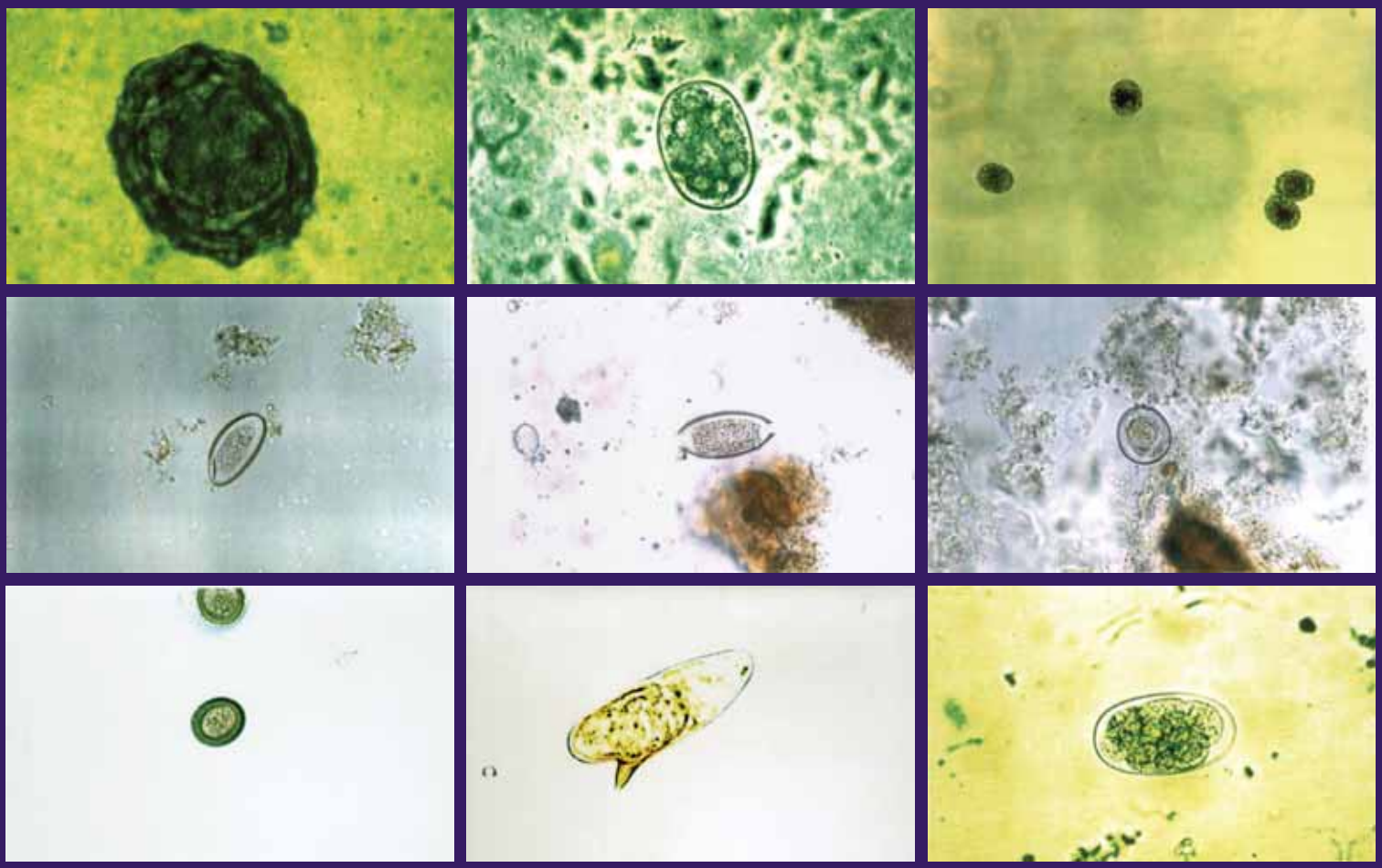
WHO-EM/CEH/121/E

Integrated Guide To

Sanitary Parasitology



World Health Organization
Regional Office for the Eastern Mediterranean
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WHO Library Cataloguing in Publication Data

WHO Regional Centre for Environmental Health Activities
Integrated Guide to Sanitary Parasitology / WHO Regional Centre for Environmental Health Activities.
p. 119
1. Sanitary parasitology – guidelines 2. Environmental health 3. Parasitic helminth 4. Water microbiology I. Title

(ISBN 92-9021-386-8) **[NLM Classification WA 671]**

ACKNOWLEDGEMENTS

This document is prepared and printed as part of the project entitled; "Improvement of Wastewater Management in some Arab Countries ", that is financed by the "Arab Fund for Economic and Social Development" (AFESD) in Kuwait.

The World Health Organization wishes to express its appreciation to all those whose efforts made possible the production of this scientific document.

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CONTENTS

ABBREVIATIONS USED IN THE TEXT

BOD	Biological Oxygen Demand
cap	capita
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
º C	Degree Celsius
d	day
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
g	gram
h	hour
ha	hectare
HRT	Hydraulic Retention Time
kg	kilogram
l	liter
log	logarithm
m	meter
max.	maximum
mg	milligram
min	minute
min.	minimum
mm	millimeter
MPN	Most Probable Number
mV	millivolte
n	Number
PFRP	Processes that Further Reduce Pathogens
PSRP	Processes that Significantly Reduce Pathogens
RH	Relative Humidity
s	second
spp.	species
t	time
TRS	Technical Report Series
TSS	Total Suspended Solids
TVS	Total Volatile Solids
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WSP	Waste Stabilization Pond
yr or y	year

Chapter

1 SANITARY PARASITOLOGY

1.1 Introduction

Over the past 40 years, there has been a considerable revival of interest in the use of wastewater for crop irrigation in arid and semi-arid regions as a result of the scarcity of alternative water supplies and the need to increase local food production. Water resources planners have come to recognize the value of this practice, in terms of both water conservation and nutrient recycling and as a method of preventing the pollution of surface and ground water. The public has not objected such practice so long as the necessary health safeguards have been included. In some countries, such as Jordan, Peru and Saudi Arabia, it is government policy to reuse all effluents from sewage-treatment plants, mainly for crop irrigation.

The "Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture (TRS, 778) published by WHO (1989), provides guidelines values for health protection. Intestinal nematodes were introduced based on the available epidemiological information supporting the conclusion that helminths present the highest risks of wastewater related disease transmission due to long latency periods with soil stage required for transmission, long persistence in the environment, low infective dose, without practical host immunity.

Preparing a document on the subject of sanitary parasitology and the contamination associated with the use of wastewater and sludge in agriculture will serve as a basis for sanitary engineers, microbiologists, and parasitologists on the subject. Based on the most recent technical and scientific information available, identification of helminth eggs is a skill that can be learned quite quickly providing the technician has access to a good microscope with an eyepiece graticule for making measurements and a source of good drawings or photographs.

Comprehensive guide on sanitary parasitology does not exist. This guide will bring this information into one volume for easy reference. This guide is intended as a comprehensive guide for the sanitary parasitology: physical, chemical and biological factors influencing the fate of helminth eggs; occurrence and survival in raw wastewater and sludge; helminth eggs indicator; guidelines; methods; viability, monitoring and compliance with guidelines and regulations.

The objective of this guide is to provide both an introduction to the material required for the identification of helminths found in wastewater and working instructions for the methods for the enumeration of parasitic helminths in wastewater, effluents and sludge. The removal of helminths by wastewater treatment plants and the effectiveness of sewage sludge treatment processes are discussed in detail in chapter 3. Appropriate references and instructions for calibrating microscopes for micrometry are given in chapter 6. The guide also covers the subject of viability determination in chapter 8. Photographs of the helminth eggs most commonly found in wastewater and sludge are given as an introductory guide. Recommendations for quality monitoring programme are given in chapter 9. Chapter 10 deals with the health aspects for the use of reclaimed wastewater in agriculture and aquaculture.

In investigations on pathogenic organisms in wastewater and sludge, parasites have received the least attention. Given the current state of knowledge, there is need for further assessment of the health

problems related to the presence of parasites in wastewater and sludges, as well as the examination of the efficacy of various wastewater and sludge treatment methods on parasite survival. A few studies were published in the 1940's and 1950's, but between 1960 and 1980 little was reported in the literature on parasite transformation through wastewater and sludges and their viability. Extensive research on sanitary parasitology has been observed since the 1980's till now.

1.2 Worms Classification of Medical Importance and Its Relation to Sanitation

1.2.1 Introduction

The most common parasitic helminthes belong to three classes of invertebrates, the cestodes or tapeworms, the trematodes or flukes and the nematodes or roundworms. The distinguishing features of each class are shown in Table 1.1 and in classification chart (Section 1.2.2).

Table 1.1. General features of helminths

FEATURES	CESTODES	TREMATODES	NEMATODES
SHAPE	Flat, tape or ribbon-like and segmented	Flat, leaf-like and unsegmented	Cylindrical and unsegmented
ANTERIOR END	Has suckers and often hooks. No mouth is present	Have suckers. A mouth is present	No suckers or hooks, a mouth is present
BODY CAVITY	Absent	Absent	Present
INTESTINE	Absent	Present but no anus	Present with anus
SEXES	Hermaphrodite	Hermaphrodite except *Schistosoma spp.*	Separate male and female worms

1.2.2 Initial Classification of Worms of Medical Importance

Table 1.2: Initial Classification of Worms of Medical Importance

SUBKINGDOM	**METAZOA**	
	Triploblastic Possess a skin Possess a mouth of sorts Body systems mainly alimentary and reproductive Posses primitive nervous and excretory Sexes may be separate, hermaphroditism frequent	
PHYLUM	**PLATYHELMINTHES**	**NEMATHELMINTHES**
	- Flattened, segmented or unsegmented, cylindrical, bilaterally symmetrical - Gut may or may not be present - No body cavity, viscera in gelatinous matrix	- Unsegmented - Possess an alimentary system - Possess a body cavity
CLASS		**CLASS**
(A) CESTODA (Tape Worms) - Segmented - Possess scolex, neck and proglottids - Hermaphroditic - Reproduction: ▪ Oviparous ▪ Sometimes multiplication within larval forms - Infection generally by encysted larvae **(B) TREMATODA (Flukes)** - Unsegmented - Leaf like or cylindrical - Generally hermaphroditic - Reproduction (digenetic): ▪ Oviparous ▪ Multiplication within larval forms - Infection mainly by larval stages entering intestinal tract, sometimes through skin		**NEMATODA (Round Worms)** - Unsegmented - Possess mouth, oesophagus and anus - In general sexes separate - Reproduction: ▪ Oviparous ▪ Larviparous - Infection by: ▪ Ingestion of eggs or ▪ Penetration of larvae through skin or ▪ Arthropod vector or ▪ Ingestion of encysted larvae.

1.2.3 Alphabetical List of Parasitic Worms Whose Eggs are Found in Stools (WHO, 1980)

The range of helminths which are important as parasites of humans and in particular those which are important in the field of sanitation and water supplies, is

Table 1.3:

	International Scientific Name (General name & Name of species)	Common Name (English)
1.	*Ancylostoma duodenale*	Hookworm [a b]
2.	*Ascaris lumbricoides*	Roundworm [1 b]
3.	*Clonorchis sinensis*	Chinese liver fluke [b]
4.	*Dicrocoelium* spp.	Lancet fluke
5.	*Diphyllobothrium latum*	Fish tapeworm [b]
6.	*Dipylidmm canssinum*	Dog tapeworm
7.	*Enterobius vermicularis*	Pinworm [b]
8.	*Fasciola hepatica*	Giant liver fluke [b]
9.	*Fasciolopsis buski*	Giant intestinal fluke [b]
10.	*Heterophyes heterophyes*	Small intestinal fluke [b]
11.	*Hymenolepis diminuta*	Rat tapeworm [b]
12.	*Hymenolepis nana*	Dwarf tapeworm [a b]
13.	*Necator americanus*	Hookworm [a b]
14.	*Metagonimus yokogawi*	Japanese fluke
15.	*Opisthorichis felineus*	Cat liver fluke
16.	*Paragonimus westermani* *	Oriental lung fluke [b]
17.	*Schistosoma bovis*	---
18.	*Schistosoma haematobium* **	---
19.	*Schistosoma intercalatum*	Schistosome (vesical) [a b]
20.	*Schistosoma japonicum*	Schistosome (rectal)[a]
21.	*Schistosoma mansoni*	Schistosome (oriental) [a b]
22.	*Strongyloides stercoralis* ***	Schistosome (intestinal) [a b]
23.	*Taenia saginata*	Threadworm [a b]
24.	*Taenia solium*	Beef tapeworm [b]
25.	*Trichostrongylus* spp.	Pork tapeworm [b]
26.	*Trichuris trichiura*	Whipworm [a b]

[a] : Commonly found in many countries

[b] : Helminths whose transmission is directly related to water, sanitation and domestic hygiene

* : Found chiefly in sputum

** : Found chiefly in urine

*** : Found chiefly as larvae in stool

1.2.4 Public Health Risks Associated with Wastewater Irrigation

The main factors that contribute to the effective transmission of pathogens by wastewater irrigation are:

1. Long persistence of the pathogen in the environment.
2. Low minimal infective dose.
3. Short or no host immunity.
4. Minimal concurrent transmission through other routes such as food, water and poor personal or domestic hygiene.
5. The need for a soil development stage.

Table 1.4 summarizes the epidemiological characteristics of the main groups of enteric pathogens as they relate to these five factors. This summary provides a simplified theoretical basis for ranking the groups of pathogens according to their potential for transmitting diseases through wastewater irrigation. On this basis, it appears that the helminth diseases are the most effectively transmitted by irrigation with raw wastewater or improper treated wastewater because they persist in the environment for relatively long periods, their minimum infective dose is small, there is little or no immunity against them, concurrent infection in the home is often limited, and latency is long and a soil development stage is required for transmission. Theoretically, the pathogens can be ranked in the following descending order of risk.

High	**Helminths** (intestinal nematodes such as *Ascaris, Trichuris,* hookworm, and *Taenia)*
Lower	**Bacterial infections** (cholera, typhoid, and shigellosis) and **Protozoan infections** (amebiasis, giardiasis)
Least	**Viral infections** (viral gastroenteritis and infectious hepatitis)

Table 1.4: Epidemiological characteristics of enteric pathogens by comparison with their effectiveness in causing infections through wastewater irrigation (Shuval. 1986)

Pathogen	Persistence in environment	Minimum infective dose	Immunity	Concurrent routes of infection	Latency/soil development stage
Viruses	Medium	Low	Long	Mainly home contact, food and water	No
Bacteria	Short/Medium	Medium/High	Short/Medium	Mainly home contact, food and water	No
Protozoa	Short	Low/Medium	None/Little	Mainly home contact, food and water	No
Helminths	Long	Low	Non/Little	Mainly soil contact outside home and food	Yes

1.2.5 Environmental Classification of Excreta-related Infections

Pathogenic enteric viruses, bacteria, protozoa and helminths escape from the bodies of infected persons in their excreta and may be passed on to others via either the **mouth** (e g, through eating contaminated vegetables) or the **skin** (as in the case of the hookworms and schistosomes) Excreta and wastewater generally contain high concentrations of excreted pathogens, especially in countries where

diarrhoeal diseases and intestinal parasites are particularly prevalent. A large number of such infections of public health importance, which are transmitted in a variety of ways, the characteristics of the causative agents also vary (see Appendix 1, Table 13), and are of great importance in determining what circumstances each infection is likely to be favoured or controlled by waste reuse practices. Feachem *et al.,* (1983) have divided infections caused by excreted pathogens into five categories according to their environmental transmission characteristics, as discussed below.

Category I infections are caused by pathogens, which are infective immediately upon excretion ("non-latent"), have a low median infective dose but cannot multiply in the environment. This category includes excreted viruses, protozoa and the helminths *Enterobius vermicularis* and *Hymenolepis nana* Transmission of these pathogens occurs predominantly through direct transmission from person to person in the immediate domestic environment, especially when crowding and low standards of personal hygiene prevail, although the survival time of excreted viruses and protozoa may be long enough for them to pose a health risk in schemes for the use of excreta and wastewater.

The pathogens causing **Category II** infections are the excreted bacteria. Like the causative agents of Category I infections, they are infective immediately on excretion. Their higher median infective dose means that they must generally be ingested in greater numbers to be capable of causing disease, but they can multiply outside their host, e g, in food or milk They are commonly transmitted in the immediate domestic environment, but their greater ability to persist in the environment means that they can survive for the longer periods involved in certain

transmission routes and therefore can pose potential health risks in schemes for the use of excreta and wastewater. There are well-documented cases pathogens in this category. For example, cholera epidemics caused by the irrigation of vegetables crops with untreated wastewater.

The diseases in **Category III** are caused by the **soil-transmitted intestinal nematodes,** which require no intermediate host. Their eggs require a latent period of development in the environment before they can cause infection. On the other hand, the minimum infectious dose is only one organism and these parasites are only weakly affected by host immunity. The most important of these are the human roundworm *Ascaris lumbricoides,* the hookworms *(Ancylostoma duodenale* and *Necator americanus),* and the human whipworm *(Trichuris trichiura)* They are all readily transmitted by the agricultural use of raw or insufficiently treated excreta and wastewater, indeed, they are the excreted pathogens of greatest public health concern in agricultural reuse schemes.

Category IV infections are caused by the tapeworms *Taenia saginata* and *T. solium.* For their successful transmission, viable eggs must first be ingested by a cow or pig (respectively) before eating the undercooked meat of infected animals can infect humans. A potential route for the transmission of these diseases is the irrigation of pasture with wastewater or using domestic sludge as fertiliser for the soil.

The infections in **Category V** are all caused by water-based helminths that require one or two intermediate aquatic hosts; the first of these is a snail, in which the pathogen multiplies asexually, and the second is either a fish or an aquatic macrophyte. Many of these helminths have a limited geographical distribution and it is only in endemic areas that their transmission is promoted by the aquaculture use of raw or insufficiently treated excreta and wastewater, together with the practice of eating raw or inadequately cooked fish and aquatic vegetables. Agricultural use is not relevant, except in so far as all irrigation schemes may facilitate the transmission of shistosomiasis.

In the above classification, infections in **Categories IIIV** are caused by excreted helminths. These all require a period of time after excretion to become infective to man, and this latency period is passed in soil, water, or an intermediate host. Most of them are environmentally persistent, with survival times usually ranging from several weeks to several years. Schemes for the use of excreta and wastewater are important mechanisms of transmission of many of these diseases. A major environmental measure for their control is therefore the effective treatment of excreta, wastewaters, and domestic sludges prior to use (Table 1.3).

1.3 Identification and Characteristics of Helminth Eggs

There are numerous texts available with drawings and photographs of eggs and larvae of all the major parasitic helminths, which may be found in raw or treated wastewater (Jeffry and Leach, 1975; Fox *et al.,* 1981; Thienpont *et al.,* 1986). The color photographs of intestinal helminth eggs are included in this guide to provide a more realistic concept of their appearance with comprehensive illustration. It is particularly important to make measurements of eggs when starting identification.

Wastewater frequently contains eggs of parasites of animals, e.g. rats and domestic animals. Although it is not necessary to identify these positively, it is important to recognize that they are not of human origin. Plates I-VIII show a number of eggs of the human parasitic helminths most frequently encountered in wastewater samples. Although these eggs are typical for each species, it must be remembered that not all eggs are absolutely uniform in size and shape. It is sometimes almost impossible; however, to determine whether eggs are of human or animal origin, e.g. the eggs of *Ascaris suum* (from pigs) and *A. lumbricoides* (from humans) are morphologically indistinguishable. Similarly, the eggs of *Trichuris* spp. are all of similar colour and shape and can only be separated from those of animal species by careful measurement (Ayres & Mara, 1996).

Table 1.5: Basic epidemiological features of sanitary helminths

Helminth	Category [a]	Transmission		Latency [c]	Persistence [d]	Health risk from untreated excreta [e]	
		Life cycle [b]	Foci of infection			Agriculture	Aquaculture
E. vermicularis	I	Human-Human	{Personal Domestic}	0	7 days	?	-
Hymenolepis spp.	I	Human-Human -Insect		0	1 month	+	-
A. lymbricoides	III	Human-Soil-Human	{Yard	10-14 days	1 year	+	-
Hookorm	III	Human-Soil-Human	Field Crops}	7 days	3 month	+	?
T. trichuira	III	Human-Soil-Human		3-4 weeks	9 months	+	-
S. stercoralis	III	Human-Human		3 days	3 weeks	+	?
Taenia spp.	IV	Human-Pig-Human Human-Cow-Human	Yard, Field, Fodder	2 months	9 months	+	-
D. latum	V	Human-Copepod-Fish-Human	Water	2 months	Life of fish	-	+
C. sinesis	V	Human-Snail-Fish-Human		6 weeks	Life of fish	-	+
P. westermani	V	Human-Snail-Fish-Human		4 months	Life of crab	-	+
S. mansoni	V	Human-Snail-Human		2 days	2 days	-	+
S. haematobium	V	Human-Snail-Human		2 days	2 days	-	+
S. japonicum	V	Human-Snail-Human		2 days	2 days	-	+

(a) Environmental classification of excreted pathogens.

(b) Life cycle from human to human, in some cases other vertebrates may also act as final hosts.

(c) Typical minimum time from excretion to mfectivity.

(d) Estimated maximum life of infective stage at 20-30°C.

(e) + presence of risk, - no risk,, ? uncertain.

1.3.1 Terms used for identification of Eggs

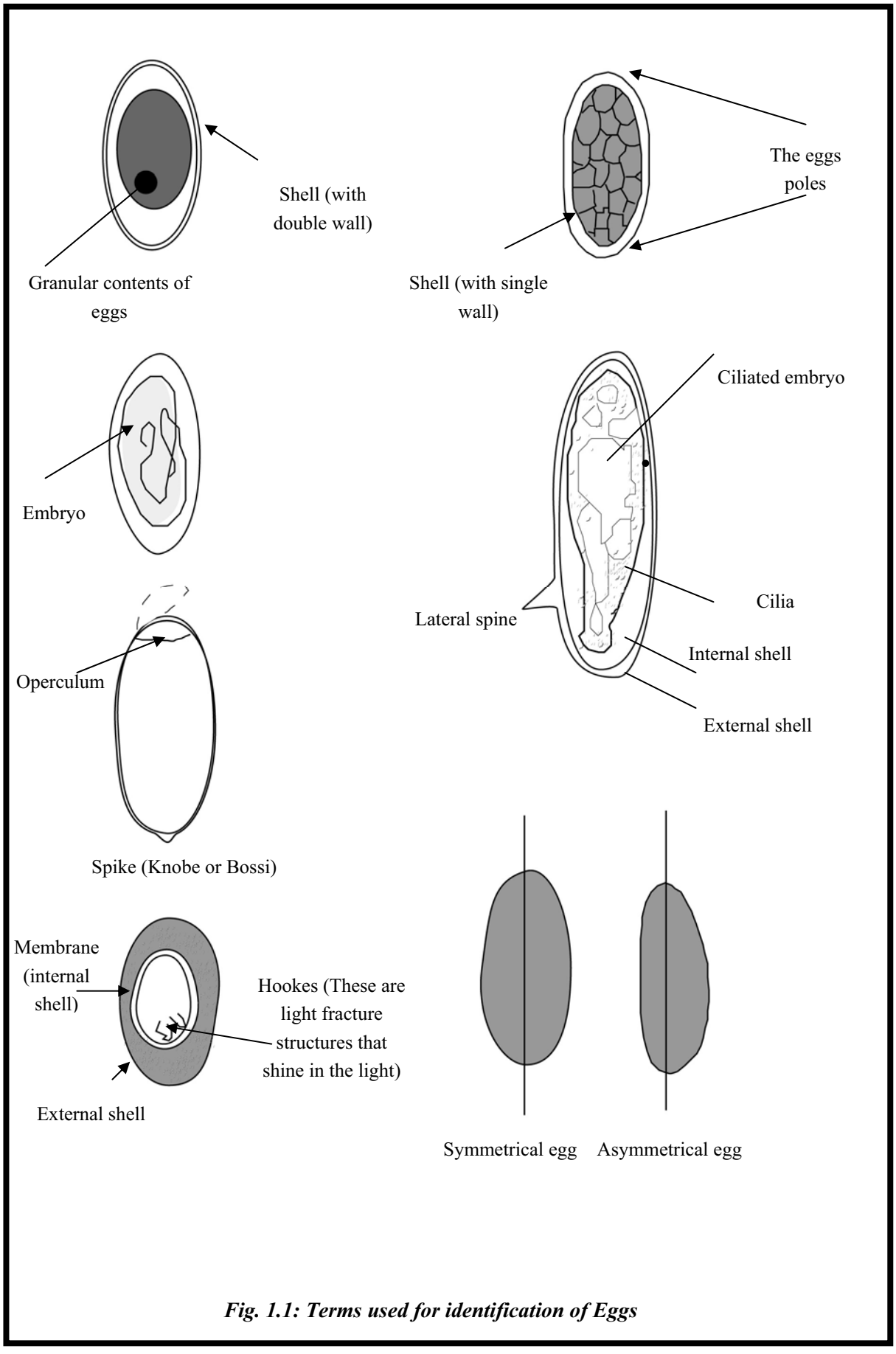


Fig. 1.1: Terms used for identification of Eggs

1.3.2 Key to the Identification of the Most Prevalent Helminth Eggs in Tropical Areas (WHO, 1980)

	Small < 35µm		Pronounced rimmed extension small knob at the wide end.		Clonorchis sinensis
	containing developed Larvae		Very large > 130 µm		Fasciola buski
					Faciola hepatica
Operculated Eggs					
	Large > 50 µm		Medium size 70-120 µm	distinct flattened operculum	Paragonimus westermani
	Without developed larvae			With an obvious rim,	
				Golden-yellow colour	
					Schistosoma japonicum
	Ciliated larvae conspicuous spine or minute knob		Minute knob		Schistosoma haematobium Schistosoma mansoni
			Terminal spine		
			Lateral spine		
Non-operculated eggs					
			Single thick dark brown shell,		Taenia
			transverse striation, spherical or subspherical		
		Eggs with 3 pairs of hooklets			
			Double shell colourless inner shell with polar filaments, oval		Hymenolepis nana
	No ciliated larvae				
	No spine				
	No knob				
			Double shell, dark brown external shell	barrel-shaped, 2 polar plugs smooth shell	Trichuris trichiura
	Eggs with no hooklets				
				Ovoid, no polar plugs,	Ascaris lumbricoides
				mamillated external shell	
				Flattened on one side	Enterobius vermicularis
			Larvae		
	Thin clear transparent shell			Not flattened	Strongyloides stercoralis
			Grey cells		Necator americanus
					Ancylostoma duodenale

1.3.3 Color Plates of Helminth Eggs

1- *Ascaris lumbricoides* (*fertilised*)

Size	:	Medium-sized worm egg 45-75 µm in length – 35-50 µm in width.
Shape	:	Ellipse-shaped to round.
Shell	:	Thick, rough albuminous outer wall, very thick colorless middle layer, inner layer contains a thin yolk membrane.
Colour	:	Golden brown, the contents of the egg are colourless or pale yellow.
Content	:	unsegmented cell with rough granules.
Unfertilised egg	:	is generally larger (88-94 µm in length by 39-44 µm), narrower and more elongate; the egg content is full of large round refractile granules.

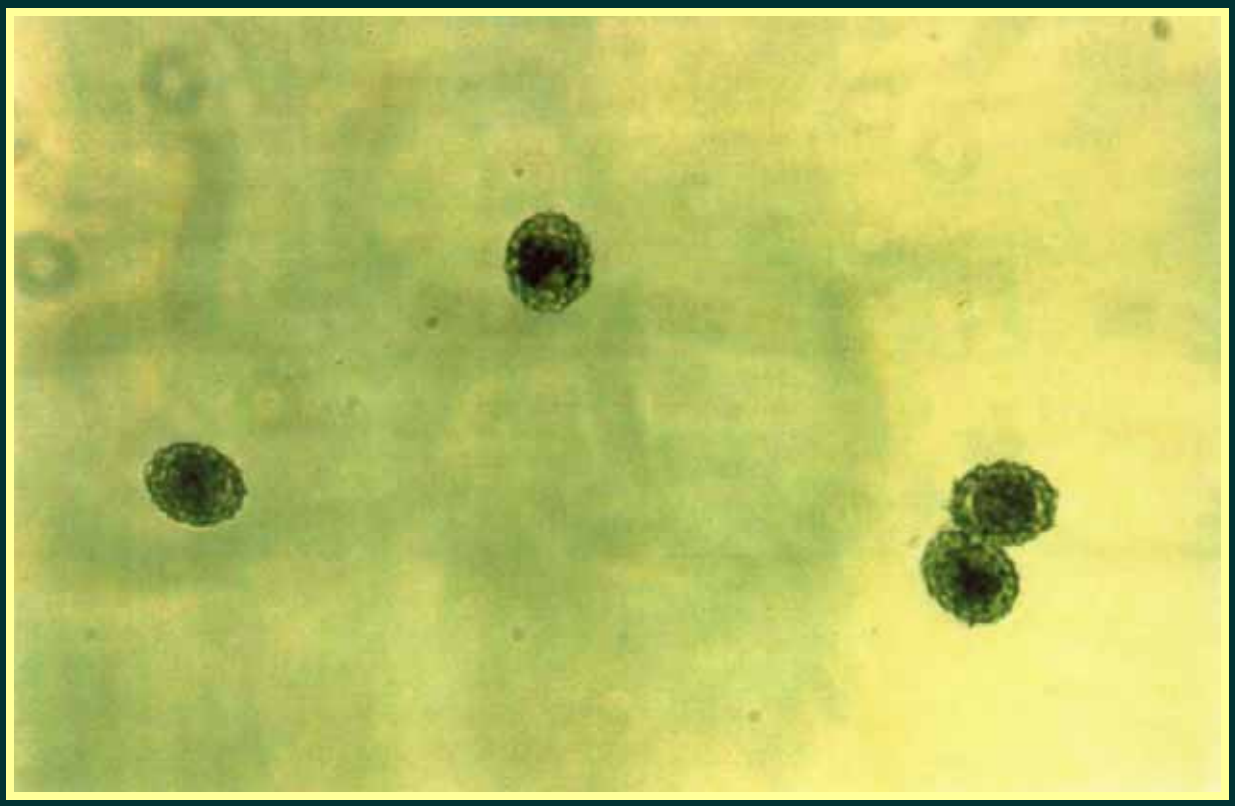


Fig 1-3: Plate 1 X200: Ascaris lumbricoides

2- Trichuris trichiura

Size	:	Medium-sized worm egg 50-58 µm in length – 22–27 µm in width.
Shape	:	Lemon-shaped, barrel-shaped
Shell	:	Thick shell with smooth surface, the inner layer is thin and transparent, the outer shell is brownish yellow.
Colour	:	Brownish; shell orange, content yellow.
Features	:	A rounded, transparent plug at each pole.
Content	:	Uniforms granular contents, unsegmented.

Fig 1-4: Plate 2 X200: Trichuris trichiura

3- Taenia saginata & Taenia solium

Size	:	Small worm egg 35-40 µm in length – 30-35 µm in width.
Shape	:	Round.
Shell	:	Thick, smooth shell with radially striated embryophore.
Content	:	A round granular mass enclosed a fine membrane, with 3 x 2 refractile lancet shaped hooklets (a hexacanth embryo).
Colour	:	Pale yellow to brown. The eggs of these two tapeworms are practically identical. Eggs are only released after the disintegration of a gravid tapeworm segment.

Fig 1-5: Plate 3 X200: Taenia saginata & Taenia solium

4- Enterobius vermicularis

Size	:	Medium-sized worm egg 50-60 µm in length – 20-32 µm in width.
Shape	:	Ovoid and asymmetrical: a similar side-walls, one side is flattened, rounded on the other.
Shell	:	Smooth and thin which consists of four layers an inner lipoid membrane, 2 chitinous layers and an albuminous, smooth outer layer.
Colour	:	Transparent, colourless.
Content	;	Either: (a) a small, granular mass in the shape of an irregular oval, or (b) contains a morula in an advanced stage of development or a L_1-larva. The egg is usually found in the folds of skin around the anus.

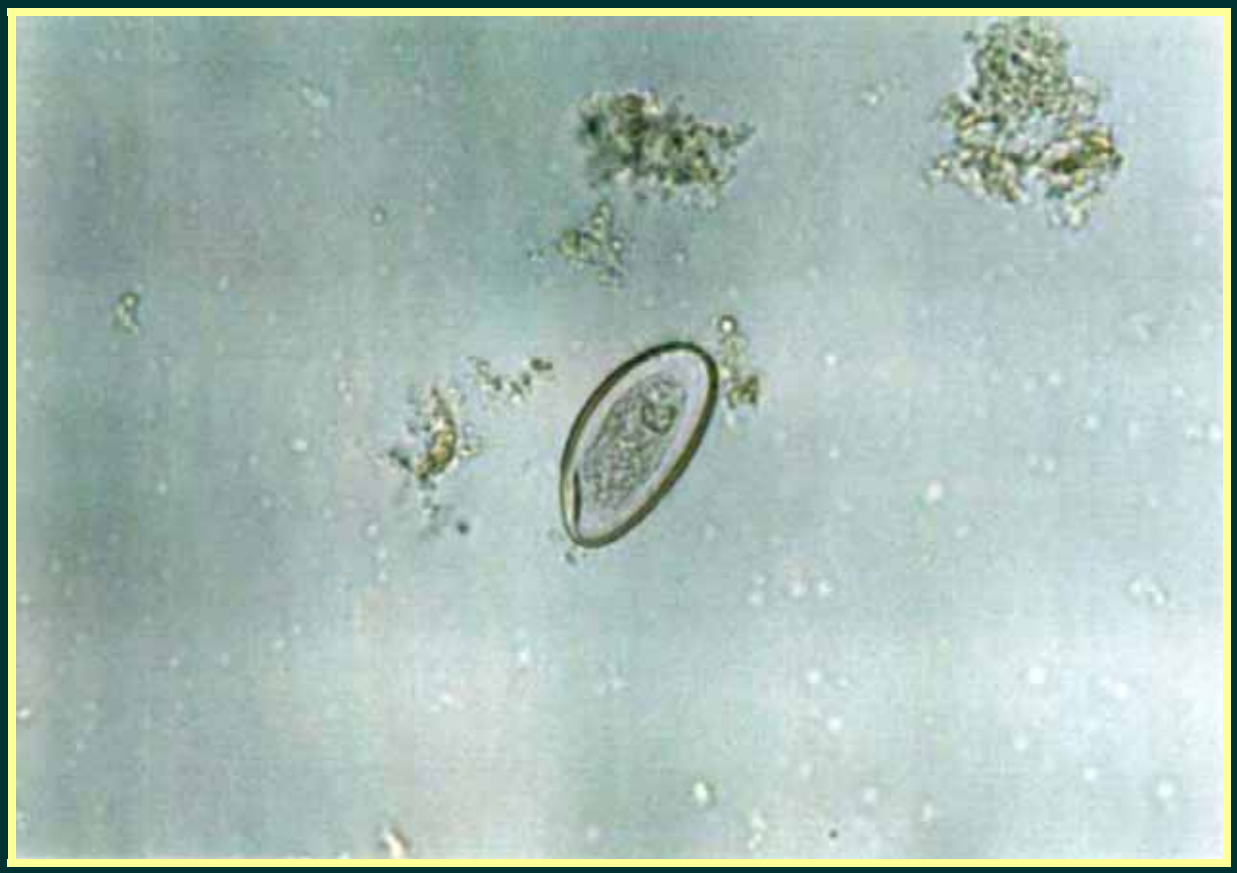


Fig 1-6: Plate 4 X200: Enterobius vermicularis

5- Hymenolepis nana

Size	:	Small worm egg 30-44 µm.
Shape	:	Round to nearly round.
Shell	:	Smooth; double; external membrane thin and internal membrane often thicker at the poles, with filaments coming away from both poles, mixed with granules occupying the space between the two membranes.
Colour	:	Very pale grey, transparent.
Content	:	Oncosphere is 24-30 µm by 16-25 µm with 6 refractile hooklets arranged in fan shape and often some well defined granules in the centre.

