E. coli.* Jordan (1926) found that *E. coli* in stored human feces became undetectable after 6–12 weeks at room temperature and after 1–3 weeks at 37°C. At 10°C they were still detectable in high concentrations after 23 weeks.

### *By anaerobic digestion*

Anaerobic digestion is the most commonly adopted method of sludge treatment at large sewage treatment works. The process is usually mesophilic (35°C) but is sometimes heated to the thermophilic range (55°C). Retention times are typically 10–60 days.

Cooke, Thackston and Malaney (1978) studied mesophilic anaerobic digestors at three treatment plants near Nashville (Tennessee, USA). Concentrations of coliforms in the raw primary sludge were $10^8$–$10^{10}$ per 100 milliliters and in the digested sludge were $10^4$–$10^9$ per 100 milliliters. All digesters were operating at 33–37°C, but retention times varied considerably. The mean removal rates at different retention times were less than 2 log units after 9 days, 3 log units, after 38 days, and nearly 4 log units after 50 days. Berg and Metcalf (1978) reported that a continuously operated mesophilic digester (35°C for 20 days) removed 95 to 99 percent of fecal coliforms and 80 to 97 percent of fecal streptococci, whereas a thermophilic digester (50°C for 20 days) removed 99.9 to >99.9999 percent of fecal coliforms and 99.8 to >99.999 percent of fecal streptococci. Studies in the USSR showed that an enteropathogenic serotype of *E.*

*coli* survived thermophilic digestion for not more than 10 days (Grigoryeva, Korchak and Bey 1969).

Continuously fed mesophilic digesters will produce sludge that still contains high concentrations of fecal indicator bacteria ($10^3$–$10^6$ per 100 milliliters), even if retention times are around 50 days. For substantially higher levels of bacterial reduction the process should be thermophilic, with retention times of at least 20 days and preferably with batch operation. Thermophilic operation is more economical in warm climates than in temperate areas because heat loss to the environment is reduced; therefore less energy input is required (from digester biogas or other sources). However, the most economical method of achieving thermophilic conditions, and thereby high levels of excreted bacterial reduction, is often by aerobic composting. Finstein and others (1980) compared anaerobic digestion with composting and found that, although digestion required less labor and produced methane, composting was advantageous with respect to pathogen kill, process stability, decomposition of industrial compounds, drying, and residue acceptability.

*By heating*

Any process that heats night soil or sludge to over 60°C for the required length of time will eliminate fecal indicator bacteria. Most of the heating processes—such as pasteurization (80°C), wet oxidation (120–370°C), incineration, and pyrolysis—will completely destroy all excreted organisms but are technically complex and require considerable energy input. More attractive is thermophilic, aerobic composting, which elevates temperatures to above 60°C without the need for any external energy source. Time-temperature combinations that will destroy enteroviruses (see chapter 9) and *Ascaris* eggs (see chapter 23) will certainly destroy the nonsporulating excreted bacteria.

*By composting*

The composting of night soil and sludge is a simple means of producing a fecal product that is safe and convenient to use in agriculture. Of greatest interest are the aerobic processes that generate sufficient heat to raise the temperature of the composting mass to 55°C or above and thus kill a large proportion of excreted pathogens.

Savage, Chase and MacMillan (1973) studied the composting of pig waste (uneaten garbage and pig feces) in four windrows, which were mechanically turned. Windrow 1 contained pig waste turned twice per week; windrow 2 contained pig waste turned 20 times per week; windrow 3 contained pig waste plus old compost turned 20 times per week; windrow 4 contained pig waste plus straw turned 20 times per week. The speed of temperature rise declined in the order of windrows 4, 3, 2, 1. All windrows eventually reached 60°C. Windrow 4 reached 60°C in 3 days, after which it rose to 72°C, stayed above 60°C for over 30 days, and fell back to ambient temperature (20–30°C) by day 38. In windrow 1, fecal coliforms and fecal streptococci numbers increased for the first 40 to 60 days until temperatures reached 50°C, after which bacterial concentrations decreased as the temperatures rose into the thermophilic range. In windrows 2 and 3, the concentrations of fecal coliforms fell from $10^7$ to $10^3$ per gram after 35 days, whereas in windrow 4 fecal coliforms became undetectable after 14 days. The combination of frequent turning and the addition of straw (which provided a source of carbon and improved the structure of the compost by furnishing more opportunity for aeration) produced a windrow that achieved very high bacterial death rates (7 log units in 14 days).

Burge, Cramer and Epstein (1978) have reviewed the composting work conducted at Beltsville Agricultural Research Center (Maryland, USA). In early experiments raw or digested sludge filter cake (20 per cent solids) was mixed with woodchips (1 volume sludge to 3 volumes of woodchips), placed in windrows, and turned daily for 2 weeks, after which it was stockpiled for a minimum of 30 days. Temperatures in the windrows rose to 50–70°C in 3 days with raw sludge, and to 40–60° in 14 days with digested sludge, except in rainy winter weather when temperatures stayed at 20–30°C. Fecal coliforms typically declined from $10^7$ per gram to undetectable levels during the windrow phase at depths of 0.8 meters and more. At depths of less than 0.4 meters, where the windrow was cooler, fecal coliform concentrations declined from $10^7$ to $10^2$–$10^4$ per gram during the windrow phase and maintained these concentrations during 30 days of stockpiling. Because of lowered temperature during heavy rain, poor bacterial kill at the edges of the windrows, and bad odor release from the raw sludge windrows, new experiments were started in which windrows (raw sludge plus woodchips) were aerated (by sucking air downwards into slotted pipes laid under the windrow) and lagged by covering with a layer of old compost. Composting by forced aeration was continued for 21 days, after which the material was stockpiled for a minimum of 30 days. Temperatures rose into the thermophilic range within 5 days, irrespective of the weather conditions. Fecal coliforms

were reduced to undetectable levels after 10 days in the windrows, and this bacterial destruction occurred at all parts of the composting mass (see also Kawata, Cramer and Burge 1977).

Unpublished data obtained by Vietnamese scientists have suggested that *E. coli* are reduced to undetectable levels after 7 weeks treatment in a sealed vault of a double-vault composting latrine (Nimpuno, personal communication). McGarry and Stainforth (1978) describe aerobic (average pile temperature 40°C) and anaerobic (average pile temperature 29°C) composting with equal parts by weight of human feces and urine, animal feces, rubbish (sweepings, brushwood, grass, ashes, weeds, leaves), and soil held for one month in China. Initial *E. coli* concentrations of the mixture were $2.5 \times 10^5$ per gram, and these were reduced to 91–233 per gram in both the aerobic and anaerobic processes.

It is clear from these and other studies (listed in the appendixes of Feachem and others 1980) that well-managed composting plants can reduce fecal indicator bacteria in night soil and sludge to undetectable levels in under 1 month. For this to occur, the process must become thermophilic rapidly. This in turn requires the addition of carbon, aeration by turning or forced ventilation, moisture control, and a good physical structure with adequate air voids for the pile. To ensure that all parts of the pile achieve a disinfecting temperature, the compost must be turned or lagged. The experiments at Beltsville have shown that forced ventilation and lagging can create high temperatures throughout a windrow even during cold and rainy winters.

*By lime treatment*

Lime treatment, resulting in high pH values, should be effective in reducing fecal indicator bacteria in night soil and sludge, although little specific information is available. Lime treatment is certainly effective in eliminating salmonellae from sludges and animal slurries (see chapter 15). Polprasert and Valencia (1981) applied various concentrations of lime to fecal samples collected from school children in Bangkok (Thailand). Initial pH was 5.8–6.0, and the fecal coliform concentrations were $4.6 \times 10^{10}$ to $1 \times 10^{11}$ per milliliter. The addition of 5,700 milligrams of CaO per liter raised the pH to 9 and reduced the fecal coliform concentration by 0–1 log units. The addition of 19,000 milligrams of CaO per liter raised the pH to 12 and reduced the fecal coliform concentration by 4–6 log units. Intermediate doses produced intermediate pH values and bacterial reductions. Fecal coliform death occurred in the first 3 hours after lime dosing, and no significant additional dieoff occurred during 2 days storage at 25°C.

