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DEPARTMENT OF CIVIL ENGINEERING

ENUMERATION AND SURVIVAL STUDIES ON HELMINTH EGGS IN RELATION TO TREATMENT OF ANAEROBIC AND AEROBIC SLUDGES IN JORDAN

BY

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Environmental Engineering

1995
NEWCASTLE UPON TYNE
ENGLAND - UK

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Mrs. L. M. Evison for her invaluable guidance, advice, close supervision and encouragement during all stages of this research. She was always ready to listen, understand and help not only on scientific matters. I am also indebted to Professor M. B. Pescod for his kindness and helpful advice during the initial stages of this research. I am grateful to Professor A. James, Dr. G. K. Anderson, Dr. M. R. Barer, Dr. M. Saqqar, Dr. I. Miqdadi, Dr. T. Curtis and Dr. N. Craine for their useful discussions during my study. The patient guidance and advice of Dr. A. Metcalfe in statistical analysis during all stages of this research is deeply appreciated. Special thanks to Professor J. Knapton and Dr. E. K. Passaris for their kindness for providing me a computer with all software facilities that I need to complete this research.

Prof. D. D. Mara and Prof. E. Stentiford of the University of Leeds, Dr. H. V. Smith and Dr. Lucy Robertson of Scottish Parasite Diagnostic Laboratory, Stobhill General Hospital, Glasgow are thanked for their useful discussions in the initial stages of this research.

Special thanks are offered to the technical staff of this department, in particular Mr. J. Hamilton, Mrs. P. Johnston, Mrs. J. Baugh, Mr. J. Edmonds and Mr. L. Hepple, whose friendship and support are most appreciated. Together with Miss A. Allen, Mr. A. Mohseni-Bandpi, M. Malik, Mr. A. Sheikh, and Mr. P. Steadman, they all made the Environmental Engineering Department a better place to work in.

The financial support provided by the European Development Fund of the European Commission administrated by The British Council, and from The Arab-British Chamber Charitable Foundation, is fully appreciated.

The Author acknowledges the co-operation and support provided by Water Authority of Jordan, specifically to Dr. Raja Jadoen, Head of the Central Laboratories; Eng. Ayman Bani-Hani Al-Samra Plant Manager; Eng. A. Tuffaha; Eng. A. Matar; E. Jamalieh Madaba WSP Manager; Jerash treatment plant Manager. Special thanks to Mr. Badowi at Al-Samra plant, and Mr. Sameer at Jerash plant for their valuable help during sampling periods. Thanks are also due to all friends in the Water Authority Central Lab. of Jordan specifically Mrs. N. Sunaa, Miss S. Zinati, Mr. A. Aloubani, Miss N. Rabei, Mr. M. Al-Kouz, Eng. Z. Taani, Eng. F. Daraousheh.

I would like to dedicate this work to my parents and my husband in order to express my utmost gratification for all that they have sacrificed and the continuous support they offer, for their encouragement and love; without their moral support this study would not have been finished. Special thanks to my sister Jummana and brother Fakhri for helping my parents to take care of my children, through all my study period.

ABSTRACT

This research involved survey, laboratory and field studies. First, an evaluation of the present status of intestinal parasitic infections was made in the Jordanian population. Second, laboratory investigations were conducted on the development of a new technique to detect the viability of *Ascaris* eggs. Third, field studies were carried out to investigate the survival and occurrence of indigenous parasite eggs and indicator pathogens in domestic waste sludges in Jordan. Field investigations were also conducted on the effect of open natural drying beds on the inactivation of parasite eggs and bacterial pathogens.

The results of this study and a survey of available literature indicated a need for a universally accepted definition of a "viable" *Ascaris* egg.

A staining technique for detecting *Ascaris* egg viability was developed in conjunction with research studies of *Ascaris* eggs in sludge. The vital stain Crystal violet showed high correlation with the incubation method, and was more precise than the other stains tested. Crystal violet showed the best spontaneous detection of changes in egg viability and, within certain limits, it was found to be a strong indicator of the state of egg viability; furthermore it did not show any evidence of toxicity. In the staining method, Crystal violet stain is added directly to an egg preparation and observations are then made immediately using a light microscope. The results are available in only 10 minutes, compared to the 30 days required for the Incubation method. Since only stained or unstained eggs were observed, the method is less subjective than the Incubation method. In order to evaluate the versatility of the staining method, the effect of UV light and temperature was also investigated.

The ultimate disposal of domestic wastewater treatment plant sludges has been recognised recently as a problem in Jordan, and has never previously been investigated from the point of view of pathogen survival and transmission. This study showed that a huge volume of sludge (36,600 m^3 dry weight basis) accumulated from 1985-1993 in six anaerobic ponds, now requires desludging, treatment and disposal. Anaerobic pond sludges displayed some physico-chemical similarities to digested primary sludge.

The average total helminth egg counts were found to be highest in Jerash sludges (313 eggs/g), followed by Al-Samra sludges (303 eggs/g), with the lowest counts observed at Madaba waste stabilisation ponds (64 eggs/g, all expressed on dry weight basis). *Ascaris lumbricoides* were the most frequently recorded helminth eggs. Anaerobic ponds were found to be more efficient than oxidation ditch treatment in destroying eggs and inhibiting cell development inside the *Ascaris* eggs in the sludge layer. The results suggested that *Ascaris* eggs are not a good indicator for other helminth egg removal in waste stabilisation ponds. Also, it was concluded that waste stabilisation pond sludges demonstrated lower numbers of pathogenic microbes than the conventional sewage treatment plant, i.e. oxidation ditch.

It was concluded that the removal of *Ascaris* eggs in anaerobic ponds may be related to the dimensions of the particles which settle towards the middle of the ponds, where high counts of eggs are also found to occur. In contrast, the bacteriological counts showed a random distribution, with no concentration gradient established along the pond (except for *Salmonella* spp.).

In this study the indicators and pathogen analysis (i.e. faecal coliforms and streptococci, *Salmonella* spp., and helminth eggs) of sludges from different treatment plants showed levels in excess of those considered acceptable for sludge applied in bulk to agricultural land, forest, public contact sites, reclamation sites, lawns, or home gardens (Class A USEPA Regulations). The sludge would therefore have to undergo a Process to Significantly Reduce Pathogens (PSRP) before it could safely be applied to agricultural land or used as a soil amendment. Except for Zn in Al-Samra anaerobic pond sludge samples, levels of trace metals in Jordanian local sludges from Al-Samra anaerobic ponds, Madaba WSP's, and Jerash wastewater treatment plant sludges were significantly lower than the recommended limit values of USEPA and EEC for application on agricultural land.