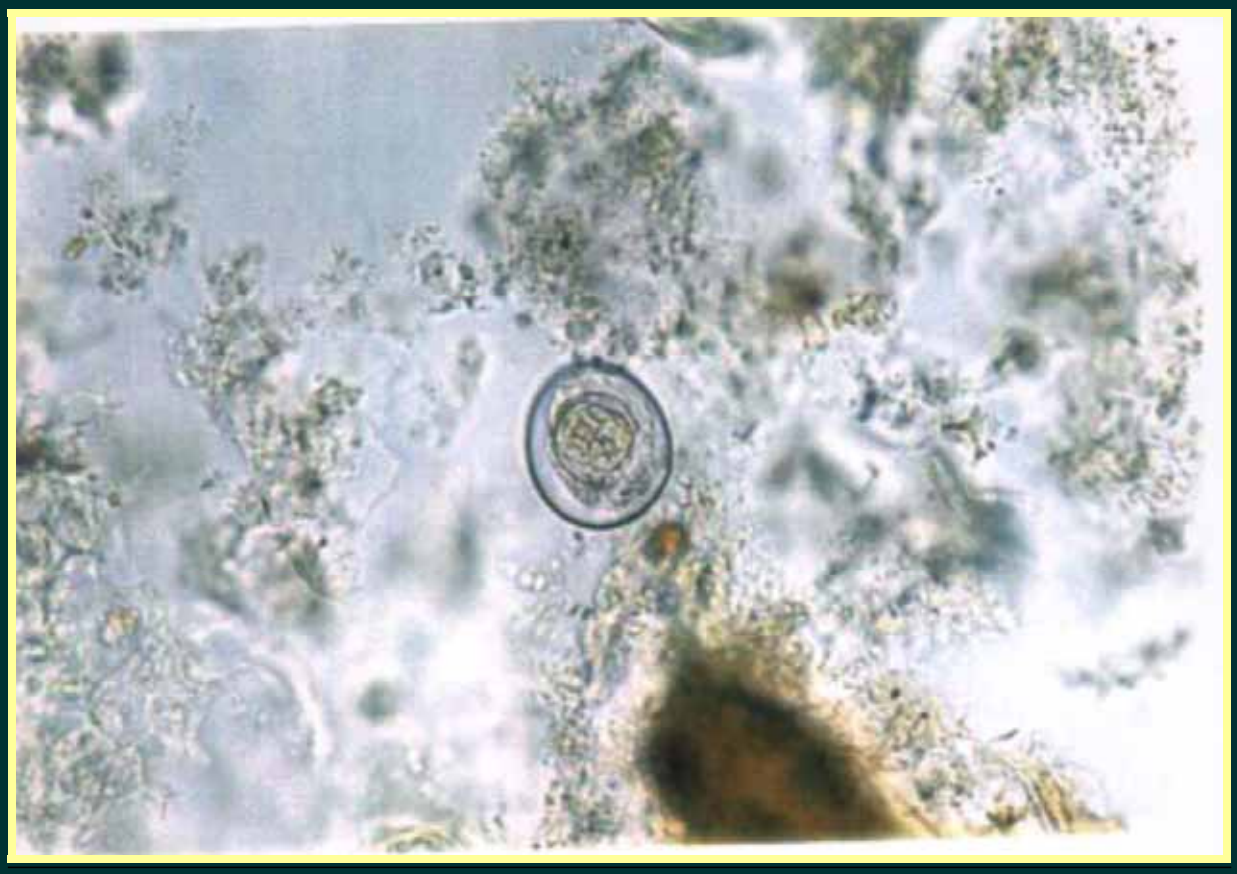


Fig 1-7: Plate 5 X200: Hymenolepis nana

6- Schistosoma mansoni

Size	:	Very large worm egg 114-175 µm in length – 45-68 µm in width.
Shape	:	Ovoid and long, with one well rounded pole and one conical pole.
Spine	:	Long thorn-like spine near the rounded pole. laterally, a slightly inclinated.
Shell	:	Transparent, smooth and very thin.
Colour	:	Golden brown, pale yellow.
Content	:	Contains a miracidium, a broad ciliated embryo surrounded by a membrane (internal shell).

Fig 1-8: Plate 6 X200: Schistosoma mansoni

7- Ancylostoma duodenale

Size	:	Medium-sized worm egg 60 µm in length – 40 µm in width.
Shape	:	Oval with similar rounded poles; barrel-shaped side-walls.
Shell	:	Thin, smooth colorless shell, appears as black line.
Colour	:	The cells inside are pale grey.
Content	:	Two to eight blastomeres, varies according to the degree of maturity. Difficult to distinguish from the egg of *Necator americanus*, which is longer.

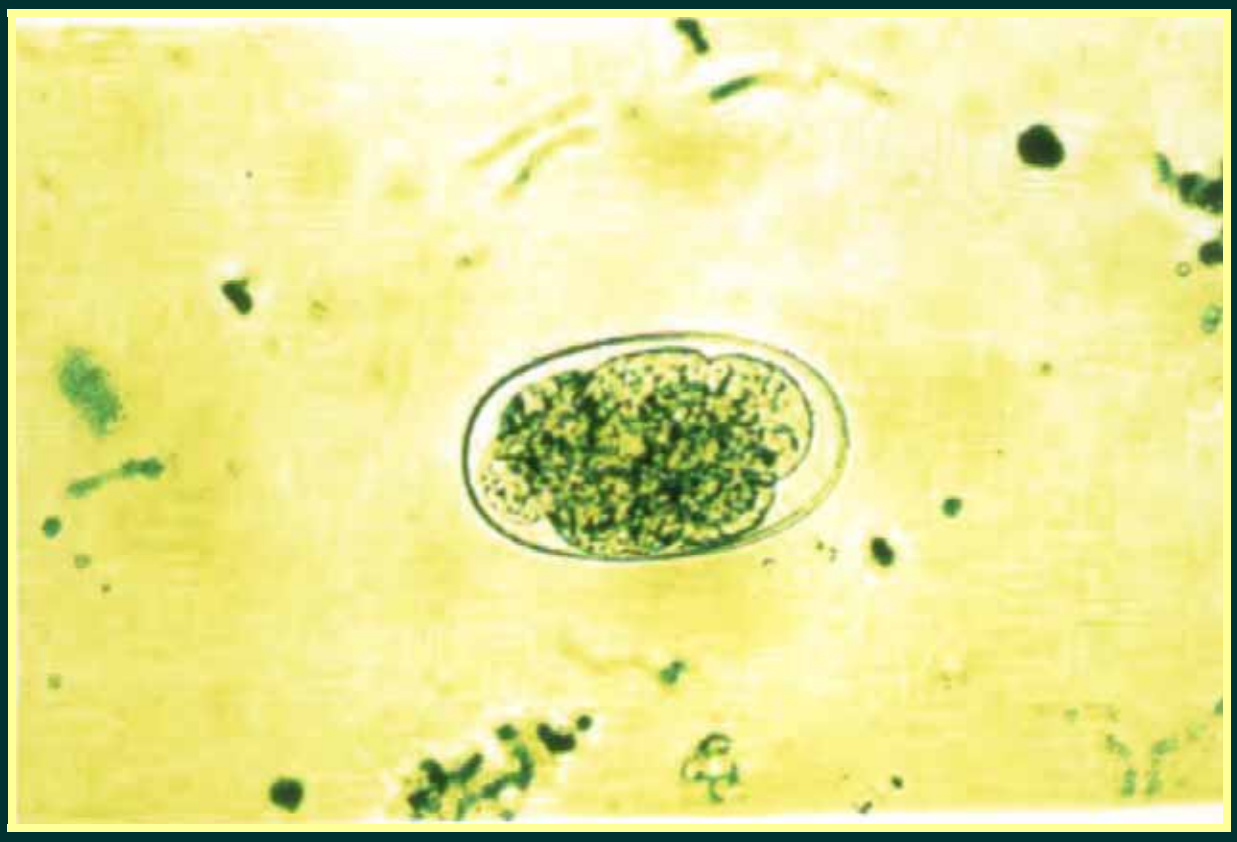


Fig 1-9: Plate 7 X640: Ancylostoma duodenale

8- Necator americanus

Size	:	Medium-sized worm egg 64-76 µm in length – 36-40 µm in width.
Shape	:	Oval with similar rounded poles and more flattened, barrel-shaped sidewalls.
Shell:	:	Thin smooth and transparent.
Content:	:	Always contains at least 8 cells (never 4 cells like *A. duodenale* in fresh stools). Difficult to distinguish from the egg of *Ancylostoma duodenale*, which is shorter.

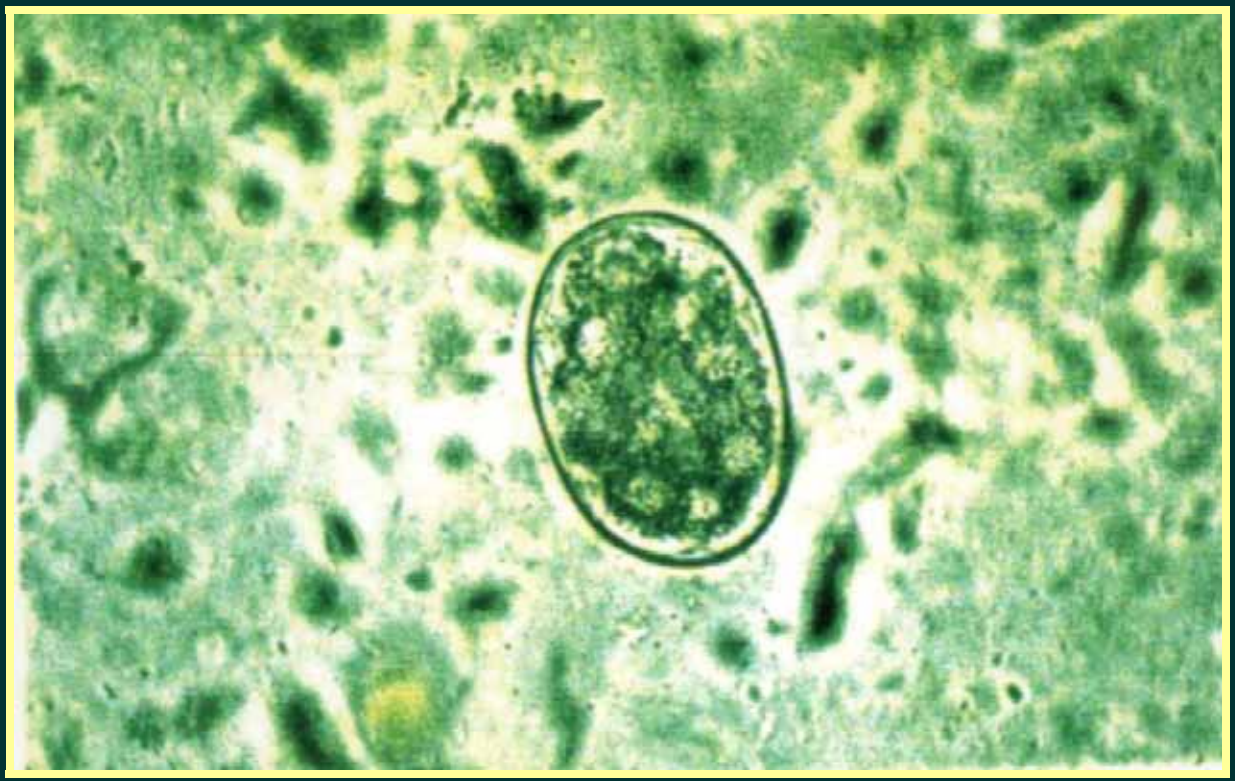


Fig 1-10: Plate 8 X640: Necator americanus

1.3.4 Free-living Helminths Larvae

Frequently, raw sewage contains living active nematode larvae or adults of free-living worms (those that do not require another animal to live in). Sometimes they are found in aerobic digestion systems and often in treated effluents. These are non-pathogenic forms and should not be considered as a subject of public health concern. These free-living nematodes feed on bacteria, algae or small organic particles.

Free-living forms of parasitic nematodes, in particular, the filariform stages of *Ancylostoma duodenale, Necator americanus* or *Strongyloides stercoralis* are considered as a subject of workers and public health concern (Al-Salem, 1996). They are extremely difficult to identify when encountered in wastewater, as there are likely to be so many free-living species present. It is therefore necessary to make detailed measurements of the relevant morphological features. To do this, individual nematodes must be mounted, stained and viewed under high-power magnification.

The larvae most commonly found in stools are those *of Strongyloides* (threadworm), and occasionally those of *Ancylostoma* (hookworm) are found; Table 1.4 shows the difference between them, which can be seen under the microscope after staining with iodine solution.

Table 1.4: Differentiating features between threadworm and hookworm larvae

Larva features	Threadworm	Hookworm
Length Breadth	200 – 300 µm 15 µm	200 – 300 µm 15 µm
Oesophagus (0)	Two swellings*	Two swellings*
Mouth (M)	Short: 4 µm	Long: 15 µm
Posterior end	Slightly tapered	Very tapered
Genital rudiment (GR)	Large and distinct (22 µm)	Small (7 µm)
Anal pore	50 µm from posterior end	80 µm from posterior end

** Larvae with two oesophageal swellings are called rhabditiform larvae.*

Free-living and plant parasitic nematode larvae can be distinguished from similar stages of pathogenic (animal/man) forms by subjecting them to a dilute acid solution. One-percent hydrochloric acid kills the larval stages of free-living or plant parasitic nematodes, but the protective sheath of third-state larva of animal parasitic forms prevents their destruction in this acid solution (Fox *et al.,* 1981).

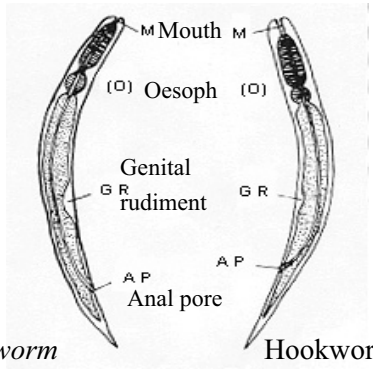


Fig 1-11: Hookworm and Threadworm

1.4 Life Cycles

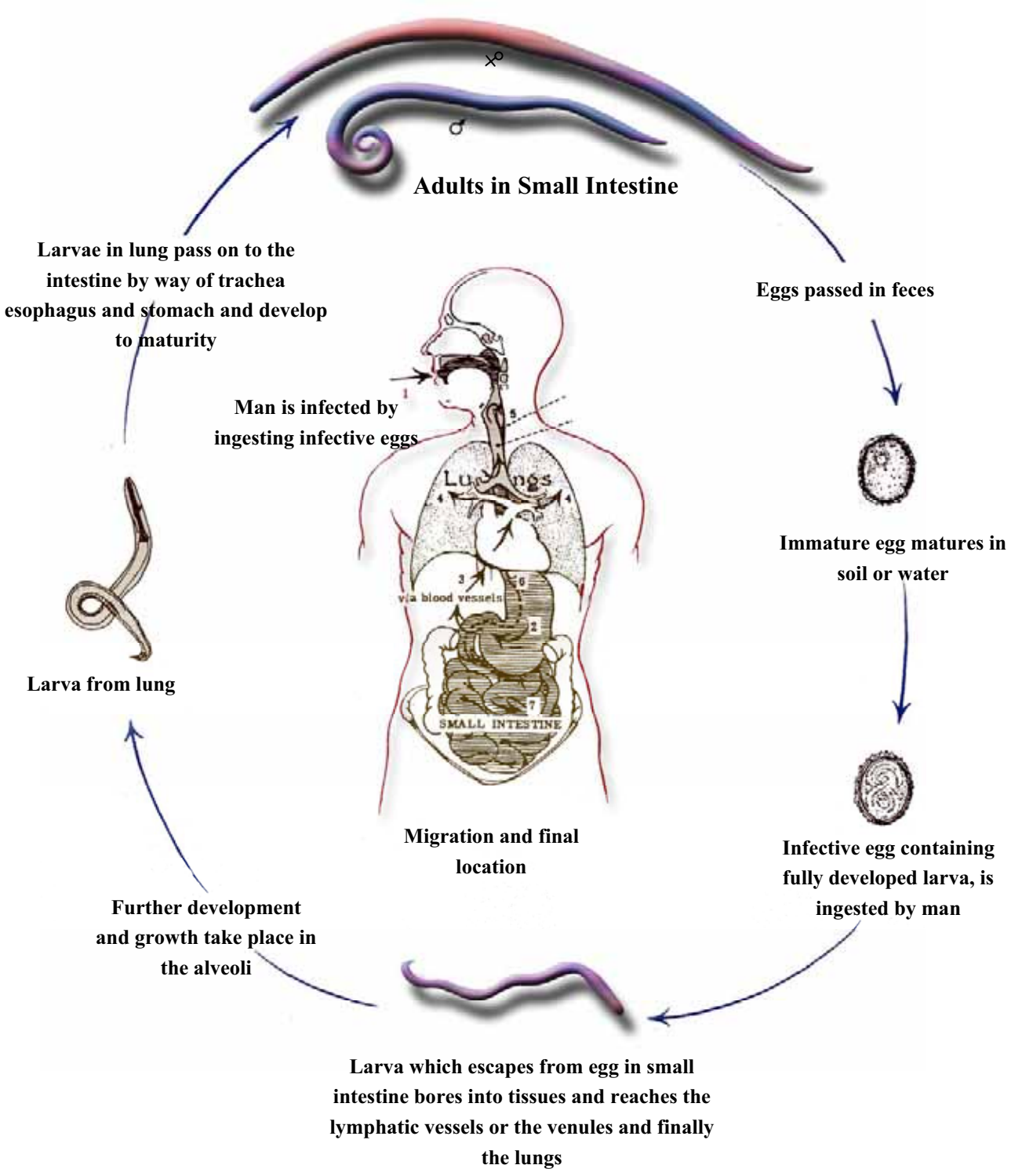


Fig. 1-12: life cycle of Ascaris lumbricoides

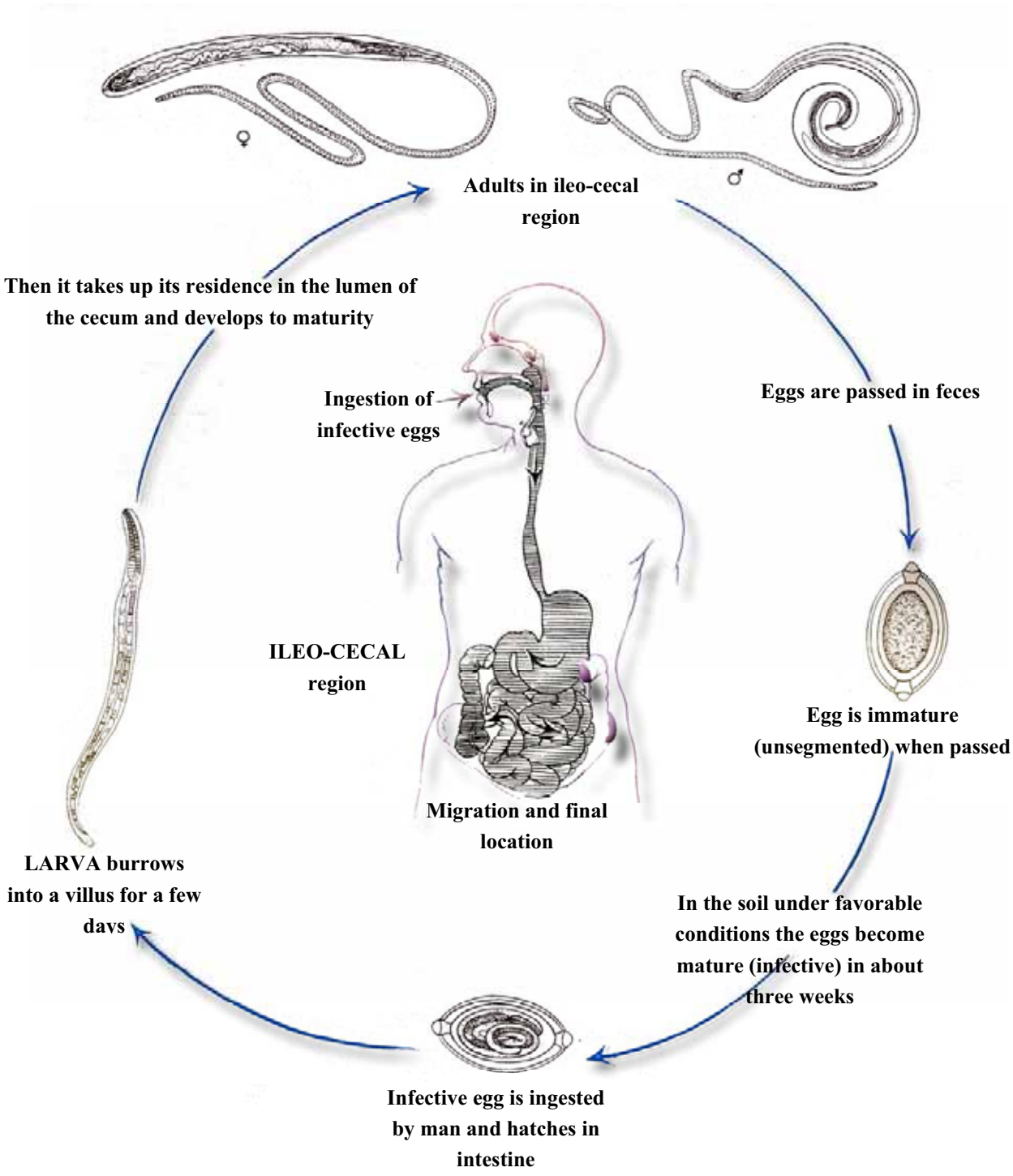


Fig. 1-13: life cycle of Trichuris trichiura

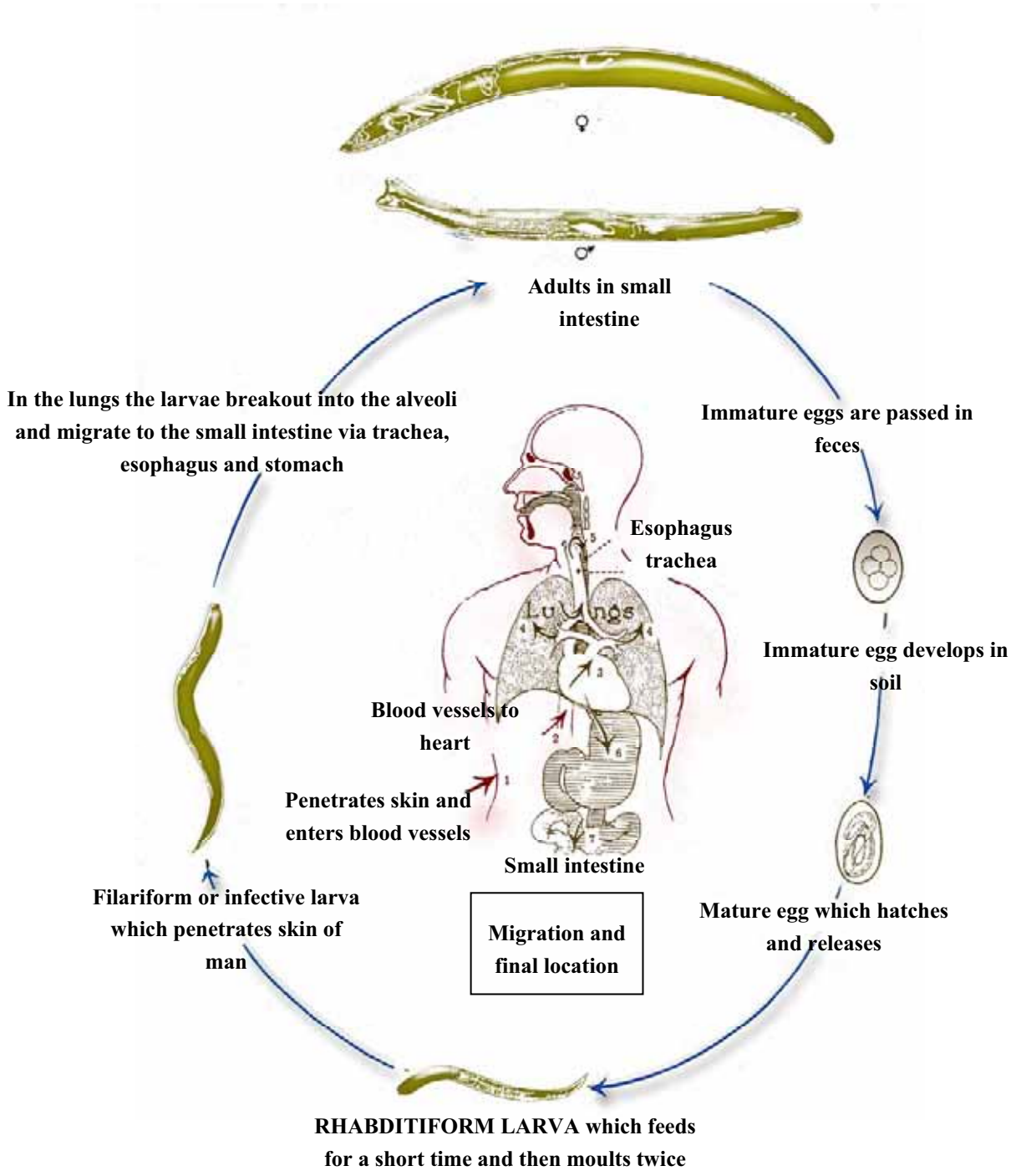


Fig. 1-14: life cycle of Hookworms

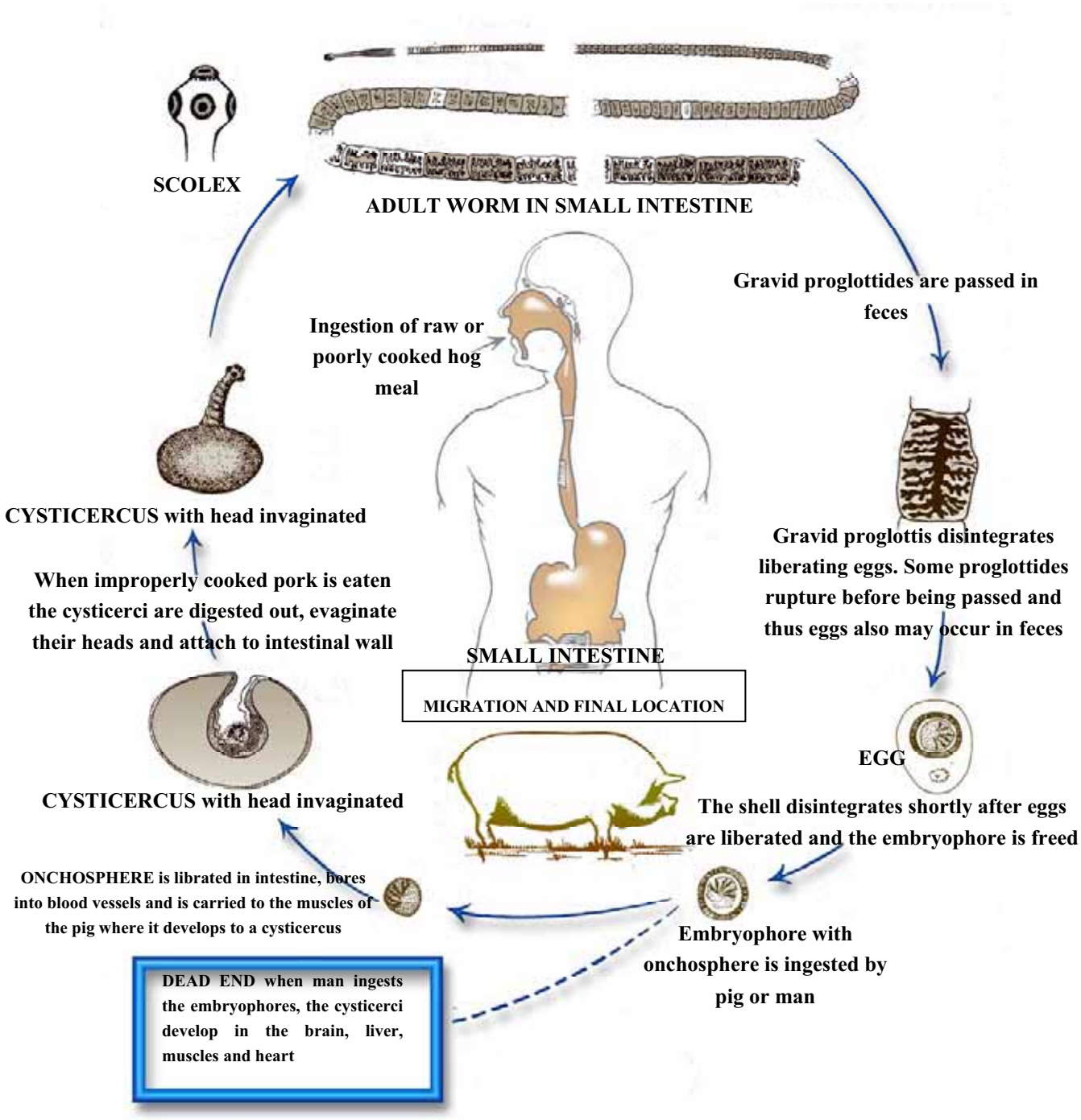


Fig. 1-15: life cycle of Taenia solium

Chapter

2 FACTORS INFLUENCING THE FATE OF HELMINTH EGGS

The survival of parasites outside the gastro-intestinal tract of the host depends on environmental conditions; e.g. the epidemiology of *Ascaris* infection depends to a large extent upon the eggs, since they must embryonate outside the body of the host. There are many abiotic and biotic variables, which may affect resistant stages of parasites. There is a great deal of interaction between these parameters.

1. In general, these parameters can be divided into three categories:
2. Physical (temperature, sunlight, seasonal, etc.);
3. Chemical (ammonia, salts, acids, etc.);
4. Biological (fungi, protozoa, and invertebrates).

The egg-shell of nematodes has been referred to as one of the most resistant biological structures (Wharton, 1986), which offers a high degree of protection to the developing embryo. The egg shell is impermeable to all substances except lipid solvents, gases and perhaps liquid water (Wharton, 1980). Nematode egg-shells consist of three basic layers, an inner lipid/ascaroside layer, a middle protein/chitinous layer and an outer vitelline layer (Foor, 1967; Bird & McClure, 1976; Matthews, 1986). *Ascaris* eggs had an outer mucopolysaccharide/tanned protein layer secreted by the uterus as the eggs are being laid (Foor, 1967). The main permeability barrier in the nematode egg shell is assumed to be the inner lipid layer (Bird, 1971). The outer chitin or vitelline layers also act as a type of sieve allowing oxygen to reach the developing larva, while preventing molecules larger than water from reaching the lipid layer during permeability changes (Matthews, 1986).

2.1 Physical Factors Affecting Helminth Egg Survival

2.1.1 Temperature

A comprehensive resume of the time-temperature requirements for the destruction of *Ascaris*, *Trichuris*, *Taenia* and hookworm eggs is given by Feachem *et al.* (1983). In general, temperatures above 60°C are rapidly lethal to eggs. The range of temperatures tolerated by *Ascaris* eggs has been the subject of much research and comment in the literature. Studies were made on factors affecting the development of *Ascaris* eggs, which showed certain adaptations for survival and development as well as some of their limitations. The influence of temperature on the development, survival, size and infectivity of free living stages of a number of animal parasitic nematodes is well-documented (Nolf, 1932; Spindler, 1936; Rogers, 1940; Barrett, 1969; Gibson, 1981; Salih, 1981, Kiff& Lewis-Jones, 1984; Udonsi & Atata, 1987; Smith & Schad, 1989). However, for the sake of simplicity the temperature effects on the development and viability of helminth eggs have been separated into three conditions as follows:

i) Optimum temperatures

The infective larvae of *Ascaris suum* develop in the egg between temperatures 16±1 °C and 34±l °C. Within this temperature range, increases in temperature increase the rate of development. The maximum rate of egg development was attained at 31±1°C. Maximum larval viability and ability to penetrate tissues *in vitro* was achieved when eggs were embryonated at 22 ± 1°C. Eggs embryonated at 28±l°C and above give rise to infective larvae that have less ability to hatch *in vitro;* and more limited ability to penetrate tissue membranes *in vitro,* when compared with those larvae from eggs embryonated at lower temperatures (Arene, 1986). However, the optimal temperatures for rate of development and larval survivability are not the same. These results may give an answer to some of the practical problems often-experienced work with *Ascaris.* For example, they may explain why it has often proved difficult to obtain heavy experimental infections in pigs while heavy natural infections commonly occur (Schartz, 1959).

Different embryonation temperatures ranging between 17°C and 32°C have been published by a number of researchers as the optimum temperature at which rapid and uniform development of *Ascaris* eggs is accomplished (Table 2.1). A review of the literature on minimum and maximum temperatures that permit full or partial development of *Ascaris* eggs are presented in Table 2.2. The maximum temperature that allowed the motile larval stage (full development) to form was around 34°C (Arene, 1986; and Seamster, 1950). While only partial development occurs without the formation of the motile larval stage, at a maximum temperature of 38°C. The figures given in the literature (i.e. Table 2.1 and 2.2) are not in complete agreement. A review of the literature that furnished information relative to optimum temperatures for development of several species of parasitic eggs is shown in Table 2.3.

ii) Low temperatures

Little work has been reported recently concerning the effects of low temperatures on the egg of *Ascaris* spp.. Low temperatures (8.9°C to 15.6°C) inhibit complete development of the cells inside the eggs; once eggs were moved to room temperature the development to the motile embryo was accomplished (Seamster, 1950). It is common practice to store eggs in a refrigerator at temperatures ranging from 7°C or 8°C upward, during which time they remain viable but uncleaved; it seems quite likely that such eggs may not be completely dormant and might undergo physiological changes.

Table 2.1. A review of the literature furnished the following information relative to the range and optimum temperatures for development of Ascaris eggs

Temperature (°C)	Reference
24	Cram (1924)
±31.3	Seamster (1950)
30-31	Fairbaim(1961)
27	Hass&Todd(1962)
17-30	Timoshin (1967)
22-26	Arfaa (1978)*
31±1	Arene (1986)
24	Fleming (1987)
29±1	Baniard *et. al.*, (1987)

* *Ascaris lumbricoides*

Table 2.2: A review of the literature on minimum and maximum temperature that permits development of Ascaris eggs

Full development	Partial development	Temperature	Reference
maximum (°C)	**maximum (°C)**	**minimum (°C)**	
34	38	16	Arene (1986)
-	-	15	Stevenson (1979)
34.4	37.2	16.7	Seamster (1950)
-	-	6-8	Zadowski & Sedorov, 1931; cited by (Seamster. 1950)
30	36	13	Timoshin (1967)

Table 2.3. A review of the literature furnished the following information relative to optimum temperatures for development of several species of parasitic eggs

Parasite species	Temperature (ºC)	Reference
Ascaris suum	31.3	Seamster (1950)
Ascaris suum	31 ± 1	Arene (1986)
Trichuris trichiura	30	Feachem *et. al.*, (1983)
Necator americanus	20 – 27	Feachem *et. al.*, (1983)
Necator americanus	30	Udonsi & Atata (1987)
Ancylostoma duodenale	28 – 32	Feachem *et. al.*, (1983)
Aspiculuris tetraptera[a]	20 – 30	Anya (1966)
Fasciola hepatica	22	Kiff& Lewis-Jones (1984)
Ostertagia ostertagi[b]	25	Pandey (1972)

a: Mouse pinworm
b: Cattle trichostrongylid nematodes

The minimum temperature that permitted development *of Ascaris suum* eggs to the motile embryo stage occurred in 37 days at 16.7°C (Seamster,1950; Arene,1986). While the development of *A. suum* eggs ceased when maximum outside temperatures were below 15°C (Table2.2) (Stevenson, 1979).

