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Health constraints on the agricultural recycling of wastewater sludges

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1 INTRODUCTION

The objective of sewage treatment is to remove solids and to reduce its biochemical oxygen demand (BOD) before returning the treated wastewater to the environment. Sewage sludge, increasingly referred to as biosolids, is an inevitable product of wastewater treatment. Conventional wastewater treatment processes comprise separate process streams for the liquid and solid fractions (sludge). The overall aim of treatment (in terms of solids) is: (i) to reduce to the minimum the amount of solids in the treated effluent in order to achieve discharge standards; and (ii) maximize the level of solids in the sludge in order to minimize the volume requiring further treatment and disposal.

Sludge is produced at various stages within the wastewater treatment process (Fig. 17.1). Usually, these solids are combined and treated as a whole. Dedicated sludge treatment may not be available at all works, particularly smaller plants. In these circumstances it is normal practice to transport the sludge to a larger works for subsequent treatment.

2 BASIC PRINCIPLES OF WASTEWATER TREATMENT

2.1 Preliminary treatment

Preliminary treatment consists of screening through bar screens to remove coarse solids and buoyant materials, such as plastics or rags, which may become trapped in pumps or other mechanical plant. The screenings are usually removed from the process stream and disposed of separately by landfilling or incineration. Occasionally, screenings may be shredded (comminuted) to reduce their size and returned to the process stream. This may cause problems with downstream processes and, as a consequence, is rarely practised at works which incorporate secondary (biological) treatment. The other component of preliminary treatment is grit removal, which is accomplished in chambers (or channels) or by centrifugation, taking advantage of the greater settling velocities of these solids. The material is largely inorganic in nature and is usually disposed of to landfill.

2.2 Primary treatment

Primary treatment is designed to reduce the load on subsequent biological (secondary) treatment processes. Although the design of primary sedimentation tanks differs, they achieve the removal of settleable solids, oils, fats and other floating material, and a proportion of the organic load. Efficiently designed and operated primary treatment processes should remove 50–70% of suspended solids and 25–40% of the BOD (organic

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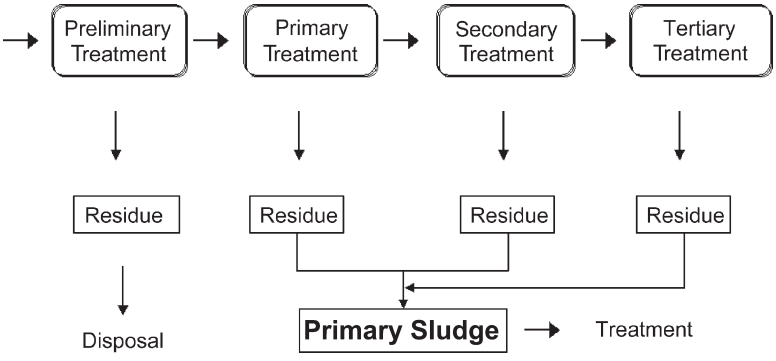

Fig. 17.1 Schematic representation of wastewater treatment showing sludge production.

load). The separated solids have a high organic content and are usually treated in order to stabilize the material prior to disposal.

2.3 Secondary treatment

Biological processes are used to convert dissolved biodegradable organic substances and colloidal material into inorganics and biological solids (biomass). There are several secondary treatment processes, but these may be divided into fixed film (e.g. trickling filters, rotating biological contactors) and suspended processes (e.g. activated sludge). Solids separation is the final stage of many of these treatment systems, which produces a sludge, the nature of which will depend on the upstream treatment process. Sludges arising from secondary treatment are usually combined with primary sludge and treated as a whole.

2.4 Tertiary treatment

Tertiary treatment will only be required for treatment works subject to specific discharge conditions. Many of these processes will not produce sludge and those that do are likely to generate only small amounts requiring dedicated treatment. Solids removed by granular media filtration will be passed to the primary sludge treatment process.

3 SLUDGE TREATMENT

The sludge obtained from the various stages is usually in the form of a liquid containing between 0.5 and 6% dry solids. The typical composition of raw (untreated) and anaerobically digested sludge is shown in Table 17.1. The nature and extent of any treatment depends on the means of final disposal or beneficial use. The aim of treatment is to reduce the water and organic content of the sludge and render it suitable for disposal or reuse. There are several commonly used methods of sludge treatment, including long-term storage (in lagoons), lime stabilization, digestion (aerobic or anaerobic), air drying, thermal drying, and incineration or gasification (for energy recovery). Detailed descriptions of these processes are outside the scope of this handbook. However, the effect of the various treatments on pathogens is described below.

Sewage sludge contains valuable amounts of plant nutrients (nitrogen and phosphorus) and trace elements (Table 17.2). For this reason sludge has historically been applied to agricultural land as part of an integrated farm management plan. Other options for disposal include energy recovery and land reclamation activities. In Europe, North America and elsewhere, the disposal of sewage sludge is subject to strict controls designed to protect soil quality while encouraging the use of sludge in agriculture. Codes of Practice, such as those published by the UK Department of the Environment (DoE, 1996) and the UK Ministry of Agriculture, Fisheries and Food (MAFF, 1998a,b), provide advice on practical aspects of utilizing sewage sludge in agriculture.

Strict limits are set on the amounts of potentially toxic elements permitted in sludge which may be used in agriculture. Application

TABLE 17.1 Chemical composition and properties of untreated and digested sludge

Constituent	*Untreated primary sludge*		*Digested primary sludge*	
	Range	*Typical*	*Range*	*Typical*
Total dry solids (TS) %	2.0–8.0	5.0	6.0–12.0	10.0
Volatile solids (% of TS)	60–80	65	30–60	40
Grease and fats (% of TS)				
Ether soluble	6–30		5–20	18
Ether extract	7–35			
Protein (% of TS)	20–30	25	15–20	18
Nitrogen (N, % of TS)	1.5–4	2.5	1.6–6.0	3.0
Phosphorus (P_2O_5, % of TS)	0.8–2.8	1.6	1.5–4.0	2.5
Potassium (K_2O, % of TS)	0.0–0.1	0.4	0.0–3.0	1.0
Cellulose (% of TS)	8.0–15.0	10.0	8.8–15.0	10.0
Iron (not as sulphide)	2.0–4.0	2.5	3.0–8.0	4.0
Silica (SiO_2, % of TS)	15.0–20.0		10.0–20.0	
pH	5.0–8.0	6.0	6.5–7.5	7.0
Alkalinity (mg/l as $CaCO_3$)	500–1500	600	2500–3500	3000
Organic acids (mg/l as HAc)	200–2000	500	100–600	200
Energy content (Kj/kg)	23 000–29 000	25 500	9300–14 000	11 500

Source: Metcalf and Eddy (1991).

rates are controlled to minimize the accumulation in the soil of toxic metals. Due to the low levels of metals in sludge, application rates are governed in practice by maximum nitrogen application rates (250 kg/ha y^{-1} or 500 kg/ha y^{-1}) and to balance phosphorus addition with crop off-take.

Information on the amounts of sewage sludge produced and its disposal is collected by a number of countries, principally the USA and the member states of the European Union. Annual sludge production in the USA is in the region of 6.8 million tonnes dry solids (M tds) of which 54% is applied to land (Bastian, 1997). The figures for the EU are 5.1 M tds and 48% respectively (CEC, 1999). Within the EU amounts of sludge produced vary considerably, with Germany producing the largest amount of treated sludge followed by the UK and France (Fig. 17.2). The proportion of treated sludge used in agriculture varies across the European Union, with just over 10% of sludge production in Ireland being applied to land compared with 66% in France (Fig. 17.3). Factors affecting the amount of sludge applied to agricultural land include topography, land use, climatic conditions, and the availability of alternative means of disposal. In the UK, sludge production is increasing, principally as a result of the EU Directive on the treatment of urban wastewater (CEC, 1991). The cessation of sea disposal has resulted in a greater proportion of sludge being used in agriculture (Table 17.3), a trend which is projected to continue in the medium term (Fig. 17.4).