*By other processes*

Various other methods have been proposed for the disinfection of sludges prior to agricultural use. Irradiation of sludges by 3–3.5 kilogray produces a 4 to 9 log reduction in Gram-negative enteric bacteria such as fecal coliforms. Gram-positive bacteria such as fecal streptococci appear to be somewhat more resistant and may be reduced by only 2 to 3 log units (Lessel and Suess 1978; Osborn and Hattingh 1978). A radiated sludge, however, is a nutrient-rich but biologically impoverished medium, and major regrowth of fecal indicator bacteria may be predicted and has been reported (Osborn and Hattingh 1978). Superchlorination of sludges (chlorine doses of 700–4,000 milligrams per liter applied under pressure) will certainly destroy fecal bacteria, but it has to be questioned seriously on environmental grounds (Kamlet 1979).

## Literature Cited

Adams, A. P., Garbett, M., Rees, H. B. and Lewis, B. G. (1980). Bacterial aerosols produced from a cooling tower using wastewater effluent as makeup water. *Journal of the Water Pollution Control Federation*, **52,** 498–501.

Allen, L. A., Pasley, S. M. and Pierce, M. A. F. (1952). Some factors affecting the viability of faecal bacteria in water. *Journal of General Microbiology*, **7,** 36–43.

Allen, M. J. (1979). Microbiology of groundwater. *Journal of the Water Pollution Control Federation*, **51,** 1743–1746.

Banerjea, R. and Sen, A. K. (1940). Study of the Eijkman test and modifications as given by coliform organisms isolated from human faeces. *Indian Journal of Medical Research*, **28,** 315–319.

Baross, J. A., Hanus, F. J. and Morita, R. Y. (1975). Survival of human enteric and other sewage microorganisms under simulated deep-sea conditions. *Applied Microbiology*, **30,** 309–318.

Barrell, R. A. E. and Rowland, M. G. M. (1979*a*). Infant foods as a potential source of diarrhoeal illness in rural West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73,** 85–90.

———(1979*b*). The relationship between rainfall and well water pollution in a West African (Gambian) village. *Journal of Hygiene*, **83,** 143–150.

Bausum, H. T., Schaub, S. A. and Kenyon, K. F. (1978). *Viral and Bacterial Aerosols at a Wastewater Spray Irrigation Site.* Technical Report 7804. Washington, DC: US Army Medical Research and Development Command.

Baylor, E. R., Baylor, M. B., Blanchard, D. C., Syzdek, L. D.

and Appel, C. (1977). Virus transfer from surf to wind. *Science*, **198,** 575–580.

Baylor, E. R., Peters, V. and Baylor, M. B. (1977). Water-to-air transfer of virus. *Science*, **197,** 763–764.

Bell, R. G. (1976). Persistence of fecal coliform indicator bacteria on alfalfa irrigated with municipal sewage lagoon effluent. *Journal of Environmental Quality*, **5,** 39–42.

Bell, R. G. and Bole, J. B. (1976). Elimination of fecal coliform bacteria from reed canarygrass irrigated with municipal sewage lagoon effluent. *Journal of Environmental Quality*, **5,** 417–418.

———(1978). Elimination of fecal coliform bacteria from soil irrigated with municipal sewage lagoon effluent. *Journal of Environmental Quality*, **7,** 193–196.

Bellair, J. T., Parr-Smith, G. A. and Wallis, I. G. (1977). Significance of diurnal variations in fecal coliform die-off rates in the design of ocean outfalls. *Journal of the Water Pollution Control Federation*, **49,** 2022–2030.

Berg, G., Dahling, D. R., Brown, G. A. and Berman, D. (1978). Validity of fecal coliforms, total coliforms and fecal streptococci as indicators of viruses in chlorinated primary sewage effluents. *Applied and Environmental Microbiology*, **36,** 880–884.

Berg, G. and Metcalf, T. G. (1978). Indicators of viruses in waters. In *Indicators of Viruses in Water and Food*, ed. Berg, G., pp. 267–296. Ann Arbor, Mich.: Ann Arbor Science Publishers.

Bettelheim, K. A., Cooke, E. M., O'Farrell, S. and Shooter, R. A. (1977). The effect of diet on intestinal *Escherichia coli*. *Journal of Hygiene*, **79,** 43–45.

Bitton, G. and Smith-Holmes, W. (1978). Bacterial aerosols generated by a package treatment plant. *Journal of Environmental Science and Health*, **A13,** 391–401.

Black, R. E., Merson, M. H., Rowe, B., Taylor, P. R., Mizanur Rahman, A. S. M., Azizal Huq, M., Abdul Aleem, A. R. M., Sack, D. A. and Curlin, G. T. (1979). Epidemiology of enterotoxigenic *Escherichia coli* in rural Bangladesh. In *Proceedings of the 14th Joint Conference, US-Japan Cooperative Medical Science Program, Cholera Panel, Symposium on Cholera*, eds. Takeya, K. and Zinnaka, Y., pp. 292–301. Tokyo: Toho University.

Blanchard, D. C. and Syzdek, L. (1970). Mechanism for water-to-air transfer and concentration of bacteria. *Science*, **170,** 626–628.

Boizot, G. E. (1941). An examination of the modified Eijkman method applied to pure coliform cultures obtained from waters in Singapore. *Journal of Hygiene*, **41,** 566–569.

Bound, W. H. and Atkinson, R. I. (1966). Bacterial aerosol from water closets. *Lancet*, **1,** 1369–1370.

Bouwer, H., Rice, R. C., Lance, J. C. and Gilbert, R. G. (1980). Rapid infiltration research at Flushing Meadows Project, Arizona. *Journal of the Water Pollution Control Federation*, **52,** 2457–2470.

Brandes, M. (1978*a*). Accumulation rate and characteristics of septic tank sludge and septage. *Journal of the Water Pollution Control Federation*, **50,** 936–943.

———(1978*b*). Characteristics of effluents from gray and black water septic tanks. *Journal of the Water Pollution Control Federation*, **50,** 2547–2559.

Brokunsult and Ross Institute (1978). *Rural Water Quality Programme in Tanzania.* Dar es Salaam: Ministry of Water Development, Energy and Minerals.

Brown, K. W., Jones, S. G. and Donnelly, K. C. (1980). The influence of simulated rainfall on residual bacteria and virus on grass treated with sewage sludge. *Journal of Environmental Quality*, **9,** 261–265.

Brown, K. W., Wolf, H. W., Donnelly, K. C. and Slowey, J. F. (1979). The movement of fecal coliforms and coliphages below septic lines. *Journal of Environmental Quality*, **8,** 121–125.

Burdyl, P. and Post, F. J. (1979). Survival of *Escherichia coli* in Great Salt Lake water. *Water Air and Soil Pollution*, **12,** 237–246.

Burge, W. D., Cramer, W. N. and Epstein, E. (1978). Destruction of pathogens in sewage sludge by composting. *Transactions of the American Society of Agricultural Engineers*, **21,** 510–514.

Cabelli, V. J. (1978). New standards for enteric bacteria. In *Water Pollution Microbiology*, **2,** ed. Mitchell, R., pp. 233–271. New York: Wiley-Interscience.

———(1979). Evaluation of recreational water quality, the EPA approach. In *Biological Indicators of Water Quality*, eds. James, A. and Evison, L., pp. 14/1–14/23. Chichester: John Wiley.

Cabelli, V. J., Dufour, A. P., Levin, M. A., McCabe, L. J. and Haberman, P. W. (1979). Relationship of microbial indicators to health effects at marine bathing beaches. *American Journal of Public Health*, **69,** 690–696.

Caldwell, E. L. and Parr, L. W. (1937). Groundwater pollution and the bored hole latrine. *Journal of Infectious Diseases*, **61,** 148–183.

Chamberlin, C. E. and Mitchell, R. (1978). A decay model for enteric bacteria in natural waters. In *Water Pollution Microbiology*, **2,** ed. Mitchell, R., pp. 325–348. New York: Wiley-Interscience.

Chandler, D. S. and Craven, J. A. (1978*a*). Bacteriological studies of land disposal of piggery effluent. *Proceedings of the Australian Society of Animal Production*, **12,** 158.