Exposure of eggs and partly developed embryos to freezing temperatures (-9°C to -12°C) resulted in a high percentage of fatality in *Trichuris* but had no apparent effect on *Ascaris.* It was also found that the further *Trichuris* eggs are developed, the less resistant they were to freezing (Nolf, 1932). As surveys and incidence reports of human helminths show, *Ascaris* is extending further into colder climates than *Trichuris.*

Ascaris eggs that had been exposed to freezing temperatures, developed into active embryos after incubation at 24°C, and neither the length of exposure to the cold nor the degree of cold itself seemed to affect the rate of development when the eggs were restored to 24°C. Although the low temperature in some cases seemed to break up the protoplasm of the egg, so that it lost its normal appearance, this evidently did not interfere with the development, as cells with abnormal appearance decreased in number or disappeared altogether during incubation. Although active embryos developed, they seemed to be short lived. Apparently this prolonged exposure impaired the vitality of the worms, and it seems probable that infectivity of such worms would be lessened, e.g. infective *Ascaris* eggs that had been refrigerated for 2 years, hatching decreased from 77% to 47% (Fairbaim, 1961).

Ascaris suum eggs remained viable for as long as forty days when exposed to temperatures ranging from -18°C to -27°C (Cram, 1924). Developed embryos in *Ascaris* eggs were killed by a 20-day exposure to temperatures of -21°C to -27°C, but not by a 10-day period at the same temperatures or by a 30-day exposure to freezing temperatures (-11°C to -8°C).

It is therefore evident that while very low temperatures may have a destructive effect upon the vitality of *Ascaris* eggs, many eggs under natural conditions are likely to survive severe winter weather. The cold of winter can not be depended upon to destroy the vitality of *Ascaris* eggs present in pens, pastures, stables, etc. It does, however, diminish their infectivity with the passage of time and may aid in controlling infection by a mechanical action in holding eggs in frozen soil and thus reducing accessibility to swine (Cram, 1924).

For each 5°C decrease in temperature, from 35°C to 8°C, the protoplasm *of Ascaris megalocephala*

practically doubled its viscosity, as judged by the length of time required to centrifuge the mitochondria down to the bottom of the egg cell. The exaggerated viscosity at the lower temperatures, with no doubt, inhibits chemical reaction, and hence development as reported by Faure-Fremiet in 1913 (cited by Brown, 1928).

Eggs of some strongylid nematodes withstand cold for a long time; winter temperatures in Canada and Britain did not kill all the eggs of horse strongyles and many hatched normally in the spring, in spite of showing inhibition in development (Parnell, 1934; and Ogbourne, 1972 cited by Salih, 1981). Moreover, eggs of some other strongylid nematodes could even resist freezing temperatures (Salih, 1981).

iii) High temperatures

The fact that temperatures above living organism optima inhibit all physiological processes indicates that a similar inhibition would be found in developing eggs exposed to high temperatures. So the simple method of achieving *Ascaris* egg elimination, without prolonged storage or adding ovicides, is by heating.

The literature contains many studies about the time-temperature survival *of Ascaris* eggs under different environmental conditions. These studies are sometimes contradictory. Many studies have been made of the heat resistance *of A. lumbricoides* eggs with different modes of killing and are summarized in Table 2.4. Ogata (1925) reported survival of the eggs at higher temperatures by using *Ascaris* eggs on matchsticks soaked in hot water. Swales and Froman (1939) studied flash pasteurization conditions. Cram (1943) used high temperature and dry heat to eliminate infective *Ascaris* spp. eggs from sludge. Reyes *et al.* (1963) determined the time/temperature necessary to completely destroy fully embryonated eggs in nightsoil. Nolf (1932) treated *Ascaris* eggs in unsealed tubes within a water bath, while Barnard *et al.* (1987) treated *Ascaris* eggs in sealed tubes within a silicone bath. Arfaa (1978) incubated eggs with fully developed larvae in various temperatures for different times.

Table 2.4: Synopsis of literature on temperature/ time experiments with Ascaris spp eggs

Temperature (ºC)	Time	Survival	Reference
55	6.5 mins	eggs developed	Barnard *et al.*, (1987)
50	10 mins	eggs dead*	Kiff & Lewis-Jones
55	10 mins	eggs dead	(1984)
60	10 mins	eggs dead	
60	10 mins	30% motile larvae	Arfaa (1978)
	15 mins	eggs dead	
65	5 mins	43% motile larvae	as above
	10 mins	eggs dead	
70	3 mins	34% motile larvae	as above
	5 mins	5% motile larvae	
	10 mins	eggs dead	
55	19.5 mins	*In vitro hatching*	Reyes *et al.* (1963)
65	2 mins	*In vitro hatching*	
54 – 55	2 hours	eggs dead	Keller (1951)
60	10 mins	motile larvae	Rudolfs *et al.* (1950)
37.8	8 days	eggs dead	Seamster (1950)
34.4	13 days	10% motile larvae	
103	3 mins	eggs dead	Cram (1943)
50	1 mins	41% motile larvae	
53	20 mins	29% motile larvae	
70	3 mins	68% motile larvae	Nolf (1932)
55	1 second	eggs dead	Ogata (1925)
70	40 seconds	motile-larvae	
60	10 seconds	eggs dead	Ohba (1923)
65	5 mins	eggs dead	

* eggs dead: means no cell cleavage and no motile embryo.

This review creates the impression that there is a great lack of uniformity in the techniques of such tests, which is reflected in the results (Table 2.4). Therefore, it is very desirable to reach greater uniformity and more consistent results. For this purpose, cultures to be used in testing the effect of temperature should be made in a more uniform way.

Trichuris eggs were killed at slightly lower temperatures (52°C to 54°C) for a shorter time than were *Ascaris* eggs (Nolf, 1932). The additional minutes exposure plus one degree rise in temperature reduced the percentage of surviving *Trichuris* eggs from 67% to 14% and the *Ascaris* eggs from 93% to 26%. Further, if the eggs of *Ascaris* and *Trichuris* are subjected to temperatures above 52°C to 54°C for even a brief period of time, most of them will lose their ability to embryonate. Death of *Taenia saginata* is reported to occur within five minutes at 71°C, and of *Necator americanus* 50 minutes at 45°C (Gotaas, 1953). *Ascaris* eggs are very heat-resistant and can be convenient indicators of the effectiveness of heat treatment, particularly concerning *Taenia* eggs, which are more difficult to assay than *Ascaris.*

Temperatures of 50°C, 55°C, and 60°C produced complete inhibition of normal egg development (Kiff and Lewis-Jones, 1984). The percentage of damaged eggs increased in relation to increased temperature and to time of exposure at a selected temperature. Five minutes exposure to 55°C resulted in obvious damage to *Monieza* spp. eggs, while 2 minutes exposure to 50°C produced total inhibition of normal development in *Fasciola* spp eggs.

After exposure to temperature above 65°C, the ascaroside membrane becomes permanently disorganised, and approaches the melting point of isolated ascarosides, which is about 82°C (Fairbairn, 1970; and Wharton, 1979). If permeability was measured by incubating the eggs in acid fuchsin at different temperatures, there was a sudden increase in permeability at 44°C (Barrett, 1976).

2.1.2 Sunlight and Ultraviolet Radiation

Extensive studies have been made on factors affecting the development of the eggs of *Ascaris* spp., but until recently little comprehensive work has been done on the effect of ultraviolet light on the development and viability of *Ascaris* eggs. The damaging effects of electromagnetic radiation on helminth eggs and larvae have been known for many years. The effects of exposure *of Ascaris megalocephala* to ultraviolet rays for 10 to 60 seconds at both 16°C and 40°C was sufficient to kill from about 10 to 80% of the eggs, varying directly with the length of time they were exposed (Dognon and Tsang 1928, cited by Nolf, 1932). Also, *Ascaris* eggs were killed by exposure to ultraviolet light of wavelength between 280 nm and 315 nm or 180 nm and 315 nm (Nolf, 1932).

A comparison of the effects of ultraviolet radiation on the infective stages of some parasitic nematodes showed that the susceptibility is directly related to the life cycle (Tromba, 1978 a, b). *Ascaris suum, Stephanurus dentatus, Strongyloides papillosus, Enterobius vermicularis,* and *Nippostrongylus brasiliensis,* all of which need shading of the infective stages for survival, are all markedly affected by a total dose of 600µ.W-min/cm^2 or less. *Chabertia ovina, Haemonchus contortus,* and *Ostertagia circumcincta,* whose preparasitic stages are exposed to relatively large amounts of sunlight, are affected only by a total dose of 8,000 to 13,000µ. W-min/cm^2.

The greatest sensitivity of *Enterobius vermicularis* eggs to radiation occurs at wavelengths below 2400A (Hollaender *et al.,* 1940). Also, found the activity of *S. mansoni* cercariae, after exposure to UV light, decreased with increasing dose level of radiation and age of cercariae. Maturation and penetration were dependent on radiation exposure levels (Ariyo & Oyerinde 1990).

Trichuris eggs were much more resistant to the effects of the light. The difference in susceptibility was not definitely understood, but it was suggested that the dark pigmentation of the outer covering of the *Trichuris* eggs probably offers them considerable protection from the shorter light rays. It was also demonstrated that a very short exposure was sufficient to prevent a large percentage *of Ascaris* eggs from reaching embryonation, and slightly longer exposure was completely lethal to them (Nolf, 1932).

Little definite information was available concerning the effect of intense sunlight on eggs of *Ascaris*

spp. It was considered that precise information on the effect of sunlight on these eggs would not only aid in explaining the low incidence of *Ascaris* in some tropical regions, but that it would also extend existing knowledge of factors that influence the spread of this and other parasites under tropical conditions. It was considered also that information of this type might explain the comparatively high incidence of *Ascaris* in temperate regions and the knowledge gained could, perhaps, be used as a basis for improving control measures for roundworms.

When fresh eggs of *A. suum* in water exposed to sunlight were killed within 4 to 6 hours, dried fresh eggs did not live more than 2 hours. Embryonated eggs in water were killed by periods of exposure ranging from 3 to 4 hours, embryonated eggs in a dried condition were killed in 1 to 2 hours. However, the temperature of the medium surrounding the eggs frequently rose above 40°C during the period of exposure and that temperature of 40°C or higher, in the absence of sunlight, effectively inhibited the development of eggs (Roberts, 1934). Also, it was found that eggs of *Ascaris suum* both in shallow water and in dried condition were killed by short continuous exposures (5 to 9 hours) to sunlight at temperature of 30-35°C (Spindler, 1940).

Longer periods of exposure to sunlight were generally required to bring about the death of fully developed *Ascaris* eggs than was required in the case of undeveloped eggs. Embryonated eggs of horse ascarids *(Parascaris equorum)* are markedly more resistant to the inimical effects of UV light than the undeveloped eggs (Shalimov, 1935).

Under natural conditions, *Ascaris* eggs would be more or less protected by the faecal mass during early stage of development. This, together with the heightened resistance to solar radiation later in development, probably accounts for long survival of some *Ascaris* eggs even under tropical conditions.

Hookworm larvae tend to prefer shaded areas, perhaps because light is a stimulus, which may increase larval activity, thus increasing lipid depletion. This may account for the decreased longevity and reduced desiccation tolerance in the presence of light. The incubation temperature of *Necator americanus* eggs affected the longevity and desiccation tolerance of resultant infective larvae. Larvae hatched at 30°C and maintained at 26°C under bright fluorescent light had a 50% survival time S_{50} of 4 days In the dark or shade, the S_{50} for larvae raised at 30°C was 5 weeks, while that of larvae hatched at 20°C was 7 weeks (Udonsi & Atata, 1987). Recent reports on the increase of ultraviolet radiation due to decrease in the earth's protective ozone layer indicate the need for further investigation of its role in the development, viability and infectivity of parasitic nematode eggs.

2.1.3 Desiccation (laboratory and field studies)

Desiccation is antagonistic to *Ascaris* eggs. Little work has been reported concerning the moisture requirements of developing *Ascaris* eggs. A summary of the literature on effects of desiccation on helminth eggs is shown in Table 2.5. Most authors state that "in the absence of moisture development is inhibited, and extreme dryness may ultimately destroy the viability of the eggs".

The minimum moisture requirements for *Ascaris* eggs were confirmed in the early part of the zoth century with 80% relative humidity being required at 22°C for egg development (Otto, 1929; and Wharton, 1979). Following culturing *Ascaris* eggs to the motile-embryo stage, it was observed that some of them were killed in 9 days and all were dead after 37 days of drying at room temperatures (Brown, 1928).

Trichuris eggs required a more highly saturated atmosphere before they could develop than did *Ascaris* eggs, and the former was less resistant to desiccation. It was evident that under fractional relative humidities, the eggs of *Trichuris* succumbed more readily than did those of *Ascaris.* The explanation for the difference may lie in two basic differences in the eggs: (1) the comparative sizes: *Ascaris* eggs are larger and have a considerably greater surface of the fibrous membrane through which the diffusion of gases occurs; (2) the difference in time required to complete embryonation under optimum conditions: *Trichuris* eggs require more time to complete their development than do *Ascaris* (Nolf, 1932).

Many early researchers noted that *Ascaris lumbricoides* degenerated rapidly on various soil types when exposed to direct sunlight (Ogata, 1925; Brown, 1928; Caldwell & Caldwell, 1928; and Otto,

1929). Studies on the effects of humidity on *Ascaris* eggs under field conditions having noted that the human ascarid eggs were rapidly killed in faecal cultures on sand in the direct sun (Brown, 1928). It was concluded that desiccation and heat were both important in killing the eggs, and the results indicated that soil type is an important factor in the rate of development and viability of the *Ascaris* and *Trichuris* eggs. The cultures on sand in the sun did not produce any embryonated eggs, while those in the shade did. Those in loam, clay and humus soils became embryonated but *Ascaris* on humus soil was slower in development due to the minimised oxygen. The temperatures on sand frequently rose above 50°C which was found to be lethal to *Ascaris* eggs while other soils used never reached those temperatures (Ogata, 1925). However, no clear distinction was made between the effects of light and desiccation. Studies showed that unembryonated *Ascaris suum* eggs, kept in shallow water at 30°C - 35°C in direct sunlight, died within 3 hours, but that fully embryonated eggs were slightly more resistant (Spindler, 1940).

The ability of embryonating eggs of *Ascaris lumbricoides* to avoid desiccation by reducing the loss of water through the egg-shell was investigated (Wharton, 1979). It was found that *Ascaris* eggs exposed to desiccation lost water at a rate dependent upon relative humidity and ambient temperature, eventually resulting in collapse of the eggs and death of the enclosed embryo. *Ascaris* eggs are relatively small with a large surface to volume ratio. A low permeability to gaseous exchange thus restricts water loss while still ensuring adequate supply of oxygen for embryonic development.

Also it was found that relative humidity apparently did not affect the rate of development. In eggs exposed to desiccation at various constant temperatures, the rate of water loss increased as an exponential function of increasing temperatures. On exposure to 63-65°C, the ability of the egg-shell to slowdown the loss of water was destroyed. Such phenomena suggest that there is not a simple "critical" and "transitional" temperature, but a gradual melting of the complex mixture of components forming the lipid layer (Wharton, 1979).

Table 2.5: Summary of literature on the effects of humidity on the survival of nematode eggs

Species (eggs)	Temperature (ºC)	Moisture content (% RH)	Remarks/ Reference
Ascaris	22	80	Minimum moisture requirements for eggs development. Otto (1929)
Ascaris and Trichuris	20 – 30 22	40 – 50 77	Unable to develop, completely destroyed after four days exposure 3.5% and 2.2% respectively, eggs reached morula stage in 11 days. Nolf (1932)
Ascaris *Ascaris*	Greenhouse (13 – 46) Greenhouse (38 – 46)	5.8 – 11.5 3.3 – 4.2	Eggs survived for 81 and 51 days respectively. Nonviable eggs after 78 days. Cram & Hicks (1944)
Ascaris *Ascaris*	26.7 – 28.9 31.1	95 80 – 95	Eggs completely developed Eggs developed only to early morula stage. Seamster (1950)
Ascaris	Open petri dish exposed to sunlight (temperature not recorded)	3.1 4.5 76 32.5	10% viable eggs after 51 drying period days. Bhaskaran *et al.* (1956)
Ascaris	30	0	Eggs collapsed after 3 days
Ascaris	16.5	0	Eggs collapsed after 3 days
Ascaris	30	75.5	Eggs developed only to blastula stage and collapsed after 7 days
Ascaris	16.5	76	No further development beyond gastrula stage after 51 days exposure
Ascaris	30	33 – 34	No development beyond 2 cell stage and collapsed after 4 days
Ascaris	16.5	32.5	No development beyond 2 cell stage and collapsed after 17 days. Wharton (1979)
Ascaris and Toxocara	Fall Winter Spring Summer	5 7 8 15	Inactivated eggs. Reimers *et al.* (1981)
*Trichostrongylus Colubriformis**	20	0 – 33 and 54.5	Poor survival
*Trichostrongylus Coltibnformis**	20	76 – 98	Eggs hatched after exposure for 104 days. Wharton (1982)

*: Plant-parasitic nematode, embiyonated eggs.

Effects of air drying on parasitic eggs in sludge

Desiccation was found to be consistently effective in destroying parasites. Little information is available on the survival of helminth eggs on drying beds. A log-log correlation was found between the density of viable parasite eggs and the moisture content in drying beds. The inactivation of parasite eggs increased with the decrease of moisture content of the drying bed sludges. The lowest moisture level at which all *Ascaris* or *Toxocara* eggs were inactivated was 5% in the fall, 7% in the winter, 8% in the spring, and 15% in the summer. Evidently, both temperature and reduction in moisture content played a part in the inactivation of these parasites (Reimers *etal.,* 1981). Studies of the inactivation of parasite eggs in dried sludge have been previously carried out with anaerobically digested sludges. Anaerobic digestion itself has been shown to have little effect on the viability of parasite eggs, but it is possible that it has a synergistic effect when coupled with air drying. This point must be kept in mind when the effects of air-drying are considered (Ward *etal.,* 1984).

The majority of the studies cited, however, is experimental and includes a step in which eggs are added in large numbers to raw sewage or digested sludge. Very few of these studies were field investigations where in the survival of indigenous parasitic forms was determined. It is difficult, therefore, to extrapolate the information from these experiments to actual sludge application sites. It is possible to determine, under controlled laboratory conditions, with accuracy, the effects of different physical factors. This information is, however, of limited value by itself on account of the difficulty in relating it to naturally occurring situations, where eggs are subjected not only to the effect of various physical factors all operating at the same time, but also to biological factors.

Air drying of sludge to very low moisture levels apparently causes complete destruction of parasite eggs. This conclusion was reached in studies conducted more than 50 years ago (Cram, 1943), and has been confirmed in recent experimentation (Reimers *etal.,* 1981; and Hindiyeh, 1995). *Ascaris* eggs survived drying to a point where the moisture content of the sludge reached 5.8 % but failed to survive when the moisture content reached a lower figure (Cram & Hicks, 1944). When sludge was dried in the sun in South Africa for 4 months in layers ranging in thickness from 37 to 150 mm, *Ascaris* eggs were completely eliminated from the 37 mm layer, in which the moisture content had fallen from 84% to below 3%. *Ascaris* eggs still remained in the thicker layers (Hogg, 1950). In laboratory experiments in India, sludge samples were kept in open dishes exposed to diffused sun light. After 51 days, the moisture content had dropped to 3.1 percent, and yet 10 percent of eggs were still viable (Bhaskaran *et. al.,* 1956). It was concluded that drying alone was not very useful because it was necessary to dry the sludge to a very low level of moisture for complete destruction of viability, which however, is not feasible in practice.

2.1.4 Survival of Helminths in Soil and on Crops

The extensive literature on the survival times of excreted pathogens in soil and on crop surfaces has been reviewed by Faechem *et al.* (1983) and Strauch (1989). There are wide variations in reported survival times, which reflect strain variation and the climatic factors, as well as differences in analytical techniques. Nevertheless, it is possible to summarize current knowledge on pathogen survival in soil and on crops in warm climates (20°C-30°C), as shown in Table 2.6.

Available evidence indicates that almost all excreted pathogens can survive in soil and ponds for a sufficient length of time to pose potential risks to farm and pond workers and also to those who handle and consume fish and aquatic macrophytes. Pathogens survive on crop surfaces for a shorter time than in soil, as they are then less well protected from the harsh effects of sunlight and desiccation. Nevertheless, survival times can be long enough in some cases to pose potential risks to crop handlers and consumers, especially when survival times are longer than crop growing cycles, as is often the case with vegetables.

Irrigation of pasture with wastewater that contains viable *Taenia saginata* eggs will induce bovine cysticercosis only if cows have access to the pasture while the eggs are still viable. An interval of at least 14 days between irrigation and grazing is often recommended and, in some countries, is obligatory. However, it is unclear how effective this is in practice as a control measure, as eggs of *Taenia* spp. have been known to survive for up to six months on grass and soil. The education of farmers and meat inspection are necessary additional control measures.

Table 2.6. Survival times of selected excreted pathogens in soil and on crop surfaces at 20-3 0°C. (Feachem et al. (1983), and WHO (1989)).

Pathiogen	Survival time	
	In soil	**On crop**
Viruses		
Enteroviruses*	Less than 100 but usually less than 20 days	Less than 60 but usually less than 15 days
Bacteria		
Faecal cliforms	Less than 70 but usually less than 20 days	Less than 30 but usually less than 15 days
Salmonella spp.	Less than 70 but usually less than 20 days	Less than 30 but usually less than 15 days
Vibrio chloerae	Less than 20 but usually less than 10 days	Less than 5 but usually less than 2 days
Protozoa		
Entamoeba histolytica cysts	Less than 20 but usually less than 10 days	Less than 10 but usually less than 2 days
Helminths		
Ascaris lumbricoides eggs		Less than 60 but usually less than 30 days
Hookworm larvae	Many months	
Teania saginata eggs	Less than 90 but usually less than 30 days	Less than 30 but usually less than 10 days
Trichuris trichiura eggs	Many months	Less than 60 but usually less than 30 days
	Many months	Less than 60 but usually less than 30 days

* Includes polio-, eco, and coxackioviruses.

2.2 Chemical Factors Affecting Helminth Egg Survival

2.2.1 pH

Parasitic eggs are considered to be highly resistant to extreme pH values. The effects of pH on the survival of helminth eggs were investigated by incubating parasite eggs in phosphate buffers at a range of pH at room temperature, 27°C and 37°C (Kiff & Lewis-Jones, 1984). Acid pH levels inhibited normal development *of Ascaris suum* eggs at all temperatures, but highly alkaline buffers allowed development to the infective larval stage. Others demonstrated that *in vitro* hatching ability but not viability of *Taenia* eggs was completely destroyed at pH 12 (Owen, 1984).

However, both human and animal hookworm species will successfully hatch and develop to the infective stage over the pH range 4.6-9.4. The ecological significance of this is that faeces and soil, while providing the optimum pH for hatching, may also provide the nutrients and electrolytes required for further development of the larvae to the infective stage. The optimal pH for *N. americanus* eggs hatching was found 6.0 (Udonsi & Atata, 1987).

2.2.2 Chemical Substances

A search of the literature revealed several references concerning the resistance of *Ascaris* eggs to chemical substances (Fairbaim, 1957; and Morishita, 1972). It has been reported that *Ascaris* eggs will

develop to the infective stage in a wide range of relatively toxic solutions such as 14% hydrochloric acid, 9% sulphuric acid, 8% acetic acid, 0.4% nitric acid, 0.3% carbonic acid, 0.5% sodium hydroxide, 1% mercuric chloride, and 4% formaldehyde. The resistance of these eggs to toxic substances is mainly due to the relatively impermeable inner membrane of the shell, which is lipoid in nature. This lipoid membrane is, however, altered by many organic solvents, including chloroform, ethyl ether, alcohol, phenols, and cresols. It is permeable to respiratory and certain noxious gases; e.g., methyl bromide, hydrogen cyanide, hydrozoic acid, ammonia, and carbon monoxide, which can kill the developing embryo. However, the charged forms of these gases will not penetrate the lipoid membrane (Fairbairn, 1957).

Dichloro-diphenyl-trichloroethane (DDT) is a relatively efficacious insecticide, which is classified as a "contact poison". DDT powder used at full strength and in direct contact with *Ascaris summ* eggs exerted no perceptible effect on their development (Seamster, 1950). Sulfanilamide, a commonly used bacteriostatic substance, was observed to produce no apparent effect on the development of *Ascaris* eggs (Table 2.7). Eggs were killed in at least 3 days by exposure to fumes of concentrated ammonium hydroxide.

Ascaris eggs from which the chitinous shell had been removed by treatment with sodium hypochlorite, hatched much faster than those in which this shell was present (Fairbaim, 1961). The digestion of a hole in the shell was, therefore, a rate-limiting step in the enzymatic response to stimulation. However, if embryonation was carried out in 1% formalin or in 2% sodium dichromate, hatching in 3 hours was reduced to 25% and 2%, respectively. Possibly these reagents, which like dilute acid are excellent inhibitors of microbial growth, reacted chemically with the shell to make it resistant to digestion by chitinase or other enzymes. In all of these solutions embryonation itself appeared to be normal. Formalin, and potassium dichromate solutions, have been used very generally as media for the embryonation of nematode eggs, because they are effective germicides but do not hinder development. If, in nematodes besides *Ascaris*, these disinfectant solutions also make the eggs difficult or impossible to hatch, they are obviously unsuitable for use in the study of infectivity and related biological problems.

Table 2.7. The effect of various chemicals on the development of Ascaris eggs

Chemicals used	Contact time days	Concentration (%)	Motile larvae (%)	Reference
$(NH_4)_2 SO_4$	8 3	5 – 15 concentrated	Motile larvae Eggs dead*	Seamster (1950)
$NH_4 Cl$	8	5 – 15	Motile larvae	As above
NH_4NO_3	8	5 – 15	Motile larvae	As above
DDT	8	5	Motile larvae	As above
sulfainilamide	8	1 – 2	Motile larvae	As above
Potassium dichromate	20	2.5	65	Arfaa (1978)
Normal sulfain	20	---	63	As above
Levamisole	20	10^{-3}	63	As above
Thiabendazole	20 20	10^{-3} 10^{-6}	Eggs dead 12	As above
Mebendazole	30	10^{-5}	Eggs dead	As above
Urea	30	10^{-3}	61	As above
Iodine	25	10^{-7}	32	As above
Cresol	5 hours	3	No embryos	Cram (1924)
Carbolic acid	10 hours	5	Developed	As above

* eggs dead: that means no cell cleavage and no motile embryo.

Decoated *Ascaris* eggs are highly resistant to the dissociated (OC1-) form of germicidal chlorine compounds, showing long survival times in concentrated NaOCI or $Ca(OCl)_2$. When exposed to undissociated HOC1 resistance was much lowered. The use of high concentrations of chlorine gas in

water under acid conditions results in more rapid death (Krishnaswami & Post, 1968).

Ozone and chlorine have been found to be capable of killing *Shistosoma mansoni* eggs when present at levels of 4.0 mg/1 and 40 mg/1, respectively (Mercado-Burgos *et al.*, 1975). However, ozone appears to have no effect on the eggs of *Ascaris* or *Hymenolepis* (Burleson & Pollard, 1976 cited by Reimers, *et. al*, 1989), and routine doses of chlorine in wastewater have no effect on parasite eggs (Liebmann, 1964). Fumigation experiments with gaseous methyl bromide indicated that this method probably would be of limited value in destroying parasites in sludge (Cram, 1924). The destruction of parasites in sludges by chemicals has had varied results.

2.2.3 Oxygen Requirements

Lack of oxygen suppresses the overall metabolism of many nematodes and influences a number of different activities. In. *Ascaris* eggs the rate of development is suppressed by low oxygen concentration (Lee & Atkinson, 1976). Developing *Ascaris lumbricoides* eggs are obligate aerobes (Passey & Fairbairn, 1955). However, unembryonated eggs will survive for several weeks at room temperature in anaerobic conditions (Brown, 1928), but the development will be inhibited. The super saturation of water with oxygen does not hasten *Ascaris* egg development. Oxygen pressures do not increase embryonic development and when sufficiently great (>506 mm) they prove lethal to the developing embryo in the very early stages of development. The amount of oxygen consumed by a single egg was very small, about $2.5x10^6$ ml during its development.

It is quite likely that *Ascaris* eggs have become adapted to developing in nature in a medium, which is not fully oxygen-saturated, with the result that higher oxygen tensions are not necessary for normal development.

It was originally thought that the rate of oxygen consumption was constant in embryonating *Ascaris* eggs (Brown, 1928); but later work by Fairbairn, (1957) showed that the rate decreases rapidly to about half its initial value during the first 36h, then increases steadily to a maximum after 10 days when the embryo is vermiform. From days 10-25 the rate decreases rapidly again, then declines more slowly to a very low level at 140 days. The initial decline was due to an oxygen debt inherited from the essentially anaerobic metabolism of the adult female worm and the second decline could be an adaptation to prolonged survival in the environment, ensuring that food reserves are not expended. The oxygen tension in faecal cultures is usually low (Fairbairn, 1957) and the initial decline could also be an adaptation to this situation.

The oxygen requirements of *Trichuris* eggs are not essentially different from those of *Ascaris* eggs. Carbon dioxide given off, if allowed to remain in close contact with the eggs, will retard their development. No nitrogen is given off during development of *Trichuris* embryos (Nolf, 1932).

2.3 Biological Factors Affecting Helminth Egg Survival

The biological factors that have been shown to affect parasite eggs include fungi and various invertebrates. The ovicidal fungi are capable of attacking and destroying *Ascaris lumbricoides* eggs under experimental conditions during several days or weeks. The rapidity of the ovicidal effect is dependent particularly on the species of ovicidal fungus and type of ovicidity. Also the heating of upper soil layers may result in a more rapid destruction of eggs by ovicidal fungi (Lysek & Bacovsky, 1979).

One fungus which has been shown to penetrate and destroy eggs is *Cylindrocarpon radicola* (Sobenina, 1978). Invertebrates, particularly insects and gastropods, can also destroy helminth eggs by mechanically breaking the eggs and ingesting them (Miller *et al.*, 1961). Experiments showed that gastropods ate large quantities of *Ascaris* eggs. Moreover, 10 to 20% *Ascaris* eggs excreted by *Planorbis planorbis, P. corneus, Bithynia tentaculata, Galba palustris, Succinea putris* and *Physa fontinalis*, were structurally damaged and incapable of further development and 8 to 10% of eggs developed only to the gastrula stage. The embryogenesis of the remaining eggs was delayed by 10 to 15 days (Asitinskaya, 1979).

Chapter

3 OCCURRENCE AND REMOVAL OF HELMINTH EGGS FROM RAW WASTEWATER AND SLUDGE

3.1 Occurrence and Survival of Helminth eggs in Raw Wastewater

Factors that affect the occurrence and concentrations of helminth eggs and protozoan cysts observed in raw wastewater, include the endemicity of disease within the indigenous animal and human population, the size and socio-economic status of the population, the percentage of population sewered, the percentage or wastewater contributed by industry, the volume of influent sampled and the recovery efficiency of the sampling method (Grimason *et al*, 1995).

Table 3.1 summarizes counts of nematode eggs in raw wastewater from a range of different countries. The extremely high concentration of nematodes eggs found in Iranian and Brazilian cities raw sewage (Table 3.1) is a direct result of the low socio-economic conditions of the country inhabitants. Partial sanitation throughout the community, poor housing and low per capita water consumption all contribute to a high level of incidence of parasitic infection in the community and to high concentrations of parasitic organisms, such as intestinal nematodes eggs, in the wastewater of such a community (Dixo *et al;* 1993). In temperate climates of Europe, about 10% of the eggs in wastewater are of human origin. This means that the sludge's load of parasites will also depend on the amount of animal waste reaching the sewage system (Liebmann, 1964).

Table 3.1 Literature survey on counts of parasitic intestinal nematode eggs in raw wastewater from different countries

Range (eggs/I)	Country	Reference
200-2130	Calcutta	Bhaskaran (1956)
10-80	Japan	Liebmann (1964)
500-13000*	Iran (Isfahan)	Sadighian *et al.* (1976)
581-838	India	Veerannan (1977)
3340	Syria (Aleppo)	Bradley & Hadidy (1981)
460	Syria (Lattakia)	Bradley&Hadidy(1981)
122-860	India	Panicker & Krishnamoorthi (1981)
100-200	Oman	Strauss (1987)
38-670	Northeast Brazil	Ayres *et a!.,* (1989)
9**	France (Nancy)	Schwartzbrod *et al.* (1989)
18-840	Morocco	Scawartzbrod *et al.* (1989)
100-800	Jordan	Al-Tarazi (1989)
33-950	Jordan	Saqqar(1990)
120-196	Kenya (Nakuru)	Ayres *et al.* (1993)
205-591	Kenya (Karatina)	Ayres *et al.* (1993)
550-8900	Brazil	Ceballos *et al.* (1993)
0-120	Marakech	Ouazzam *et al.* (1993)
17-133	Kenya	Grimason *et al.* (1995)

* : no of eggs/ g
** : mean number

3.2 Elimination of Helminths by Wastewater Treatment Plants

Most wastewater treatment processes were originally developed to remove organic matter so as to prevent deoxygenation of the receiving watercourse, and the removal of excreted pathogens was not considered important. In the present context of wastewater reuse the removal of pathogens is the principal treatment objective. The removal of excreted pathogens in wastewater treatment processes is reviewed in detail by Feachem *et al*., (1983). Table 5.2 summaries the available information for the removal of excreted helminths in various wastewater treatment processes and indicates where the Engelberg guidelines, (WHO, 1989) can be met (Mara & Cairncross, 1989).

3.2.1 Removal of Helminth Eggs by Conventional Wastewater Treatment Plants

The presence of helminth eggs is considered to be a limitation to wastewater and sludge reuse. Various biological processes have been studied to determine their effectiveness for inactivation of parasites from domestic wastewater. The efficiency of conventional treatment plants in helminth egg removal varies considerably depending on the unit processes included in the plant and on the type of helminth eggs being considered.

As shown in Table 3.2, conventional domestic wastewater treatment processes may not be totally effective in activating and/or inactivating parasites (Reimers *et al.*, 1981). Field studies have shown that trickling filters, sand filters and activated sludge processes promote embryonation of helminth eggs such as *Ascaris, Necator* and *Ancylostoma* (Cram, 1943; Newton *et al.,* 1949; Silverman & Griffiths, 1955).

Sedimentation in conventional works occurs at ambient temperatures, so the eggs are not killed, just transferred to the solid fraction for disposal by other means. The most efficient process for the removal of *Taenia* and other parasite eggs is primary sedimentation (Newton *et al.,* 1949; Bhaskaran *et al.,* 1956). The time-period for effective removal has been observed to be two hours. In India sewage treatment plants achieved 46% removal of hookworm eggs compared with 67% removal for *Ascaris* eggs by 1.5 hours sedimentation, and 75% removal for hookworm eggs and *Ascaris* eggs by two-hour sedimentation. They could also achieve 100% removal in an experimental trickling filter plant, and 81% and 96% in two activated sludge plants (Bhaskaran *et al.,* 1956).

Researchers acknowledged that tapeworm eggs settled 457 mm in two hours, but noted that most primary sedimentation tanks were 1.5 to 4m deep and were subject to turbulence from the constant inflow of sewage. They doubted whether sedimentation was capable of removing a high percentage of tapeworm eggs from primary effluent (Silverman, 1955). As *Taenia saginata* eggs have a diameter of about 40 µm and a specific gravity of 1.3, their Stoke's Law velocity of settling in water at 15°C has been calculated as 0.83m/h, compared with experimental values of about 1 m/h, and with a diameter of 50 µm and a specific gravity of 1.111.

The Stoke's Law settling velocity *of Ascaris lumbricoides* eggs in water at 15°C has been calculated as 0.48m/h (Pike, 1990). The effective liquid upward flow velocity is conventionally between 0.5 and 1.5m/h in primary and secondary settling tanks, so that eggs of *Taenia saginata* and *Ascaris lumbricoides* are unlikely to settle out completely in primary sewage treatment, unless the eggs aggregate or attach to larger and/or heavier solid particles.