TABLE 17.2 Nutrient content of sewage sludge (% dry weight)

Constituent	*Range*	*Typical*
Nitrogen	<0.1–17.6	3.0
Phosphorus	<0.1–14.3	1.5
Sulphur	0.6–1.5	1.0
Potassium	0.02–2.6	0.3

4 REGULATIONS GOVERNING THE USE OF SLUDGE IN AGRICULTURE

4.1 USA

The treatment and ultimate disposal of sewage sludge, including domestic septage, derived from the treatment of domestic sewage, is governed by 40 CFR Part 503 rule (US EPA, 1993). The regulations were developed over

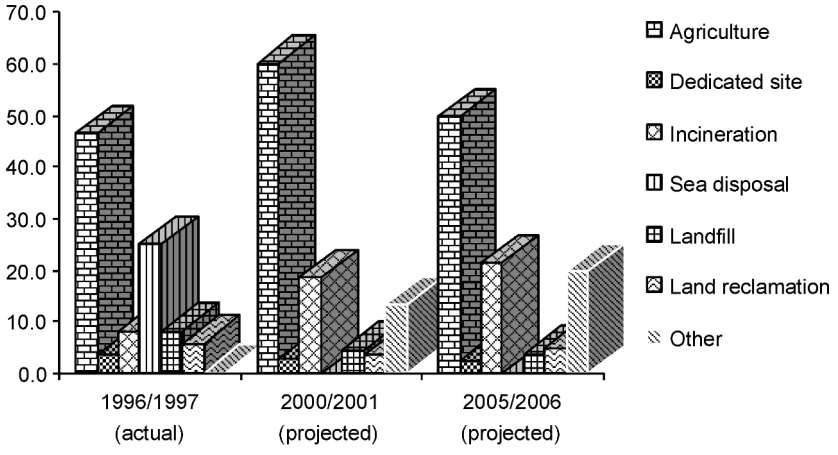

Fig. 17.2 Sludge disposal routes in the UK (Environment Agency, 1999).

a number of years and are designed for uniform application across the country over a wide range of geographic and climatic conditions, from Alaska to Hawaii. For this reason the regulations are extensive and complex. Only treated sludge is permitted to be applied to land of any type. Sludges are categorized as Class A or Class B depending on the level of treatment intended to reduce pathogens.

In order to attain Class A status sludges must have been treated by 'a process to further reduce pathogens' (PFRP). Such a process is considered capable of reducing the number of pathogens to those normally present in the soil. Provided that the treated sludge complies with end product microbiological standards (Table 17.4), it may be applied without restriction to a wide range of land types, including that intended for agricultural or horticultural

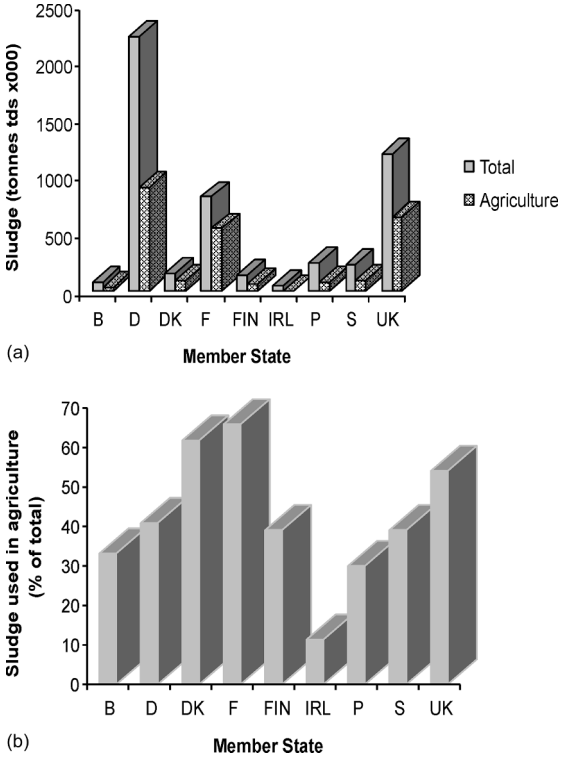

Fig. 17.3 (a) Sludge production within the European Union and amounts recycled to agricultural land; (b) Proportion of EU sludge recycled to agricultural land.

TABLE 17.3 Sludge disposal outlets in the UK

Outlet	*Quantity (%) (tds/y $^{-1}$ × 10 ^{3})*	
	1990/91	*1996/97*
Agriculture	465 (42)	520 (47)
Dedicated site	25 (2)	39 (3)
Sea disposal	334 (30)	280 (25)
Incineration	77 (7)	91 (8)
Landfill	88 (8)	91 (8)
Land reclamation		64 (6)
Forestry		1 (<1)
Horticultural compost		13 (1)
Storage (on site)	50 (5)	15 (1)
Other	68 (6)*	1 (<1)
Total	1107 (100)	1115 (100)

* More general category of 'Beneficial' used which included activities classified separately in 1996/7 survey.

Source: WRc (1998).

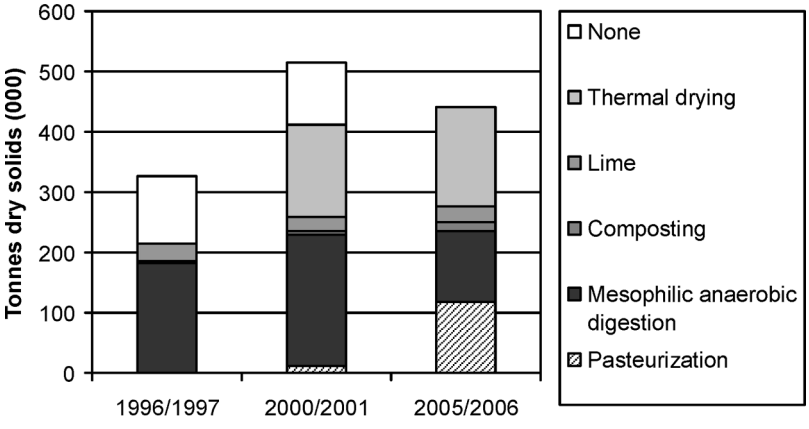

Fig. 17.4 Sludge treatment processes used in the UK.

use. Class B sludges are required to have been treated by 'a process to significantly reduce pathogens' (PSRP). With restrictions, Class B sludge may be applied to agricultural land. The sludge is required to meet end product microbiological standards (Table 17.4). The restrictions on application are:

- No grazing or harvesting of fodder crops with 30 days (of application)
- No harvesting of crops grown above ground within 14 months
- No harvesting of crops grown below ground for 20 months if the sludge remains on the soil for 4 months or longer; 38 months if the sludge remains on the soil for less than 4 months
- No harvesting of turf within 12 months
- No public access within 12 months (parks, playing fields etc.)

The implicit goal of the requirements for Class A biosolids is to reduce the number of pathogens in sewage sludge to below the level of detection (<3 MPN salmonella, <1 PFU enteric viruses, and <1 viable helminth ova – all per 4-g dry weight). The goal for the production of Class B biosolids is the reduction in the number of pathogens to levels that are unlikely to pose a public health risk (US EPA, 1999).