This research concludes that sludge drying beds can be an effective method for inactivating parasite eggs, particularly in warmer geographic locations, and thus the treated sludge can be considered safe in terms of parasite transmission for application to agricultural land. *Ascaris* eggs had degenerated when the percentage of total solids was recorded as more than 88%; this took a shorter time in sand than in gravel drying beds. The inactivation of *Ascaris* eggs in drying beds is probably due to more factors than desiccation alone. Temperature, oxygen content, solar radiation, exposure time, mould activity, type of sludge, type of media etc., may also affect survival of the eggs. Anaerobic pond sludge bacterial counts showed higher resistance to desiccation and treatment conditions in drying beds compared with oxidation ditch sludge.

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ABBREVIATIONS

A	ampere
ASG	ASWSP sludges treated on gravel drying beds
ASS	ASWSP sludges treated on sand drying beds
ASWSP	Al-Samra waste stabilisation pond/s
BOD	biological oxygen demand
cap	capital
COD	chemical oxygen demand
°C	degree Celsius
d	days
DO	dissolved oxygen
EPA	United States Environmental Protection Agency
FC	faecal coliform counts
FS	faecal streptococci counts
g	gram
h	hour
ha	hectare
HRT	hydraulic retention time
JD	Jordan Dinar
JG	JTP sludges treated on gravel drying beds
JTP	Jerash treatment plant
JS	JTP sludges treated on sand drying beds
kg	kilogram
l	litre
m	metre
max.	maximum
mg	milligram
min	minute/s
min.	minimum
mm	millimeter
mV	millivolte
n	number
ORP	oxidation-reduction potential
p	probability
PFRP	processes that further reduce pathogens
PSRP	processes that significantly reduce pathogens
r	Pearsons correlation coefficient
RH	relative humidity
s	second/s
spp.	species
t	time
TC	total coliform counts
TSS	total suspended solids
TVS	total volatile solids
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WSP	waste stabilisation pond/s
yr or y	year

DEFINITIONS OF TERMS

Definition of terms that are used in this study are as follows

- **Active eggs**: means the cells inside the eggs had the capability to complete development up to motile-larval stage, and indicates potential infectivity.
- **Chlorometric degrees** = every 10° chlorometry = 21g/l, {NaOCl = 10g Cl/l; 75 x 10 ÷ 35.5 = 21g/l} In "Van Nostrand Scientific Encyclopaedia" (Considine, 1994).
- **Complete development** = egg cells able to complete proliferation phase and morphogenesis phase.
- **Corticated eggs**: *Ascaris* eggs with shell (see Plate A).
- **Decorticated eggs**: *Ascaris* eggs with shell removed (see Plate B).
- **Developed (or embryonated) eggs**: partial development and/or complete development.
- **Partial development**: egg cells that proliferate but do not differentiate.
- It is important to emphasise that the word **viable** does not imply successful motile embryo production, but simply that the egg has not died.
- The embryonation stages of *A. lumbricoides*:

  (1) **Single cell**: Eggs always emerge from the adult female worm at the single cell stage of development, non-infective stage. This stage is easily recognised by the large single cell occupying most of the egg (see Plate A and B).

  (2) **Early morula stage**: The embryo inside the eggs will be divided from 2 to 16 cells (Plate C).

  (3) **Late morula stage**: Rapid cleavage means that individual blastomeres are not clearly seen at this stage and the embryo is known as a blastula, a compact sphere of small cells. The blastula marks the end of proliferation and beginning of morphogenesis (Plate D).

  (4) **Gastrula and tadpole stage**: At this stage the embryo elongates and becomes ventrally curved. The embryo assumes a characteristic kidney-bean shape, the anterior end being slightly thicker and often darker in colour than the posterior (Plate E).

  (5) **Motile larva stage**: from early vermiform stage to completely formed larvae, larva start to elongate and move. The completely formed larvae are narrower and more transparent than the immature larva and can move around freely within the egg. The anterior and posterior ends are distinguishable. By this stage the larva is morphologically fully differentiated and remains in this state until a suitable hatching stimulus is received, and it is potentially infective (Plate F).