Table 3.2: Effects of wastewater treatment processes on parasite eggs and cysts

Unit Operation	Effectiveness
	Removal processes (no parasite destruction)
Clarifiers (primary and secondary)	80% removal of *Ascaris*, 54% removal of *Entamoeba*; removal depends on operating conditions
Flotation	>95% removal but depends on eggs state and operating conditions
Imhoff tanks	97%
Trickling filtration	38% removal, promotes eggs development
Filtration	Retained 99% of eggs
	Stabilization processes (affecting the eggs state)
Activated sludge	Promotes egg development
Extended aeration	Promotes egg development
	Decontamination processes
Routine chlorination	No effect

Source: ***Reimers et al., 1981***

Major helminth eggs with their size, relative density and settling velocity are shown in Table 3.3. This shows that apart from *S. mansoni*, the other eggs are slightly smaller than *Ascaris suum* eggs and their relative densities are similar except for *T. saginata* eggs. The smaller size affects the settling velocity and therefore will reduce the effect of sedimentation.

Conventional treatment systems such as conventional activated sludge, rotating biological contractors, oxidation ditch, and extended aeration are not very effective in removing nematode eggs. Complete removal of helminth eggs after conventional treatment will be achieved with two or more polishing ponds in series with perhaps 5 days retention time in each pond (Al-Salem & khouri, 1991). Also using slow sand filtration after conventional treatment to remove eggs before irrigation was recommended, but the operation is costly and not simple (Al-Salem & khouri, 1991).
The effective removal of *Taenia* eggs from wastewater treatment plants effluents would reduce the risk of animals exposed to treated wastewater. Under laboratory conditions it was found that sedimentation removed only 89% of *Taenia saginata* eggs present in sewage in a three-hour period. A trickling filter in the laboratory could remove only 30 to 38% of *Taenia saginata* eggs. Sand filters of 12-inch column removed over 99% of the *Taenia saginata* eggs that had been added to settled sewage (Newton *et al*, 1949).

Table 3.3: Size, density and settling velocity of major helminth eggs species

Species	Dimensions (µm)	Density (specific gravity)	Settling (m/h)
A. suum	65 X 45	1.13	0.95
A. lumbricoides	55 X 40	1.11	0.43
S. mansoni	50 X 150	1.18	5.23
T. trichuira	22 X 50	1.15	0.48
T. saginata	40 X 35	1.3	0.83
Hookworms	60 X 40	1.055	0.26

Source: Dunn, 1991

3.2.2 Removal of Helminth Eggs in Waste Stabilization Ponds

Removal of pathogens is considered a major advantage in using waste stabilization pond (WSP) systems for domestic wastewater treatment, particularly in developing countries where public health risk from parasitic infections are high (WHO, 1989; Horan, 1990). Despite the fact that waste stabilization ponds are the simplest form of wastewater treatment system, they are the most poorly understood in terms of the reactions which take place within them. As a result of this, models for the design of WSP's tend to be purely empirical. A summary of the major types and functions of each pond is described in Table 3.4 (Horan, 1990).

Over the last decade, an increasing number of studies conducted in different countries have shown waste stabilisation pond systems to be a suitable method of wastewater treatment, especially with regard to the removal of helminth parasites (Mara & Silva, 1986; Al-Salem & Lumbers, 1987; Bartone & Arlosoroff, 1987; Schwartzbrod *et al.*, 1987; Mara *et al.*, 1990; Saqqar & Pescod, 1991; 1992 [a, b]; Ayres *etal.*,1993 [b]).

The basic mechanism of egg removal in all wastewater treatment processes is by sedimentation (plain or enhanced by adsorption to solids) (Gloyna, 1971; Panicker & Krishnamoorthi, 1978; Shephard 1978; Arthur, 1983, Feachem *et al.*, 1983). This implies that all factors influencing this process will affect helminth egg removal. This includes retention time, affected by short-circuiting due to different kinds of water currents (which can reduce the real retention time); water turbulence (which can retard the settling velocity), temperature (as exhibited by Stoke's law) with higher temperatures improving settling velocity; and the size and weight of these eggs.

Table 3.4: The principal functions of the main pond types and their typical performance and operating data (Horan. 1990)

Pond type	Depth (m)	Retention time (d)	Major role	Typical removal efficiencies
Anaerobic	2-5	3-5	Sedimentation of solids, BOD removal, stabilization of influent, removal of helinths	BOD 40-60%, SS 50-70%, fecal coliforms 1 log, Helminthes 70%
Facultative	1-2	4-6	BOD removal	BOD 50-70%, SS increases due to algae, fecal coliforms 1 log
Maturation (for three ponds)	1-2	12-18	Pathogen removal, nutrient removal	BOD 30-60%, SS 20-40%, fecal coliforms 4 log

Other factors, which can lower the removal efficiency of helminth eggs in ponds, are the

presence of detergent foam and other flotable materials (Saqqar & Pescod, 1992b). In anaerobic ponds, despite the high removal rate of suspended solids, the release of methane, hydrogen sulphide and carbon dioxide from the sludge layers leads to the resuspension of some organic and inorganic solids. It is possible that the relatively poor rate of removal of intestinal parasitic helminth eggs in anaerobic ponds is due to their resuspension by gas.

Research on full scale stabilisation ponds has shown that nematode eggs can be detected in final effluents of multi-celled pond systems with retention periods far in excess of the WHO recommended retention period of 8-10 days (Al-Salem & Lumbers, 1987; Mara *et al.,* 1990; Saqqar & Pescod, 1991, 1992 [b]; Ellis *et al.,* 1993). In Jordan, it was found that in winter complete removal of intestinal nematode eggs can be achieved in 17 days in three ponds (two anaerobic and one facultative) working in series and that to achieve the same goal in summer required only 11 days in the two anaerobic ponds working in series (Al-Salem & Terazi, 1989). However, some doubt does exist as to the ability of ponds, under various conditions, to remove all parasites from wastewater. In Brazil *Ascaris* and hookworm eggs were found in the last pond of a five-pond system with a total retention time of 17 days (Mara&Silva, 1986).

For effective helminth egg removal, the most important design parameters are probably the number of ponds in series and the mean hydraulic retention time of each pond. Many authors have reported higher efficiency of removal of intestinal parasites in a series of ponds, than in a single pond with the same overall retention time (Meiring *et al.,* 1968; Gloyna, 1971; Feachem *et al.,* 1983; Mara & Silva, 1986; Saqqar & Pescod 1992 [a]). Feachem *et al.* (1983), have provided a comprehensive review on the occurrence and survival of the most common

helminths in the environment and concluded that well-designed multicelled ponds with a total retention time of more than 20 days will achieve 100% removal of helminth eggs. Mara and Silva (1986) found in pilot-plant that a single pond could achieve complete removal of nematode eggs with a detention time of 18.9 days, or by two ponds in series with detention time of 6.8 and 5.5 days.

Bacterial removal was more efficient with several ponds in series, each with the same retention time. This is because series of ponds behave more like a plug flow reactor with each packet of water receiving repeated treatment in each pond, which individually may behave as a completely mixed reactor. The same principle may be true for helminth egg removal, where eggs, which are not removed in one pond, through resuspension or short-circuiting, may be removed in the following ponds (Marais, 1974).

A model developed to describe nematode eggs removal in waste stabilisation ponds, indicated that 14 days was needed to achieve the WHO criterion in Al-Samra WSP's in Jordan (Saqqar & Pescod, 1992). Ayres *et al.* (1992) presented an equation that can be used to design WSP's systems for egg removal, when effluent is required for restricted irrigation only. They found that the percentage removal of nematode eggs from WSP is related to the hydraulic retention time, and can be described by the equation:

% Removal of nematode eggs = 100 [1-0.14 exp (-0.38HRT)]

The expected percentage removal for any given hydraulic retention time can be predicted by using the lower 95% confidence limit as a safety margin:

% Removal of nematode eggs = 100 [1-0.41 exp (-0.49HRT + $0.0085HRT^2$)].

Although the results of Ayres *et al.* (1992 [b]) indicate that high egg removals are produced in ponds with long retention times, they suggest that a larger number of smaller ponds in series are used, so as to provide increased efficiency and to minimize hydraulic short circuiting.

The viability of *A.. lumbricoides* eggs recovered from WSP effluents was not different from freshly obtained eggs, and in aerobic conditions, some embryonation was observed. Hookworm eggs survived anaerobic WSP conditions for up to 14 days, and were found to develop and hatch in aerobic WSP. However, the hatched larvae did not develop to the filariform stage and no hookworm larvae were ever recovered from WSP effluents (Ayres, 1992 [b]).

There is some disagreement in the literature about the efficiency of waste stabilisation ponds in

removing hookworms. Veerannan (1977) and Gloyna (1971), reported 100% removal of hookworm eggs in ponds, whilst Lakshminarayana and Abdulappa (1972) and Mara and Silva (1986) found that eggs were removed but their larvae were found in the pond effluent.

In laboratory trials, hookworm larvae were found completely eliminated in less than 2 days in a facultative pond, and sludge samples did not show any viable eggs. In a maturation pond, however, filariform larvae were recovered for up to 16 days (Lakshminarayana & Abdulappa, 1972). The authors considered that the lack of oxygen was the principal lethal factor, although Cram (1943) found viable hookworm eggs in anaerobically digesting sludge after considerable periods of time.

In an operational work at Nagpur, India, consisting of a three pond series, eggs of *Ancylostoma duodenale, Hymenolepis nana* and *Ascaris lumbricoides* were regularly present, and those of *Trichuris trichiura* and *Enterobius vermicularis* occasionally present, in the raw sewage. In the final effluent the only helminths present were occasional filariform larvae of *Ancylostoma duodenale* (Shephard, 1978). A corresponding accumulation of helminth eggs in the settled sludge of the first pond was found, suggesting that removal was due to simple sedimentation. Studies in Rhodesia and South Africa have confirmed that helminth eggs are normally absent from final effluent of waste stabilisation ponds (Hodgson, 1964; Meiring *et al.*, 1968).

Little information is available on the removal of *Schistosoma* eggs in wastewater treatment processes. The effect of laboratory-simulated waste stabilisation ponds on eggs and miracidia of *Schistosoma mansoni* were studied by Kawata and Kruse (1966). Completely anaerobic ponds were found to inhibit the hatching of eggs by a mean value of 77.3%, while there was no inhibition of hatching in facultative or aerobic ponds. Eggs recovered from the sludge of the anaerobic pond after 4 hours showed only 9% hatchability, and after 8 hours hatchability was zero. Miracidia survived for a maximum of 6 hours in anaerobic pond water and 10 hours in aerobic pond water, compared with 18 hours in tap water.

Under anaerobic conditions, the schistosome snail vector *Australorbis glabratus* did not lay eggs and the mean survival period was 20 days, with none surviving beyond 42 days. In the facultative pond, the snails survived and reproduced as if under normal conditions. From their results, Kawata and Kruse recommended the inclusion of a preliminary anaerobic chamber in stabilization pond design to suppress hatching of schistosome eggs. However, since the maximum survival time of hatched miracidia is much less than the normal retention period of stabilization ponds, this alone should be sufficient to prevent transmission, provided that vector snails are absent from the maturation ponds and outflows.

Cestode eggs may be present only at very low concentration in raw wastewater. It is believed that *Taenia* eggs, for example, behave similarly to *Ascaris* eggs and settle down to the bottom of ponds (Feachem *et al.*, 1983). More research needs to be conducted on *Taenia* eggs removal in wastewater stabilisation ponds. A summary of the published information available on the removal of helminths from anaerobic ponds and other WSP's is presented in Tables 3.5 and 3.6.

Table 3.5: Literature survey on the removal of helminth eggs from anaerobic ponds

Initial concentration of eggs in raw wastewater (eggs/ L)	Depth (m)	Mean hydraulic retention time (days)	Removal of helminth eggs	Country	Reference
158	4	1.2	26.6%	Nakuru, Kenya	Ayres *et al.* (1993)
3	3	0.4	(100% sununei (79%), winter	Marrakech	Ouazzani *et al.* (1993)
307	5	(range 4.1 – 6.5)	87%	Jordan	Saqqar & Pescod (1992 b)
384	1.75	0.8	82%	Brazil	Silva (1982)
384	1.75	1.9	90%	Brazil	Silva (1982)

Table 3.6: Literature survey on the removal of helminth eggs from different types of WSP's

Type of pond	Initial concentration of eggs in raw wastewater (eggs/I)	Depth (m)	Mean hydraulic retention time (days)	Removal of helminth eggs	Country	Reference
Primary facultative	2300	2.2	61	100%	Northeast Brazil	Ceballos *et. al.* (1993)
Primary facultative	-	-	13.8	99.96%	Northeast Brazil	as above
Primary facultative	398	1.2	39.4	99.98%	Brazil	Ayrese *et. al.* (1993 b)
1A+1F+3M*	376	1.25+1+ (1+1+1)	4+3.2+ (3.2+3.2+3. 4)	100%	Karatina, Kenya	De'O1iveira (1990)
2A+4F+4M	307	5+2+1.2 5	range 32-48	100%	Jordan	Saqqar and Pescod (1992b)
Macrophytic	32	0.8	7	100%	Marrakech	Silva (1982)
Microphytic	11.7	1.6	50	100%	Marrakech	Ouazzani *et. al.* (1993)

* **Abbreviations**: A: Aerobic pond; F: Facultative pond; M: Maturation pond.

3.3 Occurrence and Survival of Helminth Eggs in Sludge

The existence of parasites in sewage sludges has long been known (Cram, 1943; Keller & Hide, 1951; Graham, 1981; Schwartzbrod *et al.*, 1989 a). Table 3.7 shows the levels of helminth eggs in raw sludges from different countries. Ayres (1992) reported finding 5,187 - 44,306 eggs/g dry weight from primary facultative pond sediment in Brazil. Whilst Sadighian *et al.* (1976) reported finding 1000 to 13000 *Ascaris* eggs per gram of raw sewage and 14000 to 25000 *Ascaris* eggs per gram of processed sludge in the sewage treatment facilities in Isfahan, Iran. This appears to be one of the highest levels of helminth eggs ever reported in sewage sludge. The number and types of parasites present appear to be influenced by the infection rate of the local population (Hays, 1977).

Table 3.7: Levels of helminth eggs in raw sludges from different countries

Counts (Range)	Unit	Country	Reference
50-243 1100-7805	eggs/ml eggs/g dry weight	Johannesburg (South Africa)	Keller & Hide (1951)
14,000-25.000	eggs/g wet weight	Iran	Sadighian *et al.* (1976)
287-1943	eggs/l00g dry weight	USA	Reimers *et al.* (1981)
83-130	eggs/kg wet weight	France(Nancy)	Schwartzbrod *et al.* (1986)
225-325	eggs/l00g wet weight	Marrakech	Schwartzbrod *et al.* (1987b)
20-340	eggs/kg wet weight	San Adrian	Schwartzbrod *et al.* (1989)
45-1015	eggs/g dry weight	Jordan	Hindiyeh(1995)
5.187-44,306	eggs/g dry weight	Brazil	Ayres (1992)

No standard method exists for the recovery and detection of helminth parasites from sludge samples, and differences in the current methodologies employed by investigators limit the degree to which accurate comparisons between studies can be made.

Parasitological contamination was rather significant in the sludge samples from Nancy treatment plant (France), since all samples proved to be positive (schwartzbrod *et al.* 1986). Concentrations varied from 113 to 135 eggs/100g depending on the sludge type that underlined the low impact of the treatment on helminth eggs. Detected eggs mainly belonged to class Nematoda and more rarely to class Cestoda (Schwartzbrod *et al.*, 1989). In france, a wide range of human and animal parasitic helminth eggs have been recovered from sludge; including *Ascaris* spp., *Toxocara* spp., *Trichuris* spp., *Hymenolepis* spp., and *Taenia* spp. were recorded (Schwartzbrod *et al.*, 1986).

Reimers *et al.*, (1981) showed in his investigation of southern USA municipal sewage sludges that most raw sludge contained viable parasite eggs and cysts; 18 species of parasite (eggs or cysts) were observed in both stabilised and raw sludges. The types of parasite eggs and their densities (an average of 1000 to 10000 eggs per kilogram of dry sludge, depending upon the parasite) were found to vary with the population served, type of industrial contribution, season of the year, and geographical region. Abattoir and packing plant wastes may significantly influence the types and densities of parasite eggs found in domestic waste sludges.

Schwartzbrod et al. (1987 [b]) found in waste stabilisation pond sediments in Marrakech, that most of the helminth eggs (nematodes and cestodes) were found in the inlet sediment of the facultative pond with numbers ranging from 40-246 eggs/g dry sediment. Also they found that cestode eggs disappeared much more rapidly than the nematodes. They stated that this decrease in numbers is probably due to several factors: anaerobic conditions in basin sediments, predative phenomena, and osmotic pressure effects as shown by Fitzgerald and Ashley (1977) and Panicker and Krishnamoorthi (1978).

Ayres (1992) found that the eggs of *A. lumbricoides* and *T. trichiura* were found to accumulate in primary facultative pond sludge. However, only 3.4% of *A. lumbricoides* eggs recovered from the sludge were viable, and it was concluded that further treatment would be necessary before WSP sludge could be safely reused for agricultural purposes.

There is evidence that the eggs of *Ancylostoma duodenale* are present in sewage (Hays, 1977), but

there appears to be none linking tothe land application of sludge with disease in man. The larvae survive six weeks in faeces (Feachem et al., 1983) and the eggs survive 60-80 days in drying sludge (Cram, 1943).

Many authors have drawn attention to the presence of *Taenia* eggs in sewage and to the possibility of egg dissemination when sewage is applied to agricultural land (Silverman & Griffiths, 1955; Gemmell & Johnstone, 1977; Crew & Owen, 1978; Burger, 1983; Kiff & Lewis-Jones, 1984; Snowdon *et al.*, 1989 Bruce *et al.*, 1990). There was a significant association between cysticercosis and the use of sludge. However, less than 5% of the affected farms had used sludge, so that route of infection other than with sludge is predominant. Nevertheless, such occurrences emphasize the importance of carefully controlled sludge treatment and disposal practice to ensure that the eggs are non-infective for cattle and to maintain assurance that sludge usage is safe (Bruce et al., 1990).

3.3.1 The Effectiveness of Sewage Sludge Treatment Processes

Conventional domestic sludge treatment processes can be divided into two categories:

1. Stabilisation processes (decreasing bulk organics, odour, and pathogen content of sludges); and
2. Inactivation processes (making the handling and disposal of sludges safer and more economical).

It has been found that these two major categories affect parasites in different ways. The effect of sludge treatment processes on the survival of helminth eggs has attracted much attention and a broad range of experimental data is available.

In sludge stabilisation processes, aerobic and anaerobic environments are produced which may or may not raise the temperature to lethal levels. Because most parasite eggs require an oxygen level for development above that in the host's gut, anaerobic digestion tends to inhibit development while aerobic digestion tends to accelerate their development. As expected, these processes will kill eggs if either the anaerobic or aerobic processes are carried out at temperatures >55°C, which are lethal to parasites. Some sludge dewatering and disinfection processes will destroy the eggs by increasing temperature, as in incineration and composting, or by greatly reducing the moisture content, as in drying beds. In more exotic methods, eggs may be destroyed by disruption using sonication, radiation, or microwaves (Reimers et al., 1981).

The information in Tables 3.8 and 3.9 was originally developed by Stem and Farrell (1977) and expanded with more recent works by Reimers et al. (1989) about the relative effectiveness of processes that are generally considered suitable for disinfecting sludges. While general methods such as pasteurisation, heat treatment, and heavy chlorination appear to be excellent for the destruction of certain pathogens, they may be undesirable due to associated costs or to resultant changes in sludge properties and/or separated liquid characteristics. Table 3.10 shows that conventional sludge treatment processes may not be totally effective in removing or destroying parasites.

Analysis of the degree and rate of inactivation of pathogens in any sludge treatment process cannot itself be used to determine or assure absolute safety from risk of infection but evidence of inactivation will suggest that the treatment is capable of providing a barrier to the spread of infection. Also, to be noted is that a count below a detectable level does not guarantee complete absence of a specific pathogen. For example, parasite eggs below the level of detection, means that the organisms are not detectable using the best isolation methods currently available (Reimers et al., 1989).

In selecting a method of sludge stabilization for a particular location the designer may use a large number of criteria on which to base a decision. Costs (both capitals and operating) will obviously be a major consideration, but there will also be various secondary criteria, which may be relevant. These include the suitability of the method for the size of works, effects of climate, effectiveness of the process for removal of pathogens, the degree of preliminary sludge treatment required, effect on dewaterability of sludge, effect on mass of sludge solids, possible operational problems, permanence of stabilization, and energy requirements.

Anaerobic digestion, lagooning, and sand-bed drying were recommended by Pescod (1971) as the most suitable sludge handling methods for use in tropical developing countries. More sophisticated techniques utilizing imported equipment and complex operational procedures are not likely to be

adopted until development affects the cost and proficiency of labour.

Table 3.8: Effectiveness of standard sludge disinfection processes

Disinfections process	Removal or inactivation				
	Indicator organisms [a]	***Salmonella***	**Regrowth problems**	**Viruses**	***Ascaris lumbricoides***
Standard Process					
Lagoon storage (pilot)	E	E	-	E	E
Drying beds [b]	G	E	R	G+	E
Long-term anaerobic storage (6 months)					
Laboratory batch test at 4 ºC	F+	E	-	P	P
Laboratory batch test at 20 ºC	E	- E	-	E	P
Temperature (heat) -time processes					
Anaerobic digestion (35 ºC)	F	F	-	P+	P
Anaerobic digestion (52 ºC)	G	E	-	G	P
Anaerobic digestion (60 ºC)	G	E	-	E (estimated)	E (estimated)
Anaerobic digestion	F	P+	-	P	P
Composting (>60 ºC)	G+	E	R	G+	E
Pasteurization (70 ºC, 0.5-1hr)	G	E	R	E	E
Pasteurization (70 ºC, 1-2hr)	E	E	R	E	E
Heat treatment (195 ºC)	E	E	R	E	E
Heat drying	E	E	R	E	E

Source: *Reimers et al., 1989*

a: Indicator organisms: total and faecal coliforms, faecal streptococci
b: Moisture content and temperature control.
E: Excellent, below detectable levels.
G: Good, more than 3 logs reduction.
F: Fair, 1-3 logs reduction.
P: Poor, less than 1 log reduction.
R: Regrowth can be a significant problem.
-: Inhibits growth.

Table 3.9: Effectiveness of innovative sludge disinfection processes

Disinfection process	Removal or inactivation		Rmovl or inactivation		
	Indicator organisms [a]	***Salmonella***	**Regrowth problems**	**Viruses**	***Ascaris lumbricoides***
Chemical treatment					
Lime treatment (pH>12)	E	E	R	E (estimated)	P
Heavy chlorination (Cl_2=1500 mg/L)	E (estimated)	E (estimated)	R (estimated)	E (estimated)	P (estimated)
Ammonification	E	E	R	E	E
Applied Fields					
Ultrasound	P to E	P to E	R	P to E	P
Gamma Irradiation (300-400 krad)	G+	E	R	F	E
Gamma Irradiation (1000 krad)	E	E	R	E	E
Gamma Irradiation (300-4000 krad, 55 ºC)	E	E	R	E	E
High energy elector irradiation (1000 krad)	E	E	R	G+	E

Source: *Reimers et al., 1989*

a = Indicator organisms: total and faecal coliforms, faecal streptococci

F = Fair, 1-3 logs reduction
E = Excellent, below detectable levels
G = Good, more than 3 logs reduction
F = fair, 1-3 logs reduction
P = poor, less than 1 log reduction
R = regrowth can be a significant problem

Table 3.10: The influence of sludge treatment processes on parasite eggs

Unit operation	Effectiveness remarks
	Stabilization processes
Anaerobic digesters	Retards egg development, increases destruction with increased temperature
Aerobic digesters	Promotes egg development (increases destruction with increased temperature)
	Decontamination processes
Incineration	100% destruction
Drying beds	100% kill at 5% moisture content; (moisture content may vary with temperature)
Composting	100% effective if all matter reaches 60 ºC for at least 2 hrs
Routine chlorination	No effect
Sonication	80% effective at 30-50 KHz and 600 Watts
Gamma radiation	100% effective at 200 KRADs
Heat	100% effective about 70 ºC and 30 minutes or in less time at higher temperature. Effectiveness depends on temperature and exposure time, but temperatures below 45 ºC appear to have no effect
Lagoon storage	50-100% (depends on time and temperature)
Lime stabilization	80-100% (depends on digestion, time, and temperature) Up to 95%
Ammonification	(depends on dosage and pH)

Source: *Reimers et al., 1989*

Chapter

4 HELMINTH EGGS AS INDICATOR IN WASTEWATER AND SLUDGE

Demonstration of the absence of specified pathogens in the end-product appears to be the obvious way of monitoring the treatment process, but unfortunately this is hindered by a number of facts. Enumeration of pathogens is either not possible at all or laborious and expensive and only possible in specialized laboratories. A more practical approach is based on the enumeration of indicator organisms. Their absence in the end product (in a specified amount) or a reduction by at least a certain factor should indicate that the process has worked satisfactorily. Removal of pathogens is the prime objective in treating wastewater for reuse. Faecal coliforms are less satisfactory as indicators of excreted viruses and are of very limited use in relation to protozoa and helminths, for which no reliable indicators exist.

There is a need for an indicator for the helminths, and the use of *Ascaris* spp. for sludge treatment is appropriate, mainly monitoring of *Ascaris* eggs serves as indicator for *Toxocara, Trichuris,* and *Hymenolepis* eggs (USEPA, 1992c). However, *Ascaris* eggs are not a good indicator for helminth egg removal in sewage treatment systems. *Ascaris* eggs are the heaviest of the helminth eggs routinely found in wastewater and therefore likely to be the species most readily removed during sewage treatment processes; eggs of human and animal hookworms, for example, may be removed by sedimentation less easily than those of *Ascaris.* It would be a risk to assume that if a particular wastewater treatment system achieved 100% removal of *Ascaris* eggs then all other helminth eggs had also been removed (Ayres, 1992).

Organisms to be considered as sludge treatment process indicators should have the following properties:

1. They should always be present in raw sludge in high numbers, be readily detectable and countable with reasonable precision and accuracy.
2. The indicator should be a single species or, at least, a small group of closely related species with similar resistance.
3. Simple, reliable and, preferably, standardised methods should be available.
4. They should possess similar or slightly greater resistance to the process than the pathogens, which they model.
5. If regrowth of pathogens after treatment is likely, the indicator should be able to model this.

These requirements show that a process indicator must be selected with care and only after study of its survival properties in the process. It should be able to demonstrate those relevant pathogens in the sludge (Pike, et. al., 1983).

Ascaris lumbricoides eggs were used as indicators in sludge studies for practical reasons; they were the most

numerous species found in raw sewage and sludge and relatively easy to handle. However, they can also be considered as an indicator for the behaviour of other parasitic helminth eggs in sludge. Meyer *et al.* (1978) suggested the use of *Ascaris* eggs as an indicator organism for sludge treatment processes for the following reasons:

1. Ascariasis is a common and ubiquitous helminthic infection.
2. *Ascaris* eggs tend to settle in sludge.
3. *Ascaris* eggs are more resistant to adverse external conditions than other enteric organisms and would provide a margin of safety in monitoring the treatment process.
4. They are readily available for experimental purposes, are larger and easier to recover and observe than others.
5. Their viability determination is more straightforward than for the cestode such as *Taenia* spp. or *Hymenolepis* spp. (Pike *et al.,* 1983). This seems to imply their use as an indicator for all enteric pathogens, although how valid is this would be is debatable.

Chapter

5 MICROBIOLOGICAL QUALITY GUIDELINES FOR WASTEWATER AND SLUDGE APPLICATION IN AGRICULTURE

The overall objective of these guidelines is to encourage the safe use of treated wastewater and excreta-derived products for agriculture and aquaculture in such a way that protects the health of workers and consumers.

5.1 Guidelines for Treated Wastewater Reuse in Crop Irrigation

Irrigation with untreated wastewater is very hazardous to health, with both fieldworkers and crop consumers being at high risk of helminthic infections; consumers are also at high risk of bacterial infection such as cholera and typhoid fever (Shuval et. al., 1986).

Table 5.1: Recommended microbiological quality guidelines for wastewater use in agriculture[a].

Category	Reuse conditions	Exposed group	Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c])	Faecal colifonm (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks [d]	Workers, consumers, public	Less or equal 1	Less or equal l000[d]	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	workers	Less or equal 1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than pnmarv sedimentation

Source World Health Organization (1989)

a In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account, and the guidelines modified accordingly

b *Ascaris* and *Trichuris* species and hookworms

c During the irrigation period.

d A more stringent guideline (Less or equal 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

e In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

The World Health Organization's (1989) guidelines for the microbiological quality of treated wastewaters to be used for crop irrigation are given in Table 5.1. They are based on a rigorous assessment of the available epidemiological evidence (Blum & Feachem, 1985; Shuval *et al.*, 1986; and Mara & Cairncross, 1989), which showed that the excreted pathogens of most concern in crop irrigation are the human intestinal nematodes and faecal bacteria. The nematode guideline of no more than one egg per liter is required for both restricted and unrestricted irrigation to protect field workers and, in the latter case, also the consumers.

There is, however, some evidence that, for restricted irrigation only, the guideline could be safely relaxed to 10 eggs per litre (see Ayres *et al.*, 1992b). Al-Salem (1992a & 1996) recommend that these guidelines are not sufficient to achieve its goals to protect the exposed workers from helminthic infection, because it does not include the threadworm larvae *Strongyloides* spp., which cause a potentially serious disease, and of a public health significance in endemic areas. The removal of excreted pathogens in watewater treatment processes is reviewed in detailed by Feachem et. al. (1983). Table 5.2 summarises the available information for the removal of excreted pathogens in various wastewater treatment processes and indicates where the Engelberg guidelines can be met (WHO, 1989; and Mara & Cairncross, 1989). Degrees of removal of viruses and cysts are also given in Table 5.2, although these are not relevant to achievement of the Engelberg guidelines.

Table 5.2: Expected removal of excreted pathogens in various wastewater treatment processes

Treatment process	Removal (log_{10} units) of			
	Bacteria	**Helminths**	**Viruses**	**Cysts**
Primary sedimentation Plant	0-1	0-2	0-1	0-1
Chemically assisted [a]	1-2	1-3 [g]	0-1	0-1
Activated sludge [b]	0-2	0-2	0-1	0-1
Biofiltration [c]	0-2	0-2	0-1	0-1
Aerated lagoon[c]	1-2	1-3 [g]	1-2	0-1
Oxidation ditch [b]	1-2	0-2	1-2	0-1
Disinfection [d]	2-6 [g]	0-1	0-4	0-3
Waste stabilisation ponds[e]	1-6 [g]	1-3 [g]	1-4	1-4
Effluent storage reservoirs [f]	1-6 [g]	1-3 [g]	1-4	1-4

a. Further research is needed to confirm performance.

b. Including secondary sedimentation.

c. Including settling pond.

d. Chlorination or ozonation.

e. Performance depends on number of ponds in series and other environmental factors

f. Performance depends on retention time, which varies with demand.

g. With good design and proper operation the Engelberg guidelines are achievable.

5.2 Guidelines for Treated Wastewater Reuse in Fishpond Fertilization

The WHO guidelines (1989) for treated wastewater effluents to be used for fishpond fertilization are an absence of trematode eggs per litre *(Clonorchis, Fasciolopsis* and *Schistosoma*; *Schistosoma* spp. are the only trematodes of interest in the Mediterranean region); and no more than 1000 Faecal Coliform per 100 ml of fishpond water. No trematode eggs are permitted because of the high asexual multiplication of the parasite in its intermediate aquatic host (water snails) (Mara and Pearson, 1998).

5.3 Regulations and Guidelines for Sludge Utilization in Agriculture

Sludge can have valuable agronomic properties and its use should be encouraged, provided that it is used correctly. Any directive should have two main objectives: first to ensure that human beings, animals, plants and the environment are fully safeguarded against the possibility of harmful effects from the uncontrolled spreading of sewage sludge on agricultural land; second to promote the correct use of sewage sludge on such land. Knowledge of the local climate, proximity to the water table, pathogen type and concentration in sludge, and soil characteristics are essential to the establishment of reasonable guidelines for sludge application to land for any country.

The risks to human and animal health from microbes in sludge applied to land and control measures were considered by a WHO Working Group of Experts (WHO 1981). Two pathogens were mentioned specifically, the *Salmonella* serotype, responsible for food poisoning in man and allied conditions in food animals, and the human beef tapeworm, *Taenia saginata*, with its larval stage in cattle, *Cysticercus bovis*. The Working Group was specifically concerned about the effects of agricultural use of sludge on human health, in which disease in animals is one link in the cycle of infection. The Working Group thought that risk to human health from other pathogens was less, although noting that those from viruses and *Sarcocystis* had not been adequately evaluated.

In a review of the disposal of sewage sludge to land in 1981, the UK Department of the Environment and the National Water Council identified four groups of pathogens as potential sources of infection in the UK. Only *Taenia sagi*nata was cited as definitely being disseminated through the disposal of sewage sludge, but eggs of other parasites, *Taenia solium, Ascaris* and *Trichuris* were a cause for concern. Among bacteria in general, *Salmonella* was specifically mentioned, enteroviruses among viruses in general, and *Giardia* among protozoan cysts.

Guidelines on sludge treatments meeting the UK regulations are given in the Code of Practice for Agricultural Use of Sewage Sludge (Department of the Environment, 1989). These were formulated to ensure that the use of sludge in agriculture does not conflict with good agricultural practice or put human, animal or plant health at risk, that water pollution and other public nuisances are avoided and that the long-term viability of agricultural activities is maintained. The UK Department of the Environment 1989 Code of Practice recognizes that it is not practicable to express the microbiological quality of sludge with numerical limits for routine monitoring purposes (Lewis-Jones & Winkler, 1991)

The constraints on grazing and harvesting specified in the 1989 UK regulations (Statutory Instrument, no. 1263) prohibit the use of sludge when fruit or vegetable crops are growing or being harvested in the soil. Fruit and vegetable crops grown in contact with the ground and normally eaten raw must not be harvested for ten months after the use of sludge. Forage crops are not to be harvested, nor animals grazed, for three weeks after the use of sludge.

Sanitised sewage sludge is specified in Article 2, paragraph 2 of the German Ordinance on Sewage Sludge. Sludge is considered as hygienically safe when treated by a technology, for which an appropriate investigation has proved that the number of indigenous or seeded *Salmonella* has been reduced by at least 4 orders of magnitude, and indigenous or seeded eggs of *Ascaris* are rendered non-infectious (Strauch, 1989). The USEPA has traditionally specified technology-based standard for pathogen reduction in municipal sludges. These technologies were classified into two broad categories known as processes that significantly reduce pathogens (PSRP), and processes that further reduce pathogens (PFRP). These treatment technologies were included in 40 CFR 257.3-6.

The USEPA recently proposed specification on the reductions in pathogenic organisms and densities of

indicator organisms that must be attained, rather than specifying the technologies that must be used. This new approach is mainly due to the difficulty in assessing the equivalency of new sludge treatment technologies to the documented processes, either PSRPs or PFRPs.

The USEPA (1992a) has proposed in Section 503.32 two classes of pathogen reduction requirements. Under these two classes, owners or operators of treatment works or distributors of sewage sludge not from treatment works will be required to monitor their sewage sludge in accordance with the methods and protocols to ensure that the pathogenic organisms or indicator organisms do not exceed the limits specified in each of the two classes proposed.

USEPA (1992c) established pathogen reduction classes: Class A and Class B. The classes stipulate the detection levels for pathogenic organisms that are not to be exceeded in sludge. "Class A" criterion is for land application and using the sludge as a fertilizer. This sludge could also be applied in bulk to agricultural land, forest, public contact sites, reclamation sites, lawns, or home gardens; or could be sold or given away in bags or other containers. Physical, biological, lagoon, air drying, or chemical addition methods, or storage for at least 1 day.