———(1978*b*). Environmental factors affecting *Escherichia coli* and *Salmonella typhimurium* numbers on land used for effluent disposal. *Australian Journal of Agricultural Research*, **29,** 577–585.

———(1980). Persistence and distribution of *Erysipelothrix rhusiopathiae* and bacterial indicator organisms on land used for disposal of piggery effluent. *Journal of Applied Bacteriology*, **48,** 367–375.

Chojnowski, K. J., Mancini, J. L. and Jeris, J. S. (1979). Influence of light on coliform mortality rates in fresh water and sea water. *Journal of the Water Pollution Control Federation*, **51,** 2538–2539.

Ciravolo, T. G., Martens, D. C., Hallock, D. L., Collins, E. R., Kornegay, E. T. and Thomas, H. R. (1979). Pollutant movement to shallow ground water tables from anaerobic swine waste lagoons. *Journal of Environmental Quality*, **8,** 126–130.

Coetzee, O. J. and Fourie, N. A. (1965). The efficiency of conventional sewage purification works, stabilization ponds and maturation ponds with respect to the survival of pathogenic bacteria and indicator organisms. *Journal of the Institute of Sewage Purification*, part 3, 210–215.

Cohen, J. and Shuval, H. I. (1973). Coliforms, fecal coliforms and fecal streptococci as indicators of water pollution. *Water, Air, and Soil Pollution*, **2,** 85–95.

Cooke, M. B., Thackston, E. L. and Malaney, G. W. (1978). Reducing coliform and *Salmonella* bacteria during anaerobic digestion. *Water and Sewage Works*, **125,** 50–53.

Crawford, G. V. and Jones, P. H. (1978). The airborne transmission of organisms emitted from wastewater treatment processes. *Water Pollution Research in Canada*, **13,** 33–43.

Cronholm, L. S. (1980). Potential health hazards from microbial aerosols in densely populated urban regions. *Applied and Environmental Microbiology*, **39,** 6–12.

Cronier, S., Scarpino, P. V. and Zink, M. L. (1978). Chlorine dioxide destruction of viruses and bacteria in water. In *Water Chlorination: Environmental Impact and Health Effects*, eds. Jolley, R. L., Gorchev, H. and Hamilton, D. H., pp. 651–658. Ann Arbor, Mich.: Ann Arbor Science Publishers.

Crozier, D. and Woodward, T. E. (1962). Armed Forces Epidemiological Board: activities of the Commission on Epidemiological Survey, 1961. *Military Medicine*, **127,** 701–705.

Dale,, D. C. and Mata, L. J. (1968). Studies of diarrheal disease in Central America. XI. Intestinal bacterial flora in malnourished children with shigellosis. *American Journal of Tropical Medicine and Hygiene*, **17,** 397–403.

Daniel, R. R. and Lloyd, B. J. (1980). Microbiological studies on two Oxfam Sanitation Units operating in Bengali refugee camps. *Water Research*, **14,** 1567–1571.

Darlow, H. M. and Bale, W. R. (1959). Infective hazards of water-closets. *Lancet*, **1,** 1196–1200.

Davenport, C. V., Sparrow, E. B. and Gordon, R. C. (1976). Fecal indicator bacteria persistence under natural conditions in an ice-covered river. *Applied and Environmental Microbiology*, **32,** 527–536.

Davis, E. M. (1979). *Bacterial Characteristics of Stormwaters in Developing Rural Areas.* Report EPA-600/2-79-050f. Cincinnati, Ohio: US Environmental Protection Agency.

Davis, E. M. and Gloyna, E. F. (1972). Bacterial die-off in ponds. *Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers*, **98,** 59–69.

Dazzo, F., Smith, P. and Hubbell, D. (1973). The influence of manure slurry irrigation on the survival of fecal organisms in Scranton fine sand. *Journal of Environmental Quality*, **2,** 470–473.

Dissanayake, M. G. (1980). Kinetics of bacterial die-off in waste stabilization ponds. Ph.D. dissertation no. EV-80-2. Bangkok: Asian Institute of Technology.

Dor, I., Schechter, H. and Shuval, H. I. (1976). Biological and chemical succession in Nahal Sareq: a free-flowing wastewater stream. *Journal of Applied Ecology*, **13,** 475–489.

Druett, H. A., Henderson, D. W., Packman, L. and Peacock, S. (1953). Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores. *Journal of Hygiene*, **51,** 359–371.

Druett, H. A., Henderson, D. W. and Peacock, S. (1956). Studies on respiratory infection. III. Experiments with *Brucella suis*. *Journal of Hygiene*, **54,** 49–57.

Dudley, D. J., Guentzel, M. N., Ibarra, M. J., Moore, B. E. and Sagik, B. P. (1980). Enumeration of potentially pathogenic bacteria from sewage sludges. *Applied and Environmental Microbiology*, **39,** 118–126.

Dudley, D. R. and Karr, J. R. (1979). Concentration and sources of fecal and organic pollution in an agricultural watershed. *Water Resources Bulletin*, **15,** 911–923.

Dunigan, E. P. and Dick, R. P. (1980). Nutrient and coliform losses in runoff from fertilized and sewage sludge-treated soils. *Journal of Environmental Quality*, **9,** 243–250.

DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., LaBrec, E. H. and Kalas, J. P. (1971). Pathogenesis of *Escherichia coli* diarrhea. *New England Journal of Medicine*, **285,** 1–9.

Dutka, B. J. and Kwan, K. K. (1980). Bacterial die-off and stream transport studies. *Water Research*, **14,** 909–915.

Dyer, B. R., Bhaskaran, T. R. and Sekar, C. C. (1945). Investigations of groundwater pollution. III. Groundwater pollution in West Bengal, India. *Indian Journal of Medical Research*, **33,** 23–62.

Echeverria, P., Blacklow, N. R., Vollett, J. L., Ulyangco, C. V., Cukor, G., Soriano, V. B., DuPont, H. L., Cross, J. H., Ørskov, F. and Ørskov, I. (1978*a*). Reovirus-like agent and enterotoxigenic *Escherichia coli* infections in pediatric diarrhea in the Philippines. *Journal of Infectious Diseases*, **138,** 326–332.

Echeverria, P., Verhaert, L., Basaca-Sevilla, V., Banson, T., Cross, J., Ørskov, F. and Ørskov, I. (1978*b*). Search for heat-labile enterotoxigenic *Escherichia coli* in humans, livestock, food and water in a community in the Philippines. *Journal of Infectious Diseases*, **138,** 87–90.

Echeverria, P., Blacklow, N. R., Zipkin, C., Vollet, J. J., Olson, J. A., Dupont, H. L. and Cross, J. H. (1979). Etiology of gastroenteritis among Americans living in the Philippines. *American Journal of Epidemiology*, **109,** 493–501.

Echeverria, P., Ho, M. T., Blacklow, N. R., Quinnan, G., Portnoy, B., Olson, J. G., Conklin, R., DuPont, H. L. and Cross, J. H. (1977). Relative importance of viruses and bacteria in the etiology of pediatric diarrhea in Taiwan. *Journal of Infectious Diseases*, **136,** 383–390.

Edmond, T. D., Schaiberger, G. E. and Gerba, C. P. (1978). Detection of enteroviruses near deep marine sewage outfalls. *Marine Pollution Bulletin*, **9,** 246–249.

Edmonds, R. L. (1976). Survival of coliform bacteria in sewage sludge applied to a forest clearcut and potential movement into groundwater. *Applied and Environmental Microbiology*, **32,** 537–546.

Edmonds, R. L. and Littke, W. (1978). Coliform aerosols

generated from the surface of dewatered sewage applied to a forest clearcut. *Applied and Environmental Microbiology*, **36,** 972–974.

Elliot, L. F. and Ellis, J. R. (1977). Bacterial and viral pathogens associated with land application of organic wastes. *Journal of Environmental Quality*, **6,** 245–251.

Enzinger, R. M. and Cooper, R. C. (1976). Role of bacteria and protozoa in the removal of *Escherichia coli* from estuarine waters. *Applied and Environmental Microbiology*, **31,** 758–763.

Essien, E. S. and Osuhor, P. C. (1979). Rural water quality control measures: an experience in the Malumfashi District of Northern Nigeria. *Public Health*, **93,** 363–370.