Plate A. *Ascaris* egg with shell
Single cell stage

Plate B. *Ascaris* egg with shell removed
Single cell stage

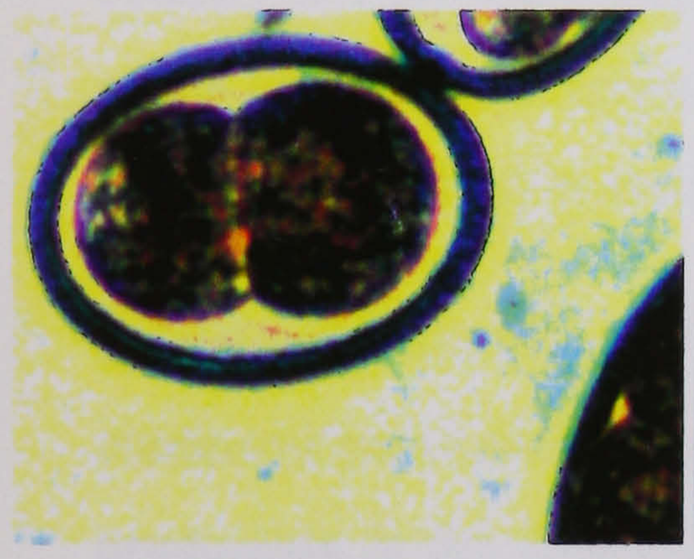

Plate C. Two cell stage

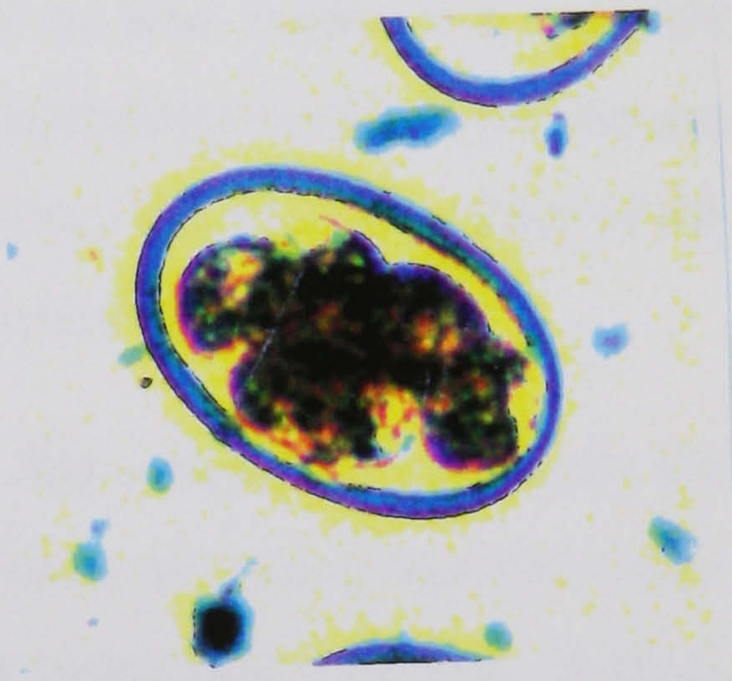

Plate D. Late morula stage

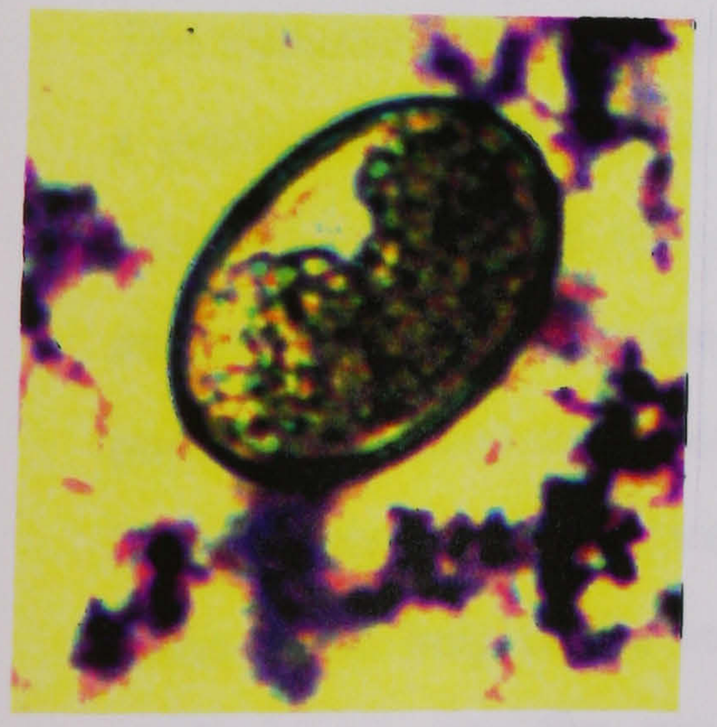

Plate E. Gastrula stage

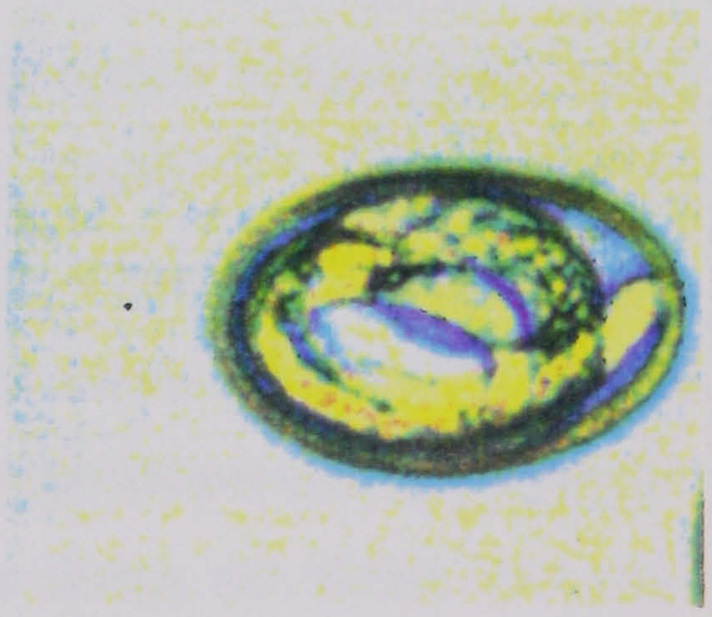

Plate F. Motile larva stage

CHAPTER ONE

INTRODUCTION AND AIMS

1.1 Introduction

Research and development is the necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. Sludges from municipal wastewater treatment plants often contain pathogens that are hazardous to humans and domestic animals. The problem of disease transmission has been a significant factor in preventing wider application of sludge disposal on land. Jordan is a semi-arid country and the reuse of sewage effluents and sludge in agriculture would extend the use of the limited water resources, allowing increased cultivation. The fertilising effect of the nutrients contained in sewage would also increase crop production.

Increased production of wastewater sludge in Jordan has resulted in part from the continued population expansion. However, the principal cause of a recent burst in sludge production is the Jordanian government requiring better wastewater treatment. Sludges from treatment plant operations are also becoming more difficult to dispose of, because of growing restrictions on use of land, air, and water.

The ultimate disposal of domestic treatment plant sludges has been recognised recently as a problem in Jordan, and has never been investigated with respect to pathogens in the past. Knowledge of the quality of the sludge in Jordan would be a major factor in assessing the unit process selection for a sludge disposal scheme. This research will particularly focus on quantifying and qualifying some of the sludge components, namely helminth eggs and their viability, pathogenic indicator microorganisms and heavy metals; these could help in the evaluation of the suitability of the sludge and sludge treatment methods to use the products as agricultural land dressing or "land amendment". Generally, the influence of sludge handling, treatment and disposal, along with the effect

of wastewater treatment processes on parasite survival is very complex and influenced by many factors. Such factors include the type of parasite, temperature, moisture content, etc.

In investigations on pathogenic organisms in sludge, parasites have received the least attention. Given the current state of knowledge, there is a need for further assessment of the health problem related to the presence of parasites in sludges, as well as the examination of the efficacy of various sludge treatment methods on parasite survival, especially from waste stabilisation ponds' sediments. 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 sewage sludges and their viability (Wright *et al.*, 1942; Cram, 1943; Cram & Hicks, 1944; Wang & Dunlop, 1954). Also little information has been written on waste stabilisation ponds sediments characteristics with respect to pathogens (Schwartzbrod *et al.*, 1987; Ayres *et al.*, 1993). Several papers were referenced describing sand drying beds design; however, specific research on drying waste stabilisation ponds sludges was notably lacking. From a review of the literature, there exists a need for the investigation of wastewater pond sludge accumulation, characterisation, and ultimate disposal, especially when considering the Mediterranean (semi-arid) region.

World Health Organisation and other researchers (EPA, 1989; WHO, 1989; Schwartzbrod, 1991; Ayres, 1992, Reimers *et al.*, 1989), identified the following as major research priorities for the future: need for the assessment of the content of pathogens, especially helminth eggs, in sludges from various sewage treatment processes, especially waste stabilisation ponds; research to develop effective techniques for the complete inactivation of pathogens in sludge from wastewater treatment processes (storage, composting, etc.); need to develop a routine technique for determining helminth eggs viability.

1.2 Aims of this Study

This study is limited to parasites, since survival information on intestinal helminth eggs in sewage sludges is relatively ill-defined as compared to bacterial contamination. This is largely due to the technical problems encountered in concentration, isolation, and identification of these organisms. It is generally accepted that the microbiological quality of waste stabilisation ponds sludges is better than that from conventional sewage treatment plants (Mara & Pearson, 1987), but there are few published data to indicate that it is safe for application on land without further treatment. Furthermore, little is known about the numbers and viability of human parasitic nematode eggs after prolonged storage in anaerobic ponds. From the helminthological point of view, we need to know the suitability of waste stabilisation pond sludges for use in agriculture, with or without further treatment.