Class A requires either that the density of faecal coliforms in the sewage sludge shall be less than 1000 Most Probable Number (MPN) per gram of total solids, or the density of *Salmonella* spp. bacteria in the sewage sludge should be less than 3 MPN per four grams of total solids. Enteric virus density should be less than one Plaque-Forming Unit per four grams of total solids, or density of viable helminth eggs should be less than one egg per four grams of total solids, at the time the sewage sludge is used or disposed, all counts being expressed on a dry weight basis. For class B sludge, the resultant sludge must have geometric mean density of faecal coliforms less than 2×10^6 MPN or Colony Forming Unit per gram of total solids.

Chapter

6 LABORATORY SKILLS

6.1 Microscope Calibration

An ability to accurately measure the size of ova or other parasitic forms through microscopic examination is often necessary in making a species identification. This measurement can be made using a calibrated scale called a stage micrometer. The ocular micrometer, a small round glass disk that is etched with a fixed scale, is inexpensive and easy to use and is strongly recommended for routine laboratory use.

Materials

Stage micrometer: a microscope slide on which 1 mm has been engraved, divided into 100 equal spaces. One space is therefore equal to 1 mm /100 = 10µm.

Ocular micrometer scale: a special ocular (eyepiece) on which a scale has been engraved. Depending upon the magnifying power of the set of objectives used in a microscope, each division in the ocular micrometer will represent different measurements. Therefore, for each set of oculars and objectives used, the ocular scale must be compared with a known calibrated scale. Not all oculars have the same size subdivisions (this depends on the manufacturer).

Ocular Micrometer Calibration

(1) Place the stage micrometer on the microscope stage and, using the lowest power dry objective, e.g. 4x or l0x, bring the scale into focus.

(2) Insert the ocular micrometer and rotate it until both scales overlap.

(3) Move the mechanical stage until both scales are aligned with the zero line.

(4) The line of the ocular micrometer, which is exactly on top of object scale line of the stage micrometer, must be chosen (Fig. 6.1).

(5) Count the number of division lines on the eyepiece micrometer between the zero line and the point where the second set of lines is superimposed.

(6) Repeat this process using each objective in turn, e.g. 4x, l0x, 40x, l00x.

(7) Calculate the precise value of each division on the eyepiece scale with each objective as follows:

With l0x objective:

It can be seen that X divisions of the ocular micrometer are exactly superimposed over Y divisions of the stage micrometer. For this objective (l0x) each space of the ocular micrometer corresponds to Y x 10 µm / X.

With 40x objective:

33 divisions of the ocular micrometer are precisely superimposed over 22 divisions of the stage micrometer. For this objective, each space of the ocular micrometer corresponds 22 X 10 / 33 = 6.7

µm (Fig. 6.1).

Each microscope and each eyepiece used must be individually calibrated because the real magnification always differs from one microscope to another. Also, beside each microscope write down a table with the magnification index for each objective.

Example:

Microscope X ... No. ....

l0x objective magnification 15 µm

40x objective magnification 6.7 µm

l00x objective magnification 1.5 µm

Index = unit of the ocular micrometer.

The eggs can be observed by magnification with a normal 10x ocular and a 10x objective. To measure an object such as a worm egg, the normal ocular is replaced by the micrometer ocular, which has been calibrated before. Superimpose the wall of the worm egg by adjusting the mechanical stage with the zero line of the micrometer ocular. The length of the egg is found by counting the number of complete spaces and estimating the amount of incomplete ones. The number is multiplied by the ocular micrometer index that was found.

6.2 Use of Centrifuges

Most methods quote centrifuge speeds as relative centrifugal forces (g). However, some papers use revolutions per minute (rpm) as the measurement of speed. The speed in rpm remains constant from centrifuge to centrifuge, but the *g* value changes with the radius of the centrifuge.

To convert rpm to force, the following formula is used:

$$RCF = \frac{r(rpm)^2}{k}$$

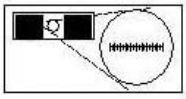
Where:

RCF = relative centrifugal force (g),

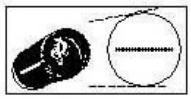
r = radius of the centrifuge from the spindle to the centre of the bucket (cm),

K = 89456.

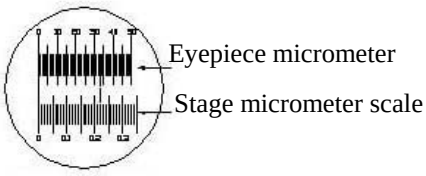
To convert force to rpm, $rpm = \sqrt{k\ RCF / r}$



A: stage micrometer scale



B: eyepiece micrometer scale



C: representing of an eyepiece micrometer superimposed over stage micrometer for microscope calibration

Fig. 6-1: Calibration of stage micrometer. Fig (A) and (B) show the stage and eyepiece micrometers, respectively .Fig (C) shows an eyepiece micrometer superimposed over a stage micrometer for calibration.

Chapter

7 METHODS FOR DETECTION AND ENUMERATION OF HELMINTH EGGS IN WASTEWATER AND SLUDGE

7.1 Introduction

The development of medical parasitology has led to a wide range of techniques for the enumeration of intestinal helminth eggs and larvae in faeces (Stoll & Hansheer, 1926; Faust *et al.,* 1938; Richie, 1948; Beaver, 1950; Bailenger, 1979) and the basic principles of these methods have been adapted for the enumeration of helminth eggs in sludge, soil and wastewater (Krige, 1964; Hays, 1977; Meyer *et al.,* 1978; Satchwell, 1986; Rude *et al.,* 1987) and compost (Steer *et al.,* 1974; Caceres *et al.,* 1987).

The enumeration of intestinal helminth eggs and larvae in wastewater and sludge, however, are not straightforward. A great variety of human and animal species, as well as free-living species, may be present ranging in size, specific gravity and surface properties. Most available enumeration methods for helminths were designed for highly contaminated faeces, sludge or soil samples. Each method has its own strengths and weaknesses.

Generally, these methods use sedimentation, filtration, flotation, or a combination of these methods to concentrate the eggs. Either the parasites are floated away from organic debris in a solution of comparatively high specific gravity or the organic matter is separated in an interphase solution, normally either diethyl ether or ethylacetate, whilst the parasite eggs sediment into a non-miscible buffer below. Both processes rely on centrifugal force.

No standard method exists for the recovery and detection of helminth parasites from sludge samples, and differences in the current methodologies employed by investigators limit the degree to which accurate comparisons between studies can be made. Also a comparative study of methods is required to evaluate the most efficient and practicable, both in relation to their deployment in laboratories and the parasite concerned (nematodes, cestodes, and trematodes).

There are advantages and disadvantages for each system, and no current method will consistently recover all the parasite eggs in sludge. One problem with a sedimentation technique (such as the one developed by Steer *et al.,* 1974), is that a relatively small sample size of sludge (about 1-3 grams wet weight) is used. Also, the sediment obtained contains relatively large amounts of material other than the parasites making it very difficult to find and identify the parasites in the sediments. They reported that pretreatment of sludge with an anionic detergent increases the recovery of eggs by neutralising electrical attraction between the egg and the particulate matter in the sludge (Steer *et al.,* 1974). Meyer *et al.* (1978) also found that the use an anionic detergent improved the recovery of eggs after testing a variety of detergents (anionic, cationic, and nonanionic). In contrast, Satchwell (1986) found that the use of detergent was not useful.

Most investigators use flotation procedures for parasitic analyses of sludges. The flotation procedures have the advantage of separating the parasites from the heavier particles in the sludges (sand, etc.) and consequently the final preparation is usually cleaner and easier to examine. A solution is used with a specific gravity that is high enough to float the parasite eggs from the sample but not so high as to cause distortion of the eggs, (thus it should be only slightly higher specific gravity than that of the heaviest eggs and low enough to leave the heavier particles in the sediment). Solutions of sucrose, zinc sulphate, sodium chloride, Na_2N0_3, $K_2Cr_2O_7$ and other salts have been used (Table 7.1). Arthurs method (described in Faust *et al.,* 1938) which employs saturated sucrose as a flotation solution was found to deform eggs rapidly whilst zinc sulphate solution (Faust *et al.,* 1938) did not concentrate *Trichuris spp.* or *Capillaria spp.* very well.

Fox *et al.* (1981) used a different type of flotation procedure for detecting parasites in sludges, i.e. a continuous sucrose gradient. The disadvantages of this technique are that only a relatively small sample size can be processed in each tube and the apparatus used to form the continuous gradient is expensive and would not normally be available in a wastewater laboratory.

A number of workers have used oxidants such as sodium hypochlorite to hydrolyse organic material and to release the parasites from other particles in the sludge (Meyer *et al.,* 1978). Sodium hypochlorite, as well as other oxidants such as perchloric acid and hydrogen peroxide, were tested by Reimers *et al.* (1981) and were found to remove the outer layer of the shell of many of the helminth eggs. Since the structure and contours of the outer layer of the shell of certain eggs like *Ascaris* and *Toxocara,* are important in the identification of the eggs, the use of sodium hypochlorite can significantly affect the appearance of eggs and consequently the ability of the worker to identify them.

Several different procedures have been used for the recovery of eggs and cysts from the surface of the flotation solution. The use of a wire loop to remove the material floating on the surface is a commonly used technique (Beaver, 1952). Meyer *et al.* (1978) passed the flotation-solution supernatant through a membrane filter and then recovered the parasites from the surface of the filter. Reimers *et al.* (1981) found it best to decant the flotation-solution supernatant, and then add water to it until the specific gravity of the diluted solution is below that of the parasites, and then centrifuge this fluid to recover, in the form of a sediment, the parasites and other particles that had originally floated in the flotation solution.

Ayres *et al.* (1989) found there was a good correlation between eggs seeded and percentage recovery, and suggested that the percentage recovery may be affected more by the quantity and quality of organic matter, rather than the absolute number of eggs present. Various different elution and flotation liquids have been used for concentrating helminth eggs from faeces, soil, sludge, and wastewater samples (Table 7.1). Recently, Gaspard and Schwartzbrod (1993) compared different elution solutions (detergents, distilled water, formaldehyde, sodium hydroxide, sodium hypochlorite) to elute *Ascaris* eggs from soil particles. The recovery analysis showed the superiority of the sodium hypochlorite solution titrating 10 chlorometric degrees, whatever the soil type. Also they found that zinc sulphate solution at 50%, 55% and 66% proved to be excellent flotation agents and can be used with equal success.

Table 7.1: Summary of different elution and flotation solutions used to concentrate parasitic eggs from different types of sewage samples

Eluting and / or flotation solutions	Reference
30% sodium hypochlorite and sodium dichromate	Otto (1929)
	Spindler (1929)
	Owen (1930)
Antiformine and Sucrose	Caldwell & Caldwell (1928)
	Storey & Philips (1982)
Formol-ether and zinc sulphate	Ritchie *et al.* (1948)
Lactalbumine hydrolysate and sucrose density gradient	O'Donnell *et al.* (1984)
Tween 40	Kazacos (1983)
Tween 60	Dubin *et al.* (1975)
Tween 80	Quinn *et al.* (1980)
Sodium Chloride, saturated	Marzochi (1977)
Zinc sulphate	Theis *et al.* (1978)
Magnesium sulphate	Ismid *et al.* (1978)
Sodium nitrate	Teichmann (1986)
Potassium iodine mercurate	Bouhoum & Schwartzbrod (1989)

7.1.1 Technique for the enumeration of parasitic helminth eggs from sewage sludge (Satchwell, 1986)

1. Sieve 250ml of liquid sludge through a 710 and 212 μm mesh sieve together with 250ml of water.
2. Macerate a 50 g sample of drying bed cake sludge with 250 ml of water and sieve with a further 250 ml of water. A bent glass rubbed gently over the surface of the mesh will facilitate sieving.
3. Centrifuge the liquid fraction at 717g for 20 seconds, after this period discards the supernatant and retain the residue.
4. The light solids and fat, which interfere with floatation, are removed by formol-ether extraction.
5. Suspend the residue in 100ml of formol saline solution (Consist or 100ml 40% HCHO solution, 9g NaCl /L) and rinse into a 500ml conical flask. Add 50ml of diethylether, shake well and leave for 10 minutes.
6. Centrifuge for 2 minutes at 728g. Discard the top three layers, leaving the residue at the bottom of each tube.
7. Wash the residue three times with water to remove all traces of ether which interferes with floatation.
8. Suspend the residues in a small volume of saturated zinc sulphate solution (sp. gr. 1.40) and transfer to four 15ml centrifuge tubes, which have had their tops, ground flat. Fill the tubes until a

slight meniscus is formed at the top and place round coverslips carefully on top. Centrifuge the tubes at 683g for 1 minute.

9. Remove the coverslips with a deliberate rapid lifting action, place on microscope slides, and examine for the presence of helminth eggs under the microscope at l00x magnification.

However, it must be borne in mind that the egg recovery of this technique gives an average of 20% recovery of *Ascaris* and about 3% for *Taenia* (Watson *et al.,* 1983).

7.2 Quantitative Determination of Helminth Eggs in Wastewater

The enumeration of helminth eggs in wastewater is not straightforward: they are not normally present in such large numbers that they can be found by making a simple direct smear, and thus it is necessary to use a technique which harvests them from a relatively large sample material. Different helminth eggs and samples of wastewater from different treatment processes vary in their properties and therefore it is not possible to recommend one "perfect" multipurpose technique (Ayres *et al.* 1989).

Nematode eggs are usually removed but not killed by sedimentation in wastewater treatment, whereas in the treatment of excreta they are usually killed but not removed. Thus, in wastewater examination it is not necessary to ascertain whether the eggs are viable, whereas this is the primary concern when examining samples of excreta.

A useful summary of some techniques available for the enumeration of helminth eggs in wastewater is shown in Table 7.2. Recently, four methods for the enumeration of human parasitic nematodes in treated wastewater stabilisation ponds were compared by Ayres *et al.* (1991). The recovery of eggs was found to be higher using the method currently recommended by WHO (1989), but only when 10 litre samples, rather than 1 litre samples, were processed.

Many methods for the enumeration of helminth eggs in wastewater are described in the literature. Each method has its own advantages and disadvantages: some have a high percentage recovery, but are very time-consuming; many are not reported in sufficient detail for replication to be possible, or their recovery rate is unknown; some require prohibitively expensive chemicals or are otherwise unsuitable for use in laboratories with limited equipment; and others only recover a limited range of species. It is clear that there is no one method that is universally useful, recovers all the helminth eggs of medical importance, and has a known rate of recovery.

Table 7.2: Tentative methods for quantitative determination of helminth eggs in wastewater

Method	Principle	Volume (L)	Settling	Centrifuge	Buffer or detergent	Flotation	Calculation	Recovery rate	Notes	Reference
WHO (1989)	Sedimentation	≥ 1	Over night	1000 g for 15 min	Acetic acid (pH 4.5)	Zinc sulphate (relative density 1.18)	$*N = \frac{X.\ V}{P.\ S}$	---	Raw & effluent samples	WHO (1989)
WHO (1989)	Centrifugation and flotation	1	Over night	700 g for 10 min	---	Sodium nitrate (relative density 1.3)	Total No. recovered from 1L	70% 100 eggs 50% 10 eggs 33% 1 egg	Raw & effluent samples	WHO (1989)
Leeds I	Sedimentation	1	(all centrifuge)	2500 rpm for 10 min	0.01% Triton X 100	$MgSO_4$ or NaCl (relative density 1.3)	---	24 ± 4%	Raw sewage	Ayres *et al*. (1989)
Leeds II	Sedimentation	4	1 hr	2500 rpm for 10 min	0.01% Triton X 100	NaCl (relative density 1.04)	Doncaster counting dish	80%	Effluent of stabilisation ponds	Ayres *et al*. (1989)
Stien-schwarts brod	Sedimentation and flotation	25	2 hr	1000 g for 15 min	Ether/ butanol/ acetic acid (pH 4.5)	♦Janeckso Urbanyi reagent (relative density 1.42)	$♠N = \frac{M.\ A}{P.\ V}$	50%	Raw & effluent samples	Stein & Schwartzbrod (1988)

***N** : No. of eggs/ L
X : No. of eggs counted
V : Total volume of product (ml)

♠N : No. of eggs / L
A : No. of eggs counted
M : Volume of the meniscus (ml)

♦100g mercuric iodide
80g potassium iodide
250ml distilled water

The enumeration of intestinal helminth eggs and larvae in wastewater, however is much less straightforward. A great variety of human and animal parasite species, as well as free-living species, may be present, varying in size, specific gravity and surface properties, and a much lower concentrations than in faeces, sludge or compost.

Bouhoum & Schwartzbrod (1989) compared a range of methods for faecal analysis with a view to adapting them for wastewater samples. Of the wide range of flotation solutions tested, they found that iodomercurate (Janeckso & Urbany, 1931) concentrated the greatest range of species of parasitic helminth eggs, but concluded that the reagent was too corrosive and expensive for routine use. Arthur's method (described in Faust *et al*, 1938), in which saturated sacruse is used as a flotation solution, was found to deform eggs rapidly, while zinc sulfate solution (Faust *et al,* 1938) did not concentrate *Trichuris* spp or *Capillaria* spp. very well. Bouhoum & Schwartzbrod (1989) concluded that Bailenger's method (Bailenger, 1979), which they adapted for wastewater, was the best method overall, it requires relatively inexpensive reagents and successfully concentrates the full range of species routinely found in wastewater.

This modified Bailenger method is generally useful, simple and cheap. However, its limitations are well recognized (see below), and there is still a need for its further evaluation. Nevertheless, of all the methods available, it reliably recovers the eggs of the intestinal nematodes mentioned in Table 5.1, is replicable, and is already widely used in laboratories around the world.

7.2.1 TECHNIQUE FOR THE ENUMERATION OF PARASITIC HELMINTH EGGS IN WASTEWATER (WHO, 1989)

This method relies on a sedimentation-floatation procedure selected from more than 20 different procedures tested under field conditions in several countries (Bouhoum & Schwartzbrod, 1989; Ayres & Mara, 1996).

ADVANTAGES AND DISADVANTAGES

The modified Bailenger method has the following advantages:

- Sample collection and preparation are straightforward.
- Minimum laboratory equipment is needed. A few special chemical reagents are required but are usually both locally available and inexpensive.
- Microscopic counting cells (McMaster or Sedgwick-Rafter) are routinely used in parasitology laboratories, and should be readily available from laboratory supply companies.
- For greater accuracy and to check the homogenization, replicate samples can be examined from each sample and the arithmetic mean count calculated.
- The method has the following disadvantages:
- The percentage recovery of eggs is not known, but it has been shown that it compares favourably with that in all other methods (Ayres *et al,* 1991; Bouhoum & Schwartzbrod, 1989). This method would successfully recover a wide range of helminth eggs including *Ascaris* spp , *Trichuris* spp , *Capillaria* spp , *Enterobius vermicularis, Toxocara* spp , *Taenia* spp., hookworm and *Hymenolepis* spp.
- The method is not suitable for many of the operculated or trematode eggs Some of these eggs may float in the zinc sulfate flotation solution but sink again quickly or become distorted, making accurate identification difficult.
- Ether is highly flammable and tox/c Ether can be replaced by ethyl acetate for the extraction of parasite eggs from faeces without any loss in efficiency (Rude *et al,* 1987). Ethyl acetate is much safer than ether; it has lower boiling and flash points and is less toxic.

Equipments and Consumables.

Reagents:

The reagents required are the following:

Aceto-acetic buffer (pH 4.5): 15g of sodium acetate trihydrate with 3.6 ml of glacial

acetic acid made up to 1 litre with distilled water.

Ether or ethyl acetate

Saturated zinc sulfate solution: 33%, relative density 1.18

Detergent solution: 1 ml triton X-100 or Tween 80, made up to 1 litre with tape water

Equipment

The following will be required.

Centrifuge: capable of generating l000g

Centrifuge tubes

Vortex mixer

Siphon

Graduated cylinder

Microscope

Hydrometer: Specific gravity 1.000 to 1.620, of clear polycarbonate, for testing

liquids heavier than water.

Sedgwick-Rafter Cell: Glass counting cell 50 mm x 20 mm x 1 mm deep is formed by four matte surfaced strips cemented in 76 x 34 mm slide, overall thickness 3.5 mm.

Plastic containers for sample collection, open-topped, straight-sided should be used.

1. Collect a grab sample of wastewater of known volume (V litres), usually 1 litre for raw or partially treated wastewaters and 10 litres for final treated effluents.
2. Allow the sample to settle for 8 hours, sedimentation can occur overnight and the procedure is continued the next day.
3. The supernatant is carefully removed and discarded without disturbing the sediment, using a suction pump or siphon.
4. Transfer the sediment to one or more centrifuge tubes. The wall of the sedimentation container should be washed thoroughly using a spray bottle with detergent solution, and add the rinsing to the sediments in the centrifuge tubes. All the recovered material is centrifuged at 1000 g for 15 minutes.
5. The supernatant is removed and discarded. Transfer all the sediments to one tube. Remember to rinse thoroughly with detergent solution to ensure that no sediment is discarded. Recentrifuge at 1000 g for 15 minutes.
6. Remove the supernatant, and suspend the pellet in an equal volume of acetoacetic buffer (pH 4 5) and stir the mixture.
7. Ether is then added at a volume equal to twice that of the buffer and the mixture is stirred for 10 minutes.
8. Centrifuge the sample at 1000 g for 15 minutes. The sample will now have separated into three distinct phases. All the non-fatty, heavier debris, including helminth eggs, larvae and protozoa, will be in the bottom layer. Above this will be the buffer, which should be clear (by controlling the pH, the hydrophilic-lipophilic balance of the parasite eggs in relation to the extraction medium will be modified so as to optimize the concentration of parasite eggs. Acetoacetic buffer at pH 4.5 was found to be the most suitable for the concentration of a wide range of helminth eggs

(Bailenger, 1979)). The fatty material moves into the ether and forms a thick dark plug at the top of the sample.

9. Discard the supernatant and resuspend the pellet in five volumes of saturated zinc sulfate solution. Record the volume of the final product (X ml). Mix the sample thoroughly, preferably using a vortex mixer.
10. Remove an aliquot with a Pasteur pipette and transfer to microscopic counting cell for final examination.
11. Leave the full slide to stand on a flat surface for 5 min before examination. This allows all the eggs to float to the surface.
12. Place the counting slide on the microscope stage and examine under 10x or 40x magnification. For greater accuracy, the mean of two slides, or preferably three, should be recorded.
13. The total number of eggs per litre (N) present in the original sample of wastewater is determined from the equation:

$$N=AX/PV$$

N = number of eggs per litre of sample

A = number of eggs counted in the counting slide or the mean of counts from two or three slides

X = volume of the final product (ml)

P = volume of the microscopic counting cell, e.g., McMaster cell (0.3 ml), Sedgwick-Rafter cell (1 ml).

V = original wastewater sample volume.

Notes:

- The sample containers should have clean smooth internal walls and be prewashed with a detergent to remove any grease and thus decrease the chances of helminth eggs adhering.
- Remove the supernatant carefully using a water siphon or pump as it is very easy to suck up eggs with other debris in the pellet. A glass pipette or plastic pipette tip may be inserted into the siphon tube for greater control.
- The supernatant must not be poured off as eggs can be lost in this way.

Chapter

8 DETERMINATION OF HELMINTH EGGS VIABILITY

8.1 Introduction

Several techniques have been reported in the literature for viability determination of *Ascaris* spp. eggs, and somewhat fewer for other helminths. The public health significance of dead eggs is minimal; however, when organisms are infective or viable, the risk to public health can be enormous. *Ascaris* eggs are environmentally persistent, with survival times usually in excess of one year; the minimum infective dose is only one egg. Hence, information about the viability of *Ascaris* eggs is of considerable importance.

One of the major difficulties in the work of helminthologists has been to differentiate between living and dead nematodes. Techniques used for assessing the viability include:

1. Morphological criteria;
2. Incubation technique (Hass and Todd, 1962; Meyer *et al.,* 1978; Cacares *et al.,* 1987) (Table 8.1);
3. Exclusion or inclusion of vital dyes (Shepherd, 1962; Kagei, 1982; Kaneshiro & Stem, 1985; Zohu *et al.,* 1985; and Hindiyeh, 1995) (Table 8.2);
4. Differential flotation technique used to separate fertile from non-fertile eggs (Stien & Schwartzbrod, 1988);
5. Induced larval motility using sodium hypochlorite as a possible viability test for embryonated *Ascaris* eggs (Keller, 1951 b; Smith, 1991); and
6. Infectivity in animal models (Reimers *et al.,* 1989). The cost, time and large numbers of eggs required to perform animal infectivity studies make the method impractical for routine use.

(1) Morphological Criteria

The direct morphological microscopic method is simple, but it lacks objective standards, requires skill and experience; and is not easy for beginners. Failure of larvae to move may not signify death; living and dead eggs cannot be distinguished by direct observation. Only when degeneration is apparent can death be confirmed, and this may take many weeks or months. Reimers *et al.* (1989) found a good correlation between the estimation of viability of *Ascaris* eggs using morphologic criteria and the method of culturing the eggs, while Ayres (1992) found that the morphological criteria method was not as accurate as the culturing method.

Some of morphological changes appearing in dead *Ascaris* eggs are as follows:

- Vacuolation in the cytoplasm of egg cells, presumably due to fatty degeneration;

- Cytolysis;
- Shrinkage of egg cells;
- Caving in of a portion of the cell surface of the egg shell or of the protein coat; and
- Formation of large retractile granules within the cell.

(2) Incubation Technique

Different researchers for culturing *Ascaris* eggs, to detect their development and viability (Table 8.1) have used wide variations of culture media. Oksanen *et al.* (1990) found that using 0.1N H_2S0_4 or tap water as a culture media for *Ascaris* eggs embryonation, in which they are left for incubation at 25-30°C for 21-30 days, did not alter the rate of embryonation and the eggs were equally infective after culturing, while in 1% buffered formalin eggs developed slightly more slowly.

Table 8.1. Culture media used to study the development and viability of Ascaris eggs

Culture media	Reference
10% potassium bichromate	Cram (1924)
0.1% Formalin	Brown (1928)
1% sodium carbonate	Passy & Faibaim (1995)
Normal saline solution + 5% formalin	Bhaskaram *et al.* (1956)
0.1 N sulphuric acid	Fairbairn(1961)
Distilled water	Kiff& Lewis-Jones (1984)
Tap water	Arfaa (1978)
2% sodium dichromate	Fleming (1987)

The technique described by Hass and Todd (1962), Meyer *et al.* (1978), as modified by Carrington and Hannan (1981), was used to enumerate and determine the viability of helminth eggs in sewage sludge by incubation. The technique is relatively simple, as eggs in sewage are extracted and removed immediately into a solution of 0.1 N H_2SO_4 in which they are left for incubation at 25-30°C for 21-30 days. The main disadvantage of the incubation technique is that it takes several weeks for embryonation, making the method impractical for routine use.

(3) Vital Stains Method

Several authors have reported the use of stains for the determination of viability, although in the past these have not been considered sufficiently reliable to be used (WHO, 1967). The two main disadvantages of the staining process are that some of the stains used have a toxic effect on the eggs, and that staining does not always take place immediately after death (e.g. for potato root eelworm larvae using iodine and Potassium iodide solution; Boyd, 1941). Eosin and Acridine orange were also tested by Tennant (1964) but were found to be relatively toxic. Trypan blue has been used for cell viability but may be inaccurate in the identification of dead cells; cells must be counted within 3-5 min because the number of blue-staining cells increases with time (Hudson & Hay, 1980).

Table 8.2. Summary of literature reported to distinguish dead and live helminth eggs and larvae using vital stains

Vital Stain	Concentration	Helminth	Reference
Sudan III	In 75% alcohol	*Ascaris* ggs	Ogata (1925) Kagei (1982)
Iodine and Potassium iodide	0.025 g 1%	*Heterodera* spp. larvae[a, b]	Boyd (1941)
Chrysoidin	50 mg/ L	*Heterodera* spp. larvae	Doliwo (1956) [c]
Phloxine B	5%	*Meloidogyne* eggs[b]	Fenner (1962)
New blue R	0.05%	*Heterodera* eggs	Shepherd (1962)
Eosin Y	0.67%	Free-Living nematodes	Chaudhuri *et al.* (1966)
Methylene blue-cosin borax	d	*Ascaris* eggs	Shou *et al.* (1985)
Methylene blue	0.05%	*Ascaris suum* infective larvae	Arene (1986)
Tetrazolium salts	0.25%	*Taenia* eggs	Owen (1984)
Mendola's blue	0.1%	*Taenia* eggs	Storey (1987)
Crystal violet	c	*Ascaris* eggs	Hindiyeh (1995)

a) Potato root eelworm larvae

b) Plant parasitic nematode spp.

c) Cited by Chaudhuri *et al.* (1966)

d) A detail in the Appendix 2 for preparation working solutions.

Keller (1951 a) used Trypan blue, Thionine blue, Methyl green, Neutral red, Congo red, Eosin malachite green, Sudan III and Kresofuchin stains to detect the viability of *Ascaris* eggs, and did not find any to be satisfactory. Kaneshiro and Stern (1985) concluded after testing different vital dyes that none of these dyes proved satisfactory because no single dye was able to differentiate between viable and non-viable eggs of *Ascaris*, *Toxocara*, *Trichuris* and *Hymenolepis* spp. all together. They conclude that several vital stains that were excluded from live cells, were capable of discriminating between viable and non-viable eggs of each species of parasite separately. Crystal violet and Nile blue sulphate were excluded from live and absorbed by dead eggs of *Ascaris suum.* Benzopurpurin, Saphranin O, Neutral red, methanol, ethanol, and acetone: ethanol fractions of the Nile blue sulphate mixture were capable of distinguishing between viable and non-viable eggs of *Trichuris vulpis.* Benzopurpurin was taken up by dead *Toxocara canis* eggs and excluded from living ones, whereas Nile blue sulphate was taken up by viable *Toxocara* eggs and excluded from non-viable ones. Saphranin O, Brilliant cresyl blue and Neutral red were all capable of discriminating between viable and non-viable eggs of *Hymenolepis diminuta.*

Arene (1986) confirmed the judgement of dead *Ascaris suum* larval-stage by using 0.05% Methylene blue for 5 minutes; living larvae remained unstained, while dead larvae stained deep blue and retained the blue coloration even after two washings by centrifugation in distilled water.

Sudan III staining was used by Ogata (1925) for the purpose of distinguishing between live and dead *Ascaris* eggs. The red-stained granules of "fat-corpuscles" gradually decreased in size as the size of the larva increased at each stage of its development. Also, he found that unfertilised eggs were stained with red granular spots, while healthy fertilised eggs were not stained at all. Fertilised eggs killed by boiling water were seen to have fine red colouring.

To differentiate between living and dead nematodes or eggs over a wide range of genera and species, Shepherd (1962) soaked nematodes in 0.05% aqueous solution of the New blue R basic vital stain for varying periods, for up to 24 hours. *Heterodera* eggs were soaked for up to 7 days; after the requisite

time, dead specimens were darkly stained but live ones were unstained.

Fenner (1962) determined nematode mortality following heat treatment by adding a drop of 5% aqueous Phloxine B to nematodes in a few ml of water. Dead specimens and *Meloidogyne* eggs stained immediately but dying larvae were slow to accept stain. Chaudhuri *et al.* (1966) used Eosin-Y and found that a 0.67% aqueous solution stained 99% of heat killed, free-living nematodes within 30 minutes, but not live ones; it also stained nematodes killed by freezing or by various chemicals.

Vital stain tests on eggs of *Heterodera schachtii* by Moriarty (1964) led to the conclusion that phloxine B was unsatisfactory for determining viability. However, chrysoidin was satisfactory, and gave an accurate measure of viability as measured by the hatching test, although it had the disadvantage of needing high magnification, because the colour difference cannot be observed at low magnification. New blue R may be useful for estimating viability of nematodes subjected to heat or chemicals but not populations that have died from "natural causes".

In research about the survival of tapeworm eggs during simulated sewage treatment processes. Storey (1987) used Mendola's blue vital stain with an efficiency of 74% to distinguish between dead and living *Taenia* eggs. Owen (1984) suggested that staining of viable eggs with tetrazolium salt after treatment with bile and pepsin provided a positive and conclusive test of the viability of *Taenia* eggs. Under microscopic examination viable embryos will show deposition of dark, formazan dye whilst the non-viable embryos remain colourless.

8.2 Basic Vital Stains as a Presumptive Method for Determining *Ascaris* Eggs Viability (Hindiyeh, 1995)

Using Crystal violet as a presumptive vital staining method has the potential for use in the rapid assessment of *Ascaris* eggs viability.

The use of vital stains to investigate the viability of *Ascaris* eggs has several advantages over previously used methods. First the vital staining procedure is simple to perform, reliable, inexpensive, rapid (5 to 10 min), and practical for routine use. Second, Crystal violet method does not need to be performed carefully to obtain optimum staining, except at extreme pH values. The third advantage is that no particular attention need to be paid to the concentration of Crystal violet stain used as a function of the number of eggs and or storage of the working solution. Fourth, direct results on the viability of eggs can be obtained on groups and individual eggs, which can be subjected to further experimentation without compromising the sample. Fifth, the method is inexpensive, only needing a phase contrast microscope, which is available in most laboratories. The sixth advantage is that, Crystal violet stain is not toxic and acts as good culture media for embryonation and hatching eggs. Seventh, the fact that eggs can still be stained after one month storage at 4°C will help to ensure a constant supply of suitable reference material for teaching and diagnosis. Lastly, the effects of disinfectants and various environmental factors on the viability of *Ascaris* eggs can be tested with vital stains on the same population of eggs both before and after exposure to chemical and physical agents.

The dye exclusion test for egg viability depends upon the fact that those viable eggs has cells that do not take up certain dyes, whereas non-viable cells do. It has been shown that non-viable cells, which take up the dye, do not respire and glycolyse, and cannot decolourize basic dyes (Phillips, 1973).

Preparation of Crystal Violet Working Solution:

The working solutions made up in ammonium oxalate solution consisting of 2g of crystal violet, 20 ml 95% alcohol, mixed with 80 ml of 1% aqueous ammonium oxalate (Lillie, 1977).

Technique

1. Extract *Ascaris* eggs form wastewater, sludge, uterus, etc. Mix *Ascaris* eggs with 7% sodium hypochlorite for 30 minutes, to remove the eggshell.
2. Wash the eggs at least five times with distilled water until neutral pH was achieved.
3. Mix 2-3 drops of crystal violet solution with an egg suspension, or place one drop from the egg

suspension on a clean glass slide; the end of a wire loop touched a drop of vital stain and mixed it thoroughly with the eggs.

4. After 10 minutes, use light or phase contrast microscope to distinguish between non-viable *Ascaris* eggs that accumulated the stain (blue), whereas viable eggs excluded the dye and were colorless.
5. It is suggested that if all the eggs in the specimen take up the stain it can be assumed that approximately 100% of the egg sample under examination are dead, in this case no confirmation by the incubation method is needed. If one egg or more are unstained (viable) in the specimen, the confirmation of the egg mortality must be obtained by using incubation method. The resulting unstained eggs should incubated at 26 °C with 0.1 N H_2 SO_4 or tap water until control ova of *Ascaris lumbricoides* or *A. summ* are fully embryonated. The concentrate is then microscopically examined for parasite ova using a Sedgwick-Rafter counting chamber.

8.3 Use of N- Butanol for Determination of *Ascaris* Eggs Viability

The Stien-Schwartzbrod technique (1988) uses n-butanol as part of the procedure to separate fertile and infertile eggs. A change in the structure of the coat in fertilised eggs allows the esterfication of lipids by the alcohol and so increases the specific gravity of the eggs causing them to sediment, whilst unfertilised eggs remain in suspension. Laboratory work with *Ascaris suum* has shown a very good correlation between fertile and viable eggs, so the technique can be assumed to enumerate viable *Ascaris* eggs. It is not known if the same procedure applies to other helminth eggs. n-Butanol cannot be used in samples of sludge or compost as it becomes absorbed to the solid materials and makes final examination of material impossible even if the samples thoroughly washed.