TABLE 17.4 End product microbiological standards for Class A and Class B sludges (US EPA, 1993)

Standard	*Class A*	*Class B*
Faecal coliforms/g ds	Less than 1000	Less than 2 000 000*
Salmonellae 4/g ds	Less than 3	
Enteroviruses pfu 4/g ds	Less than 1	
Parasite ova 4/g ds	Less than 1	

ds Dry solids.
* Geometric mean of seven samples.

4.2 European Union

The controls on the application of sewage sludge to agricultural land within member states derive from Council Directive 86/278/EEC published in 1986 for implementation within 3 years (CEC, 1986). The principal rationale of the Directive was to minimize the accumulation in the soil of heavy metals or other potential toxic elements (PTE) with the objective of protecting soil fertility and public health. However, the Directive included measures for controlling transmissible disease by introducing constraints on the use of sludge. Article 7 of the Directive requires Member States to prohibit the use of sludge or the supply of sludge for use on:

- grassland or forage crops if the grassland is to be grazed or the forage crops to be harvested before a certain period has elapsed. This period, which shall be set by the Member States, taking particular account of their geographical and climatic situation, shall under no circumstances be less than 3 weeks:

TABLE 17.5 Examples of effective sludge treatment processes as defined in the UK Code of Practice

Process	*Conditions*
Pasteurization	Minimum 30 min at 70°C; or Minimum 4 h at 55°C Followed in all cases by mesophilic anaerobic digestion
Mesophilic anaerobic digestion	Mean retention of at least 12 days at 35°C ± 3°C; or Mean retention of at least 20 days at 25°C ± 3°C. Followed in each case by secondary digestion with a mean retention time period of at least 14 days
Thermophilic aerobic digestion	Mean retention of at least 7 days. All sludge to be subjected to a minimum of 55°C for at least 4 h
Composting (windrows or aerated piles)	Compost must be retained at 40°C for at least 5 days including a period of 4 h at a minimum of 55°C. Followed by a period of maturation
Alkaline stabilization (with lime)	pH to be 12 or greater for a period of at least 2 h
Liquid storage	Storage for at least 3 months.
Dewatering and storage	Dewatering and storage for at least 3 months. Storage at least 14 days if sludge previously subjected to mesophilic anaerobic digestion

Source: DoE (1966).

- soil in which fruit and vegetables are growing, with the exception of fruit trees
- ground intended for the cultivation of fruit and vegetable crops which are normally in direct contact with the soil and normally eaten raw, for a period of 10 months preceding the harvest of crops and during the harvest itself
- sludge shall be treated before being used in agriculture[1]. Member States may nevertheless authorize, under conditions laid down by them, the use of untreated sludge if it is injected or worked into the soil.

In the UK, The Sludge (Use in Agriculture) Regulations 1989 directly implement the provisions of the Directive (Anon, 1989). This was accompanied by a Code of Practice (DoE, 1996) which provided practical guidance on how the requirements of the Directive could be met. It recognizes that pathogens may be present in untreated sludges and that their numbers can be reduced significantly by appropriate treatment. Examples of effective treatment processes are given in the Code (Table 17.5).

[1] Treated sludge is defined in Article 2(b) of the Directive as '*sludge which has undergone biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and other health hazards resulting from its use.*'

At the time that the Code was prepared the pathogens of concern were considered to be salmonellae, *Taenia saginata* (human beef tapeworm), potato cyst nematodes (*Globodera pallida* and *Globodera rostochiensis*) and viruses.

The guidance was based on the concept of multiple barriers to the prevention of transmission of pathogens when sludge was applied to agricultural land. The barriers are:

- Sludge treatment, which will reduce pathogen content
- Restrictions on which crops may be grown on land to which sludge has been applied
- Minimum intervals before grazing or harvesting.

The scientific and public health principles which underpin this concept are valid. They recognize that for certain crops the risk of disease transmission is unacceptable, i.e. salad items which have a short growing period and which are to be consumed raw. For other crops the combination of treatment and a suitable period of no harvesting will result in the numbers of pathogenic microorganisms being reduced below a minimum infective dose (MID). The concept of MID is important – it relates to the number of organisms which must be ingested to cause

TABLE 17.6 Minimum infective dose (MID) for a range of gastrointestinal pathogens

Organism	*Minimum infective dose*
Salmonella spp.	10^4–10^7
Salmonella typhi	10
Escherichia coli O157:H7	10–10^2
Vibrio cholerae	10^3
Giardia intestinalis	10–10^2
Cryptosporidium parvum	10–10^2
Entamoeba histolytica	10–10^2
Hepatitis A virus	1–10 PFU

PFU, plaque forming unit

disease. It varies widely depending not only on the particular pathogen but also on the susceptibility of the host (Table 17.6). For example in the young, elderly, pregnant or those whose immunity is reduced the minimum number of organisms required to initiate disease is much smaller.

Despite the current concerns surrounding the risks to food safety, it is important to recognize that there have been no instances documented in which disease transmission to man or animals has occurred where the provisions of the relevant UK Regulations and Codes of Practice were followed.

5 PATHOGENS

Pathogens are microorganisms that are capable of causing disease in the host species (man, animals or plants). All the major groups of microorganisms contain species which are pathogenic including viruses (e.g. hepatitis virus), bacteria (e.g. salmonellae), fungi (e.g. *Aspergillus*), protozoa (e.g. *Cryptosporidium*) and helminths (e.g. *Taenia*). Although there are several plant pathogens which may potentially be present in sewage sludge (e.g. brown rot, potato root eelworm and beet rhizomania), this review will deal with pathogens affecting man and animals. Many of these are described as zoonotic, i.e. directly transmissible to man from animals. Examples of zoonotic infections include salmonellosis and cryptosporidiosis. This is a particularly important factor when considering the risks to human health arising from the use of sludge in agriculture.

The type and number of pathogens that are likely to be present in untreated sewage will depend on the inputs to the sewerage system. The spectrum of human pathogens will mirror the incidence of infection in the community. People suffering from diseases of the gastrointestinal tract will excrete large numbers of the pathogen in their faeces. Industrial sources of pathogens include meat processing plants, abattoirs and livestock facilities. The World Health Organization in its review of health risks arising from sewage sludge applied to land described a wide range of pathogens that could be present in sludge (WHO, 1981). This was subsequently updated and expanded by Strauch (1991) and the United States EPA who collated the data shown in Table 17.7 (USEPA, 1989).

The list is extremely comprehensive and, in reality, the risk from many of these microorganisms is very small. The organisms shown in bold are those identified by the US EPA as posing a significant risk to human health and which were taken into account in the development of the current Part 503 Regulations (US EPA, 1992). It is interesting to note that at that time they did not consider *Escherichia* as posing a significant risk to health. It is now known that certain shiga toxin-producing strains, such as *E. coli* O157[2], are capable of being transmitted by contaminated foodstuffs (Armstrong *et al*., 1996; Tauxe, 1997; Mead and Griffin, 1998; Parry and Palmer, 2000).

In practice, the list of microorganisms that we need to be concerned with is relatively small. The pathogens of concern will vary from region to region depending on the nature and prevalence of endemic infectious intestinal disease within the indigenous population. For example, data for England and Wales collated by the PHLS Communicable Disease Surveillance Centre reveal that over half of all notified infections are due to *Campylobacter* (Fig. 17.5).

In contrast, intestinal parasites, particularly *Ascaris*, are a major disease burden in the developing world. These parasites form cysts or ova which are especially robust and resistant to environmental conditions, attributes which contribute to high levels of re-infection.

[2] Previously referred to as verotoxigenic *E. coli* or VTEC.