This research included three phases: survey, laboratory and field studies. First, an evaluation of the present status of intestinal parasitic infections, especially the incidence of *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia* spp. in the Jordanian population. Second, laboratory investigations were conducted on the development of a new technique to detect the viability of *Ascaris* eggs, by comparing 4 different vital stains, and evaluating the versatility of the staining method, and the advantages and disadvantages inherent in the method.

The field studies consisted of a year-long investigation of indigenous parasites and indicator pathogens in domestic waste sludges in Jordan. This investigation has resulted in new information concerning: (1) the types and concentrations of resistant stages of parasitic helminth eggs and bacterial indicators in Jordanian sludges, especially from anaerobic waste stabilisation pond sediments; (2) characterisation and quantity of sludge from anaerobic ponds at the Al-Samra system; (3) the distribution of sludge and pathogens over the bottom of the pond; (4) the level of heavy metals in sludge. This information will help in making a preliminary evaluation of the amenability of these sludges for disposal on land. (5) field investigations were also conducted on the effect of

sludge treatment processes on the inactivation of parasite eggs and bacterial pathogens by using open natural drying beds.

Details of the minor objectives for each major objective will be found in the introduction of each chapter.

1.3 Unique Characteristics of this Study

The uniqueness of the present study can be characterised by the following main features:

1. Extensive research is still necessary to advance knowledge on the assessment of pathogens (specifically intestinal parasites) in sludge of different wastewater treatment plant processes (conventional and unconventional).

2. Research studies on the helminth and bacterial content in sludges in Jordan and the Eastern Mediterranean Region are rare.

3. Jordan can be considered as representative of the Eastern Mediterranean Region, and the findings of such studies may be applied to other countries in the region.

4. The study has been carried out on real wastewater treatment plants (conventional and waste stabilisation ponds) without artificial seeding of pathogens. A major part of the study was carried out on the Al-Samra waste stabilisation pond system in Jordan; this complex is one of the largest pond installations in developing countries. The value of laboratory-scale experimental systems is always questionable. This is due to the fact that laboratory-scale and pilot plants units may not behave as, or contain the ecosystems found in, real systems.

CHAPTER TWO

LITERATURE REVIEW

2.1 FACTORS INFLUENCING THE FATE OF HELMINTH EGGS

The survival of parasites outside the host's gastro-intestinal tract depends on environmental conditions. There are many abiotic and biotic variables which may affect resistant stages of parasites. In general, these parameters can be divided into three categories:

(1) Physical (temperature, sunlight, seasonal, etc.);

(2) Chemical (ammonia, salts, acids, etc.);

(3) Biological (fungi, protozoa, and invertebrates).

The effects of these have been studied by several workers, notably Brown (1927, 1928), Cram (1928), Otto (1929), Seamster (1950), Arfaa (1978), Stevenson (1979), Arene (1986), Barnard *et al.* (1987), Ayres (1992) and Grimason *et al.* (1995). There is a great deal of interaction between these parameters; however, for the sake of simplicity the factors have been separated through the outcoming discussion. Factors that are more related to this study will be more comprehensively discussed.

The epidemiology of *Ascaris* infection depends to a large extent upon the eggs, since they must embryonate outside the body of the host. 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. Wharton (1980) found that the egg shell is impermeable to all substances except lipid solvents, gases and perhaps liquid water. 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). Matthews (1986) suggests that 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 even reaching the lipid layer during permeability changes.

2.1.1 Physical Factors Affecting Helminth Egg Survival

2.1.1.1 Temperature

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). 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. 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±1°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±1°C and above give rise to infective larvae which have less ability to hatch *in vitro*, shorter longevity when aged in phosphate buffered saline (pH 7.2) at 37°C, and more limited ability to penetrate tissues membranes *in vitro*, when compared with those larvae from eggs embryonated at lower temperatures

(Arene, 1986). 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 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 which permit full or partial development of *Ascaris* eggs are presented in Table 2.2, showing the maximum temperature that allowed the motile larval stage (full development) to form was around 34°C (Arene, 1986; Seamster, 1950), while only partial development occurs without the formation of the motile larval stage, at a maximum temperature of 38°C. In conclusion the figures given in the literature (i.e. Table 2.1 and 2.2) are not in complete agreement. A review of the literature which 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 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	Fairbairn (1961)
27	Hass & Todd (1962)
17-30	Timoshin (1967)
22-26	Arfaa (1978)*
31±1	Arene (1986)
24	Fleming (1987)
29±1	Barnard *et al.* (1987)

**Ascaris lumbricoides*

TABLE 2.2. A review of the literature on minimum and maximum temperature which permit development of *Ascaris* eggs

Full development maximum (°C)	Partial development maximum (°C)	Temperature minimum (°C)	Reference
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	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**	20-30	Anya (1966)
Fasciola hepatica	22	Kiff & Lewis-Jones (1984)
Ostertagia ostertagi◊	25	Pandey (1972)

*Mouse pinworm
◊Cattle trichostrongylid nematodes

Seamster (1950) was studying the rates of development of eggs exposed to a series of accurately controlled low temperatures. He stated that the minimum temperature which permitted development of *Ascaris suum* eggs to the motile embryo stage occurred in 37 days at 16.7°C, the same results as reported by Arene (1986). Also he reported that the threshold of development for *A. suum* eggs under laboratory conditions was 14.5°C which is in agreement with the findings of Stevenson (1979), who reported that the development of *A. suum* eggs ceased when maximum outside temperatures were below 15°C (Table 2.2).

Exposure of eggs and partly developed embryos to freezing temperatures (-9 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*.

Cram (1924) in reporting on the resistance of *Ascaris suum* to low temperatures, observed that such eggs remained viable for as long as forty days when exposed to temperatures ranging from -18 to -27°C. The results showed *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. This was similar to Fairbairn's (1961) observation, that in infective *Ascaris* eggs which had been refrigerated for 2 years, hatching decreased from 77% to 47%.

Developed embryos in *Ascaris* eggs were killed by a 20-day exposure to temperatures of -21 to -27°C, but not by a 10-day period at the same temperatures nor by even a 30-day exposure to freezing temperatures (-11 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, and 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).

Faure-Fremiet (1913) cited in Brown (1928) found that 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 no doubt inhibits chemical reaction, and hence development as well.

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

iii) High temperatures

The fact that all physiological processes are inhibited by temperatures above their optima 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 into 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 mode of killing and are summarised in Table 2.4. Ogata (1925) reported survival of the eggs at higher temperatures by using *Ascaris* eggs on match sticks, soaked in hot water. Swales and Froman (1939) studied flash pasteurisation 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 temperature for different times.