Evans, M. R. and Owens, J. D. (1972). Factors affecting the concentration of faecal bacteria in land-drainage water. *Journal of General Microbiology*, **71,** 477–485.

Evison, L. (1979). Microbial parameters of raw water quality. In *Biological Indicator of Water Quality*, eds. James, A. and Evison, L., pp. 16/1–16/19. Chichester: John Wiley.

Evison, L. and James, A. (1973). A comparison of the distribution of intestinal bacteria in British and East African water sources. *Journal of Applied Bacteriology*, **36,** 109–118.

Evison, L. and Tosti, E. (1980). Bathing water quality in the North Sea and the Mediterranean. *Marine Pollution Bulletin*, **11,** 72–75.

Fannin, K. F., Gannon, J. J., Cochran, K. W. and Spendlove, J. C. (1977). Field studies on coliphages and coliforms or indicators of airborne animal viral contamination from wastewater treatment facilities. *Water Research*, **11,** 181–188.

Faust, M. A., Aotaky, A. E. and Hargadon, M. T. (1975). Effect of physical parameters on the in situ survival of *Escherichia coli* MC-6 in an estuarine environment. *Applied Microbiology*, **30,** 800–806.

Feachem, R. (1973). The Raiapu Enga pig herd. *Mankind*, **9,** 25–31.

———(1974). Faecal coliforms and faecal streptococci in streams in the New Guinea Highlands. *Water Research*, **8,** 367–374.

———(1975). An improved role for faecal coliform to faecal streptococci ratios in the differentiation between human and non-human pollution sources. *Water Research*, **9,** 689–690.

———(1977). Water supplies for low income communities: resource allocation, planning and design for a crisis situation. In *Water, Wastes and Health in Hot Climates*, eds. Feachem, R., McGarry, M. and Mara, D., pp. 75–95. Chichester: John Wiley.

Feachem, R., Burns, E., Cairncross, S., Cronin, A., Cross, P., Curtis, D., Khan, M. K., Lamb, D. and Southall, H. (1978). *Water Health and Development: An Interdisciplinary Evaluation.* London: Tri-Med Books.

Feachem, R. G. A., Bradley, D. J., Garelick, H. and Mara, D. D. (1981). *Health Aspects of Excreta and Sullage Management: A State-of-the-Art Review.* Appropriate Technology for Water Supply and Sanitation, vol. 3. Washington, DC: The World Bank, Transportation, Water and Telecommunications Department.

Fedorak, P. M. and Westlake, D. W. S. (1980). Airborne bacterial densities at an activated sludge treatment plant. *Journal of the Water Pollution Control Federation*, **52,** 2185–2192.

Feliciano, D. V. (1979). Wastewater aerosols and health risks. *Journal of the Water Pollution Control Federation*, **51,** 2573–2576.

Ferguson, W. W. and June, R. C. (1952). Experiments on feeding adult volunteers with *Escherichia coli*. III $B_4$, a coliform organism associated with infant diarrhea. *American Journal of Hygiene*, **55,** 155–169.

Finstein, M. S., Cirello, J., Suler, D. J., Morris, M. L. and Strom, P. F. (1980). Microbial ecosystems responsible for anaerobic digestion and composting. *Journal of the Water Pollution Control Federation*, **52,** 2675–2685.

Foster, D. M., Emerson, M. A., Buck, C. E., Walsh, D. S. and Sproul, O. J. (1980). Ozone inactivation of cell- and fecal-associated viruses and bacteria. *Journal of the Water Pollution Control Federation*, **52,** 2174–2184.

Freiji, L., Sterky, G., Wadström, T. and Wall, S. (1979). Child health and diarrhoeal disease in relation to supply and use of water in African communities. *Progress in Water Technology*, **11,** 49–55.

Gallagher, T. P. and Spino, D. F. (1968). The significance of numbers of coliform bacteria as an indicator of enteric pathogens. *Water Research*, **2,** 169–175.

Gameson, A. L. H. and Gould, D. J. (1975). Effects of solar radiation on the mortality of some terrestrial bacteria in sea water. In *Proceedings of the International Symposium on Discharge of Sewage from Sea Outfalls*, ed. Gameson, A. L. H., pp. 209–219. Oxford: Pergamon.

Gameson, A. L. H. and Saxon, J. R. (1967). Field studies on effect of daylight on mortality of coliform bacteria. *Water Research*, **1,** 279–295.

Gangarosa, E. J. (1978). Epidemiology of *Escherichia coli* in the United States. *Journal of Infectious Diseases*, **137,** 634–638.

Geldreich, E. E. (1976). Faecal coliform and faecal streptococcus density relationships in waste discharges and receiving water. *CRC Critical Reviews in Environmental Control*, **5,** 349–368.

———(1978). Bacterial populations and indicator concepts in feces, sewage, stormwater and solid wastes. In *Indicators of Viruses in Water and Food*, ed. Berg, G., pp. 51–97. Ann Arbor, Mich.: Ann Arbor Science Publishers.

Geldreich, E. E., Best, L. C., Kenner, B. A. and Van Donsel, D. J. (1968). The bacteriological aspects of stormwater pollution. *Journal of the Water Pollution Control Federation*, **40,** 1861–1872.

Geldreich, E. E. and Bordner, R. H. (1971). Fecal contamination of fruits and vegetables during cultivation and processing for market. A review. *Journal of Milk and Food Technology*, **34,** 184–195.

Geldreich, E. E. and Clarke, N. A. (1966). Bacterial pollution indicators in the intestinal tract of freshwater fish. *Applied Microbiology*, **14,** 429–437.

Geldreich, E. E., Huff, C. B., Bordner, R. H., Kabler, P. W.

and Clark, H. F. (1962). The faecal coli-aerogenes flora of soils from various geographical areas. *Journal of Applied Bacteriology*, **25,** 87–93.

Geldreich, E. E. and Kenner, B. A. (1969). Concepts of fecal streptococci in stream pollution. *Journal of the Water Pollution Control Federation*, **41,** R336–R352.

Geldreich, E. E., Kenner, B. A. and Kabler, P. W. (1964). Occurrence of coliforms, fecal coliforms and streptococci on vegetation and insects. *Applied Microbiology*, **12,** 63–69.

Gerasimenko, D. D. (1977). On survival rate of colon bacillus in oil-polluted sea water. *Mikrobiologicheskii Zhurnal Kiev*, **39,** 726–728.

Gerba, C. P., Goyal, S. M., Smith, E. M. and Melnick, J. L. (1977). Distribution of viral and bacterial pathogens in a coastal canal community. *Marine Pollution Bulletin*, **8,** 279–282.

Gerba, C. P. and McLeod, J. S. (1976). Effect of sediments on the survival of *Escherichia coli* in marine waters. *Applied and Environmental Microbiology*, **32,** 114–120.

Gerba, C. P., Wallis, C. and Melnick, J. L. (1975*a*). Fate of wastewater bacteria and viruses in soil. *Journal of the Irrigation and Drainage Division, Proceedings of the American Society of Civil Engineers*, **101,** 157–174.

———(1975*b*). Microbiological hazards of household toilets: droplet production and the fate of residual organisms. *Applied Microbiology*, **30,** 229–237.

———(1977). Disinfection of wastewater by photodynamic oxidation. *Journal of the Water Pollution Control Federation*, **49,** 575–583.

Gilbert, R. G., Gerba, C. P., Rice, R. C., Bouwer, H., Wallis, C. and Melnick, J. L. (1976). Virus and bacteria removal from wastewater by land treatment. *Applied and Environmental Microbiology*, **32,** 333–338.

Gilman, R. H., Spira, W. M., Rabbani, G. H. and Al-Mahomod, A. (1980). Invasive *E. coli* and *V. parahaemolyticus*: a rare cause of dysentery in Dacca. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74,** 688–689.

Glantz, P. J. and Krantz, G. E. (1965). *Escherichia coli* serotypes isolated from fish and their environment. *Health Laboratory Science*, **2,** 54–63.

Goff, G. D., Spendlove, J. C., Adams, A. P. and Nicholas, P. S. (1973). Emission of microbial aerosols from sewage treatment plants that use trickling filters. *Health Services Reports*, **88,** 640–652.