TABLE 17.7 Pathogenic microorganisms which may be present in sewage sludge

Bacteria	*Viruses*	*Protozoa*	*Yeasts*
Salmonella	**Hepatitis A**	**Cryptosporidium**	Candida
Shigella	**Enteroviruses**	**Entamoeba**	Cryptococcus
Yersinia	**Poliovirus**	**Giardia**	Trichosporon
Escherichia	**Coxsackie viruses**	**Balantidium**	
Pseudomonas	**Echoviruses**	Toxoplasma	**Fungi**
Clostridia	**Rotavirus**	Sarcocystis	Aspergillus
Bacillus	Adenovirus		Phialophora
Listeria	Reovirus	**Cestodes**	Geotrichum
Vibrio	Astrovirus	**Taenia**	Trichophyton
Mycobacterium	Calicivirus	Diphyllobothrium	Epidermophyton
Leptospira	Coronavirus	Echinococcus	
Campylobacter	Norwalk and Norwalk-like viruses		
Staphylococcus		**Nematodes**	
Streptococcus		**Ascaris**	
		Toxocara	
		Trichuris	
		Ancylostoma	
		Necator	
		hymenolepsis	

Source: US EPA (1999).

The literature contains numerous reports of surveys and investigations into the occurrence of pathogens and indicator bacteria in wastewater and sludge treatment plants. Because of methodological and geographical differences and, in some instances, lack of details regarding treatment processes, many of these data are not directly comparable. However, the data shown in Table 17.8 provide an indication of the likely numbers of indicators and pathogens in domestic wastewater and sludges.

6 EFFECTS OF SLUDGE TREATMENT ON PATHOGENS

It must be recognized at the outset that treatment is designed to stabilize sewage sludge and reduce its putrescence. Pathogens may be inactivated as a consequence of the particular treatment applied. It has not been normal practice to optimize sludge treatment processes for pathogen reduction. Indeed to do so may reduce the effectiveness of the stabilization process.

A review of the literature by Ward and colleagues (1984) showed that the range of pathogen inactivation reported was large, depending on the extent of the treatment process and variation between operating conditions, even for the same generic treatment process (Table 17.9).

There are limited data on the effect of some of these processes on certain pathogens. In practice, research in this area has been restricted to

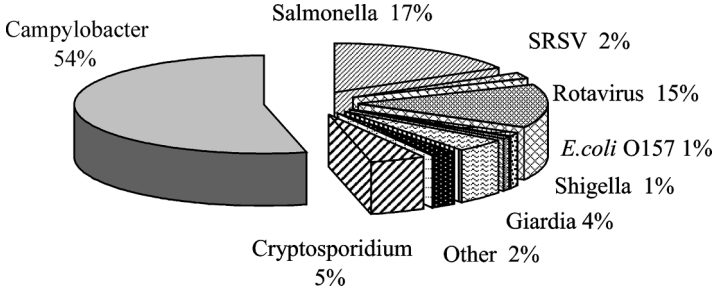

Fig. 17.5 Infectious intestinal disease in England and Wales by aetiological agent.

TABLE 17.8 Typical numbers of microorganisms found in various stages of wastewater and sludge treatment

Microorganism	*Number per 100* ml			*Number per gram*		
	Crude sewage	*Primary treatment*	*Secondary treatment*	*Tertiary treatment*[a]	*Raw*	*Treated*[b]
Faecal coliforms	10^8	10^7	10^6	<2	10^7	10^6
Salmonellae	10^3	10^2	10	<2	10^3	10^2
Shigella	10^3	10^2	1	<2	10^2	3
Listeria	10^4					10^3
Campylobacter	10^5				10^4	10
Enteric virus	5×10^4	1×10^4	10^3	0.002	10^3	10^2
Helminth ova	8×10^2	10	0.08	<0.08	10	10
Giardia cysts	10^4	5×10^3	2.5×10^3	3	10^2	10

[a] Including coagulation, sedimentation, filtration, disinfection.
[b] Mesophilic anaerobic digestion.
Source: Jones *et al*. (1990); Metcalf and Eddy (1991); National Research Council (1996); De Luca *et al*. (1998); Watkins and Sleath (1981).

those pathogens with a high prevalence and likely to cause disease (e.g. salmonellae) and those that are more likely to exhibit resistance to the sludge treatment process (e.g. *Ascaris*). In comparison little is known about the pathogens which have only recently emerged as public health issues, most notably *E. coli* O157. The recent emergence of this pathogen means that there is little information available on the fate of *E. coli* O157 (and other shiga toxin-producing *E. coli* (STEC)) during the treatment of wastewater and sewage sludge. Despite the highly infectious nature of STEC and the presence of multiple virulence factors there is evidence that it is no more resistant to inactivation during sludge treatment than the indigenous populations of *E. coli* in sewage and sludge (Horan, personal communication).

TABLE 17.9 Summary of pathogen reduction during sludge treatment

Treatment	*Log reduction*		
	Bacteria	*Viruses*	*Parasites*
Mesophilic anaerobic digestion	0.5–4	0.5–2	0
Aerobic digestion	0.5–4	0.5–2	0
Composting	2–>4	2–>4	2–>4
Air drying	0.5–4	0.5–>4	0.5–>4
Lime stabilization	2–>4	>4	0

Source: Ward *et al*. (1984)

The inactivation of indigenous *E. coli* in full-scale sludge treatment processes was investigated during a 3-month study, which looked at nine different sludge treatment processes at 35 sites in the UK (UKWIR, 1999). All of the processes surveyed reduced the numbers of *E. coli*. So-called 'enhanced' treatment processes, such as composting, lime addition and thermal drying, were capable of reducing numbers of *E. coli* to the detection limit of the analytical method. For all of these methods, over 90% of results showed bacterial reductions of 6 log or greater. Lagooning of sludge was capable of significantly reducing numbers of *E. coli* and, depending on the method of operation, reductions in the order of 5 log were observed. Mesophilic anaerobic digestion (MAD), the process carried out at the majority of sites surveyed, reduced numbers of *E. coli* by, on average, between 1.4 and 2.3 log depending on the solids content of the product. For sites producing a liquid product (2–4% ds) 78% of all reductions for were in the range 1 to 2 log. Where digested sludge was subsequently dewatered to produce a cake, 89% of results showed reductions in the range 2 to 4 log. The one vermiculture site in the survey showed results intermediate between MAD and the 'enhanced' treatment processes (Table 17.10).

Cryptosporidium parvum is another pathogen of increasing importance. Wastewater discharges and run off from agricultural land are

TABLE 17.10 Effect of sludge treatment processes on numbers of *E. coli*

Treatment	*n*	*Log reduction in E. coli*		*Log reduction in E. coli treated sludge (100/g dry wt)*	
		Mean	95%ile	Mean	95%ile
Lagooning	36	2.65	6.00	5.93	8.32
MAD, liquid	208	1.39	2.36	7.41	8.27
MAD, cake	93	2.29	3.64	6.65	7.46
Vermiculture	14	5.12	6.54	4.50	5.07
Composting	31	6.71	9.10	2.43	4.70
Lime addition	32	7.10	9.05	1.45	3.00
Thermal drying	70	7.14	8.90	1.67	3.56

Source: UKWIR (1999).

an important source of *Cryptosporidium* oocysts found in watersheds. The transmission of cryptosporidiosis is zoonotic and the possibility exists of foodborne infection arising from the use of sewage sludge in agriculture. Stadterman *et al*. (1995) found that a laboratory activated sludge plant removed 98.6% of seeded *Cryptosporidium parvum* oocysts. In a comparison of different treatment regimes, activated sludge and anaerobic digestion were found to be the most effective means of removing oocysts, the latter destroying 99.9% in 24 hours.