The review however 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.

TABLE 2.4. Synopsis of literature on temperature / time experiments with *Ascaris* spp. eggs.

Temperature (°C)	Time	Survival	Reference
55	6.5 mins	eggs developed	Barnard *et al.* (1987)
50	10 mins	eggs dead*	Kiff & Lewis-Jones (1984)
55	10 mins	eggs dead	
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)
	1 mins	41% motile larvae	
50	20 mins	29% motile larvae	
53	3 mins	68% motile larvae	Nolf (1932)
70	1 second	eggs dead	Ogata (1925)
55	40 seconds	motile-larvae	
70	10 seconds	eggs dead	Ohba (1923)
60	5 mins	eggs dead	

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

Nolf (1932) showed that *Trichuris* eggs were killed at slightly lower temperatures (52 to 54°C) for a shorter time than were *Ascaris* eggs. The results showed that on 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 to 54°C for even a brief period of time, most of them 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*.

Kiff and Lewis-Jones (1984) found that temperatures of 50°C, 55°C, and 60°C produced complete inhibition of normal egg development. 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.

Above 65°C the ascaroside membrane becomes permanently disorganised and this approaches the melting point of isolated ascarosides which is about 82°C (Fairbairn, 1970; 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).

2.1.1.2 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. Most recent studies on the effect of radiation on the host-helminth system have focused on the development of immunity. However, only in 1970's has electromagnetic radiation been used to attenuate infective stages of helminths for use as vaccines. Tromba (1978) investigated the effects

of UV on *A. suum* and *Stephanurus dentatus* as a necessary prelude to immunisation trials with attenuated infective stages of these parasites.

The damaging effects of electromagnetic radiation on helminth eggs and larvae have been known for many years. Dognon and Tsang (1928, cited by Nolf, 1932) reported that the effects of exposure of *Ascaris megalocephala* to ultraviolet rays for 10 to 60 seconds at both 16 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. Nolf (1932) found that *Ascaris* eggs were killed by exposure to ultraviolet light of wavelength between 280 and 315 mμ, or 180 and 315mμ. *Trichuris* eggs were much more resistant to the effects of the light. The difference in susceptibility was not definitely understood, but the author 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.

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). *Ascaris suum*, *S. 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 600μW-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 13,000μW-min/cm^2.

For *Enterobius vermicularis* eggs, Hollaender *et al.* (1940) have reported that the greatest sensitivity of these eggs to radiation occurs at wavelengths below 2400A. Ariyo and Oyerinde (1990) 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.

Pigs vaccinated with UV-irradiated *Ascaris suum* eggs developed immunity to challenge exposure which reduced significantly both the number of larvae migrating to the lungs

(Tromba, 1978a) and the number of adults that developed in the small intestines (Tromba, 1978b). The immunity was induced by attenuated 1st-molt larvae and L 2 from eggs that developed *in vivo* only to L 4 (Tromba, 1978c). These stages, therefore, should be sources of protective antigens.

In view of the fact that 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.

Roberts (1934) reported that fresh eggs of *A. suum* in water exposed to sunlight were killed in from 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, Roberts observed that the temperatures of the medium surrounding the eggs frequently rose above 40°C during the period of exposure and he later found that temperatures of 40°C or higher, in the absence of sunlight, effectively inhibited the development of eggs.

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). He found also that 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. This is in agreement with the findings of Shalimov (1935), who reported that the embryonated eggs of horse ascarids (*Parascaris equorum*) are markedly more resistant to the inimical effects of UV light than the undeveloped eggs.

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. Udonsi and Atata (1987) found that 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.

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.

2.1.1.3 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. In the early part of this century, Otto (1929) stated that a minimum of 80% relative humidity was required at 22°C for egg development, and so far as the present author can determine, this is the only experimental evidence recorded for the minimum moisture requirements for *Ascaris* eggs, which was confirmed later by Wharton (1979). Brown (1928) cultured *Ascaris* eggs to the motile-embryo stage and observed that some of them were killed in 9 days and all were dead after 37 days of drying at room temperatures. Most authors state that "in the absence of moisture development is inhibited, and extreme dryness may ultimately destroy the viability of the eggs".

TABLE 2.5. Summary of literature on the effects of humidity on the survival of nematode eggs

Species (eggs)	Temperature (°C)	Moisture content (% RH)	Remarks	Reference
Ascaris	22	80	minimum moisture requirements for eggs development	Otto (1929)
Ascaris and *Trichuris*	20-30	40-50	unable to develop, completely destroyed after four days exposure	Nolf (1932)
Ascaris and *Trichuris*	22	77	3.5% and 2.2%, respectively eggs reached morula stage in 11 days	
Ascaris	greenhouse (13-46)	5.8-11.5	eggs survived for 81 and 51 days, respectively	Cram & Hicks (1944)
Ascaris	greenhouse (38-46)	3.3-4.2	nonviable eggs after 78 days	
Ascaris	26.7-28.9	95	eggs completely developed	Seamster (1950)
Ascaris	31.1	80-95	eggs developed only to early morula stage	
Ascaris	open petri dish exposed to sunlight (temperature not recorded)	3.1	10% viable eggs after 51 drying period days	Bhaskaran *et al.* (1956)
Ascaris	30	0	eggs collapsed after 3 days	Wharton (1979)
Ascaris	16.5	0	eggs collapsed after 9 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	
Ascaris and *Toxocara*	Fall	5	inactivated eggs	Reimers *et al.* (1981)
	Winter	7		
	Spring	8		
	Summer	15		
*Trichostrongylus colubriformis**	20	0-33, and 54.5	poor survival	Wharton (1982)
*Trichostrongylus colubriformis**	20	76-98	eggs hatched after exposure for 104 days	

* Plant-parasitic nematode, embryonated eggs

Nolf (1932) observed that *Trichuris* eggs required a more highly saturated atmosphere before they could develop than did *Ascaris* eggs, and that the former were less resistant to desiccation. He claimed "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. and (2) the difference in time required to complete embryonation under optimum conditions: *Trichuris* eggs require more time to complete their development than do *Ascaris*.

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 were made by Brown (1928), having noted that the human ascarid eggs were rapidly killed in faecal cultures on sand in the direct sun. He concluded that desiccation and heat were both important in killing the eggs, and his results indicated that soil type is an important factor in the rate of development and viability of the *Ascaris* and *Trichuris* eggs. He found that 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, he thought, to the minimised oxygen. The temperatures on sand frequently rose above 50°C which Ogata (1925) found to be lethal to *Ascaris* eggs while other soils used never reached those temperatures. Also Caldwell and Caldwell (1928) studied the development of *Ascaris* eggs under field conditions and concluded that a) other conditions being equal, the type of soil has no appreciable influence on development, and b) desiccation is the greatest lethal factor to the development of *Ascaris* eggs.