Goldshmid, J. (1974). Water quality aspects of ground-water recharge in Israel. *Journal of the American Water Works Association*, **5,** 163–166.

Goncharuk, E. I., Grigoryeva, L. V., Bey, T. V., Shulyak, E. V. and Korchak, G. M. (1970). Removal of intestinal viruses and bacteria from sewage by a circulating oxidizing canal. *Gigiena i Sanitariya*, **35,** 36–41.

Gorbach, S. L., Nahas, L., Lerner, P. I. and Weinstein, L. (1967). Studies in intestinal microflora. I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. *Gastroenterology*, **53,** 845–855.

Gorden, R. W. and Fliermans, C. B. (1978). Survival and viability of *Escherichia coli* in a thermally altered reservoir. *Water Research*, **12,** 343–352.

Gordon, J. E. (1964). Acute diarrheal disease. *American Journal of the Medical Sciences*, **248,** 345–365.

Goyal, S. M., Gerba, C. P. and Melnick, J. L. (1977). Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. *Applied and Environmental Microbiology*, **34,** 139–149.

———(1978). Prevalence of human enteric viruses in coastal canal communities. *Journal of the Water Pollution Control Federation*, **50,** 2247–2256.

———(1979). Human enteroviruses in oysters and their overlying waters. *Applied and Environmental Microbiology*, **37,** 572–581.

Grabow, W. O. K., Bateman, B. W. and Burger, J. S. (1978). Microbiological quality indicators for routine monitoring of wastewater reclamation systems. *Progress in Water Technology*, **10,** 317–327.

Grabow, W. O. K. and Isaacson, M. (1978). Microbiological quality and epidemiological aspects of reclaimed water. *Progress in Water Technology*, **10,** 329–335.

Grabow, W. O. K., Middendorff, I. G. and Basson, N. C. (1978). Role of lime treatment in the removal of bacteria, enteric viruses and coliphages in a wastewater reclamation plant. *Applied and Environmental Microbiology*, **35,** 663–669.

Grabow, W. O. K. and Nupen, E. M. (1972). The load of infectious microorganisms in the waste water of two South African hospitals. *Water Research*, **6,** 1557–1563.

Gracey, M., Ostergaard, P., Adnan, S. W. and Iveson, J. B. (1979). Faecal pollution of surface waters in Jakarta. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73,** 306–308.

Gray, E. A. (1975). Survival of *Escherichia coli* in stream water in relation to carbon dioxide and plant photosynthesis. *Journal of Applied Bacteriology*, **39,** 47–54.

Grigoryeva, L. V., Korchak, G. I. and Bey, T. V. (1969). Survival of bacteria and viruses in sewage sludges. *Mikrobiologichnyi Zhurnal*, **31,** 659–664.

Guélin, A. (1962). Polluted waters and the contamination of fish. In *Fish as Food*, **2,** ed. Borgstrom, G., pp. 481–502. New York: Academic Press.

Guerrant, R. L., Moore, R. A., Kirschenfeld, P. M. and Sande, M. A. (1975). Role of toxigenic and invasive bacteria in acute diarrhea of childhood. *New England Journal of Medicine*, **293,** 567–573.

Guy, E. M. and Small, J. A. (1977). Survival of streptococci and coliforms of bovine faecal origin in drainage water and soil stored at different temperatures. *New Zealand Journal of Agricultural Research*, **20,** 13–18.

Hagedorn, C., Hansen, D. T. and Simonson, G. H. (1978). Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. *Journal of Environmental Quality*, **7,** 55–59.

Hakim, K. E. (1978). *Study of Relationship of Microbial Indicators to Health Effects at Alexandria Bathing Beaches Progress Report, 1976–1977*. Alexandria, Egypt: High Institute of Public Health.

Hanes, N. B. and Fragala, R. (1967). Effect of seawater

concentration on survival of indicator bacteria. *Journal of the Water Pollution Control Federation*, **39,** 97–104.

Hanes, N. B., Sarles, W. B. and Rohlich, G. A. (1964). Dissolved oxygen and survival of coliform organisms and enterococci. *Journal of the American Water Works Association*, **56,** 441–446.

Harper, G. J. and Morton, J. D. (1953). The respiratory retention of bacterial aerosols: experiments with radioactive spores. *Journal of Hygiene*, **51,** 372–385.

Hendricks, C. W. (1971). Enteric bacterial metabolism of stream sediment eluates. *Canadian Journal of Microbiology*, **17,** 551–556.

——— (1972). Enteric bacterial growth rates in river water. *Applied Microbiology*, **24,** 168–174.

Hendricks, D. W., Post, F. J. and Khairnar, D. R. (1979). Adsorption of bacteria on soils: experiments, thermodynamic rationale, and application. *Water, Air and Soil Pollution*, **12,** 219–232.

Hoff, J. C. and Becker, R. C. (1969). The accumulation and elimination of crude and clarified poliovirus suspensions by shellfish. *American Journal of Epidemiology*, **90,** 53–61.

Hughes, J. M., Merson, M. H. and Gangarosa, E. J. (1977). The safety of eating shellfish. *Journal of the American Medical Association*, **237,** 1980–1981.

Irving, T. E. (1980). *Sewage Chlorination and Bacterial Regrowth.* Technical Report TR 132. Stevenage, UK: Water Research Centre.

Jackson, G. B. (1979). Conventional chlorination found nonfeasible for lagoons. *Journal of the Water Pollution Control Federation, Deeds & Data*, **16**(1), 9 and 14–16.

Jamieson, W., Madri, P. and Claus, G. (1976). Survival of certain pathogenic microorganisms in sea water. *Hydrobiologia*, **50,** 117–121.

Janssen, W. A. (1974). Oysters: retention and excretion of three types of human waterborne disease bacteria. *Health Laboratory Science*, **11,** 20–24.

Jegathesan, M., Lim Teong Wah, Lim Eng Soon, Ding Su Har and Lim Boo Liat (1976). Bacterial enteropathogens in Malaysian shellfish. *Tropical and Geographical Medicine*, **28,** 91–95.

Jordan, E. O. (1926). The changes in bacterial content of stored normal and typhoid feces. *Journal of Infectious Diseases*, **38,** 306–322.

June, R. C., Ferguson, W. W. and Worfel, M. T. (1953). Experiments in feeding adult volunteers with *Escherichia coli* 55, $B_5$, a coliform organism associated with infant diarrhea. *American Journal of Hygiene*, **57,** 222–236.

Kamlet, K. S. (1979). Superchlorination of sewage sludge: adding injury to insult? *Sludge*, **2,** 16–18 and 27–31.

Kampelmacher, E. H., Fonds, A. W. and van Noorle Jansen, L. M. (1977). Reduction of *Salmonella, E. coli*, coliforms and faecal streptococci by chlorination of sewage treatment plant effluents. *Water Research*, **11,** 545–550.

Karthegisan, J. and Pugh Thomas, M. (1980). The effect of tidal heights on the distribution and abundance of coliform bacteria in the sediments of two sites in the Mersey estuary. *International Journal of Environmental Studies*, **14,** 299–308.

Katugampola, D. C. and Assim, T. H. (1958). Coliform organisms in domestic water supplies in Ceylon. *Ceylon Journal of Medical Science*, **9,** 95–101.

Katzenelson, E., Buium, I. and Shuval, H. I. (1976). Risk of communicable disease infection associated with wastewater irrigation in agricultural settlements. *Science*, **194,** 944–946.

Katzenelson, E. and Teltch, B. (1976). Dispersion of enteric bacteria by spray irrigation of wastewater. *Journal of the Water Pollution Control Federation*, **48,** 710–716.

Katzenelson, E., Teltch, B. and Shuval, H. I. (1977). Spray irrigation with wastewater: the problem of aerosolization and dispersion of enteric microorganisms. *Progress in Water Technology*, **9,** 1–11.

Kawata, K., Cramer, W. N. and Burge, W. D. (1977). Composting destroys pathogens. *Water and Sewage Works*, **124,** 76–79.