Studies of anaerobic mesophilic digestion under laboratory conditions showed that oocysts added to the contents of a digester operating at 35°C rapidly lost viability (as measured by excystation), decreasing to 17% after 3 days from an initial 81% viability (Whitmore and Robertson, 1995). Losses of viability in distilled water and anaerobic sludge at 35°C were similar, amounting to 90% after 18 days, indicating that the principal effect on viability was temperature. Oocysts exposed to mesophilic anaerobic digestion for 3 days and then stored for a further 14 days were completely inactivated. Aerobic digestion or pasteurization, both at 55°C, caused 92% loss of viability in 5 minutes. Thermophilic anaerobic digestion at 50°C resulted in complete inactivation within the first 24 hours (Whitmore and Robertson, 1995).

7 ROUTES OF TRANSMISSION

Recently expressed concerns over the use of sewage sludge in agriculture have focused on the risks to human health arising from the production of foods on land to which sludge has been applied (Anon, 1998a). A number of exposure pathways whereby foodstuffs become contaminated with pathogens can be envisaged. The exposure pathways relevant to sewage sludge in agricultural production are:

1. Sludge → soil → plant → human
2. Sludge → soil → animal → human
3. Sludge → soil → plant → animal → human
4. Sludge → soil → drinking water → human
5. Sludge → soil → irrigation water → plant → human.

These may be developed into a conceptual model which describes the framework for a microbiological risk assessment (Fig. 17.6). Other routes of exposure, which do not involve the food chain, can be identified. These include direct contact with sludge-treated soil or indirect contact via companion animals. The risk of exposure by this route (direct contact) is probably greatest among children.

It can be seen that there are several pathways that are unrelated to the use of sewage sludge, probably the most important of which is the application to land of organic wastes such as animal slurries and manures. The use of such materials in agriculture is less regulated than for sewage sludge and accounts for the majority of organic waste spread to land (Table 17.11). Despite this, the focus of attention has been on the human health risks via the food chain from the application of sewage sludge to agricultural land (RCEP, 1996; Anon, 1998a).

As previously mentioned, at the time that existing controls on the agricultural use of sewage sludge were being formulated, the pathogens of concern were salmonellae and *T. saginata*. In the intervening period, pathogens such as shiga toxin-producing *Escherichia coli* and *Cryptosporidium* have been recognized as important causes of intestinal infectious disease in humans. Any assessment of health

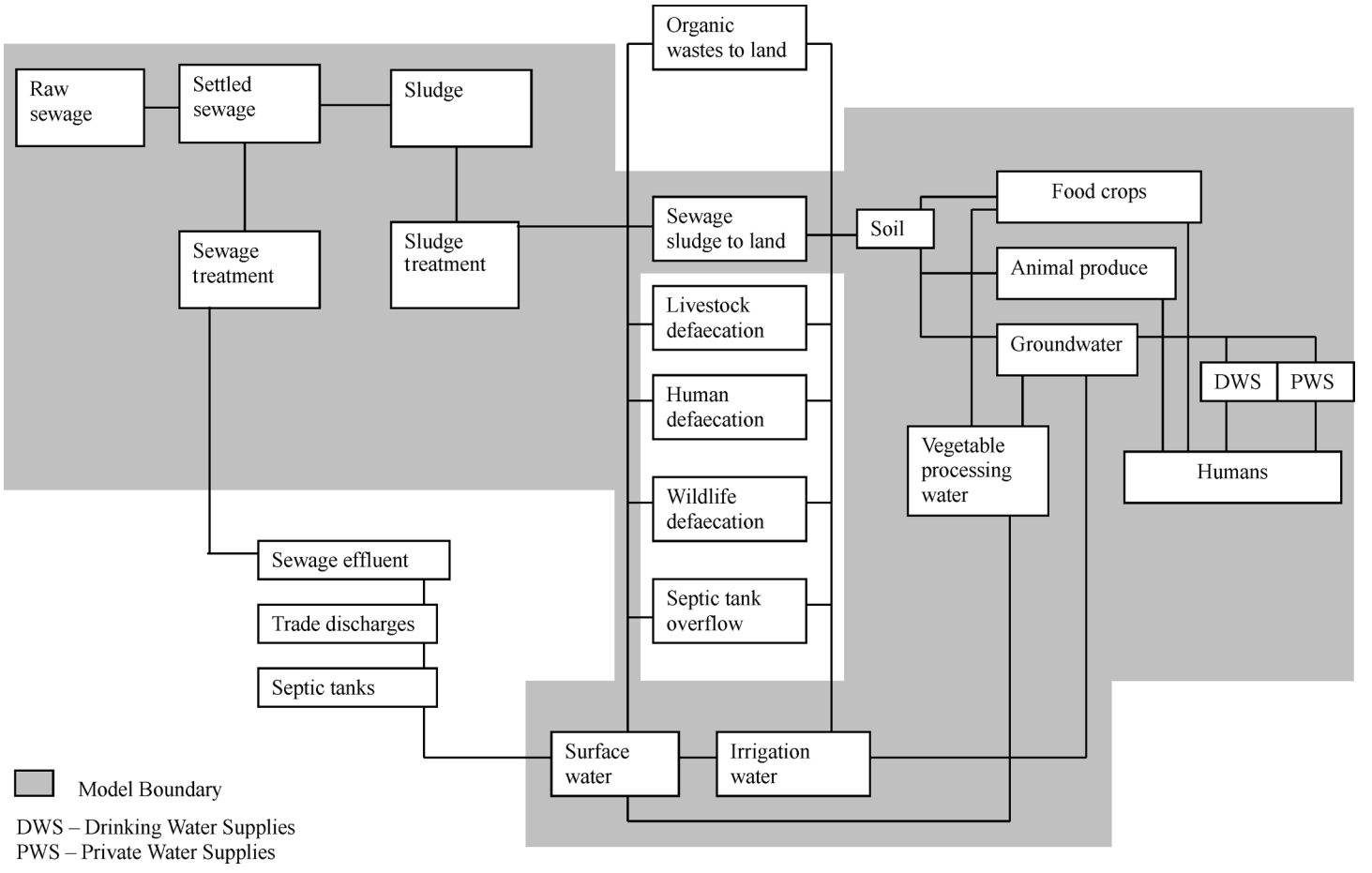

Fig. 17.6 Conceptual model for a microbiological risk assessment into the application of sludge to agricultural land (Pollard, personal communication).

TABLE 17.11 Estimates of the quantities of organic materials applied to land in the UK

Origin	*Quantity (tonne × 10^3 dw)*
Farm animal	21 000
Sewage sludge	430
Paper industry	520
Food industry	600
Sugar industry	200
Others	150

dw: Dry weight.
Source: WRc (1998).

risks associated with the beneficial use of sewage sludge in agriculture must consider these two pathogens.

7.1 Shiga toxin-producing *E. coli*

Shiga toxin-producing *E. coli* (STEC) are now recognized as an important group of enteric pathogens. Although there are many serotypes capable of producing shiga toxins, *E. coli* O157:H7 is the most widely known. This organism was first described in 1982 following an outbreak of haemorrhagic colitis in the USA (Riley *et al*., 1983). Outbreaks have been associated with the consumption of foodstuffs, drinking water, and swimming in natural surface waters. Zoonotic infections have also been reported. The majority of cases are believed to be foodborne, with an estimated 85% of cases (n = 110 220) in the USA suspected to be food-related (Mead *et al*., 1999).