However, no clear distinction was made between the effects of light and desiccation. Spindler (1940) 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.

The ability of embryonating eggs of *Ascaris lumbricoides* to avoid desiccation by reducing the loss of water through the egg shell was investigated by Wharton (1979). He 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 Wharton 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. These 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).

i) Effects of air drying on parasitic eggs in sludge

Desiccation was found to be consistently effective in destroying parasites. These results suggested that the drying of sludges on drying beds located in warm climates might be an effective way of inactivating parasite eggs and other pathogens, and in part led to the initiation of this present study.

Little information is available on the survival of helminth eggs on drying beds. Reimers *et al.* (1981) found a log-log correlation 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.

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 *et al.*, 1984).

The majority of the studies cited, however, are experimental and include a step in which eggs are added in large numbers to raw sewage or digested sludge. Very few of these studies are field investigations wherein the survival of indigenous parasitic forms is 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 *et al.*, 1981). *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). He 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. These studies are valuable from a public health standpoint, but they reveal little concerning the actual relationship between development and humidity.

2.1.2 Chemical Factors Affecting Helminth Egg Survival

2.1.2.1 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 Kiff and Lewis-Jones (1984) by incubating parasite eggs in phosphate buffers at a range of pH at room temperature, 27°C and 37°C. Acid pH levels inhibited normal development of *Ascaris suum* eggs at all temperatures, but highly alkaline buffers allowed development to the infective larval stage. Owen (1984) demonstrated that *in vitro* hatching ability but not viability of *Taenia* eggs was completely destroyed at pH 12.

Udonsi and Atata (1987) showed that pH 6.0 was optimum for *N. americanus* hatching. 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.

2.1.2.2. Chemical substances

A search of the literature revealed several references concerning the resistance of *Ascaris* eggs to chemical substances. The destruction of parasites in sludges by chemicals has had varied results. The effects of various chemicals on the eggs of *Ascaris* have been studied by numerous workers. These studies which demonstrate the remarkable resistance of *Ascaris* eggs to chemicals have been reviewed by Fairbairn (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 (Fairbairn, 1957). 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". Seamster (1950) proved that DDT powder used full strength and in direct contact with *Ascaris suum* eggs exerted no perceptible effect on their development. Sulfanilamide, a commonly used bacteriostatic substance, was observed to produce no apparent effect on the development of *Ascaris* eggs (Table 2.6). 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 (Fairbairn, 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.

Decoated *Ascaris* eggs are highly resistant to the dissociated OCl^- form of germicidal chlorine compounds, showing long survival times in concentrated NaOCl or $Ca(OCl)_2$. When exposed to undissociated HOCl resistance was much lower. The use of high concentrations of chlorine gas in water under acid conditions results in more rapid death (Krishnaswami & Post 1968).

TABLE 2.6. The effect of various chemicals on the development of *Ascaris* eggs

Chemicals used	Contact time days	Concentration (%)	motile larvae (%)	Reference
$(NH_4)_2SO_4$	8 3	5-15 concentrated	motile larvae eggs dead*	Seamster (1950)
NH_4Cl	8	5-15	motile larvae	as above
NH_4NO_3	8	5-15	motile larvae	as above
DDT	8	50	motile larvae	as above
Sulfanilamide	8	1-2	motile larvae	as above
Potassium dichromate	20	2.5	65	Arfaa (1978)
Normal saline	20	-	63	as above
Levamisole	20	10^{-3}	36	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	7/10	32	as above
Cresol	5 hours	3	no embryos	Cram (1924)
Carbolic acid	10 hours	5	developed	

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

Ozone and chlorine have been found to be capable of killing *Shistosoma mansoni* eggs when present at levels of 4.0 mg/l and 40 mg/l, 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, 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).

2.1.2.3 Oxygen requirement

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) 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 2.5×10^{-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 indeed at 140 days. Fairbairn suggested that the initial decline was due to an oxygen debt inherited from the essentially anaerobic metabolism of the adult female worm and that 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.

Nolf (1932) conclude that 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.

2.1.3 Biological Factors Affecting Helminth Egg Survival

The biological factors which 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. 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. 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).

2.2 REVIEW OF QUANTIFICATION, QUALIFICATION AND VIABILITY METHODS FOR DETECTION OF HELMINTH EGGS IN WASTEWATER AND SLUDGE

2.2.1 Methods for Detecting Types and Enumerating of Helminth Eggs

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.*, 1984; Caceres *et al.*, 1987).

The enumeration of intestinal helminth eggs and larvae in 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.

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).

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.

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. He 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 (1989) 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_2NO_3, $K_2Cr_2O_7$ and other salts have been used (Table 2.7). Arthurs method (described in Faust *et al.*, 1938) which employs saturated saccharose 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 continious 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 they found it 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 (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 2.7). 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.

A useful summary of some techniques available for the enumeration of helminth eggs in wastewater is shown in Table 2.8. 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, but only when 10 litre samples, rather than 1 litre samples, were processed.

TABLE 2.7. 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 & Phillips (1982)
Formol-ether and zinc sulphate	Ritchie *et al.* (1948)
Lactalbumin 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)

TABLE 2.8. 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	1000g 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	700g for 10min	-	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 X100	$MgSO_4$ 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 X100	NaCl (relative density 1.04)	Doncaster counting dish	80%	effluent of stabilisation ponds	Ayres *et al.* (1989)
Stien - Schwartz brod	sedimentation and flotation	25	2 hr	1000g/15min	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)
P = volume of product in the counting cell (ml)
S = volume of wastewater sample (litre)

♠N = no. of eggs/L
A = no. of eggs counted
M = volume of the meniscus (ml)
P = volume in McMaster counting chamber

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

2.2.2 Determination of Helminth Egg Viability

Most methods that are used to enumerate parasitic eggs use relatively inexpensive reagents and successfully concentrate the full range of species routinely found in wastewater sludges. However methods using ether, for example the Satchwell method (1986), might kill helminth eggs, thus making viability assessments impossible.

Several techniques have been reported in the literature for viability determination of *Ascaris spp.* eggs, and somewhat fewer for other helminths. These are categorised as follows:

I. Morphological criteria;

II. Vital stains;

III. Infectivity;

IV. Incubation.

All these categories will be discussed comprehensively in Chapter Four.

Wide variations of culture media have been used by different researchers for culturing *Ascaris* eggs, to detect their development and viability (Table 2.9). Oskanen *et al.* (1990) found that using 0.1N H_2SO_4 or tap water as a culture media for *Ascaris* eggs embryonation 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.

(i) Viability determination using n-butanol

The Stien and 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 esterification 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 material and makes final examination of material impossible, even if the samples are thoroughly washed.