Keswick, B. H., Fujioka, R. S., Loh, P. C. and Burbank, N. C. (1980). Comparative disinfection by bromine chloride and chlorine of viruses and bacteria in wastewater. *Journal of the Water Pollution Control Federation*, **52,** 2581–2588.

Kibbey, H. J., Hagedorn, C. and McCoy, E. L. (1978). Use of fecal streptococci as indicators of pollution in soil. *Applied and Environmental Microbiology*, **35,** 711–717.

Kinney, E. C., Drummond, D. W. and Hanes, N. B. (1978). Effects of chlorination on differentiated coliform groups. *Journal of the Water Pollution Control Federation*, **50,** 2307–2312.

Klock, J. W. (1971). Survival of coliform bacteria in wastewater treatment lagoons. *Journal of the Water Pollution Control Federation*, **43,** 2071–2083.

Koornhof, H. J., Robins-Browne, R. M., Richardson, N. J. and Cassel, R. (1979). Etiology of infantile enteritis in South Africa. *Israel Journal of Medical Sciences*, **15,** 341–347.

Kott, Y., Roze, N., Sperber, S. and Betzer, N. (1974). Bacteriophages as viral pollution indicators. *Water Research*, **8,** 165–171.

Kudoh, Y., Matsushita, S., Yamada, S., Tsuno, M., Ohta, K., Kai, A., Sakai, S. and Ohashi, M. (1979). Travelers' diarrhea and enterotoxigenic *Escherichia coli*—a survey in 1977–1979 in Tokyo. In *Proceedings of the 15th Joint US–Japan Cooperative Medical Science Program, Conference on Cholera*, pp. 225–236. Bethesda, Md.: National Institutes of Health.

Kudryavtseva, B. M. (1972). An experimental approach to the establishment of zones of hygienic protection of underground water sources on the basis of sanitary bacteriological indices. *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, **16,** 503–511.

Lance, J. C., Rice, R. C. and Gilbert, R. G. (1980). Renovation of wastewater by soil columns flooded with primary effluent. *Journal of the Water Pollution Control Federation*, **52,** 381–388.

Lee, J. A. and Kean, B. H. (1978). International conference on the diarrhea of travelers. New directions in research. *Journal of Infectious Diseases*, **137,** 355–369.

Lessel, T. and Suess, A. (1978). Hygienization of sewage

sludge by gamma irradiation, experience with an operating pilot plant. *Progress in Water Technology*, **10,** 641–652.

Loedolff, C. J. (1965). The function of Cladocera in oxidation ponds. In *Advances in Water Pollution Research*, **1,** ed. Jaag, O., pp. 307–325. Oxford: Pergamon.

Loh, P. C., Fujioka, R. S. and Lau, L. S. (1979). Recovery, survival and dissemination of human enteric viruses in ocean waters receiving sewage in Hawaii. *Water, Air and Soil Pollution*, **12,** 197–217.

Longley, K. E., Moore, B. E. and Sorber, C. A. (1980). Comparison of chlorine and chlorine dioxide as disinfectants. *Journal of the Water Pollution Control Federation*, **52,** 2098–2105.

McCambridge, J. and McMeekin, T. A. (1979). Protozoan predation of *Escherichia coli* in estuarine waters. *Water Research*, **13,** 659–663.

McCoy, E. L. and Hagedorn, C. (1979). Quantitatively tracing bacterial transport in saturated soil systems. *Water, Air and Soil Pollution*, **11,** 467–479.

McCoy, E. L. and Ziebell, W. A. (1976). The effects of effluents on groundwater: bacteriological aspects. In *Second National Conference on Individual Onsite Wastewater Systems*, pp. 67–75. Ann Arbor, Mich.: National Sanitation Foundation.

McFeters, G. A., Bissonnette, G. K., Jezeski, J. J., Thomson, C. A. and Stuart, D. G. (1974). Comparative survival of indicator bacteria and enteric pathogens in well water. *Applied Microbiology*, **27,** 823–829.

McFeters, G. A., Stuart, S. A. and Olson, S. B. (1978). Growth of heterotrophic bacteria and algal extracellular products in oligotrophic waters. *Applied and Environmental Microbiology*, **35,** 383–391.

McGarry, M. G. and Stainforth, J., eds. (1978). *Compost, Fertilizer and Biogas Production from Human and Farm Wastes in the People's Republic of China.* Ottawa: International Development Research Centre.

Mancini, J. L. (1978). Numerical estimates of coliform mortality rates under various conditions. *Journal of the Water Pollution Control Federation*, **50,** 2477–2484.

Mara, D. D. (1976). *Sewage Treatment in Hot Climates.* Chichester: John Wiley.

Mara, D. D. and Silva, S. A. (1979). Sewage treatment in waste stabilization ponds: recent research in northeast Brazil. *Progress in Water Technology*, **11,** 341–344.

Marais, G. v. R. (1974). Faecal bacterial kinetics in stabilization ponds. *Journal of the Environmental Engineering Division, Proceedings of the American Society of Civil Engineers*, **100,** 119–139.

Marzouk, Y., Goyal, S. M. and Gerba, C. P. (1980). Relationship of viruses and indicator bacteria in water and wastewater of Israel. *Water Research*, **14,** 1585–1590.

Mata, L. J., Carrillo, C. and Villatoro, E. (1969). Fecal microflora in healthy persons in a preindustrial region. *Applied Microbiology*, **17,** 596–602.

May, K. R. and Druett, H. A. (1953). The pre-impinger, a selective aerosol sampler. *British Journal of Industrial Medicine*, **10,** 142–151.

Mehlman, I. J., Fishbein, M., Gorbach, S. L., Sanders, A. C., Eide, E. L. and Olson, J. C. (1976). Pathogenicity of *Escherichia coli* recovered from food. *Journal of the Association of Official Analytical Chemists*, **59,** 67–80.

Menon, A. S. and Bedford, W. K. (1973). *A Study of the Seasonal Effects on the Microbiology of a Northern Pulp and Paper Mill Aeration Lagoon.* Report No. EPS 4-AR-73-1. Ottawa: Environmental Protection Service, Environment Canada.

Merson, M. H., Morris, G. K., Sack, D. A., Wells, J. G., Feeley, J. C., Sack, R. B., Creech, W. B., Kapikian, A. Z. and Gangarosa, E. J. (1976). Travellers' diarrhea in Mexico: a prospective study of physicians and family members attending a congress. *New England Journal of Medicine*, **294,** 1299–1305.

Metcalf, T. G. (1978). Indicators of viruses in shellfish. In *Indicators of Viruses in Water and Food*, ed. Berg, G., pp. 383–415. Ann Arbor, Mich.: Ann Arbor Science Publishers.

Mitchell, J. R., Presnell, M. W., Akin, E. W., Cummins, J. M. and Liu, O. C. (1966). Accumulation and elimination of poliovirus by the Eastern oyster. *American Journal of Epidemiology*, **84,** 40–50.

Mitchell, R. (1968). Factors affecting the decline of non-marine micro-organisms in seawater. *Water Research*, **2,** 535–543.

Mitchell, R. and Chamberlin, C. (1975). Factors influencing the survival of enteric microorganisms in the sea: an overview. In *Proceedings of the International Symposium on Discharge of Sewage from Sea Outfalls*, ed. Gameson, A. L. H., pp. 237–251. Oxford: Pergamon.

———(1978). Survival of indicator organisms. In *Indictors of Viruses in Water and Food*, ed. Berg, G., pp. 15–37. Ann Arbor, Mich.: Ann Arbor Science Publishers.

Mitchell, R. and Yankofsky, S. (1969). Implication of a marine ameba in the decline of *Escherichia coli* in seawater. *Environmental Science and Technology*, **3,** 574–576.

Moeller, J. R. and Calkins, C. (1980). Bactericidal agents in wastewater lagoons and lagoon design. *Journal of the Water Pollution Control Federation*, **52,** 2442–2451.

Moore, J. L., Perin, D. E. and Maiden, B. G. (1979). Estimating the effect of water quality improvement on public swimming. *Water Resources Research*, **15,** 1323–1328.

Moussa, R. S. (1965). Type distribution of coliforms isolated from faecal and non-faecal habitats. *Indian Journal of Medical Research*, **53,** 629–637.