The egg suspension was mixed with an equal volume of n-butanol and centrifuged at 1,000 g for 5 minutes This separates fertile eggs, which sediment, from non-fertile ones, which float. The pellet of fertile eggs are carefully recovered and washed twice with distilled water.

Note: This method for *Ascaris* eggs and wastewater only.

8.4 Method for the Enumeration and Viability Determination of Helminth Eggs in Sewage Sludge

The technique described by Meyer *et al.* (1978), with modifications by Carrington and Harman (1981), is used as detailed below. The technique is convenient as eggs are extracted and removed immediately into a solution of 0.IN H_2SO_4 in which they are left for incubation.

Method:

1. 100 ml of 2.62% sodium hypochlorite (50% dilution of Chlorox) is added to 75 ml of sludge in a plastic bottle and mixed thoroughly by swirling for 2-3 min.
2. The solution is left to stand for 5-10 min while the foam subsides; then the total volume is made up to about 225 ml with dilute sodium hypochlorite solution and allowed to stand at room temperature for 50 min.
3. The floating scum is removed by suction.
4. The sample is centrifuged at 800g for 2 min and the supernatant fluid is removed by aspiration.
5. Two ml of anionic detergent (Tween 80) is mixed with the pellet by shaking and the volume then made up to 225 ml with distilled water and the mixture is centrifuged as before.
6. The pellet is washed twice more in the same way with distilled water.
7. The pellet is suspended in 75 ml of zinc sulphate solution, sp. Gr. 1.2 (approximately 33.2%) and centrifuged again at 800g for 2 min. leaving the eggs in the supernatant.
8. The centrifuged sample is allowed to stand for 2 min to ensure the flotation of all the eggs, longer standing time results in eggs becoming adhered to the sides of the container.

9. The supernatant is decanted onto a 45 mm diameter membrane with a 0.45 µrn pore size and filtered by negative pressure.
10. The filter holder is flushed with a stream of water to dislodge any eggs adhering to the walls and the membrane is also washed well with water.
11. The membrane is placed in a petri dish filled with 0.1 N H_2SO_4 and the eggs removed from the membrane by scraping gently with a glass cover slip and then rinsing the membrane on each side with 0.1 % H_2SO_4. At this point the technique differs slightly from the published technique.
12. In order to count the eggs before incubation the solution containing the eggs is centrifuged at 800g for 2-3 min and the supernatant removed leaving a pellet of 3-4 ml (with less than 0.25 ml solids) The final volume is recorded (Y).
13. The sample is thoroughly homogenised and a 1 ml subsample immediately pipetted into a Sedgwick Rafter counting chamber Sedgwick Rafter counting chambers contain 1 ml and are divided into 1000 x l µm squares.
14. Eggs per ml are calculated by counting the number of eggs in 20 squares chosen randomly using a random numbers table to avoid areas of uneven distribution within the chamber.
15. Two chambers are counted for each sample and the final result expressed as a mean of the two counts.

Calculation No. of eggs (N)/litre sludge = (X x 50) Y 13.333.

Where 1000ml/75ml = 13.333

8.5 Analytical Method for Viable Helminth Ova in Sludge

The described test procedure was developed for solid and semi-solid samples. It is not suitable for water or sewage. A total solids analysis is also required to express the final results as ova/g dry weight.

Procedure 388, 389, 390, 391, 392, 393: Helminth Ova (11-25-91)

1. Scope and Application

- This procedure determines (CSDLAC) parameter numbers 388, Total Parasites; *389, Total* Ascaris, *390, Viable* Ascari; *391,* Trichuris, *392;* Hymenolepis; 393, *Toxocara.*
- This procedure is applicable to composted sewage sludge and other solid and semi-solid materials.

2. Summary of Procedure

This procedure identifies, quantifies and determines the viability of several types of ova from intestinal parasites. Solid samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particles. The solids in the screened portion are allowed to settle out and the supernatant decanted off. The sediment is subjected to density gradient centrifugation using zinc sulfate (specific gravity 1.20). This flotation procedure yields a layer most likely to contain *Ascaris* and some other parasitic. Proteinaceous material is removed using an acid-alcohol/ether extraction step and the resulting concentrate is incubated at 26 °C until control ova *of Ascaris lumbricoides* var. *suum* are fully embryonated. The concentrate is then microscopically examined for parasite ova using a Sedgwick-Rafter counting chamber.

3. Advantages and Limitations

- Concentration of the sample increases the probability that ova will be detected if they are in the

sample.

- *Ascaris* ova as an indicator are advantageous since they are relatively large and easy to identify.
- Seeded studies have indicated the recovery for this test to be approximately ninety percent.
- The test uses standard microbiological equipment.
- The test requires intensive training to enable the analyst to identify ova in a complex mixture of debris and to determine the viability.
- The test may take up to 5 weeks to complete including sample processing and incubation.
- Numerous transfers of the sample to new vessels may decrease the recovery of indigenous ova.

1. Sample Handling and Preservation

- Solid samples are collected in sterile bags such as Whirl-Pack bags Liquid sludge samples are collected in clean screw cap containers such as Nalgene bottles or jars.
- Samples not analyzed promptly are stored at 0 °C to 4 °C.

5. Apparatus

- Standard light microscope.
- Sedgwick-Rafter cell.
- 2 L Pyrex beakers.
- Table-top centrifuge.
- Rotor to hold four 100ml centrifuge tubes, preferably glass or Teflon.
- Rotor to hold eight 15 ml conical centrifuge tubes, preferably glass or Teflon.
- 48 mesh Tyier sieve.
- Large plastic funnel to support sieve.
- Teflon spatula.
- Large test tube rack to accommodate 100 ml centrifuge tubes.
- Small test tube rack to accommodate 15 ml conical centrifuge.
- Number "0" rubber stoppers.
- Wooden applicator sticks.
- Vacuum source.
- Vacuum flask, 2 L or larger.
- Stopper to fit vacuum flask fitted with glass or metal tubing as a connector for ¼ inch tygon tubing.
- Pasteur pipettes.
- Incubator at 26°C.

6. Reagents

- Phosphate-buffer solution: Prepare stock phosphate buffer solution by dissolving 34.0 g potassium dihydrogen phosphate (KHO_4) in 500 ml distilled water, adjusting to pH 7.2 ±0.5 with 1 N NaOH, and diluting to 1 L with distilled water. Add 1.25 ml stock phosphate buffer solution and 5.0 ml magnesium chloride solution (81.1 g $MgCl_2.5H_2O$/L distilled water) to 1 L distilled water. Prepare phosphate buffer working solution containing 0.1%

(v/v) Tween 80. Adjust the pH to 7.2 ± *0*.1 with 1 N NaOH.

- Tween 80.
- Zinc sulfate solution, sp. gr. 1.20. Weigh 454 g $ZnSO_4$ into distilled water. Dissolve and check specific gravity with a hydrometer. Adjust specific gravity to 1.2 as necessary.
- 0.1 N H_2SO_4 in 35% ethyl alcohol.
- Ethyl ether, reagent grade.

7. Procedure

- Weigh 50 g (wet weight of compost and blend at high speed for 1 min. with 450 ml phosphate buffered water containing 0.1 percent Tween 80 to achieve a ten percent suspension. If the sample is a liquid sludge, pour it directly into a blender jar (400 to 500 ml) and add Tween 80 to 0.1% v/v prior to blending as above. Record volume tested.
- The moisture content (%) of the sample is for use in the final calculation of ova/g dry weight. The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.
- Pour the homogenized sample through a 48 mesh Tyier sieve held on a large funnel over a 2 L beaker.
- Wash the sample through the sieve with several rinses of warm tap water. Washings are caught in the beaker.
- Allow the screened and washed sample to settle overnight.
- Siphon off the supernatant to just above the settled layer of solids.
- Mix the settled material by swirling and then pour it into two 100ml centrifuge tubes.
- Rinse the beaker two or three times and pour the rinsing into two 100ml centrifuge tubes.
- Balance the tubes and centrifuge at 400 g for 3 min.
- Pour off the supernatant and resuspend the pellet thoroughly in zinc sulfate solution, specific gravity 1.20.
- Centrifuge the zinc sulfate suspension at 400g for 3 min.
- Pour the zinc sulfate supernatant into a 500 ml Erlenmeyer flask, dilute to at least half the concentration with deionized water, cover and allow to settle 3 hour or overnight.
- Aspirate the supernatant to just above the settled material.
- Resuspend the sediment by swirling and pipette into two to four 15 ml conical centrifuge tubes.
- Rinse the flask two to three times with deionized water and pipette the rinse water into the tubes.
- Centrifuge the tubes at 480g for 3 min.
- Combine the pellets into one tube and centrifugee at 480g for 3 min.
- Resuspend the pellets in 7 ml acid alcohol solution (0.1 NH_2SO_4 in 35% ethanol) and add 3 ml of ether.
- Cap the tube with a rubber stopper and invert several times, venting each time.
- Centrifuge the tube at 660g for 3 min.
- Resuspend the pellet in 4 ml 0.1 N H_2SO_4 and pour into Nalgene tubes with loose caps.
- Incubate the tubes at 26 °C for three to four weeks.
    - Simultaneously incubate control ova dissected form an adult *Ascaris suum*.

- When the majority of control ova are embryonated, samples are ready to be examined.
- Examine concentrates microscopically using a Sedwick-Rafter cell to enumerate detected ova.
- Note viability is based on the presence of embryonated ova whose larval forms can be induced to move when the light intensity is increased.
- Identify the ova and report as ova/g dry weight.

8. Calculation

- Calculate % total solids using the % moisture result: % Total Solids = 100% - % moisture.
- Calculate number of ova per dry weight in the following manner:

$$\textbf{Ova/g dry wt.} = \frac{(\text{No. ova}) \times (\text{Cell factor/ transects}) \times (\text{Final volume ml})}{(\text{Conc. \%}) \times (\text{Sample screened ml}) \times (\text{Total solids \%})}$$

Transect = One microscope field diameter width across the length of a Sedgwick-Rafter cell.

Cell factor = Number of transects to examine entire Sedgwick-Rafter. This is dependent on microscope model and magnification. Cell factor is also equal to the number of transects per ml since the Sedgwick-Rafter cell contains 1 ml.

9. Quality Assurance Guidelines

Run duplicate tests every tenth sample.

10. Precision and Accuracy

- Precision criterion was established as per standard Methods, 17th ed., 1989, 9020B.4b,pp. 9-17 to 9-18.
- The current established precision criterion is 0.5702.
- There is currently no means of assessing the accuracy of the method. (Theis *et al.,* 1978; Reimers *et al.,* 1981; Yanko, 1987; and EPA, 1992).

This is an expanded version of the Yanko (1987) method referenced in the Part 503 regulation. This expanded version provides additional detail and presents the method in a step-by-step fashion as provided in pages 393-1 to 393-6 of "Laboratory Section Procedures for the Characterization of Water and Wastes". 4th edition. Published by the Sanitation Districts of Los Angeles, California, 1989.

Chapter

9 MONITORING AND SURVEILLANCE OF THE RECLAIMED WATER AND SLUDGE FOR AGRICULTURE USE

9.1 Introduction

The compliance with standards and bylaws must be the prime concern of the regulatory agencies (Ministry of Health, Ministry of Environment, etc.). On it depends the protection of health and environment and the sustainable development. A comprehensive monitoring and surveillance programme is required to insure that proper treatment of wastewater is achieved and that environmental degradation is not occurring, and protection of the designated use of the water is achieved. Minimising adverse health impact, and at the same time, increasing agricultural production in sustainable manner with complete health and environmental safety is the main target of monitoring and surveillance. Therefore, the physical properties, as well as, the chemical and biological constituents of wastewater and sludge are important in the design and operation, collection, treatment and reuse of the treated effluent. Appropriate aspects for regular monitoring and surveillance must include checking the enforcement of the legal and administration measures by routine surveys. Disease surveillance focusing on farm workers and the population adjacent to the reclaimed watercourse is playing major role in updating the water quality surveillance programmes.

9.2 Pollutants and Guidelines

The usual guidance of the regulatory agency consists of three components:

- **Magnitude:** The level of pollutant (or pollutant parameter) generally expressed as allowable concentration.
- **Duration:** The period of time (average period over the in stream concentration is averaged for comparison with criteria concentration).
- **Frequency:** How often criteria can be exceeded.

The constituents of concern in wastewater treatment and reclaimed wastewater irrigation are listed in (Table 9.1). Surveillance and monitoring usually is based on both the technology available to treat the pollutants (i.e. technology-based effluent limits), and limits that are protective of the designated uses of the receiving water/reuse (water quality based effluent limits). The USA Clean Water Act (CWA) designated the following as conventional pollutants:

- Five day biochemical oxygen demand (BOD_5),
- Total suspended solids (TSS),
- PH,
- Faecal coliform, and
- Oil and grease (O&G).

The EPA guidelines for reclaimed water use in agriculture are summarised in (Table 9.2). USEPA requires that all Public Owned Treatment Works (POTW) must achieve Secondary Treatment Standard (Table 9.3), (USEPA, 1996). The Chemical oxygen demand (COD) and the total organic carbon (TOC) laboratory tests can provide an accurate measure of the organic content of wastewater in a shorter time frame than a BODs test (i.e, several hours versus 5 days). The regulation authority may substitute COD or TOC monitoring for BOD_5 when long-term BOD COD or BOD TOC correlation has been demonstrated. These secondary treatment standards must be applied as mass based limits using the operational flow of the plant. The concentration-based effluent limitation for both 30-day and 7-day average limitation can be applied (Table 9.3).

Table 9.1: Constituents of concern in wastewater treatment and irrigation with reclaimed wastewater

Constituents	Measured parameter	Reason for Concern
Suspended solids	Suspended solids including volatile and fixed solids	Suspended solids can lead to the development of sludge deposits and anaerobic conditions when untreated wastewater is discharged m the aquatic environment. Excessive amounts of suspended solids cause plugging m irrigation systems.
Biodegradable organics	Biochemical oxygen demand, chemical oxygen demand	Composed principally of proteins carbohydrates, and fats. If discharged to the environment, their biological decomposition can lead to the depletion of dissolved oxygen m receiving waters and to the development of septic conditions.
Pathogens	Indicator organisms, total and fecal coliform bacteria	Communicable diseases can be transmitted by the pathogens in wastewater bacteria. viruses, parasites.
Nutrients	Nitrogen, phosphorus, potassium	Nitrogen, phosphorus and potassium are essential nutrients for plant growth, and their presence normally enhances the value of the water for imgation. When discharges to the aquatic environment, nitrogen and phosphorus can lead to the growth of undesirable aquatic life. When discharged in excessive amounts on land, nitrogen can also lead to the pollution of groundwater.
Stable (refractory) organics	Specific compounds (e.g. phenols, pesticides, chlorinated hydrocarbons)	These organics tend to resist conventional methods of wastewater treatment. Some organic compounds are toxic in the environment, and their presence may limit the suitability of the wastewater for irrigation.
Hydrogen ion activity	pH	The pH of wastewater effects metal solubility as well as alkalinity of soils. Normal range in municipal wastewater is pH=6.5-8.5, but industrial waste can alter pH significantly.
Heavy metals	Specific elements (e.g. Cd, Zn, Ni, Hg)	Some heavy metals accumulate in the environment and are toxic to plants and animals. Their presence may limit the suitability of the wastewater for irrigation.
Dissolved inorganic	Total dissolved solids, electrical conductivity, specific elements (e.g. Na, Ca, Mg, Cl, B)	Excessive salinity may damage some crops. Specific ions such as chloride, sodium, boron are toxic to some crops. Sodium may pose soil permeability problems.
Residual chlorine	Free and combined chlorine	Excessive amount of free available chlorine (>0.05 mg/1 Cl_2) may cause leaf-tip burn and damage some sensitive crops. However, most chlorine in reclaimed wastewater is in combined form, which does not cause crop damage. Some concerns are expressed as to the toxic effects of chlorinated organics in regard to groundwater contamination.

Source: Pettygrove & Asano, 1988

Table 9.2: Summary of EPA guidelines for reclaimed water reuse in agriculture

Type of reuse	Treatment requirement	Water quality
Food crops not commercially processed	Secondary Filtration Disinfection	< 2.2 Fecal coliform/100 ml, 1 mg/1 Cl_2 residual after 30 min contact time (minimum). Turbidity 2 NTU/ 10 mg/L BOD.
Food crops commercially processed	Secondary Filtration Disinfection	200 Fecal coliform/100 ml, 1mg/ L Cl_2 residual after 30 min. contact time (minimum)/ 30 mg/ L BOD/ 30 mg/L SS.
Nonfood crops pasture, fodder, fiber and seed	Secondary Filtration Disinfection	200 Fecal coliform/100 ml, 1 mg/1 Cl_2 residual after 30 min. contact time (minimum)/ 30 mg/L BOD/ 30 mg/L SS.

Table 9.3 Secondary treatment standards

Parameter	30- day average	7- day average
5-day BOD $CBOD_5$ [a] Equivalent-to-secondary[b, c]	30 mg/L 25 mg/L 45 mg/L	45 mg/L 40 mg/L 65 mg/L
TSS	30 mg/L	45 mg/L
pH	6-9 (instantaneous)	---
Removal / Equivalent-to-secondary treatment definition[2,3]	85% BOD_5 and TSS 65% BOD_5	---

a Carbonaceous BOD_5

b Water quality must not be adversely affected by the application of equivalent-to-secondary treatment definition

c Trickling filter or waste stabilization ponds

9.3 Monitoring and Surveillance Guidelines

It is of vital importance to depend on perfectionism of the design to achieve the health requirement than depending on quality of operation and maintenance. The **helminth egg guideline** value (WHO, 1989) is intended as a design goal for wastewater treatment systems, and **not** as a standard requiring routine testing of effluent quality.

9.3.1 Sludge Application Guidelines

US EPA requires that all sludges applied to agricultural land, a forest, a public contact site, or a reclamation site, a lawn, or home garden (class A) must meet all the requirements to reduce pathogen densities to below detectable limits, which are:

Salmonella spp	Less than 3 per 4 grams total solid sewage sludge
Enteric viruses	less than 1 per 4 grams total solids sewage sludge
Viable helminth ova	less than 1 per 4 grams total solid sewage sludge
Viable helminth ova	less than 1 per 4 grams total solids sewage sludge and one of the vector attraction reduction requirements is achieved.

These vector attraction reduction requirements are designed to either reduce the attractiveness of sewage sludge to vector or to prevent the vectors from coming in contact with sewage sludge. EPA regulation contains 12 options for demonstrating reduced vector attraction of sewage sludge, summarized in Table 9.4 (US EPA 1992[c]). The potential for regrowth of pathogenic bacteria in Class A sludges makes it important to insure that substantial regrowth has not occurred. For this reason, all the Class A pathogen requirements alternatives require that:

- Either the density of fecal coliform in the sewage sludge be less than 1000 MPN per gram total solids (dry weight basis) or
- The density *of Salmonella* sp. bacteria in the sewage be less than 3 MPN per gram of total solids (dry weight basis).

These requirement must be met either:

- At the time of use or disposal
- At the time the sewage sludge is prepared for sale or given away in a bag or containers for agricultural uses (USEPA, 1992b).

9.3.2 Chemical Guideline for Sewage Sludge Application in Agriculture

WHO/ EOS/ 95,20. (1995) "Developing Human Health-related Chemical Guidelines for Reclaimed Wastewater and Sewage Sludge Application in Agriculture." This guideline is illustrated in Table 9.5, which stated that "the pollutant concentration of soil is more suitable global reference point than pollutant mass loading rate assessing potential negative impact of pollutant in soil, primarily because crop uptake of pollutants is a function of pollutant concentration in soil and because soil properties and environmental conditions are variable around the world. The same pollutant mass loading to soil with different background concentrations may result different soil pollutant concentrations."

9.3.3 Monitoring

Monitoring approaches for irrigation systems are different than for systems that discharge to streams. The objective of an irrigation site-monitoring program is to provide for early detection of problems. In most cases, simple adjustments can be made to the operation to avoid polluting ground or surface water. As a minimum, monitoring should occur at four spots in the system: 1) the treatment plant effluent, 2) storage, 3) irrigation system, 4) soil (and in some cases the vegetation and groundwater). The frequency of monitoring as suggested in Table 9.6 depends on public access to the irrigation site and the system size.

9.3.4 Treatment Plant Effluent

The treatment plant effluent should be monitored to ensure that 1) minimum treatment levels are achieved before it is discharged to the storage facility; 2) Test the reliability of reclaimed water to ensure efficiency of the treatment method in removing the pollution load; 3) Check whether any operational or maintenance works are needed, based on any change occurring on the parameters determined.

4) Test the reliability of sewage treatment plant. The effluent should be monitored for $CBOD_5$ and total coliform bacteria. Treatment systems using chlorine for disinfection may choose to monitor chlorine residual as an early warning for problems in the disinfection system. Total metal analysis is necessary for treatment plants receiving industrial wastewater.

9.3.5 Storage System

The storage system requires only limited monitoring. A weekly record of storage volume will help in managing the system to avoid future problems. A simple, easy to read staff gauge with cross-arms is an excellent way to measure liquid levels. Red markings at the top of the gauge give an easy indication that water levels are too high.

9.3.6 Irrigation System

For the irrigation system, precipitation and water applied by the system need to be monitored. Simple rain gauges placed in and near the application site can capture both precipitation and irrigation water.

Table 9.4: Summary of requirement for vector attraction reduction under US EPA 40 CFR part 503

Requirement	What is required	Most Appropriate for
Option 1	At least 38% reduction in volatile solids during sewage sludge treatment	Sewage sludge processed by - Anaerobic biological treatment - Aerobic biological treatment - Chemical oxidation
Option 2	Less than 17% additional volatile solids loss during bench-scale anaerobic batch digestion of the sewage sludge for 40 additional days at 30° C to 37 ºC (86 °F to 99 °F)	Only for anaerobically digested sewage sludge that cannot meet the requirements of Option 1
Option 3	Less than 15% additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20 °C (68 °F)	Only for aerobically digested sewage sludge with 2% or less solids that cannot meet the requirements of Option 1, e.g. sewage sludges treated in extended aeration plants
Option 4	SOUR at 20° C (68° F) is ≤1 5 mg oxygen/hr/g total sewage sludge solids	Sewage sludges from aerobic processes (should not be used for composted sludges)
Option 5	Aerobic treatment of the sewage sludge for at least 14 days at over 40 °C (104° F) with an average temperature of over 45 °C (113 °F)	Composted sewage sludge (Options 3 and 4 are likely to be easier to meet for sludges from other aerobic processes)
Option 6	Addition of sufficient alkali to raise the pH to at least 12 at 25° C (77° F) and maintain a pH ≥12 for 2 hours and a pH ≥ 11 5 for 22 more hours	Alkali-treated sewage sludge (alkalies include lime, fly ash, kun dust, and wood ash)
Option 7	Percent solids ≥ 75% pnor to mixing with other materials	Sewage sludges treated by an aerobic or anaerobic process (i.e. sewage sludges that do not contain unstabilized solids generated in primary wastewater treatment)
Option 8	Percent solids ≥ 90% pnor to mixing with other materials	Sewage sludges that contain unstabilized solids generated in primary wastewater treatment (e g any heat-dned sewage sludges)
Option 9	Sewage sludge is injected into soil so that no significant amount of sewage sludge is present on the land surface 1 hour after injection, except class A sewage sludge which must be injected within 8 hours after the pathogen reduction process	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, a forest, or a reclamation site, or placed on a surface disposal site
Option 10	Sewage sludge is incorporated into the soil within 6 hours after application to land or placement on a surface disposal site, except Class A sewage sludge which must be applied to or placed on the land surface within 8 hours after the pathogen reduction process	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, forest, or a reclamation site, or placed on a surface disposal site
Option 11	Sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day	Sewage sludge or domestic septage placed on a surface disposal site
Option 12	pH of domestic septage must be raised to ≥ 12 at 25° C (77° F) by alkali addition and maintained at ≥ 12 for 30 minutes without adding more alkali	Domestic septage applied to agricultural land. a forest, or a reclamation site or placed on a surface disposal site

*Source: Reference adapted from Control of Pathogens and Vector*Attraction in Sewage sludge EPA/625/R-92/013 1992

Table 9.5. WHO recommended pollutant concentration of soil for irrigating cropland with reclaimed wastewater and applying sewage sludge as fertilizer

Inorganic Elements	
Constituent	**Concentration in soil (mg/ kg DW)**
Aresnic	9
Barium	2900
Beryllium	20
Cadmium	7
Chromium	3200
Fluorine	2600
Lead	150
Mercury	5
Nickel	850
Selenium	140
Silver	3
Organic Compounds	
Compound	**Concentration in soil (mg/ kg DW)**
Aldrin	0.2
Benzene	0.03
Benzo (a) pyrene	3
Chlorodane	0.3
Chlorobenzene	ND
Chloroform	2
Dichlorophenols	ND
2,4-D	10
DDT	ND
Dieldrin	0.03
Heptachlor	1
Hexachlorobenzene	40
Hexachloroethance	2
Pyrene	480
Lindane	0.6
Methoxychlor	20
Pentachloroethance	320
PCBS	30
Tertrachloroethane	4
Tetrachloroethylene	250
Toluene	50
Toxahene	9
2,4,5-T	ND
2,3,7,8 TCDD	30

Source: WHO, 1995

9.3.7 Soil

The soil within the irrigation site is one of the integrators of all the material being applied. Soil samples can be analysed at the certified laboratory and collect a soil sample before irrigation begins and each year at the beginning of the application season. For systems over 500 m^3d, samples should be collected twice a year.

By testing a sample of soil from the same spot each year any possible accumulations of minerals and metals can be monitored. Monitoring tests should include the 1) biological tests to detect any pathogen incidence in the soil. Helminths here probably the greatest danger to human health of labourers, crops handlers and consumers. 2) Chemical tests to indicate undesirable or hazardous accumulation of elements, especially heavy metals in the soil. This will act as an early warning for possible surface or ground water contamination. If levels begin to get high, simple adjustments can be made in irrigation scheduling to avoid problems.

9.3.8 Crops

The crops/vegetation is a biological integrator of all of the material being applied. Both information on yield and plant tissue nutrient levels can act as an early warning system for problems. Plant tissue samples can also be analysed at a certified laboratory. Plant tissue tests can reveal nutrient imbalances and the need to add soil amendments such as lime, potassium, or phosphorus. Testing also should include the quality parameters of these crops to insure that the crops are always free from all pathogens or hazards of concern to public health.

9.3.9 Groundwater

Monitoring of groundwater quality is necessary especially whenever the soil and groundwater conditions are favourable of artificial recharge done through infiltration basins. The following groundwater quality parameters are to be monitored: suspended solids, total dissolved solids. nitrogen, phosphate, faecal coliform. Groundwater should be monitored up-gradient and down-gradient of large irrigation systems. Monitoring wells should be sampled at the beginning and end of the irrigation season for indicators of wastewater contamination.

9.4 Monitoring Programs

Monitoring programs for systems greater than 2,000 m^3d would be similar, but it needs to be developed individually to meet local conditions and wastewater characteristics. While much of the monitoring occurs during the irrigation period, some monitoring must continue year-round. Records of wastewater flow and storage volumes, for example, need to be recorded throughout the year. Depending on the pre-treatment system used, the effluent may also need to be monitored throughout the year.

9.5 Surveillance and Reporting Requirements

The following section is adapted mainly from U.S. EPA NPDES Permit Writers' Manual EPA-8333-B-96-003 (USEPA, 1996).

A typical regulatory agency requirements regarding the municipal WWTP contains the following:

- Effluent Limitations
- Effluent Monitoring Requirements
- Influent Monitoring Requirements
- Biosolids Monitoring Requirements
- Monitoring for Toxic Substances and Biomonitoring Requirements

- A Schedule of Compliance (includes actions needed and deadlines)
- Compliance Maintenance Annual Reports
- Special Report Requirements (including information required and submittal deadlines)
- General Conditions. This includes standard language for all wastewater dischargers
- Other Special Conditions as appropriate

A typical industrial regulatory requirements contains the following:

- Numeric Discharge Limitations
- Effluent Monitoring Requirements
- Biomonitoring Requirements
- Special Report Requirements (including information required and submittal deadlines):
- A Schedule of Compliance (includes actions needed and deadlines)
- General Conditions. This includes standard language for all wastewater dischargers

Periodic surveillance and reporting also serve to remind the operating body of its compliance responsibilities and provides feedback regarding the performance of the treatment facility(s). The monitoring and reporting procedures should contain specific requirement for the following items:

- Sampling location
- Sample collection methods
- Monitoring frequencies
- Analytical methods
- Reporting and record keeping requirements

Basic factors that may affect sampling location, sampling methods and sampling frequency are:

- Applicability of "effluent limitations guidelines" (ELG)
- Effluent and process variability
- Effect of flow and/or pollutant load on the receiving water
- Characteristics of pollutants and discharges
- WWTP compliance history

These factors must be carefully considered by the regulatory agency, as error could lead to inaccurate compliance determination, misapplication of national ELGs and/or misapplication of the national quality standards.

9.5.1 Establishing Surveillance Conditions

The regulating agency describes how surveillance conditions can be incorporated in the discharge/use permits. This requires that the implementing agency to monitor pollutant mass (or other applicable unit of measures), effluent volume, monitor all pollutants and report data at least once per year.

9.5.2 Monitoring Location

The regulatory agency is responsible for determining the most appropriate monitoring location and explicitly specifying those in the discharge regulation/permit. Specifying the appropriate monitoring location is critical to producing valid compliance data. Important factors to consider is selecting a monitoring location include:

- The wastewater flow should be measurable;
- The location should be easily and safely accessible;
- The sample must be representative of the expected effluent during the time period that is monitored;
- Permitting well mixing such as near a parshall flume or at location in a sewer with hydraulic turbulence. Weirs tend to enhance the settling of solids immediately upstream and the accumulation of floating oil or grease immediately downstream. Such location should be avoided for sampling.

The most logical monitoring point for an effluent is just prior to discharge/use to the receiving water. This is particularly true for ensuring compliance with water quality-based effluent limits.

9.5.3 Monitoring Frequency

The intent of the frequency is to detect most events of noncompliance without requiring needless or burdensome monitoring. The following factors that should be considered when establishing appropriate monitoring frequencies include:

- Estimated variability of the concentration of the parameter by reviewing effluent data for the facility;
- Design capacity of treatment facility;
- Type of treatment method used;
- Post compliance record/history;
- Cost of monitoring relative to discharger's capabilities(example of analytical cost is shown in Table 9.7);
- Frequency of the discharge;
- Number of monthly samples used in developing discharge/use regulatory limit;
- Tiered limits-where the regulatory agency has included "tiered" limits in the discharge/use permit, consideration should be given to varying the monitoring.

frequency requirements to correspond to the applicable tiers. For example, if a facility has seasonal discharge limits (irrigation), it may be appropriate to increase the monitoring frequency during the higher production season, and reduce the frequency during the off-season.

Table 9.6: Frequency of monitoring for land application and surface disposal

Amount of Sewage Sludge (metric to dry solids per 365-day period	Frequency
Greater than Zero but less than 290 dry weight basis	Once per year
Equal to or greater than 290 but less than 1.500	Once per quarter (four times per year)
Equal to or greater than 1. 500 but less than 15. 000	Once per 60 days (six times per year)
Equal to or greater than 15.000	Once per month (12 times per year)

Reference: Environmental Regulation and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-92/013:(1992)

Table 9.7. Estimated costs for common analytical procedures[1]

Parameters	JORDAN / RSS[3]US$	USA/US$
BOD_5	35.7	30
TSS	12.9	15
TOC	31.4	60
Oil and Crease	23.6	35
Odor	5.7	30
Color		30
Turbidity	4.3	30
Fecal coliform	31.4	15
Metals (each)	17.1	15
Cyanide	47.1	35
Gasoline (Benzene, Toluene, Xylene)		100
Purgeable Halocarbons (EPA Method 601)		113
Acrolein and Acrylonitrile (EPA Method 603)		133
Purgeables (EPA Method 624)		251
Phenols (EPA Method 604)	107.1	160
Organochlorine Pesticides and PCBs (EPA Method 608)		157
Polynuclear Aromatic Hydrocarbons (EPA Method 610)		175
Dioxin (2, 3, 7, 8 – TCDD) (EPA Method 613)		400
Base/Neutrals and Acids (EPA Method 625)		434
Priority Pollution Scan[2]		2.000
Acute WET		750
Chronic WET		1.500

Reference: U. S. EPA NPDES permit Writer's Manual (1996)

1. Based on 1994-1995 costs.
2. Includes 13 metals, cyanide, dioxin, volatiles (purgeables), base/neutral and acids, pesticides and PCBs, and asbestos.
3. These prices taken from Royal Scientific Society/Jordan 1999 rates.

9.5.4 Sample Collection Methods:

There are two basic sample collection methods, which must be specified in the monitoring programme including "grab" and "composite". The grab samples must be collected for pH, temperature, dissolved oxygen, chlorine, purgeable organics, sulfides, oil and grease, coliform bacteria and cyanide. The reason grab samples must be taken for these parameters is that they evaluate characteristics that may changed during the time necessary for composting.

A "grab" sample is a single sample collected at a particular time and place that represent the composition of the wastestream only at that time and place. When the quality and flow of wastewater being sampled is not likely to be changed over time, a grab sample is appropriate. Grab sample should be used when:

- The wastewater characteristics are relatively constant;
- The parameters to be analyzed are likely to change with storage such as temperature, residual chlorine, soluble sulfide, cyanides, phenols, microbiological parameters, and pH.

- The parameters to be analyzed are likely to be affected by composting process such oil and grease and volatiles;
- Information on variability over a short time period is desired;
- Composite sampling is practical or the compositing process is liable to introduce artifacts of sampling.
- The spatial parameter variability is to be determined. For example variability through the cross section and/or depth of stream or large body of water;
- Effluent flows are intermittent from well-mixed batch process tank. Each batch-dumping event should be sampled.

Grab samples can measure maximum effect only when the sample is collected during flows containing the maximum concentration of pollutant toxic to the test organism. Another type of grab sample is sequential. A special type of automatic sampling device collects relatively small amount of a sampled wastestream, with the interval between sampling either time or flow proportioned.

Unlike the automatic composite sampler, the sequential sampling device automatically retrieves a sample and holds in a bottle separate from other automatically retrieved samples. Many individual samples can be stored separately in the unit, unlike the composite sample, which combines in a common bottle. This type of sampling is effective for determining variation in effluent characteristics over short period of time.

A "composite" sample is a collection of individual samples obtained at regular interval, usually based upon time or flow volume. A composite sample is desirable when the materiel being sampled varies significantly overtime either as a result of flow or quality changes. There are two general types of composites and the regulatory agency should clearly express which type is required to be adapted:

- Time composite samples collect a fixed volume at equal time intervals and are acceptable when flow variability is not excessive. Automatically timed composited samples are usually preferred over manually collected composites. Composite samples collected by hand are appropriate for infrequent analyses and screening, composite samples can be collected manually if samples have a fixed volume at equal time intervals when flow variability is not excessive.
- Flow-proportional composting is usually preferred when effluent flow varies appreciably over time; the equipment and instrumentation for flow-proportional compositing have more downtime due to maintenance problems. When manually compositing effluent samples according to flow where no flow measuring device exist, use the effluent flow measurement without any correction for time lag. The error in the effluent and effluent flow measurement is insignificant except in those cases where extremely large volumes of water impounded, as in reservoirs.

There are numerous cases where composites are inappropriate. Samples for some parameters should not be composited (pH, residual chlorine, temperature, cyanides, volatile organics, microbiological tests, oil, and grease, total phenols). They are also not recommended for sampling batch or intermittent processes. Grab samples are needed in these cases to determine fluctuations in effluent quality.

For whole effluent toxicity (WET), composite samples are used unless it is known that the effluent is most toxic at a particular time. Some toxic chemicals are short-lived, degrade rapidly, and will not be present in the most toxic form after lengthy compositing even with refrigeration or other forms of preservation. Grab samples should be required for bioassays to be taken under those circumstances.