There is very little information concerning the presence of STEC in sewage and sewage sludge. It is reasonable to assume that domestic sewage will contain STEC, if not continuously then intermittently, reflecting the incidence of infection in the community. The likelihood of STEC being present will be greater for those wastewater treatment works receiving wastes from animal-handling facilities, such as markets, abattoirs and meat processing plants. Surveillance of *E. coli* O157 in animals presented for slaughter carried out in northern England revealed that 15.7% of cattle, 2.2% of sheep and 0.4% of pigs were positive for the organism (Chapman, 2000).

Survival of *E. coli* O157 in the environment has been investigated by a number of workers. Maule (1997, 2000) showed that survival of the organism was found to be greatest in soil cores containing rooted grass. Under these conditions viable numbers were shown to decline from approximately 10^8/g soil to between 10^6 and 10^7/g soil after 130 days. When the organism was inoculated into cattle faeces it remained detectable at high levels for more than 50 days. In contrast, the organism survived much less readily in cattle slurry and river water where it fell in numbers from more than 10^6/ml to undetectable levels in 10 and 27 days, respectively. Survival of *E. coli* O157:H7 in bulk manures may be prolonged, with the organism being detected for more than one year in static piles of ovine manure (Kudva *et al*., 1998). Survival was reduced if the manure piles were aerated.

The fate of *E. coli* O157 present in animal slurry applied to pasture was investigated by Fenlon and colleagues (2000). Following application, numbers of both *E. coli* and *E. coli* O157 declined steadily with greater than 2 log reduction within 29 days. Relatively few cells (2% of total) were transported away from the soil surface and into the deeper layers of the soil. Run-off following heavy rainfall resulted in a loss from the soil of 7% of the *E. coli* applied in the slurry. A recent ecological study on predation of *E. coli* O157 by *Acanthamoeba polyphaga* has shown that the bacterial cells are capable of surviving and even replicating within this common environmental protozoan (Barker *et al*., 1999). This may be important in the dissemination and survival of STEC within the environment.

7.2 Cryptosporidium

Human infection with *Cryptosporidium* was first reported in 1976 (Fayer *et al*., 2000). It is now apparent that *Cryptosporidium* is a significant cause of infectious intestinal disease (IID), accounting for about 5% of IID in which the causative organism is identified. In England and Wales, during 1999, there were nearly 5000 laboratory confirmed cases of cryptosporidiosis (n = 4759) (Anon, 2000a); in the USA

the estimated number of cases is 300 000 of which 10% are believed to be foodborne (Mead *et al*., 1999).

Oocysts of *C. parvum* are environmentally hardy and, under certain conditions, may remain viable for many months. Survival studies in microcosms containing untreated river water showed that oocysts are extremely persistent, the times for 10 log reductions in viability being 160 days at 15°C and 100 days at 5°C (Medema *et al*., 1997). Investigations into the survival in soils treated with sewage sludge showed that viability declined by 20–40% at 20°C over 44 days. Temperature was the principal factor affecting oocyst survival (Whitmore and Robertson, 1995).

Little is known about the movement of oocysts through the soil. The most relevant data were reported by Mawdsley and colleagues (1996a) who studied the transport of *Cryptosporidium* oocysts through soil following the application of slurry to a poorly draining silt clay loam soil. Bovine slurry seeded with 5×10^9 *C. parvum* oocysts was applied to the surface of soil blocks (80 cm × 56 cm × 20 cm) removed from a perennial ryegrass ley at an application rate equivalent to 50 m^3/ha. The blocks were irrigated 24 h following slurry application and periodically thereafter. Samples of run-off (at 4 cm depth) and leachate (at 20 cm depth) were collected and the number of oocysts enumerated. After 70 days the blocks were destructively sampled and examined for the presence of oocysts. Experiments were carried out in triplicate. Numbers of oocysts leaching from the blocks declined from 8.4×10^6 on day 1 to 2.3×10^4 at day 70. Oocysts levels were consistently lower in run-off compared with the leachate from the base of the soil blocks. Numbers fell below the limit of detection after 21 days and 28 days in two blocks, but were detectable in the third block for the duration of the experiment (70 days). These results suggest that oocysts tend not to become associated with soil particles, being either transported away in run-off or moving vertically downwards through the soil column. The majority of oocysts were retained in the top 2 cm of soil (Mawdsley *et al*., 1996b).

8 ASSESSMENT OF HUMAN HEALTH RISKS

The process of microbiological risk assessment is now considered to comprise three phases: problem formulation, analysis, and risk characterization (ILSI, 2000). The analysis phase consists of two elements: characterization of exposure and characterization of human health effects (Fig. 17.7).

Characterization of exposure requires an evaluation of the interaction between the pathogen, the environment and the human population. Factors that need to be considered include the virulence of the pathogen, survival in the environment, route of infection, numbers of pathogen present, effectiveness of control/treatment processes, infectious dose, severity of illness and size of exposed population.

Unlike the field of chemical toxicology, microbiological risk assessment is in its infancy. There exist major gaps in our knowledge about the organisms of concern. This is particularly the case for the emerging pathogens such as STEC and *Cryptosporidium*. There have been attempts to assess the risks posed by *Cryptosporidium* in drinking water supplies (Gale, 1996, 1999; Haas, 2000). However, the foodborne route has not been modelled, probably because of the received view that cryptosporidiosis is primarily waterborne, despite evidence demonstrating the potential for foodborne transmission. *Cryptosporidium* oocysts have been found on the surface of fresh, raw vegetables obtained from retail markets (Ortega *et al*., 1991; Monge *et al*., 1996). In the UK, an outbreak of cryptosporidiosis affecting 50 school children was linked to the consumption of improperly pasteurized milk (Gelletli *et al*., 1997). In the USA, outbreaks have been associated with the drinking fresh-pressed apple juice (non-alcoholic cider) (Millard *et al*., 1994). Outbreaks of cryptosporidiosis associated with infected food-handlers demonstrate clearly the potential for significant foodborne transmission of *Cryptosporidium* (Besser-Wiek *et al*., 1996; Quiroz *et al*., 2000).

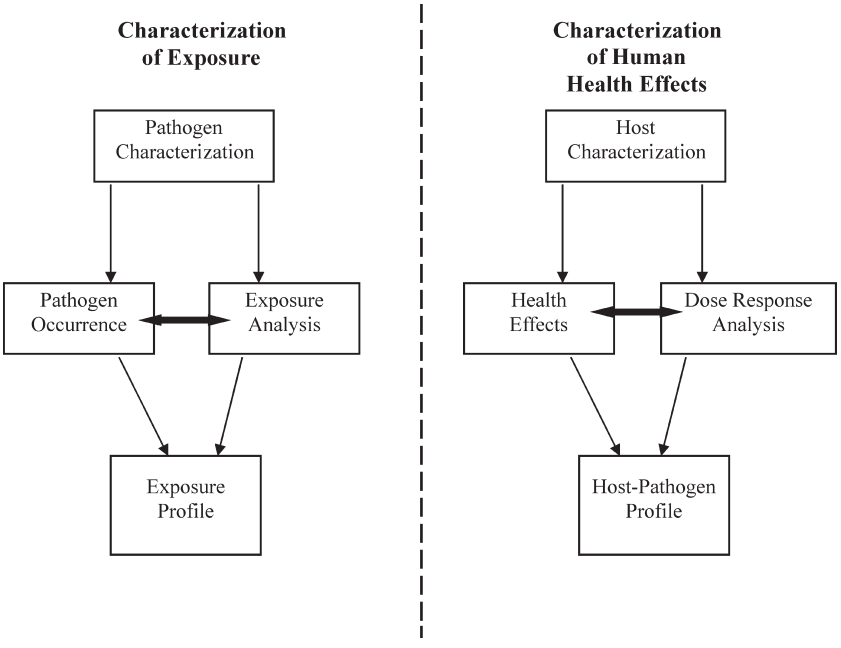

Fig. 17.7 Analysis phase of a microbiological risk assessment for foodborne pathogens (ILSI, 2000).