Munger, S. F., Heyward. A. A. and Dutton, R. T. (1979). A survey of the microbiological quality of shellfish on metropolitan Seattle area beaches. Abstract Q14. *Abstracts of the Annual Meeting of the American Society of Microbiology.* Washington, DC.

Myhrstad, J. A. (1979). Disinfection of sewage by ultraviolet irradiation. *National Institute of Public Health Annals*, **2,** 11–16.

Newson, S. W. B. (1972). Microbiology of hospital toilets. *Lancet*, **2,** 700–703.

Olivieri, V. P., Kawata, K. and Kruse, C. W. (1978). Relationship between indicator organisms and selected pathogenic bacteria in urban waterways. *Progress in Water Technology*, **10,** 361–379.

Oluwande, P. A. (1980). Public health implications of chemical and bacteriological characteristics of treated water in Western State of Nigeria. *Nigerian Medical Journal*, **7,** 86–89.

Osborn, D. W. and Hattingh, W. H. J. (1978). Disinfection of sewage sludge: a review. *Water South Africa*, **4,** 169–178.

Parhad, N. M. and Rao, N. U. (1974). Effect of pH on survival of *Escherichia coli*. *Journal of the Water Pollution Control Federation*, **46,** 980–986.

Parker, C. D. (1962). Microbiological aspects of lagoon treatment. *Journal of the Water Pollution Control Federation*, **34,** 149–161.

Parker, D. T., Spendlove, J. C., Bondurant, J. A. and Smith, J. H. (1977). Microbial aerosols from food-processing waste spray fields. *Journal of the Water Pollution Control Federation*, **49,** 2359–2365.

Petr, T. (1980). Bacterial densities and faecal pollution in the Purari River catchment, Papua New Guinea. In *Purari River (Wabo) Hydroelectric Scheme Environmental Studies. Vol II Aquatic Ecology of the Purari River Catchment*, ed. Petr, T., pp. 41–58. Konedobu, Papua New Guinea: Department of Minerals and Energy.

Petrilli, F. L., de Renzi, G. P., Palmerini Morelli, R. and de Flora, S. (1979). Survey of the pollution in a coastal area of the Tyrrhenian Sea. Aerial photography, physico-chemical and microbiological investigations and mutagenic monitoring. *Water Research*, **13,** 895–904.

Pichot, G. and Barbette, J. (1978). Estimation des taux moyens de disparition des bacteries fecales dans les eaux cotieres Belges de la Mer du Nord. *Revue Internationale d'Oceanographie Medicale*, **51/52,** 115–126.

Pike, E. B. and Carrington, E. G. (1979). The fate of enteric bacteria and pathogens during sewage treatment. In *Biological Indicators of Water Quality*, eds. James, A. and Evison, L., pp. 20/1–20/32. Chichester: John Wiley.

Polprasert, C. and Valencia, L. G. (1981). The inactivation of faecal coliforms and *Ascaris* ova in faeces by lime. *Water Research*, **15,** 31–36.

Poon, C. P. C. (1968). Viability of long-storaged airborne bacterial aerosols. *Journal of the Sanitary Engineering Division, American Society of Civil Engineers*, **94,** 1137–1146.

Poynter, S. F. B. and Stevens, J. K. (1975). The effects of storage on the bacteria of hygienic significance. In *The Effect of Storage on Water Quality*, pp. 69–90. Medmenham, UK: Water Research Centre.

Qasim, S. R. (1978). Treatment of domestic sewage by using solar distillation and plant culture. *Journal of Environmental Science and Health*, **A13,** 615–627.

Raghavachari, T. N. S. and Iyer, P. V. S. (1940). The occurrence of the *Aerogenes* group of coliform organisms in faeces and its significance in water analysis. *Indian Journal of Medical Research*, **28,** 55–60.

Rahe, T. M., Hagedorn, C. and McCoy, E. L. (1979). A comparison of fluorescein dye and antibiotic resistant *Escherichia coli* as indicators of pollution in groundwater. *Water, Air and Soil Pollution*, **11,** 93–103.

Randall, C. W. and Ledbetter, J. O. (1966). Bacterial air pollution from activated sludge units. *American Industrial Hygiene Association Journal*, **27,** 506–519.

Raveendran, O., Gore, P. S. and Unnithan, R. V. (1978). Observations on faecal contamination of Cherai Beach in Kerala. *Indian Journal of Marine Science*, **7,** 128–129.

Reneau, R. B. and Pettry, D. E. (1975). Movement of coliform bacteria from septic tank effluent through selected coastal plain soils in Virginia. *Journal of Environmental Quality*, **4,** 41–44.

Reneau, R. B., Pettry, D. E., Shanholtz, M. I., Graham, S. A. and Weston, C. W. (1977). Distribution of total and fecal coliform organisms from septic effluent in selected coastal plain soils. *Public Health Reports*, **92,** 251–259.

Roller, S. D., Olivieri, V. P. and Kawata, K. (1980). Mode of bacterial inactivation by chlorine dioxide. *Water Research*, **14,** 635–641.

Roper, M. M. and Marshall, K. C. (1979). *The Survival of Coliform Bacteria in Saline Sediments*. Australian Water Resources Council Technical Paper 43. Canberra: Australian Government Publishing Service.

Rosenberg, M. L., Kaplan, J. P., Wachsmuth, I. K., Wells, J. G., Gangarosa, E. J., Guerrant, R. L. and Sack, D. A. (1977). Epidemic diarrhea at Crater Lake from enterotoxigenic *Escherichia coli*, a large waterborne outbreak. *Annals of Internal Medicine*, **86,** 714–718.

Rudolfs, W., Falk, L. and Ragotzkie, R. A. (1950). Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, sewage and sludges, and on vegetation. 1. Bacterial and viral diseases. *Sewage and Industrial Wastes*, **22,** 1261–1281.

Ryder, R. W., Sack, D. A., Kapikian, A. Z., McLaughlin, J. C., Chakraborty, J., Rahman, A., Merson, M. and Wells, J. (1976). Enterotoxigenic *Escherichia coli* and reovirus-like agent in rural Bangladesh. *Lancet*, **1,** 659–663.

Sack, D. A., Kaminsky, D. C., Sack, R. B., Itotia, J. N., Arthur, R. R., Kapikian, A. Z., Ørskov, F. and Ørskov, I. (1978). Prophylatic doxycycline for travellers' diarrhea: results of a prospective double-blind study of Peace Corps volunteers in Kenya. *New England Journal of Medicine*, **298,** 758–763.

Sack, R. B., Hirschhorn, N., Brownlee, I., Cash, R. A., Woodward, W. E. and Sack, D. A. (1975*a*). Enterotoxigenic *Escherichia coli* associated diarrheal disease in Apache children. *New England Medical Journal*, **292,** 1041–1045.

Sack, R. B., Hirschhorn, N., Woodward, W. E., Sack, D. A. and Cash, R. A. (1975*b*). Antibodies to heat-labile *Escherichia coli* enterotoxin in Apaches in Whiteriver, Arizona. *Infection and Immunity*, **12,** 1475–1477.

Sack, R. B., Sack, D. A., Mehlman, I. J., Ørskov, F. and Ørskov, I. (1977). Enterotoxigenic *Escherichia coli* isolated from food. *Journal of Infectious Diseases*, **135,** 313–317.

Sadovski, A. Y., Fattal, B. and Goldberg, D. (1978). Microbial contamination of vegetables irrigated with

sewage effluent by the drip method. *Journal of Food Protection*, **41,** 336–340.

Sadovski, A. Y., Fattal, B., Goldberg, D., Katzenelson, E. and Shuval, H. I. (1978). High levels of microbial contamination of vegetables irrigated with wastewater by the drip method. *Applied and Environmental Microbiology*, **36,** 824–830.

Saleh, F. A. (1980*a*). Bacteriological quality of Nile Water before and after impoundment (1963–1973): a review. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, II*, **135,** 123–129.

———(1980*b*). Isolation and enumeration of faecal streptococci from Nile water (1975–1976). *Water Research*, **14,** 1669–1678.

Sandhu, S. S., Warren, W. J. and Nelson, P. (1979). Magnitude of pollution indicator organisms in rural potable water. *Applied and Environmental Microbiology*, **37,** 744–749.