Eight types of composite samples and advantages and disadvantages of each are shown in Table 9.8. As shown samples may be composited by time or flow and a representative sample will be assured. However, where both flow and pollutant concentration fluctuates dramatically, a flow-proportioned composite sample should be taken because a greater quantity of pollutant will be discharges during these periods. As an alternative, time-proportioned samples may be taken with flow records for

weighing the significance of various samples.

Continuous monitoring is another option for limited number of parameters such as flow, total organic carbon (TOC), temperature, pH, conductivity, fluoride and dissolved oxygen. Reliability, accuracy, and cost of continuous monitoring vary with the parameter. Continues monitoring can be expensive, so continues monitoring will usually only be appropriate requirement for the most significant discharges with variable effluent. The environmental significance of the variation of any of these parameters in the effluent should be compared to the cost of continuous monitoring.

Table 9.8: Compositing methods

Method	Advantages	Disadvantages	Comments
Time Composite			
Constant sample volume, constant time interval between samples	Minimal instrumentation and manual effort, requires no flow measurement	May lack representativness, especially for highly variable flows	Widely used in both automatic samplers and manual handling
Flow-Proportional Composite			
Constant sample volume, time interval between samples proportional to stream flow	Minimal manual effort	Requires accurate flow measurement reading equipment, manual compositing from flowchart	Widely used in automatic as well as manual sampling
Constant time interval between samples, sample volume proportional to total stream Flow at time of sampling	Minimal instrumentation	Manual compositing from flowchart in absence of prior information on the ratio of minimum to maximum flow, chance of collecting too small or too large individual discrete samples for a given composite volume	Used in automatic samplers and widely used as manual method
Constant time interval between samples. sample volume proportional to total stream flow since last sample	Minimal instrumentation	Manual compositing from flow chart in absence of prior information on the ration of minimum to maximum flow, chance of collecting either too small or too large individual discrete samples for a given composite volume	Not widely used in automatic samplers but may be done manually
Sequential Composite			
Series of short period composites, constant time intervals between samples	Useful if fluctuations occur and time history is desired	Requires manual compositing of aliquots based on flow	Commonly used. however, manual compositing is labor intensive
Series of short period composites, aliquots taken at constant discharge increments	Useful if fluctuations occur and time history is desired	Requires flow totalizer, requires manual compositing of aliquots based on flow	Manual compositing is labor intensive
Continuous Composite			
Constant sample volume	Minimal manual effort, requires no flow measurement	Requires large sample capacity, may lack representativeness for highly variable flows	Practical but not widely used
Sample volume proportional to stream flow	Minimal manual effort, most representative especially for highly variable flows	Requires accurate flow measurement equipment large sample volume variable pumping capacity and power	Not widely used

Reference adapted from EPA 1996

9.6 Analytical Methods

The regulatory agency must specify the analytical methods to be used for monitoring. These are usually indicated as standards conditions. In particular, analytical methods for industrial and municipal wastewater pollutant must be conducted in accordance with the method specified in the regulation, which references one or more of the following:

- Test methods stated by the country bylaw or regulating agency.
- Standard Methods for the examination of water and wastewater. According to American Public Health Association, American Water Work Association, and Water Pollution Control Federation 1992.
- Standard Methods of the Examination of water and wastewater. 18th ed.
- Methods for the Chemical Analysis for Water and Wastewater ((USEPA 1979). Method for chemical Analysis of Water and Wastewater. EPA-600/ 4-79-020. Environmental Monitoring and Support laboratory.
- Test methods: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater. USEPA 1982 Test Methods: methods for Organic Chemical analysis of Municipal and Industrial Wastewater. EPA-600/4-82-057, in addition to test methods presented in chapter 7 and chapter 8 of this Guide.

In the absence of analytical methods for other parameters, the regulatory agency must still specify the analytical methods to be used. An excellent source of analytical methods information is the Environmental Monitoring Methods Index (EMMI).

9.7 Other Consideration in Establishing Monitoring Requirements

The regulatory agency should consider the cost of sampling, which can be imposed on the implementing body. The sample frequency and analyses impact the analytical cost. The estimated costs (USA 1994-1995) for analytical procedures are shown in Table 9.7 (USA EPANPDES, Permit Writers' Manual. EPA-833-B-96-003 96).

If simple or inexpensive indicator parameters (e.g., BOD_5 acts as an indicator for the priority pollutants in the treatment works) or alternate parameters will produce data representative of the pollutant present in the discharge, then the indicators or surrogate pollutants or parameters should be considered. Complex and expensive sampling requirements may not be appropriate if the regulatory agency cannot justify the need for such analyses.

9.7.1 Establishing Monitoring Conditions for Unique Discharges

There are a variety of discharges that are regulated under the regulatory agency monitoring program that are different than traditional wastewater discharges. Regulatory agency needs to account for these unique discharges in establishing monitoring requirements. This section discusses several of these unique discharges including storm water, combined sewer and sanitary sewer overflows, WET, and municipal sludge. Monitoring requirements vary according to the type of requirements regulating the storm water discharge and the activity. State programs may regulate storm water discharges.

9.7.2 Monitoring Combined Sewer Overflows and Sanitary Sewer Overflows

These monitoring conditions should require monitoring of a representative number of Combined Sewer Overflows (CSOs) for a representative number of wet weather event for certain key parameters along with ambient water quality monitoring to ascertain attainment with water quality standards. A

facility's regulations may also contain monitoring requirements for sanitary sewer overflows (SSOs). These can be developed on a case-by-case basis.

9.7.3 Whole Effluent Toxicity Monitoring

Whole effluent toxicity monitoring (WET) conditions included in regulatory requirement should specify the particular biomonitoring test to be used, the test species, required test endpoint, and QA/QC procedures. (Quality Assurance and Quality Control.) USAEPA has published recommended toxicity test protocols in the following four manuals:

- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. USEPA (1992) Methods for measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms.
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. USEPA (1991.)
- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA-600/4-91-003. Environmental Monitoring and Support Laboratory. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. USEPA 991. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; Third Edition; EPA-600/4-91-002. Environmental Monitoring and Support Laboratory.
- National Pollutant Discharge Elimination System (NPDES) Compliance Monitoring Inspector Training: Biomonitoring. USEPA (1990) NPDES Compliance Monitoring Inspector Training. Biomonitoring Office of Water.
- Samples for WET may be composite or grab samples. Twenty-four hour composite samples are suggested except when (1) the effluent is expected to be more toxic at a certain time of day; (2) toxicity may be diluted during compositing; and (3) the size of the sample needed exceeds the composite sampler volume (e.g., 5 gallons).
- WET tests are relatively expensive (Table 9.7 on costs). Therefore, the test frequency should be related to the probability of any discharge having whole effluent toxicity. Samples should be evenly spaced throughout the year so that seasonal variability can be ascertained.

9.8 Municipal Sludge Monitoring

The purpose of monitoring municipal sludge is to ensure safe use or disposal. The USEPA 40 CFR Part 503 sludge regulations require monitoring of sewage sludge that is applied to land, placed on a surface disposal site, or incinerated. The frequency of monitoring is based on the annual amount of sludge that is used or disposed by these methods. POTWs that provide the sewage sludge to another party for further treatment (such as composting) must provide that party with the information necessary to comply with regulation. Table 9.6 shows the minimum monitoring requirements for sewage sludge prior to use and disposal established in 40 CFR Part 503. More frequent monitoring for any of the required or recommended parameters is appropriate when the POTW:

- Influent load of toxics or organic solids is highly variable.
- Has a significant industrial load.
- Has a history of process upsets due to toxics, or of adverse environmental impacts due to sludge use or disposal activities. The sampling and analysis method specified in EPA 40 CFR 503.8 is recommended to be followed for monitoring the required parameters. In the absence of any specific methods in 40 CFR Part 503, guidance on appropriate methods is contained in *Part 503 Implementation Guidance, Control of Pathogens and Vector Attraction in Sewage Sludge,* and *POTW Sludge Sampling and Analysts Guidance Document.*

9.9 Reporting and Recordkeeping Requirements

The regulatory agency must specify that the implementing agency to keep records and periodically report on monitoring activities. Discharge Monitoring Reports (DMRs) must be used by implementing agency to report self-monitoring data. Data reported include both data required by the regulation and any additional data the implementing agency has collected consistent with regulation requirements. All facilities are required to submit reports (on discharges and sludge use or disposal) at least annually. POTWs with pretreatment programs are required to submit a pretreatment report at least. However, that monitoring frequency and reporting should be dependent on the nature and effect of the discharge/sludge use or disposal. Thus, the regulatory agency can require more frequent than annual reporting.

Records must be kept by the implementing agency for at least 3 years and this time may be extended by the regulatory agency upon request. An exception is for sewage sludge records that must be kept 5 years or longer if required. The regulatory agency should designate where records should be located. Monitoring records include:

- Date, place, time
- Name of sampler
- Date of analysis
- Name of analyst
- Analytical methods used
- Analytical results

Monitoring records must be representative of the discharge. Records, which must be retained, include continuous strip chart recordings, calibration data, copies fall reports for the regulatory agency, and copies of all data used to compile reports and applications. Sludge regulations usually establish recordkeeping requirements that vary depending on the use and disposal method for the sludge. The same recordkeeping requirements should be applied to other sludge monitoring parameters. Minimum frequency of monitoring for pathogen requirement depends on the amount of sewage sludge used or disposed annually (Tables 9.6 and 9.9).

Table 9.9: Minimum requirements for sewage sludge monitoring, based on method of sludge use or disposal

Method	Monitoring Requirements	Frequency
Land application.	1. Sludge weight and % total solids Metals Ax, Cd, Cu, Pb, Hg, Mo, Ni, Se, and Zn Pathogen reduction Vector attraction Reduction.	1.0 < and < 290*, annually 290 < and < 1,500, quarterly 1,500 < and <15,000, bimonthly 15,000>, monthly.
Co-disposal in municipal solid waste landfill.	1. Sludge weight and % total solids 2. Passes Paint-filter liquid test 3. Suitability of sludge used as cover 4. Characterize in accordance with hazardous waste rules.	1, 2, 3 and 4 Monitoring requirements or frequency not specified by EPA. Determined by local health authority or regulatory agency.
Surface disposal: lined sites with leachate collection and unlined sites	1. Sludge weight and % total solids Pathogen reduction Vector attraction reduction Metals As, Cr, Ni (unlined sites only) 2. Methane gas.	1. Based on sludge quantity (as above) 2. Continuously.
Incineration	1. Sludge weight and % total solids Metals As, Cd, Cr, Pb, and N1 2. Be and Hg (National emissions standards) 3. THC or CO, Oz, moisture, combustion temperatures 4. Air pollution control device operating parameters	1. Based on sludge quantity (as above) 2. As required by subparts C and E of 40 CFR Part 61 as may be specified by permitting authority (local air authority) 3. Continuously 4. Daily

Reference: EPA 1992a

* Dry weight of sludge in metric tons per year.

Notes:

- Monitoring frequencies required may be reduced after 2 years of monitoring but in no case shall be less than once per year.
- A successful land application program may necessitate sampling for other constituents of concern (such as nitrogen) in determining appropriate agronomic rates. This will be determined by the regulatory agency

Chapter

10

HEALTH ASPECTS FOR USE OF RECLAIMED WASTEWATER IN AGRICULTURE AND AQUACULTURE

10.1 Introduction

Reuse of the effluent is a very old human practice. The first irrigation farm recorded was constructed in Germany 1531. Interest in wastewater farming or land application increased particularly in Europe after the First Royal Commission on Sewage Disposal in England gave its official blessing to the practice. In its report of 1865 the Commission stated. “The right way to dispose of town sewage is to apply it continuously to the land and it is by such application that" the pollution of rivers can be avoided”. (Shuval *et. al.,* 1986). Another old system for sewage disposal is to discharge wastewater or effluent to surface or ground water. As an example, the populated area of the Rhein River Basin is estimated to be about 6 million inhabitants. These people are drinking from this river of which 20-40% of its flow is wastewater effluents. These figures can approach 100% in periods of extremely low flow. In the United Kingdom, the River Thames, which provides two-thirds of the water supply for the Greater London Area, is about 14% sewage effluent, when flowing at any average rate (WHO, 1973). In Greater Amman Area (Jordan) the septage percolation contributed a significant element of the total groundwater recharge. It appears that about one-third or more of the recharge in 1980 may have been via cesspools (Lawrance et. al., 1982).

The above proves the importance of the following statement of UNESCO, (1958): “There is no new water of the face of the earth and probably there is no previously unused water either”. In the Middle East area, as other developing countries, water is very scarce. For this reason each available drop of water must be used and managed in a safe and feasible way. "No higher quality water, unless there is a surplus of it, should be used for a purpose that can tolerate a lower grade." United Nations Economic and Social Council, (1958).

10.2 Health Aspects

The reuse of wastewater for irrigation is associated with biological agents (pathogenic viruses, bacteria, protozoa, helminths), and eventually reach others whom they enter via the mouth (for example, through the eating of contaminated vegetables as with case of *Ascaris*) or the skin (as in the case of the hookworms and schistosomes) (WHO, 1988). These kinds of agents are of serious concern in countries, which have diarrhea diseases, and nematodes infections; like the case in East Mediterranean Region (EMR). It is very important to understand transmission vectors and the health risk factors involved in the excreted pathogens. There are thirty known excreted infections of public health importance and these may be conveniently grouped into five categories of similar environmental transmission characteristics and pathogen properties as demonstrated in Table 10.1 (WHO, 1988).

Factors affecting the transmission of diseases depend on the followings:

- Survival time of the pathogen in soil, on crops or in water.

- The presence of pathogen in the required intermediate host or hosts, as in categories IV and V (WHO, 1988).
- The mode and frequency of excreta or wastewater application.
- The type of crop to which the excreta or wastewater is applied.
- The nature of exposure of a human host to the contaminated soil, water or crop.

The survival time of excreted pathogens in different environment for a temperature range of 20-30 °C are shown in Table 10.2 (Feachem *et. al.,* 1983). Table 10.2 indicates that nearly all excreted pathogens can survive in water, soil, and crops for a sufficient length of time to pose potential risks to farm and pond workers (Mara and Silva, 1986). Figures 10.1 and 10.2 demonstrate pathogen survival on crops and in soil which can be sufficient, in some cases, to pose potential risks to handlers and consumers, especially when survival time, are longer than a crops (principally vegetables) growing cycle (Mara and Pearson 1987).

Table 10.1: Environmental classification of excreted infections

Category epidemiological factors	Infection	Environmental transmission factors	Measure control measure
Non-latent; medium or high infective dose (I)	Amebiasis, Balantidiasis, Enterobiasis, Enteroviral infections	Personal Domestic	Domestic
Non-latent; moderately; persistent able to multiply (II)	Campylobacter infection cholera Pathogenic *Escherichia coli* infection Salmonellosis Shigellosis Typhoid Yersiniosis	Personal Domestic Water Crop	Domestic water supply Health Education Improved housing Provision of toilet Treatment of excreta prior to discharge or reuse
Latent and persistent; no intermediate host (III)	Ascariasis Hookworm infection Strongyloidiasis Trichuriasis	Yard Field Crop	Provision of toilets Treatment of excreta prior to land application
Latent and persistent; cow or pig as intermediate host (IV)	Taeniasis	Yard Field Crop	Provision of toilets Treatment of excreta prior to land application Cooking, meat inspection
Latent and persistent; aquatic intermediate host(s) (V)	Clonorchiasis Diphyllobothriasis Fascioliasis Fasciolopsiasis Gastrodiscoidiasis Heterophyiasis Metagonimiasis Opisthorchiasis Paragonimiasis Schistosomiasis	Water	Provision of toilets Treatment of excreta prior to discharge control of animal reservoirs Control of intermediate host Cooking of water plants and fish Reduction water contact

Source: Feachem *et. al.,* 1983.

10.3 Epidemiological Evidence

Crop irrigation with untreated wastewater causes excess infection with intestinal nematodes in both consumers and farm workers as shown in Figures 10.3 and 10.4.

Table 10. 2: Survival of excreted pathogens (at 20-30°C).

Type of Pathogen	Survival times in days			
	In faeces night soil and sludge	**In fresh water and sewage**	**In the soil**	**On crops**
Viruses				
Enteroviruses	< 100 (< 20)*	< 120 (< 50)	<100(<20)	< 60(<15)
Bacteria				
Faecal Coliform	< 90(<50)	< 60(< 30)	< 70 (< 20)	<30(<15)
Salmonella spp.	< 60 (< 30)	< 60 (< 30)	< 70 (< 20)	< 10 (< 5)
Shigella spp.	< 30(<10)	< 30 (< 10)	-	<5(< 2)
Vibrio cholera	< 30 (< 5)	<30(<10)	<20(<10)	< 10 (< 2)
Protozoa				
Entamoeba				
histolytica				
Cysts	<30(< 15)	<30(< 15)	< 20 (< 10)	< 60 (< 30)
Helminths				
Ascaris	Many months	Many months	Many months	
lumbricoides				
Eggs				

Source: Feachem *et. al.* (1983)

* Figures in brackets show the usual survival time source.

10.4 Guidelines for Reuse of Wastewater in Agriculture and Aquaculture

From the previous sections regarding survival times and potential risk of different pathogens, and frequency of infection or disease, evidence indicates that pathogens can be classified according to their relative health risks by using untreated excreta and wastewater in agriculture and aquaculture as shown in Table 10.3 (Mara and Person, 1987).

The recommended Microbiological Quality Guidelines for wastewater reuse in agriculture adapted by WHO (1988) are given in Table 10.4, which shows that the high actual risks are associated with intestinal nematodes and bacteria, but viruses have little or no actual risk.

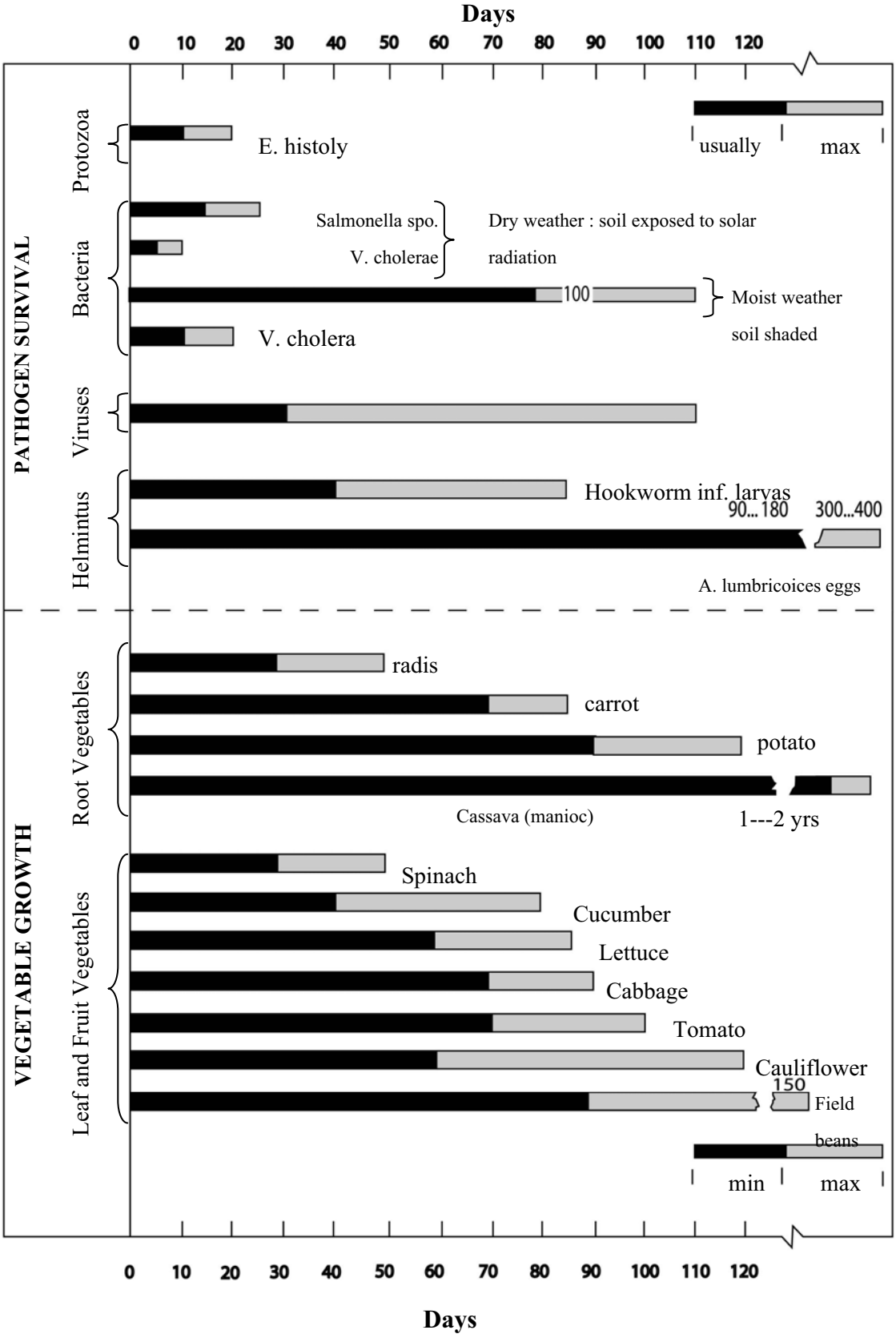


Fig10-1: Pathogen survival in soil compared with vegetable growth periods in warm climates.

Source: Strauss, 1985

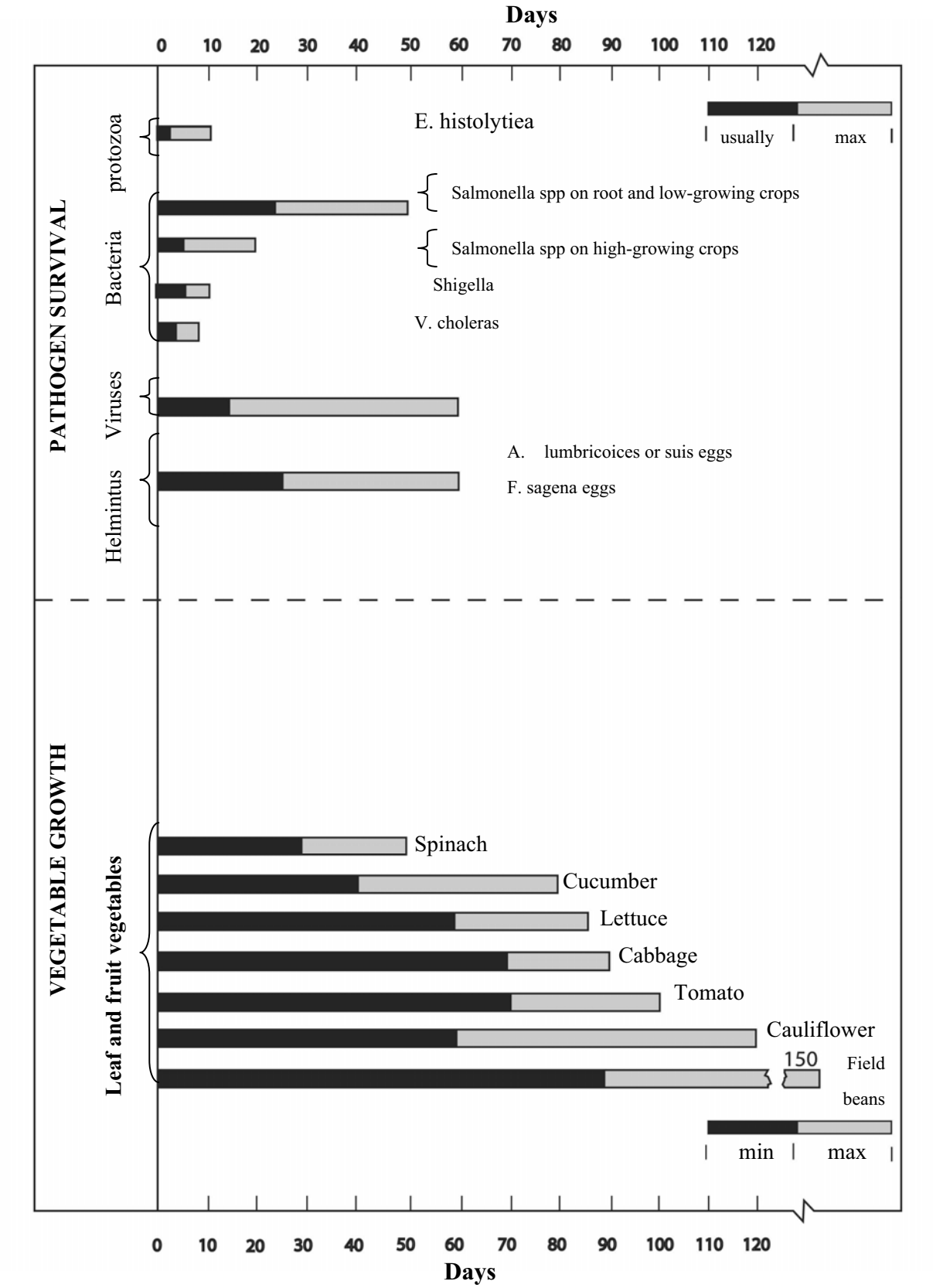


Fig. 10-2: Pathogen survival on crops compared with vegetable growth periods in warm climates.

Source: Strauss, 1985

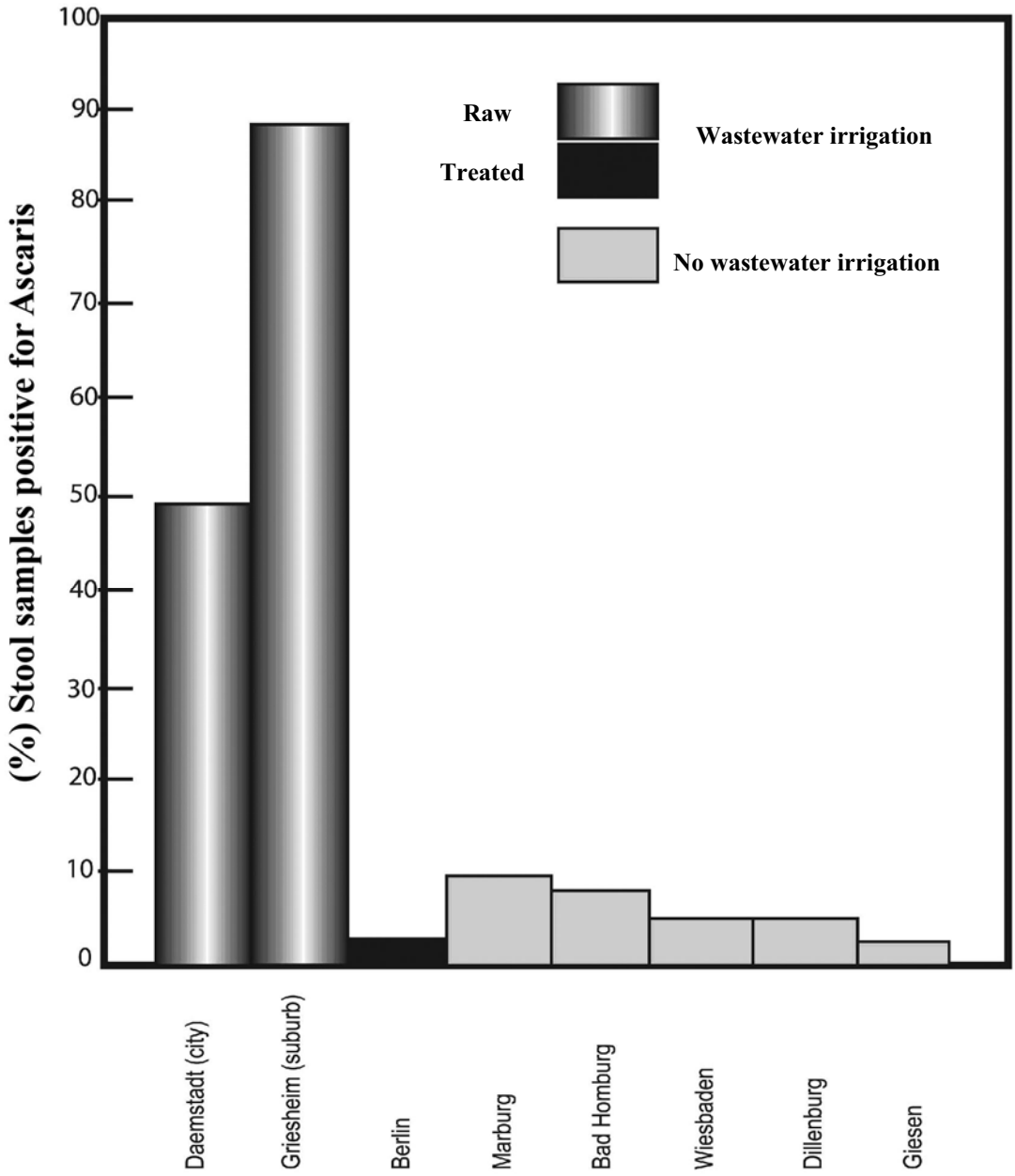


Fig. 10-3: Wastewater irrigation of vegetable and ascariasis prevalence in Berlin (West) and a number of cities in the Federal Republic of Germany in 1949.

From Shuval et al. (1986) reproduced by permission of the World Bank.

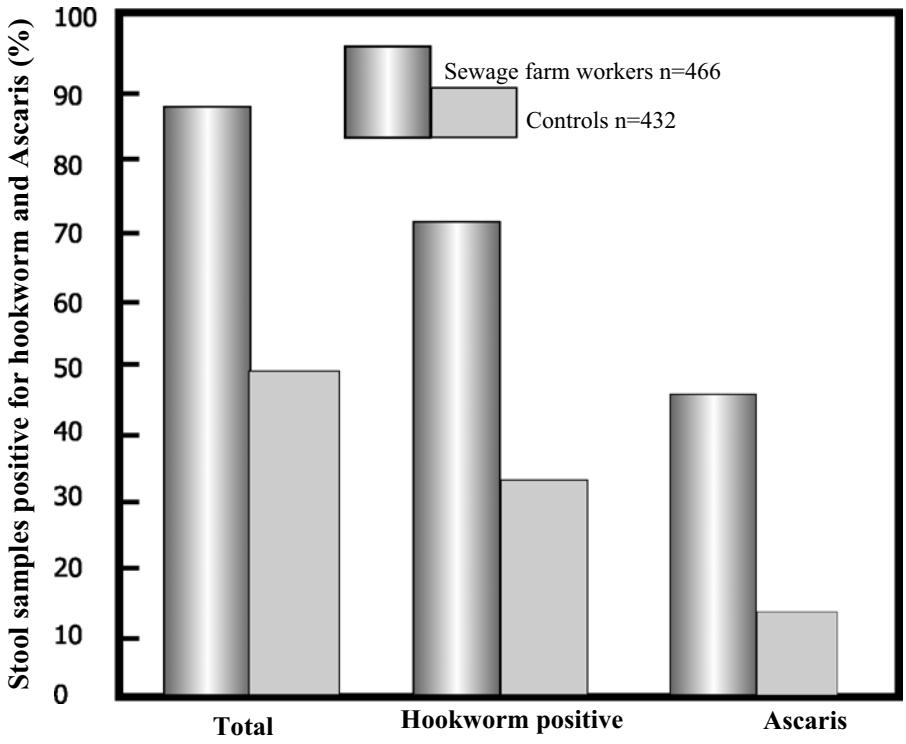


Fig. 10.4: Prevalence of hookworm and Ascaris infections in sewage farm workers and control groups in various regions of India.

From Shuval et al. (1986) reproduced by permission of the World Bank.

Table 10.3: Relative health risks from use of untreated excreta and wastewater in agriculture and aquaculture

Class of pathogen	Relative amount of excess frequency of infection of disease
Intestinal nematodes: *Ascaris* *Trichuris* *Ancylostoma* *Necator*	High
Bacteria infections: Bacterial diarrhoeas (e.g. cholera, typhoid)	Lower
Viral infections: Viral diarrhoeas Hepatitis A	Lower
Trematode and cestcode Infections: Schistosomiasis Clonorchiasis Taeniasis	From high to nil, depending upon particular Excreta use practice and local circumstances

Studies have virtually ignored low level or endemic occurrence of waterborne viral diseases for several reasons (USEPA, 1992c):

- A significant body of information exist indicating that viruses are reduced or inactivated to low or immeasurable levels via appropriate wastewater treatment, including filtration and disinfection, .
- Current virus detection methods are not sufficiently sensitive to accurately detect low concentrations of viruses even in large volumes of water.
- Enteric virus infections are often not apparent, thus making it difficult to establish the endemicity of such infections.
- There is no consensus among virus experts regarding the health significance of low levels of viruses in reclaimed water.
- The apparently mild nature of most enteric virus infections preclude reporting by the patient or the physician.
- Current epidemiological techniques are not sufficiently sensitive to detect low level transmission of viral diseases through water. The laboratory culturing procedure to determine the presence or absence of viruses in a water sample takes about 14 days, and another 14 days are required to identify the viruses; the complexity and high cost of laboratory procedures, and the limited number of facilities having the personnel and equipment necessary to perform the analyses.
- Damage due to enteroviral infections may not become obvious for several months or years. Once introduced into a population, person-to-person contact becomes a major mode of transmission of an enteric virus, thereby obscuring the role of water in its transmission, and;
- There have been no documented cases of viral disease resulting from the reuse of wastewater at any of the water reuse operation in the USA.

Table 10.4: Recommended microbiological quality guidelines for wastewater use in agriculture [a,e]

Category	Reuse conditions	Exposed group	Intestinal nematodes (arithmetic mean no. of eggs per litre[b]	Faecal coliform (geometric mean no. per 100 ml)[c]	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked sports fields, public parks	Workers, consumers, public	< or = 1	< or = 1000[d]	A series of stabilization ponds designed to achieve the microbiological quality shown, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, pasture and trees [d]	Workers	< or = 1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminths and faecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

a. In specific cases, local epidemiological, socio-cultural and environmental factors should be considered, and the guidelines modified accordingly.

b. *Ascaris* and *Trichuris* species and hookworm.

c. A more stringent guideline (< or = 200 faecal coliform per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

d. In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

e. Regional consultation convened in CEHA on 20-22 October 2003 suggested a new guidance for wastewater reuse in agriculture. The new guide is attached as Appendix IV.

10.4.1 Environmental Contamination with Intestinal Parasites

The degree of contamination of the environment with the products of intestinal parasites is enormous and depends largely on inadequate excreta disposal. About 20 percent of the region's population lack safe water, and more than 30 percent lack safe sanitation. The contamination of the environment is uneven. In the case of Aascariasis, it is concentrated around houses where small children are the most important disseminators of the infection. Hookworm eggs are disseminated by adolescents and adults contamination is heaviest around the edges of cultivated fields. The reproductive potential of each *Ascaris* female worm is extremely high - about 240,000 eggs per day, which counterbalances the heavy losses in the viability and infectivity of these eggs in the environment.

From the various ecological factors, (landscape, weather, and type of soil) which are regulating the population *of Ascaris* eggs outside the human host, the most important factor is the radiation from sunlight. Depending on the action or absence of one or more of these factors, *A. lumbricoides* eggs can survive for more than six years in a temperate climate but only for a few hours under some tropical conditions. The results of a soil examination for helminths in a focus of ascariasis in Poland showed *Ascaris* eggs with a mean of 1.8 - 2.8 eggs per gram of soil (WHO, 1981).