TABLE 2.9. 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	Passey & Fairbairn (1955)
Normal saline solution + 5% formalin	Bhaskaran *et al.* (1956)
0.1N sulphuric acid	Fairbairn (1961)
Distilled water	Arfaa (1978)
Tapwater	Kiff & Lewis-Jones (1984)
2% sodium dichromate	Fleming (1987)

2.3 REGULATIONS AND GUIDELINES FOR SLUDGE UTILISATION

Sludge can have valuable agronomic properties and its use should be encouraged, provided 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 watertable, 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 (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 saginata* 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 recognises 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, 1988).

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 (Tables A and B, Appendix 2.1).

The USEPA (WPCF, 1989) 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 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 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 requires either that the density of faecal coliforms in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids, or that the density of *Salmonella* spp. bacteria in the sewage sludge should be less than 3 Most Probable Number per four grams of total solids, enteric virus density less than one Plaque-Forming Unit per four grams of total solids, 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 CFU per gram of total solids. Class B sludges result from sludge treatment by the following processes: physical, biological, lagoon, air drying, or chemical addition methods, or storage for at least 1 day.

2.4 OCCURRENCE AND SURVIVAL OF HELMINTH EGGS IN RAW WASTEWATER

Factors that affect the occurrence and concentrations of eggs and 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 wastewater contributed by industry, the volume of influent sampled and the recovery efficiency of the sampling method (Grimason *et al.*, 1995).

Table 2.10 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 2.10), which is a direct result of the low socio-economic conditions of the countries 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).

TABLE 2.10. Literature survey on counts of parasitic intestinal nematode eggs in raw wastewater from different countries

Range (eggs/l)	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)
122-860	India	Panicker & Krishnamoorthi (1981)
38-670	Northeast Brazil	Ayres *et al.* (1989)
9**	France (Nancy)	Schwartzbrod *et al.* (1989)
18-840	Morocco	Schwartzbrod *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	Ouazzani *et al.* (1993)
17-133	Kenya	Grimason *et al.* (1995)

*no. of eggs/g
** mean number

TABLE 2.11. Parasites which may be encountered in sewage in the United Kingdom (Alderslade, 1981)

Protozoa		
	Amoebae	
		Entamoeba histolytica
	Flagellates	
		Giardia lamblia
Helminths		
	Cestodes	
		Echinococcus granulosus
		Hymenolepis nana
		Taenia saginata
	Trematodes	
		Fasciola hepatica
	Nematodes	
		Ascaris lumbricoides
		Enterobius vermicularis
		Strongyloides spp.
		Trichuris trichiura
		Toxocara canis
		Trichostrongyles

Alderslade (1981) reported parasites which might occur in sewage in the UK (Table 2.11). Liebmann (1964) believes that 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.

2.5 ELIMINATION OF HELMINTHS BY WASTEWATER TREATMENT PLANTS

2.5.1 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 2.12 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. Bhaskaran *et al.* (1956) found in their study on sewage treatment plants in India that 46% removal of hookworm eggs was achieved compared with 67% removal for *Ascaris* eggs by 1.5 hours sedimentation, and 75% removal for hookworm eggs and *Ascaris* eggs by two hours sedimentation. They could also achieve 100% removal in an experimental trickling filter plant, and 81% and 96% in two activated sludge plants.

TABLE 2.12. Effects of wastewater treatment processes on parasite eggs and cysts (Reimers *et al.*, 1981)

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 egg state and operating conditions
Imhoff tanks	97% removal
Trickling filtration	38% removal, promotes egg development
Filtration	Retained 99% of eggs
	Stabilisation processes (affecting the eggs state)
Activated sludge	Promotes egg development
Extended aeration	Promotes egg development
	Decontamination processes
Routine chlorination	No effect

Silverman (1955) 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. Silverman doubted whether sedimentation was capable of removing a high percentage of tapeworm eggs from primary effluent.

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 1m/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.

Major helminth eggs with their size, relative density and settling velocity are shown in Table 2.13. 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 effects the settling velocity and therefore will reduce the effect of sedimentation.

Tarazi (1989) found that conventional treatment systems such as activated sludge, RBC, oxidation ditch, and extended aeration are not very effective in removing nematode eggs. However, the efficiency of these systems falls in the range (21-100%) for nematode eggs and (0-100%) for *Ascaris* eggs.

The use of sand filtration to remove eggs before irrigation was recommended, because the effective removal of *Taenia* eggs from wastewater treatment plants effluents would reduce the risk of animals exposed to treated wastewater

TABLE 2.13: Size, density and settling velocity of major helminth eggs species

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

(Dunn, 1991), page 171

Under laboratory conditions Newton *et al.* (1949) found sedimentation removed only 89% of *Taenia saginata* eggs present in sewage in a three hour period. They reported that only 30 to 38% of *Taenia saginata* eggs could be removed by a trickling filter in the laboratory. Also 12-inch column sand filters removed over 99% of the *Taenia saginata* eggs that had been added to settled sewage.

Panicker and Krishnamoorthi (1981) investigated parasite egg and cyst reduction in oxidation ditches and aerated lagoons. They reached several conclusions: parasites were reduced by 90 to 100% in the oxidation ditch in optimum operation. Most of the cysts and eggs were removed by settling, with the protozoal cysts settling slowly. Lagooning removed 75 to 100% of parasites, and so the removal efficiency in the aerated lagoon was less than that in the oxidation ditch. It was, however, suggested that the removal levels would have improved, had effluent from the lagoon been allowed to settle in a settling tank.

2.5.2 Removal of Helminth Eggs in Waste Stabilisation Ponds

Removal of pathogens is considered a major advantage in using waste stabilisation pond 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 stabilisation ponds are the simplest form of wastewater treatment system, but 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 (Horan, 1990). A summary of the major types and functions of each pond is described in Table 2.14 (Horan, 1990).

In 1995, Almasi and Pescod describe comprehensively the treatment mechanisms in anoxic ponds which operate in the grey area of organic loading between anaerobic and strictly facultative conditions. They conclude anoxic ponds optimize land area requirements and reduce the risk of odour nuisance, compared with facultative and anaerobic ponds, respectively.

TABLE 2.14. 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, stabilisation of influent, removal of helminths	BOD 40-60%, SS 50-70% faecal coliforms 1 log, helminths 70%
Facultative	1-2	4-6	BOD removal	BOD 50-70%, SS increases due to algae, faecal coliforms 1 log
Maturation (for three ponds)	1-2	12-18	Pathogen removal, nutrient removal	BOD 30-60%, SS 20-40%, faecal coliforms 4 log, nitrogen 40-60%, helminths 100%

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; Ayres *et al.*, 1993).

The basic mechanism of eggs 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.

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; Ellis *et al.*, 1993).