Savage, J., Chase, T. and MacMillan, J. D. (1973). Population changes in enteric bacteria and other microorganisms during aerobic thermophilic windrow composting. *Applied Microbiology*, **26,** 969–974.

Schaub, S. A. and Sorber, C. A. (1977). Virus and bacteria removal from wastewater by rapid infiltration through soil. *Applied and Environmental Microbiology*, **33,** 609–619.

Sebodo, T., Soenarto, Y., Rohde, J. E., Ryan, N. J., Taylor, B. J., Luke, R. J. K., Bishop, R. F., Barnes, G. L., Holmes, I. H. and Ruck, B. J. (1977). Aetiology of diarrhoea in children aged less than two years in Central Java. *Lancet*, **1,** 490–491.

Shuval, H. I. (1978). Studies on bacterial and viral contamination of the marine environment. *Revue Internationale d'Oceanographie Medicale*, **50,** 43–50.

Shuval, H. I., Cohen, J. and Kolodney, R. (1973). Regrowth of coliforms and fecal coliforms in chlorinated wastewater effluent. *Water Research*, **7,** 537–546.

Silvey, K. G., Abshire, R. L. and Nunez, W. J. (1974). Bacteriology of chlorinated and unchlorinated wastewater effluents. *Journal of the Water Pollution Control Federation*, **46,** 2153–2162.

Slade, J. S. and Edworthy, K. J. (1981). Virology of wastewater recharge of the chalk aquifer. II. Microbiology and water quality. In *Viruses and Wastewater Treatment*, eds. Goddard, M. and Butler, M. Oxford: Pergamon.

Slanetz, L. W., Bartley, C. H., Metcalf, T. G. and Nesman, R. (1970). Survival of enteric bacteria and viruses in municipal sewage lagoons. In *Proceedings of the Second International Symposium for Waste Treatment Lagoons*, ed. McKinney, R. E., pp. 132–141. Lawrence, Kansas: University of Kansas.

Slanetz, L. W., Bartley, C. H. and Stanley, K. W. (1968). Coliforms, fecal streptococci and *Salmonella* in seawater and shellfish. *Health Laboratory Science*, **5,** 66–78.

Small Scale Waste Management Project (1978). *Management of Small Waste Flows.* Report EPA-600/2-78-173. Cincinnati, Ohio: US Environmental Protection Agency.

Snow, G. E. (1977). The effects of chlorinated wastewater effluent on in situ river coliform bacteria. Abstract N60. *Abstracts of the Annual Meeting of the American Society for Microbiology*. Washington, DC.

Sorber, C. A., Bausum, H. T., Schaub, S. A. and Small, M. J. (1976). A study of bacterial aerosols at a wastewater irrigation site. *Journal of the Water Pollution Control Federation*, **48,** 2367–2379.

Spencer, H. C., Wells, J. G., Gary, G. W., Sondy, J., Puhr, N. D. and Feldman, R. A. (1980). Diarrhea in a non-hospitalized rural Salvadoran population: the role of enterotoxigenic *Escherichia coli* and rotavirus. *American Journal of Tropical Medicine and Hygiene*, **29,** 246–253.

Stenquist, R. J., Parker, D. S., Loftin, W. E. and Brenner, R. C. (1977). Long-term performance of a coupled trickling filter-activated sludge plant. *Journal of the Water Pollution Control Federation*, **49,** 2265–2284.

Stiles, C. W. and Crohurst, H. R. (1923). The principles underlying the movement of *Bacillus coli* in groundwater, with resulting pollution of wells. *Public Health Reports*, **38,** 1350–1353.

Teltch, B. and Katzenelson, E. (1978). Airborne enteric bacteria and viruses from spray irrigation with wastewater. *Applied and Environmental Microbiology*, **35,** 290–296.

Tjostem, J. L., Young, J., Hoilien, C. and Elson Iverson, R. (1977). Bacterial and nitrate contamination of well water in northeast Iowa. *Proceedings of the Iowa Academy of Science*, **84,** 14–22.

Tomkins, A. M., Drasar, B. S., Bradley, A. K. and Williamson, W. A. (1978). Water supply and nutritional status in rural Northern Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72,** 239–243.

Tomkins, A. M., Bradley, A. K., Oswald, S. and Drasar, B. S. (1981). Diet and the faecal microflora of infants, children and adults in rural Nigeria and urban UK. *Journal of Hygiene*, **86,** 285–293.

Train, R. E. (1979). *Quality Criteria for Water*. London: Castle House.

Van der Drift, C., van Seggelen, E., Stumm, C., Hol, W. and Tuinte, J. (1977). Removal of *Escherichia coli* in wastewater by activated sludge. *Applied and Environmental Microbiology*, **34,** 315–319.

Van Donsel, D. J., Geldreich, E. E. and Clarke, N. A. (1967). Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. *Applied Microbiology*, **15,** 1362–1370.

Vasconcelos, G. J. and Swartz, R. G. (1976). Survival of bacteria in seawater using a diffusion chamber apparatus in situ. *Applied and Environmental Microbiology*, **31,** 913–920.

Vaughn, J. M., Landry, E. F., Baranosky, L. J., Beckwith, C. A., Dahl, M. C. and Delihas, N. C. (1978). Survey of human virus occurrence in wastewater-recharged groundwater on Long Island. *Applied and Environmental Microbiology*, **36,** 47–51.

Vaughn, J. M., Landry, E. F., Thomas, M. Z., Vicale, T. J. and Penello, W. F. (1979). Survey of human enterovirus occurrence in fresh and marine surface waters on Long Island. *Applied and Environmental Microbiology*, **38,** 290–296.

Viraraghavan, T. (1978). Travel of microorganisms from a septic tile. *Water, Air and Soil Pollution*, **9,** 355–362.

Wehner, J. F. and Wilhelm, R. H. (1956). Boundary conditions of flow reactor. *Chemical Engineering Science*, **6,** 89–93.

Wheater, D. W. F., Mara, D. D., Jawad, L. and Oragui, J. (1980). *Pseudomonas aeruginosa* and *Escherichia coli* in sewage and fresh water. *Water Research*, **14,** 713–721.

Wheater, D. W. F., Mara, D. D. and Oragui, J. (1979). Indicator systems to distinguish sewage from stormwater run-off and human from animal faecal material. In *Biological Indicators of Water Quality*, eds. James, A. and Evison, L., pp. 21/1–21/27. Chichester: John Wiley.

White, G. F., Bradley, D. J. and White, A. U. (1972). *Drawers of Water*. Chicago: University of Chicago Press.

WHO (1971). *International Standards for Drinking Water Quality*, 3rd ed. Geneva: World Health Organization.

Williams Smith, H. and Crabb, W. E. (1961). The faecal bacterial flora of animals and men: its development in the young. *Journal of Pathology and Bacteriology*, **82,** 53–66.

Wood, P. C. (1979). Public health aspects of shellfish from polluted waters. In *Biological Indicators of Water Quality*, eds. James, A. and Evison, L., pp. 13/1–13/18. Chichester: John Wiley.

Woodbridge, D. D. and Cooper, P. C. (1979). Synergistic effects for irradiation of fecal coliforms. *Journal of the Water Pollution Control Federation*, **51,** 2717–2723.

Wyatt, T. D. and Wilson, T. S. (1980). An investigation of the bacteriological water quality in two pools disinfected with ozone. *Baths Service and Recreation Management*, Jan./Feb., 22–24.

Yde, M. and de Maeyer-Cleempoel, S. (1980). Faecal pollution of Belgian coastal waters. *Marine Pollution Bulletin*, **11,** 108–110.

Yeager, C. H. (1929). Well pollution and safe sites for bored-hole latrines. *Malayan Medical Journal*, **4,** 118–125.

Zanoni, A. E., Katz, W. J., Carter, H. H. and Whaley, R. C. (1978). An in situ determination of the disappearance of coliforms in Lake Michigan. *Journal of the Water Pollution Control Federation*, **50,** 321–330.

Zubrzyoki, L. and Spaulding, E. H. (1962). Studies on the stability of the normal human fecal flora. *Journal of Bacteriology*, **83,** 968–974.