On the other hand, it is clear that STEC infection is primarily foodborne (Mead *et al*., 1999). Data are required on the levels of STEC in treated sludge, their survival following land application and the potential for transfer to food crops before a microbiological risk assessment can be performed. Research is currently being undertaken to address these issues.

9 PRECAUTIONARY PRINCIPLE

Against a background of concern over methods of food production in the UK, the water industry, under the auspices of Water UK, and representatives of the food suppliers agreed a set of guidelines matching the level of sewage treatment with the crop under cultivation (Anon, 1998b).

The Safe Sludge Matrix (Anon, 2000b, Tables 17.12 and 17.13) forms the basis of the agreement and consists of a table of crop types, together with clear guidance on the minimum acceptable level of treatment for any sewage sludge (biosolids) based product, which may be applied to that crop or rotation. The agreement was driven by the desire to ensure the highest possible standards of food safety and to provide a framework that gives the retailers and food industry confidence that sludge reuse on agricultural land is safe. The matrix enables farmers and

TABLE 17.12 The safe sludge matrix

Crop group	*Untreated sludges*	*Treated sludges*	*Enhanced treated sludges*
Fruit	✗	✗	✓‡
Salads	✗	✗ (30 month harvest interval applies)	✓‡
Vegetables	✗	✗ (12 month harvest interval applies)	✓‡
Horticulture	✗	✗	✓‡
Combinable and animal feed crops	✗	✓	✓
Grass – grazing	✗	✗† (Deep injected or ploughed down only)	✓†
Grass – silage	✗	✓†	✓†
Maize – silage	✗	✓†	✓†

† 3 week no grazing and harvest interval applies.
‡ 10 months harvest interval applies.

TABLE 17.13 Cropping categories within the Safe Sludge Matrix

Fruit	*Salad (e.g. ready to eat crops)*	*Vegetables*	*Horticulture*	*Combinable and animal feed crops*	*Grassland and maize*	
					Silage	*Grazing*
Top fruit (apples, pears, etc.)	Lettuce	Potatoes	Soil based glasshouse and polythene tunnel production (including tomatoes, cucumbers, peppers, etc.)	Wheat	Cut grass	Grass
	Radish	Leeks	Mushrooms	Barley	Cut maize	Forage Swedes/turnips
Stone fruit (plums, cherries, etc.)	Onions	Sweetcorn	Nursery stock and bulbs for export	Oats	Herbage	Fodder mangolds/ beet/kale
	Beans (including runner, broad and dwarf French)	Brussels sprouts	Basic nursery stock	Rye	Seeds	Forage rye and triticale
Soft fruit (currants and berries)	Vining peas	Parsnips		Triticale		Turf production
	Mange tout	Swedes/turnips	Seed potatoes for export	Field peas		
Vines	Cabbage	Marrows	Basic seed potatoes	Field beans		
Hops	Cauliflower	Pumpkins		Linseed/flax		
	Calabrese/broccoli	Squashes	Basic seed production	Oilseed rape		
Nuts	Courgettes	Rhubarb		Hemp		
	Celery	Artichokes		Sunflower		
	Red beet			Borage		
	Carrots			Sugar beet		
	Herbs					
	Asparagus					
	Garlic					
	Shallot					
	Spinach					
	Chicory					
	Celeriac					

growers to continue to utilize the beneficial properties in sewage sludge as a valuable and cost effective source of nutrients and organic matter.

The main impact was the cessation of raw or untreated sewage sludge being used on agricultural land. As from the end of 1999, all untreated sludges have been banned from application to agricultural land used to grow food crops. Treated sludge[3] can only be applied to grazed grassland where it is deep injected into the soil. The regulations require that there will be no grazing or harvesting within 3 weeks of application. Where grassland is reseeded, sludge must be ploughed down or deep injected into the soil.

More stringent requirements apply where sludge is applied to land growing vegetable crops and, in particular, those crops that may be eaten raw (e.g. salad crops). Treated sludge can be applied to agricultural land which is used to grow vegetables provided that at least 12 months has elapsed between application of the sludge and harvest of the vegetable crop. Where the crop is a salad, which might be eaten raw, the harvest interval must be at least 30 months. Where enhanced treated sludges[4] are used, a 10-month harvest interval applies.

10 CONCLUSIONS

From an environmental perspective there is a persuasive argument that, of the disposal options available, recycling nutrients by means of applying sewage sludge to land, with appropriate safeguards, is the Best Practicable Environmental Option (BPEO) (CEC, 1986; RCEP, 1996). The risks to human, animal and plant health were taken into account when developing the current regulations and codes of practice. The fundamental principle (for reducing disease transmission risk) implicit to these controls on the use of sludge in agriculture is the concept of imposing multiple barriers to the recycling of pathogens from sludge to their hosts. The effectiveness of this approach is borne out in practice as noted by the Royal Commission on Environmental Pollution (RCEP, 1996) which concluded that, 'There are no instances in the UK in which a link has been established between the controlled application of sewage sludge and occurrence of disease in the general population through water or food contamination'. However, it is the case that the current controls predate the emergence of pathogens such as *Cryptosporidium* and STEC and may not sufficiently reduce the risk associated with these microorganisms.

More data are required on the numbers and fate of these emergent pathogens before a meaningful microbiological risk assessment can be carried out. Research is being undertaken in the UK, USA and elsewhere to generate these data. The likelihood is that the controls on the use of sewage sludge in agriculture will be strengthened as results of this research and in the light of public perception and expectations about food safety and environmental risks.

[3] There is a range of different treatment processes used to reduce the fermentability and possible health hazards associated with sewage sludge. These rely on biological, chemical or heat treatment. The most common form of treatment is anaerobic digestion.

[4] Enhanced treatment, originally referred to as 'Advanced Treatment', is a term used to describe treatment processes which are capable of virtually eliminating any pathogens which may be present in the original sludge.

REFERENCES

Anon (1989). The Sludge (Use in Agriculture) Regulations 1989. SI No. 1263 as amended by SI No. 880 (1990).

Anon (1998a). House of Commons Environment, Transport and Regional Affairs Committee. Sewage Treatment and Disposal, Second Report. Stationery Office, London.

Anon (1998b). The 'ADAS Matrix': the food and water industry in agreement. *Wastes Management*, December 1998, 28–29.

Anon (2000a). Data on infectious disease in England and Wales. Available on line at http://www.phls.co.uk/

Anon (2000b). The Safe Sludge Matrix. Available on line at http://www.adas.co.uk/matrix/SSM.pdf

Armstrong, G.L., Hollingsworth, J. and Morris, J.G. (1996). Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiologic Reviews* **18**(1), 29–51.

Barker, J., Humphrey, T.J. and Brown, M.W. (1999). Survival of *Escherichia coli* O157 in a soil protozoan:

implications for disease. *FEMS Microbiology Letters* **173**(2), 291–295.

Bastian, R.K. (1997). The biosolids (sludge) treatment, beneficial use, and disposal situation in the USA. *European Water Pollution Control* **7**(2), 62–79.

Besser-Wiek, J.W., Forfang, J., Hedberg, C.W. *et al.* (1996). Foodborne outbreak of diarrheal illness associated with *Cryptosporidium parvum* – Minnesota, 1995. *Morbidity and Mortality Weekly Report* **45**(36), 783–784.

CEC (1991). Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment. *Official Journal of the European Communities* **L135**/40 (30 May).

CEC (1986). Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *Official Journal of the European Communities* **L181**/6 (4 July).