10.4.2 Prevalence and Intensity of Helminthic Infections in the Region

In Faizabad City, Afghanistan, it was found that the prevalence rate of Ascariasis for the male students (7-12 years old) had reached level of 96.6%, whereas the prevalence rate for female schoolchildren of the same age group was 79.5%. The highest prevalence was within the female age seven years (87.05%) and the lowest prevalence was within the girls age twelve which was 55.5% (Al Salem,1996). A study (Al-Shtayeh *et. al.,* 1989) summarizes six years of accumulated data on 22,970 specimens in Nablus, West Bank, Palestinian Authoring:

The infection rates were as follows:

Ascaris 177 per 1000

Trichuris 13 per 1000

In Jordan, the only available statistics (for 1988) report on cases of *Ascaris* found in samples from patients who were visiting the hospital for non-parasitic medical care and whose stool samples were tested for helminth eggs. One percent positive samples were found in the patients whose stools were tested at the Central Laboratories of the Ministry of Health. In Amman City, the concentration of intestinal nematodes in 1988 was 297 eggs per liter, of which 245 eggs per liter were *A.. lumbricoides.* (Al-Salem *et. al.,* 1989.). While the *Ascaris* eggs concentration in the Amman Waste Stabilization ponds influent in 1998 was undetectable. As study from the Gaza Strip (Smith, 1990) showed that more than 50% of the children under the age of 10 are infected by *Ascaris.*

A study from Riyadh, Saudi Arabia (Abdel-Hafez *et al.,* 1986) on 5,727 stool specimens from three hospitals during 1986 showed:

Ascaris	30 per 1000
Trichuris	25 per 1000
Hookworm	4 per 1000

In a rural community in Iran (Croll *et al.* 1982), a sample of 252 people showed that 216 people were infected with ascarisis (85.7%). A study of parasitic infestation and the use of untreated sewage for irrigation of vegetables in Syria (Bradley *et. al.,* 1981) shows that the domestic sewage of Aleppo contained 3340 *Ascaris* eggs/liter, which represents an *Ascaris* infestation rate of 42% of the total Aleppo population excreting an average of 800,000 eggs daily per person. The correlation between the number of parasites in Aleppo and the irrigation of vegetables with sewage is that irrigation completes the cycle by returning the parasites back to the community.

On the other hand, a sample of untreated sewage from the Syrian coastal town of Lattakia contained 460 *Ascaris* eggs/liter. Untreated sewage is not used for irrigation in Lattakia and this is reflected in the lower parasite count of Lattakia sewage. In Egypt, a village with an improved water supply, latrines and refuse collection had a lower prevalence (50%) and intensity (4200 eggs/gram) of ascariasis than a village without improved sanitation (prevalence of 76% and intensity of 6900 eggs/gram) (Chandler, 1954). In South Batinah Region (Oman) epidemiological study of intestinal parasitic infestation among schoolchildren showed that 19% of the examined schoolchildren was infected with *Hymenolepis nana.* The percentage of children infected with *Ascaris lumbricoides* was relatively low (0.1%) while infection with strongyloides was 5 per 1000 of examined schoolchildren (Al Salem, 1998).

10.4.3 Tentative Effluent Quality Guidelines for Aquaculture

The tentative effluent quality guidelines for aquaculture are as follows:

Fecal coliform [a b]	<103 = 104 per 100 mL
Viable trematode eggs [c]	zero [d]

a. A further public health measure is necessary to ensure that high standards of hygiene are maintained during fish handling and especially gutting.

b. Geometric mean number

c. Absence of viable trematode eggs, whose life cycle involve fish and aquatic macrophytes.

d. Readily achieved by stabilization pond treatment

These guidelines show the followings:

- The most important factors indicating the suitability of wastewater reuse are the intestinal nematodes and fecal coliforms. Therefore, the designer of wastewater treatment plant and reuse systems must take these factors into consideration as a principal consideration of his design.
- The guidelines depend on the reuse conditions and accordingly are divided into three categories:

  Category A: Direct contact between the crop or water and the workers, consumers and public.

  Category B: Direct contact is only between the workers and the water or soil.

  Category C: There is no contact between workers and water or soil (such as forests).
- The only wastewater treatment method noted in the guidelines, which satisfies the criteria for categories A and B, is the wastewater stabilization pond system.

10.5 Measures to Reduce the Health Hazards of Wastewater Reuse

The major control measures that protect consumers and workers from health hazards related to wastewater reuse are:

1. Type and level of treatment
2. Application method

3. Crop Selection
4. Human exposure

10.5.1 Type and Level of Treatment

The type and level of treatment must satisfy guideline criteria for the type of reuse system. The main object of wastewater treatment for reuse is to reduce pathogens to the acceptable levels and this can be achieved only by properly designed and operated wastewater stabilization pond systems. Since these systems have relatively long detention times and very low horizontal velocities, pathogens die-off and precipitation of helminths eggs are maximized.

10.5.2 Application Method

The type of the irrigation system such as localized irrigation can prevent some contamination. This irrigation system can give the highest degree of health protection as well as using water more efficiently and often producing higher yields.

10.5.3 Crop Selection

The health hazards associated with crops selection affect the consumers and the workers. A very high level of pathogen reduction is needed when crops grown are for consumption without processing.

10.5.4 Human Exposure

Careful selection and fencing of the site is needed to prevent people from coming into direct contact with pathogens in the irrigation water. Wearing suitable clothing can protect the field worker and crop handlers.

10.6 Integration of the Various Measures for Health Protection (WHO, 1998)

To the planners and decision-makers concerned with wastewater reuse, wastewater treatment appears as a more straightforward and "visible" measure for health protection, seconded only by crop restriction. Both measures, however, are relatively difficult to implement fully: the first limited by costs and problems of operation and maintenance, and the second by lack of appropriate markets for specific products or by legal and/or institutional constraints.

It should be considered that the application of isolated measures while not economical may have only partial effects in terms of health protection. Crop restriction for instance if applied may protect the consumers of crops but does not provide protection to farm workers and their families. In order to analyze the various measures under an integrated fashion aiming at the optimization of the health protection scheme a generalized model has been proposed. It was conceived to help in decision making, exposing the range of options for protecting agricultural workers and the crop-consuming public allowing for flexibility of response to different situations.

Each situation can be considered separately and the most appropriate option chosen taking into account economic, cultural and technical factors. The graphical conception of the model is shown in Figure 10.5.

It was assumed that pathogens flow to the center of the circle going through the five concentring bands which represents wastewater of excreta, field or pond, crop, workers and consumers. The thick black circle represents a barrier beyond which pathogens should not pass if health is to be protected. The level of contamination of wastewater, field, or crop or the level of risk to consumer or worker is shown by the intensity of shading. White areas in the three outer bands means zero or no significant level of contamination and in the inner bands a presumed absence of risk to human health and therefore indicates that the strategy leads to "safe" use of wastewater.

If no protective measures are taken, both workers and consumers will be at the highest risk of contamination. Assuming crop restriction is applied (Regime A, Fig 10.5) consumers will be safe but workers will still be at high risk. Regime B assumes that application of wastewater is made through subsurface of localized irrigation avoiding crop contamination and consequently maintaining both workers and consumers virtually free of contamination.

If human exposure control is the only protective measure assumed both consumers and workers will still be submitted to the same level of risk since such measures are rarely fully effective in practice. Regime D assumes partial treatment of wastewater through ponding (D-l) or conventional systems (D-II). Stabilization ponds with an average detention time of 8-13 day provides good helminth egg removal providing protection to the workers. However, reduction of bacteria is not enough to meet the proposed guidelines so the risk to consumers will remain high. With conventional systems, it does not provide a sufficient helminth egg removal and a small level of risk still remains for both workers and consumers. The following three regimes, E, F and G are examples of the combination of protective measures. Regime E includes partial treatment and crop restriction. In this case, full protection is provided to consumers but only treatment by ponding systems will provide full protection to workers. In Regime F, human exposure control is added to partial treatment. The combination of the two measures may lead to complete protection of the workers but some low level of risk still remains to the consumers.

Associating crop restriction and human exposure control (Regime G) will provide full protection to consumers but still some risks will be remaining to the workers. Finally, Regime H includes full treatment of wastewater, which will provide complete protection to both workers and consumers. The feasibility and efficacy of any combination of measures will depend on several local factors, which must be carefully considered before a final choice is made.

Some of these factors are the following:

- Availability of resources (institution, staff, funds);
- Existing social and agricultural practice, and;
- Existing patterns of excreta-related diseases.

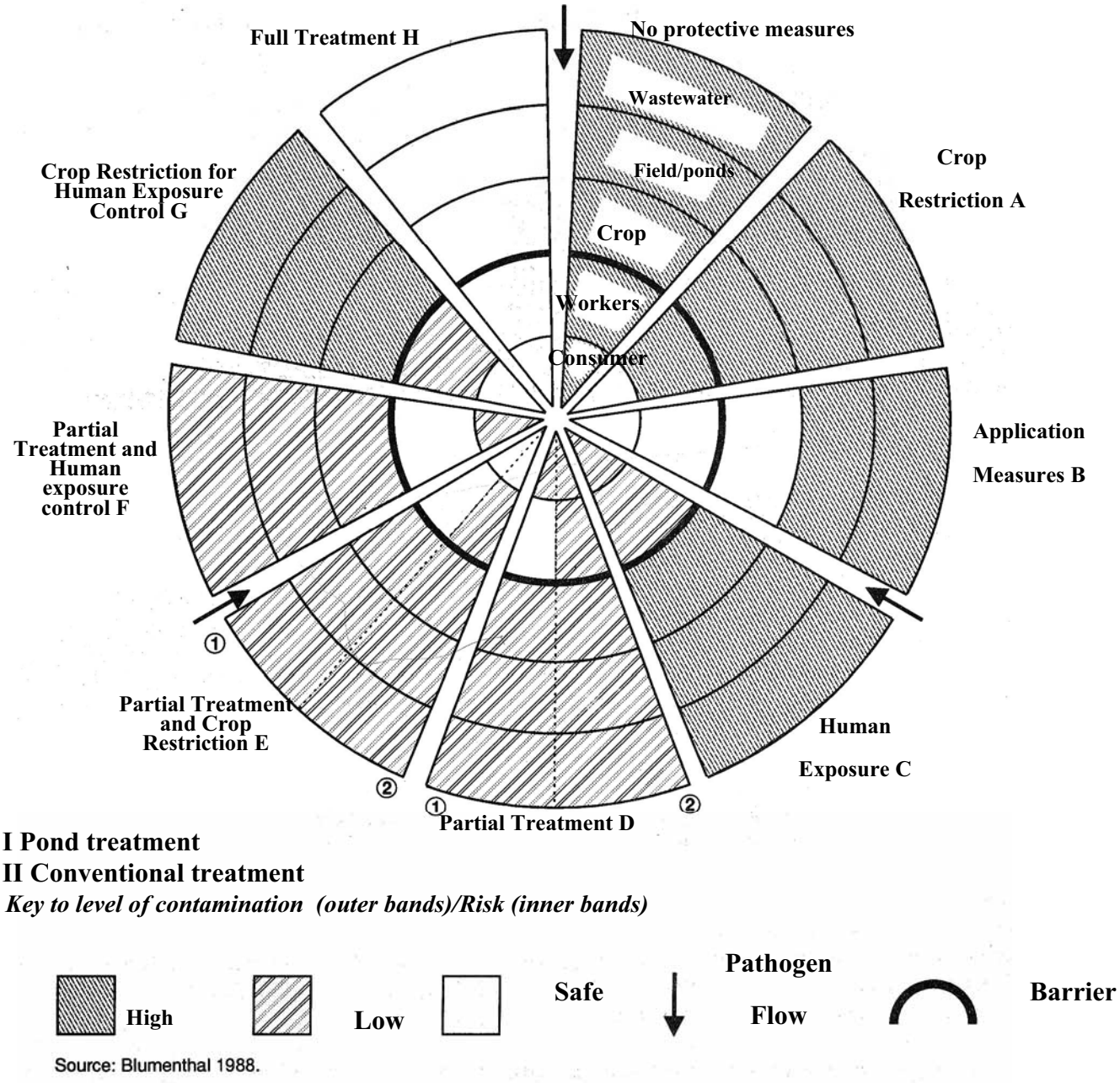


Fig. 10-5: Generalized model illustrating the effect of different control measures in reducing health risks from wastewater reuse

Source: WHO, (1989)

10.7 Special Health Issues

The 1989, WHO published new guidelines for wastewater use in agriculture and aquaculture Table 10.4 (WHO, 1989). The guidelines included a new dimension, which was not considered in the previous WHO report on reuse (WHO, 1973). The new guidelines set microbiological quality criteria for wastewater use in irrigation of crops to be eaten cooked or eaten raw; sport fields; public parks; cereal crops; industrial crops; fodder, and trees. The new dimension in the guidelines required that wastewater should contain less than 1 nematode egg per liter. In addition to nematode eggs the fecal coliform criteria has been revised and required that wastewater should contain less than 1000 fecal coliforms per 100 mL for vegetables eaten raw.

The WHO Guidelines for Use of Wastewater in Agriculture Aquaculture (WHO, 1989) stated that:

The presence of free-living nematode. larvae stages, sometimes in large numbers, in stabilization pond effluents IS OF **NO** PUBLIC HEALTH SIGNIFICANCE because they are not pathogenic to human beings (emphasis added).

This statement is valid for all helminthic pathogens excreted in faeces except for *Strongyloides stercoralis* (threadworm) and *Enterobius vermicularis* (pinworm), since their eggs are NOT normally excreted in the faeces. The pinworm is of minor public health importance because it is an infection that does not commonly cause serious illness. *Strongyloides* is potentially serious particularly in malnourished of immune-suppressed individuals. When the body's immune responses are deficient, disseminated strongyloidiasis may occur, with larvae attaching most organs of the body; such cases are usually fatal (Feachem *et al.,* 1983).

The mode of transmission of Strongyloidiasis infective filariform larvae, which develop in most soils, contaminated with faeces is; penetrate the skin (usually of the foot), enter the venous circulation, and are carried to the lungs. The eggs hatch and liberate non-infective, rhabditiform larvae which migrate into the lumen of the intestine, leave the host in the faeces and develop wither into infective filariform larvae, which may infect the same or a new host, or into free-living adults after reaching the soil (Benenson 1985). In addition, Feachem *et al* (1983) stated that "*Strongyloides stercoralis* is a minute nematode parasitizing man. The adult females, only 2-2.5 millimeters long, live embedded in the mucosa of the small intestine."

The eggs are ovoid and measure 50-60 by 30-35 micrometers but are seldom seen because larvae hatch out and are passed in the faeces. *S. stercoralis* exists in night soil and sludge as a delicate larva, not as a robust egg. A new infection can be initiated by the penetration of single larvae. Since S*trongyloides* represent a high actual risk, it is recommended to eliminate or remove 100% of its concentration. This would mean to have zero *S. stercoralis* larva/liter, because infection can only be initiated by skin penetration of a single *S. Stercoralis* larva. The period of communicability is as long as there are living worms in the intestine which may extend up to 35 years (Benenson, 1985) concerning inactivation of strongyloides in sewage treatment processes, there are no studies reported (Feachem, 1983).

However, it is suggested that sludge pasteurization as currently applied in Switzerland and Germany at 70 C for 30 minutes may offer a considerable safety. "Pathogens may be reduced in rapid sand filtration but not substantially and probably insufficiently to justify investment in this filtration method by the health benefits it yields and most helminth eggs will be totally unharmed by effluent chlorination" (Feachem *et al.,* 1983). This was confirmed by a study carried out in Jordan and by the performance of the Bahrain tertiary treatment plant operating on dual media filtration, chlorination, and ozonation. (Al Salem, 1992 c). So far, there is no guaranteed **feasible** method of inactivation neither for sewage or sludge treatment processes. It is recommended to take protection measures by wearing shoes and gloves, burying the sludge at least 0.5 m below ground surface, and stopping irrigation of crops at least 3 weeks before harvesting.

GLOSSARY

Reference: (Fox *et al.*, 1981)

ACTIVATED SLUDGE: A common method of biological sewage treatment. Settled sewage is supplied either by mechanical agitation or by diffused aeration. The bacteria that grow in the medium, together with other solids, are removed as sludge in a secondary sedimentation tank and recycled to the aeration tank inlet.

ADULT STAGE: A reproductive mature organism.

AERATION TANK: A tank in which a mixture of sewage or other wastewater and activated sludge is aerated.

AEROBIC: Requiring, or not destroyed by, the presence of free elemental oxygen.

AEROBIC DIGESTION: A biological process by which primary and/or activated or humus sludge is subjected to prolonged aeration so that its organic content is partially oxidized and the amount reduced by a combination of endogenous respiration, cryptic growth, predator activity, and slow oxidation of residual organic matter.

ALUM: Hydrated aluminium sulphate, $Al_2(S0_4)_3$. $18H_2O$; used as a coagulant.

ANAEROBIC: Not requiring oxygen to sustain life.

ANAEROBIC DIGESTION: Normally a controlled process of anaerobic decomposition of sludge or of a strong organic waste. The process may be carried out at ambient temperature (cold digestion), at about 35°C (mesophilic digestion), or at about 55°C (thermophilic digestion).

ANNELIDA: A phylum of invertebrate animals containing segmented worms (Oligochaeta) and leeches (Hirudinea).

AQUATIC: Organism that lives or grows in water.

***ASCARIS* spp..:** The generic name of a large parasitic nematode (roundworm) commonly found in humans, swine, and other animals. The eggs of which can be disseminated by way of sewage effluents.

BIOCHEMICAL OXYGEN DEMAND (BOD): The amount of dissolved oxygen consumed by microbiological action when a sample is incubated in the dark, usually for 5 days at 20°C. Aeration Tank Unit (ATU) may be used to suppress consumption by nitrification.

BRIGHTFIELD MICROSCOPY: Conventional microscopy using a compound microscope and transmitted light.

BURDEN: The number of parasitic worms with which a person is infected. This is also called the intensity of infection.

CENTRIFUGE: A mechanical device employing centrifugal force for separating solids from liquids, e.g. for concentrating or dewatering sewage sludge. Used in the laboratory for hastening the separation of suspended solids from a sample under test.

CENTRIFUGATION: The act of separating materials in suspension by using centrifugal force.

CERCARIA: The aquatic distributive stage of a parasitic trematode worm (fluke). A water snail is the intermediate host of the worm and the cercaria passes from the snail into the water. The cercaria then enters a second intermediate hostor a mammal, which in some cases, e.g. *Schistosoma,* is man.

CHEMICAL TREATMENT: The use of a chemical or chemicals in the treatment of wastewater or sludge, e.g. coagulation, neutralization, sludge conditioning.

CHLORINE: A greenish-yellow gas with a density about 2.5 times that of air, which may be dissolved in water by means of a chlorinator and added to a domestic or industrial wastewater to prevent septicity, or to an effluent or water for the purpose of disinfection.

CLARIFICATION: The removal of turbidity and suspended matter from sewage or water, rendering it more transparent.

COMPOSITE SAMPLE: A combination of individual samples taken at selected intervals, often hourly for 24 hours, to obtain from the bulked sample a figure representative of the composition over a period and thus avoid analysis of a large number of samples taken at intervals during that period. Individual samples may have equal volumes, or preferably be proportional to the flow at the time of sampling.

COMPOST: The humus-like product of the decomposition of excreta mixed with organic material rich in carbon.

COMPOSTING: The aerobic fermentation of waste organic matter, including organic house refuse. Sewage sludge can be added to a bulking agent and carbon source such as straw, sawdust, or wood-chips up to the liquid limitation of the process.

CONDITIONING: The physical or chemical treatment of sludge to facilitate dewatering. Methods of conditioning include the addition of inorganic or organic chemicals, mechanical thickening, elutriation, heat treatment and wet-air oxidation.

CONTAMINATION: The presence of "foreign" or unwanted materials in a substance. Water may be rendered unfit for its intended use because of the presence above acceptable concentrations of pollutants, microorganisms, or chemicals.

CONVENTIONAL TREATMENT: Wastewater treatment processes routinely used in Europe, including biofiltration, activated sludge and oxidation ditches. The retention time of these processes is normally no more than a few hours. Generally, not effective for helminth egg removal.

COPEPODA: An order of the class, Crustacea, e.g. *Cyclops.*

COVERSLIP: A thin glass cover that is placed over a specimen mounted on a glass microscope slide.

CYSTICERCOSIS: The bovine effect of infection by *Taenia.*

***CYSTICERCUS BOVIS*:** The level stage of the parasitic beef tapeworm *Taenia saginata.*

DECANTATION: The withdrawal of the upper layer after settlement of a liquid containing solids or after separation of a liquid of higher density.

DENSITY: The mass of a material per unit volume.

DESLUDGE: Remove accumulated sludge from septic tanks, etc.

DEVELOPMENTAL STAGE: A stage of development in the life cycle of organisms that occur in several forms (i.e., egg, larva, adult).

DEWATERING; A process by which water is removed from sludge to form a slurry or cake. After dewatering, the sludge may still contain up to 80 per cent of water. Methods include drainage by gravity and air-drying on beds, pressure filtration, centrifugation and vacuum filtration.

DIGESTED SLUDGE: Sludge which has been subjected to either aerobic or anaerobic digestion, whereby the sludge is rendered innocuous and the concentration of organic matter had been reduced.

DITCH, OXIDATION: A channel, also known as the Pasveer ditch, in which wastewater circulates in the course of the treatment process and is aerated by a large rotor.

DOSE, INFECTIOUS: The number of pathogens that must simultaneously enter the

body, on average, to cause infection.

EFFICIENCY: The ratio of the total output to the total input, expressed as a Percentage.

EFFLUENT: The treated sewage that leaves a sewage treatment plant.

EGG: Reproductive stage that forms following the union of gametes.

EMBRYONATED EGG: An egg, which contains a living miniature organism.

ENTEROBIUS: A nematode worm, parasitic in man, the eggs of which can be found in sewage.

EXCRETA: Faeces and urine In this guide the term is used also to refer to sludge, septage and nightsoil.

EXTENDED-AERATION PROCESS: A modification of the activated-sludge process whereby the sewage and activated sludge are subjected to prolonged aeration, the sludge being returned at a high rate, with the aim of bringing about considerable oxidation and aerobic digestion of the organic matter in the activated sludge.

FLOTATION: A process in which the specific gravity of particles of suspended matter is modified, causing them to rise to the surface so that they may be removed by skimming applied to the thickening of activated sludge by injecting fine bubbles of air which adhere to the sludge floes and cause them to rise Also applied to the separation of oil from oily wastes.

FLUKES: Parasitic flatworms of the phylum Platyhelminthes, class Trematoda, e.g. *Schistosoma,* the cause schistosomiasis (bilharzia) in man They have larval stages usually with an alternation of host, one of which is an aquatic snail.

GEOMETRIC MEAN: The mean calculated on a logarithmic scale.

GRAB SAMPLE: A sample taken at no set time or rate of flow, also termed a "spot sample".

HELMINTH: A nematode, cestode or trematode worm.

HERMOPHRODITE: Male and female organs found in individual or in the case of the cestodes, each segment.

HOOKWORM: Nematodes having mouth parts armed with hooks In the adult stage they are parasitic on mammals, including man, attaching themselves to the wall of the intestine.

HYDROMETER: Meter with specific gravity 1 000 to 1 620, of clear polycarbonate, for testing liquids heavier than water.

HYPOCHLORITE: Chlorine compound used in household bleach.

IMHOFF TANK: A deep two-storied tank introduced in Germany by Karl Imhoff in 1906 and formerly much used in Germany and USA It consists of an upper or continuous-flow sedimentation chamber slopes steeply to trapped slots through which solids may slide into the lower chamber. The lower chamber receives no fresh sewage directly, but is provided with gas vents and with means for withdrawing digested sludge from near the bottom.

INCIDENCE: The number of cases of a specified disease diagnosed or reported during a defined period of time, divided by the number of persons at risk in the population in which the disease occurred.

INDICATOR ORGANISM: An organism whose presence indicates a potential risk from one or more species of pathogen.

INFLUENT: Water, sewage or other liquid, untreated or partially treated, flowing into a section of the treatment plant.

INVERTEBRATES: Animals not possessing a backbone.

LAGOON: An artificial lake constructed by excavation and using the excavated soil for forming embankments, or employing a natural depression. Used for storing and consolidating sludge or, where land is cheap and the climate is suitable, for stabilizing organic matter in crude or biologically-treated sewage by providing a relatively long period of retention.

LARVA: A pre-adult development stage in which feeding is independent of the parent.

LATENCY: Minimum time from excretion by man to potential reinfection of man. The latent period of a pathogen is the time it requires to develop in the environment before it can cause infection.

MATURATION POND: A large shallow basin used for the further treatment of sewage which has already received biological treatment and from which the solids synthesized in biological treatment have been removed.

McMASTER CHAMBER: A special microscopic chamber (0.3 ml) for counting parasite stages in flotation media.

MENISCUS: The concave or convex upper surface of a column of liquid.

NEMATODE: An unsegmented roundworm of the Phylum Nematoda that may be parasitic or free-living.

NIGHTSOIL: Human excreta transported without flushing water.

NO-GRAZING" PERIOD": The stipulated period for the non-grazing of animals following an application of sewage sludge.

OBJECTIVE LENS: A microscope lens at the end nearest the specimen.

OVUM (p1. ova): Female reproductive cell (this term is often used interchangeably with egg).

PARASITE: An organism that lives in or on another organism and derives its sustenance from it.

PATHOGEN: An organism, which is capable of producing disease.

PERSISTENCE: Maximum survival time of final infective stage.

PHASE CONTRAST: Microscopy using a special condenser to produce indirect lighting.

POND, FACULTATIVE: A pond that is aerobic near the surface but anaerobic lower down.

POND, MATURATION: The final pond in a series of waste stabilization ponds. Maturation ponds are entirely aerobic.

PREVALENCE: The number of persons sick or exhibiting a certain condition at a particular time (regardless of when that illness or condition began) divided by the number of persons at risk in the population in which it occurred.

PRIMARY SLUDGE: Sludge formed from sewage solids removed by settlement in a primary sedimentation

tank.

RAW SEWAGE: Sewage as it enters a sewage treatment facility prior to undergoing any treatment.

RESTRICTED IRRIGATION: Refers to irrigation of edible crops, sports fields and public parks.

RETENTION TIME: The period of time wastewater takes to pass through a pond or other treatment process, calculated by dividing its volume by the flow of wastewater.

RISK, ACTUAL: Probability of an individual's developing a particular disease over a specified period.

RISK, POTENTIAL: The chance of infection or disease that might occur but that does not at present occur.

SEDGWICK-RAFTER CHAMBER: A counting chamber (1.0 ml) that can be used on a microscope to count microorganisms. Glass counting cell 50 mm x 20 mm x 1 mm deep is formed by four matte surfaced strips cemented in 76 x 34 mm slide. Overall thickness 3 5 mm.

SEDIMENTATION: Process of allowing solid particles to settle out of suspension in a fluid.

SEWAGE: Human excreta and wastewater, flushed along a sewer pipe.

SEWER: A pipe containing wastewater or sewage.

SEWERAGE: A system of sewer pipes

SLUDGE: The accumulated solids separated from sewage during processing.

SLUDGE TREATMENT: The processing of sludge to render it suitable for disposal It may include one or more of the following processes digestion, conditioning, dewatering, drying and incineration.

SODIUM CHLORIDE: Common table salt can be used to make an excellent flotation medium.

SODIUM NITRATE: Compound used as fertilizer that is highly soluble in water and will produce an excellent levitation medium.

SPECIFIC GRAVITY: Ratio of the density of an aqueous salt solution to the density of pure water.

SUPERNATANT: The fluid or upper portion of a sample after the solids has settled out.

SURFACTANT: A contraction of "surface-active agent" Surfactants are essential constituents of detergent formulations and include both soap and synthetic materials synthesized from petroleum or natural oils such as coconut oil A wetting agent, which, by reducing the surface tension of a liquid, improves the wetting action.

***TAENIA* spp:** Generic name of a parasitic tapeworm.

TAPEWORM: A parasitic flatworm that has a segmented, ribbon-like body.

TRANSMISSION STAGE: Resistant stage of a parasite that is transmitted to a new host.

TREMATODE: Parasitic flatworm with an unsegmented body, including the parasitic worms called flukes. Trematodes of medical importance have intermediate stages in snails, e.g. ***Schistosoma.***

***TRICHURIS* spp.:** Generic name of a parasitic roundworm commonly called "whipworms".

UNRESTRICTED IRRIGATION: Refers to irrigation of trees, fodder and industrial crops, fruit trees and pasture.

WASTEWATER: Refers to the liquid waste discharged from homes, commercial premises and similar sources to individual disposal systems or to the municipal sewer pipes, and consists mainly of human excreta and used water. It may contain small amounts of industrial waste.

ZINC SULFATE: Chemical compound that makes an efficient levitation medium when mixed with water.

Appendix 1

BASIC EPIDEMIOLOGICAL FEATURES OF EXCRETED PATHOGENS BY ENVIRONMENTAL CATEGORY

Source: FEACHEM, F G *et al.*, 1983. Reprinted by permission of the World Bank

Pathogenic	Excreted load[a]	Latency [b]	Persistence[c]	Multiplication outside human host	Medium infective dose (LD_{50}) [d]	Significant immunity?	Major nonhuman reservoir?	Intermediate host
Category I								
Enteroviruses	10^7	0	3 months	No	L	Yes	No	None
Hepatitis a virus	10^6 (?)	0	?	No	L(?)	Yes	No	None
Rotavirus	10^6 (?)	0	?	No	L(?)	Yes	No(?)	None
Balantidium coli	?	0	?	No	L(?)	No(?)	Yes	None
Entamoeba histolytica	10^5	0	25 days	No	L	No(?)	No	None
Giardia lamblia	10^5	0	25 days	No	L	No(?)	Yes	None
Enterobius vermicularis	Not usually found in faeces	0	7 days	No	L	No	No	None
Hymenolepis nana	?	0	1 month	No	L	Yes(?)	No(?)	None
Category II								
Campylobacter fetus spp jejuni	10^7	0	7 days	Yes [(F)]	H(?)	?	Yes	None
Pathogenic *Escherichia coli* [g]	10^8	0	3 months	Yes [(F)]	H	Yes(?)	No(?)	None
Salmonella *S. typhi*	10^8	0	2 months	Yes [(F)]	H	Yes	No	None
Other salmonellae	10^8	0	3 months	Yes [(F)]	H	No	Yes	None
Shigella spp	10^8	0	1 month	Yes [(F)]	M	No	No	None
Vibrio cholerae	10^7	0	1 month (?)	Yes	H	Yes(?)	No	None
Yersinia enterocolitica	10^7 10^5	0	3 months	Yes	H(?)	No	Yes	None
Category III								
Ascaris lumbricoides	10^4	10 days	1 year	No	L	No	No	None
Hookworms	10^2	7 days	3 months	No	L	No	No	None
Strongyloides stercoralis	10	3 days	3 weeks (free-living stage much longer)	Yes	L	Yes	No	None
Trichuris trichiura	10^3	20 days	9 months	No	L	No	No	None

Pathogenic	Excreted load[a]	Latency [b]	Persistence[c]	Multiplication outside human host	Medium infective dose ($1D_{50}$) [d]	Significant immunity?	Major nonhuman reservoir?	Intermediate host
Category IV								
Teania saginata and *T. solium* [j]	10^4	2 months	9 months	No	L	No	No	Cow (*T.saginata* or pig (*T.solium*)
Category V								
Clonorchis sinensis [j]	10^2	6 weeks	Life of fish	Yes[k]	L	No	Yes	Snail and fish
Diphyllobothrium latum [j]	10^4	2 months	Life of fish	No	L	No	Yes	Copepod and fish
Fasciola hepatica [j]	?	2 months	4 months	Yes[k]	L	No	Yes	Snail and aquatic plant
			?					Snail and aquatic plant
Fasciolopsis buski [j]	10^3	2 months		Yes[k]	L	No	Yes	Snail and aquatic plant
			?					Snail and fish
Gastrodiscoides hominis [j]	?	2 months (?)		Yes[k]	L	No	Yes	
			Life of fish					Snail and fish
Heterophyes	?	6 weeks		Yes[k]	L	No	Yes	Snail and crab or crayfish
Heterophyes [j]			Life of fish					
Metagonimus yokogawail [j]	?	6 weeks (?)	Life of fish	Yes[k]	L	No	Yes	
Paragonimus Westermani [j]	?	4 months		Yes[k]	L	No	Yes	Snail
			2 days					
Schistosoma								
S. haematobium [j]	4 /ml of urine	5 weeks		Yes[k]	L	Yes	No	Snail
			2 days					Snail
			2 days					None
			7 days					
S. japonicum [j]	40	7 weeks		Yes[k]	L	Yes	Yes	
S. mansoni [j]	40	4 weeks		Yes[k]	L	?	No	
Leptospira spp	urine (?)	0		No	L	Yes (?)	Yes	

a Typical average number of organisms per gram of faeces (except of *Schistosoma haematobium* and *Leptospira* species, which occur in urine)
b Typical minimum time from excretion to infectivity
c Estimated maximum life of infective stage at 20-30 ºC
d L Low (<102): M medium (104): H high (106): uncertain
e Includes polio-, echo-, and coxsackieviruses
f Multiplication takes place predominantly on food
g Includes enterotoxigenic, enteroinvasive, and enteropathogenic *E. coli*
h *Ancylostoma duodenale* and *Necator americanus*
I Latency is minimum time from exuretion by man to potential reinfection of man. Persistence here refers to maximum survival tune of final infective stage. Life cycle involves one intermediate host
j Latency and persistence as for *Taenia* species Life cycle involves two intermediate hosts.
k Multiplication takes place in intermediate snail host.

Appendix II

Preparation of Working Solutions of Vital Stains

Crystal Violet Stain: 2g crystal violet (sigma Co.), 20 ml 95% ethanol, mixed with 80 ml of 1% aqueous ammonium oxalate (Lillie, 1977).

Methylene Blue-Eosin-Borax Stain: mix 0.2g methylene blue (Sigma Co.) into 100mL distilled water, boil 10 minutes then mix 0.5g borax. Boil for an additional 10 minutes; after dissolving, filter. pH (10-11) should be obtained (Zhou et al., 1985).

Appendix III

Van Veer Grab Sampler

The Van Veer Grab Sampler is a simple sampling device, which does not require any messenger. The grab should be opened prior to lowering. This is achieved by spreading apart the lever arms that are attached to the buckets. A latch will engage which holds the jaws of the buckets apart in the open position. The bridle should be tensioned, and the grab can be lowered to take the sample. When the grab makes contact with the sediment, the bridle is then no longer under tension. The latch then releases the buckets. The grab can then be hauled to the surface. This action causes the bridle to pull the two levers together, which closes the buckets, a position which remains whilst the bridle is under tension

Appendix IV

RECOMMENDED GUIDANCE FOR TREATED WASTEWATER USE IN AGRICULTURE[a]

Category	Reuse conditions	Exposed group	Irrigation technique	Intestinal nematodes[b] and larvae of Strongyloides Stercoralis (Larvae/eggs per litre[c])	Thermo-tolerant coliforms[h] (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve required microbiological quality
A	Unrestricted irrigation For vegetable and salad crops eaten uncooked, sports fields, public parks[d]	Workers, consumers, public	Any	< detection	$\leq 10^3$	Waste stabilization ponds with a retention time of 21 days or secondary treatment followed by equivalent storage or slow sand filtration or equivalent
B	Restricted irrigation					
	Cereal crops, industrial crops, fodder crops, pasture and trees[f]	Workers, nearby communities	Spray or sprinkler	< detection	$\leq 10^5$	As for category A
		Workers, nearby communities	Flood/furrow	< detection	$\leq 10^{3g}$	As for category A
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Trickle, drip or bubbler	Not applicable	Not applicable	Pre-treatment as required by the irrigation technology, but not less than primary sedimentation

[a] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.

[b] *Ascaris* and *Trichuris* species and hookworms; the guidelines limit is also intended to protect against risks from parasitic protozoa.

[c] Guideline values are given for design purposes. They should be achieved during the planning and design stages for effluent reuse projects, and not used as a standard specification for monitoring effluent quality and samples collection.

[d] A more stringent guidelines limit ($\leq$ 200 thermotolerant coliforms/100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

[f] In the case of fruit trees, irrigation should stop two weeks before fruit is picked, and no fruit should be picked off the ground. Spray/sprinkler irrigation should not be used.

[g] In cases where the treatment method fails to achieve the guideline limit, use thermotolerant coliforms < 10^5, provided that precautions are taken such as protective clothes, crop restriction and providing a buffer zone between the irrigated area and nearby communities.

[h] Scientific studies suggested that *Escherichia coli* (*E.coli*) is the preferred indicator of fecal contamination. *E. coli* is approximately equivalent to 90% of the Fecal coliforms. (TTA/ CEHA)

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