CEC (1999). Report from the Commission to the Council and the European Parliament on the implementation of community waste legislation for the period 1995–1997. Commission of the European Communities, Brussels, COM 752 (final).

Chapman, P.A. (2000). Sources of *Escherichia coli* O157 and experiences over the past 15 years in Sheffield, UK. *Journal of Applied Microbiology*, **88** Supplement, 51S–60S.

De Luca, G., Zanetti, F., Fateeh-Moghadm, P. and Stampi, S. (1998). *Zentrablatt für Hygiene und Umweltmedizin* **201**(3), 269–277.

DoE (1996). *Code of Practice for Agricultural Use of Sewage Sludge*, revised edition. HMSO, London.

Environment Agency (1999). UK Sewage Sludge Survey. *R&D Technical Report P165*. Environment Agency, Bristol.

Fayer, R., Morgan, U. and Upton, S.J. (2000). Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal of Parasitology* **30**(12–13), 1305–1322.

Fenlon, D.R., Ogden, I.D., Vinten, A. and Svoboda, I. (2000). The fate of *Escherichia coli* and *E. coli* O157 in cattle slurry after application to land. *Journal of Applied Microbiology* **88** Supplement, 149S–156S.

Gale, P. (1996). Developments in microbiological risk assessment models for drinking water – a short review. *Journal of Applied Bacteriology* **81**, 403–410.

Gale, P. (1999). Assessing the risk of cryptosporidiosis. *Journal of American Water Works Association* **91**(3), 4.

Gelletli, R., Stuart, J., Soltano, N. *et al.* (1997). Cryptosporidiosis associated with school milk. *Lancet* **350**, 1005–1006.

Haas, C.N. (2000). Epidemiology, microbiology, and risk assessment of waterborne pathogens including *Cryptosporidium*. *Journal of Food Protection* **63**(6), 827–831.

ILSI (2000). *Revised Framework for Microbial Risk Assessment*. International Life Sciences Institute, Washington DC.

Jones, K., Betaieb, M. and Telford, D.R. (1990). Correlation between environmental monitoring of thermophilic campylobacters in sewage effluent and the incidence of Campylobacter infection in the community. *Journal of Applied Bacteriology* **69**(2), 235–240.

Kudva, I.T., Blanch, K. and Hovde, C.J. (1998). Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.* **64**, 3166–3174.

MAFF (1998a). *Code of good agricultural practice for the protection of water*. HMSO, London.

MAFF (1998b). *Code of good agricultural practice for the protection of soil*. MAFF Publications, London.

Maule, A. (1997). Survival of the verotoxigenic strain *E. coli* O157:H7 in laboratory-scale microcosms. In: *Coliforms and E. coli: Problem or solution?* D. Kay and C.R. Fricker (eds) Royal Society of Chemistry, London.

Maule, A. (2000). Survival of verocytotoxigenic *Escherichia coli* O157 in soil, water and on surfaces. *Journal of Applied Microbiology* **88** Supplement, 71S–78S.

Mawdsley, J.L., Brooks, A.E., Merry, R.J. and Pain, B.F. (1996a). Use of a novel soil tilting table apparatus to demonstrate the horizontal and vertical movement of the protozoan pathogen *Cryptosporidium parvum* in soil. *Biology and Fertility of Soils* **23**(2), 215–220.

Mawdsley, J.L., Brooks, A.E. and Merry, R.J. (1996b). Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. *Biology and Fertility of Soils* **21**, 30–36.

Mead, P.S. and Griffin, P.M. (1998). *Escherichia coli* O157:H7. *Lancet* **352**(9135), 1207–1212.

Mead, P.S., Slutsker, L., Dietz, V. *et al.* (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases* **5**(5), 607–625.

Medema, G.J., Bahar, M. and Schets, F.M. (1997). Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochthonous microorganisms. *Water Science and Technology* **35**(11–12), 249–252.

Metcalf and Eddy Inc. (1991). *Wastewater Engineering: Treatment, Disposal and Reuse*. McGraw-Hill, New York.

Millard, P.S., Gensheimer, K.F., Addiss, D.G. *et al.* (1994). An outbreak of cryptosporidiosis from fresh-pressed apple cider. *Journal of the American Medical Association* **272**(20), 1592–1596.

Monge, R. and Chinchilla, M. (1996). Presence of *Cryptosporidium* oocysts in fresh vegetables. *Journal of Food Protection* **59**, 1866–1870.

National Research Council (1996). *Use of Reclaimed Water and Sludge in Food Crop Production*. National Academy Press, Washington DC.

Ortega, Y.R., Sheehy, R.R., Cama, V.A. *et al.* (1991). Restriction fragment length polymorphism analysis of *Cryptosporidium parvum* isolates of bovine and human origin. *Journal of Protozoology* **38**(6), 40S–41S.

Parry, S.M. and Palmer, S.R. (2000). The public health significance of VTEC O157. *Journal of Applied Microbiology*, **88** Supplement, 1S–9S.

Quiroz, E.S., Bern, C., MacArthur, J.R. *et al.* (2000). An outbreak of cryptosporidiosis linked to a foodhandler. *Journal of Infectious Diseases* **181**, 695–700.

RCEP (1996). Sustainable use of Soil, Royal Commission on Environmental Pollution Nineteenth Report, Cmnd 3165. HMSO, London.

Riley, L.W., Remis, R.S., Helgerson, S.D. *et al.* (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* **308**(12), 681–685.

Stadterman, K.L., Sninsky, A.M., Sykora, J.L. and Jakubowski, W. (1995). Removal and inactivation of *Cryptosporidium*

oocysts by activated sludge treatment and anaerobic digestion. *Water Science and Technology* **31**(5–6), 97–104.

Strauch, D. (1991). Microbiological treatment of municipal sewage waste and refuse as a means of disinfection prior to recycling in agriculture. *Studies in Environmental Science* **42**, 121–136.

Tauxe, R.V. (1997). Emerging foodborne diseases: an evolving public health challenge. *Emerging Infectious Disease* **3**(4), 425–434.

UKWIR (1999). *A Survey of E. coli in UK Sludges*, Report No. 99/SL/06/3. UK Water Industry Research, London.

US EPA (1989). *Technical Support Document for Pathogen Reduction in Sewage Sludge*. NTIS No. PB89-136618. National Technical Information Service, Springfield VA.

US EPA (1992). *Technical Support Document for Reduction of Pathogens and Vector Attraction in Sewage Sludge. Report No. 822/R-93-004*. US EPA, Cincinatti.

US EPA (1993). Part 503 – Standards for the use and disposal of sewage sludge. *Federal Register* **58**(32), 9387–9401.

US EPA (1999). *Control of Pathogens and Vector Attraction in Sewage Sludge, Report No. EPA/625/R-92/013*. US EPA, Cincinatti.

Ward, R., McFeters, G. and Yeager, J. (1984). *Pathogens in Sewage Sludge: Occurrence, Inactivation and Potential Regrowth*. Sandia Report; DAND83-0557, TTC-0428, UC-71, Sandia National Laboratories, Alberquerque.

Watkins, J. and Sleath, K.P. (1980). Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *Journal of Applied Bacteriology* **50**, 1–9.

Whitmore, T.N. and Robertson, L.J. (1995). The effect of sewage sludge treatment processes on oocysts of *Cryptosporidium parvum*. *Journal of Applied Bacteriology* **78**(1), 34–38.

WHO (1981). *The Risk to Health of Microbes in Sewage Sludge Applied to Land. EURO Reports and Studies No 54*. World Health Organisation, Copenhagen.

WRc (1998). *Review of the Scientific Evidence relating to the Controls on the Agricultural Use of Sewage Sludge*. WRc, Medmenham.