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). Saqqar and Pescod (1991) found that 88% of nematode eggs present were removed in the anaerobic ponds at Al-Samra, with a nominal retention period of 8 days, with only a further 6% being removed by a series of four facultative ponds during the additional 15 days. Nematodes were found to be absent of the effluent from the final maturation pond after a total retention period of 34 days. The authors pointed out that these results were obtained during the winter period with a water temperature varying between 12 and 15°C. They concluded that whenever high stormwater flows are received by the pond systems, lower removal efficiencies for the microbiological parameters should be expected at the design stage.

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, 1968; Gloyna, 1971; Feachem *et al.*, 1983; Mara & Silva, 1986; Saqqar & Pescod 1992). 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 ponds that 88-98% of *Ascaris* and 91-97% of hookworm eggs were removed in an anaerobic pond while 99-100% of *Ascaris* and 98-100% of hookworm eggs were removed in four facultative ponds. Also they found that complete removal of nematode eggs can be achieved by a single pond with a detention time of 18.9 days, or by two ponds in series with detention time of 6.8 and 5.5 days.

Marais (1974) showed that 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.

A model developed to describe nematode eggs elimination in waste stabilisation ponds, indicated that 14 days was needed to achieve the WHO criterion in Al-Samra WSP's (Saqqar & Pescod, 1992). They found that the removal efficiency of nematode eggs from WSP depends on the retention time (θ), and described it as a non-linear regression equation:

removal efficiency of nematode eggs = $1 - \exp - (0.2RT) / (1 - \beta)$

β = *system-dependent coefficient.*

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 = $100 [1-0.14 \exp (-0.38HRT)]$

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

Although the results of Ayres *et al.* (1992) 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 minimise hydraulic short circuiting.

Ayres (1992) found that 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.

There is some disagreement in the literature about the efficiency of waste stabilisation ponds in removing hookworms. Veerannan (1977) and Gloyna (1971), report 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, Lakshminarayana and Abdulappa (1972) found that hookworm larvae were 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. 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 works 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 *Shistosoma* 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 stabilisation 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 stabilisation 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). No studies have been conducted on their 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 2.15 and 2.16.

TABLE 2.15. 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)
4	3	0.4	(100% summer) (79% winter)	Marrakech	Ouazzani *et al.* (1993)
307	5	range 4.1-6.5	87%	Jordan	Saqqar & Pescod (1992)
384	1.75	0.8	82%	Brazil	Silva (1982)
384	1.75	1.9	90%	Brazil	Silva (1982)

TABLE 2.16. 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/l)	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%	Karatina, Kenya	De'Oliveira (1990)
1A+1F+3M	376	1.25+1+ (1+1+1)	4+3.2+ (3.2+3.2+3.4)	100%	Brazil	Ayres *et al.* (1993)
2A+4F+4M	307	5+2+1.25	range 32-48	100%	Jordan	Saqqar and Pescod (1992)
Macrophytic	32	0.8	7	100%	Marrakech	Silva (1982)
Microphytic	11.7	1.6	50	100%	Marrakech	Ouazzani *et al.* (1993)

2.6 SURVIVAL OF HELMINTH EGGS IN SLUDGE

The existence of parasites in sewage sludges has long been known (Cram, 1943; Keller, 1951; Graham, 1981; Schwartzbrod *et al.*, 1989). Table 2.17 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 13,000 *Ascaris* eggs per gram of raw sewage and 14,000 to 25,000 *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.

TABLE 2.17. Levels of helminth eggs in raw sludges from different countries

Counts (Range)	Unit	Country	Reference
50-243	eggs/ml	Johannesburg (South Africa)	Keller & Hide (1951)
1100-7805	eggs/g dry weight		
14,000-25,000	eggs/g wet weight	Iran	Sadighian *et al.* (1976)
287-1943	eggs/100g dry weight	USA	Reimers *et al.* (1981)
83-130	eggs/kg wet weight	France(Nancy)	Schwartzbrod *et al.* (1986)
225-325	eggs/100g wet weight	Marrakech	Schwartzbrod *et al.* (1987b)
20-340	eggs/kg wet weight	San Adrian	Schwartzbrod *et al.* (1989)
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. The parasites that commonly may be present in sewage sludge under European conditions are listed in Table 2.18 (Hannan, 1981).

TABLE 2.18. Some parasites of public health importance likely to be present in sludges in Europe (Hannan, 1981)

Protozoa	*Entamoeba histolytica* *Giardia lamblia* *Toxoplasma gondii* *Sarcocystis spp.*
Cestodes	*Taenia saginata* *Taenia solium* *Diphyllobothrium latum* *Echinococcus granulosa*
Nematodes	*Ascaris lumbricoides* *Ancylostoma duodenale* *Toxocara canis* *Toxocara cati* *Trichuris trichiura*

Schwartzbrod *et al.* (1989), showed that parasitological contamination was rather significant in the sludge samples from Nancy treatment plant since all samples proved to be positive. Concentrations varied from 113 to 135 eggs/100g depending on the sludge type which underlined the low impact of the treatment on helminth eggs. Detected eggs mainly belonged to the Nematode family and more rarely to the Cestode family.

A wide range of human and animal parasitic helminth eggs have been recovered from sludge; in France Schwartzbrod *et al.* (1986) have recorded *Ascaris spp.*, *Toxocara spp.*, *Trichuris spp.*, *Hymenolepis spp.*, and *Taenia spp.* Hays (1977) has reviewed the literature on the prevalence of parasites in sewage sludges in United States. The number and types of parasites present appear to be influenced by the infection rate of the local population.

Reimers *et al.* (1981) from his investigation of southern USA municipal sewage sludges showed 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 10,000 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) 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 agricultural purposes.

There is evidence that the eggs of *Ancylostoma duodenale* are present in sewage (Hays, 1977), but there appears to be none linking the land application of sludge with disease in man. The larvae survive six weeks in faeces (Feachem *et al.*, 1978) 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., 1989a*; Bruce *et al.*, 1990. Bruce *et al.* (1990) concluded that 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 routes of infection other than with sludge are predominant. Nevertheless, such occurrences emphasise 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.

2.7 INDICATORS OF PATHOGENS IN 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 specialised 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 (Havelaar, 1980).

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, 1984).

Strauch (1989) stated that faecal streptococci are to be ruled out as indicators for the hygienic quality of sewage sludge according to his results. The indifferent behaviour of the faecal streptococci indicates that high numbers do not necessarily indicate a

bacteriological hazard whereas their low concentration does not exclude this danger. Faecal streptococci appear to be useful for monitoring sanitation processes such as pasteurisation, radiation and composting. Bacterial endospores generally are too resistant, so that neither spore-counts nor total counts will give information about the process conditions applied. In the composting process, special problems arise due to the regrowth of certain indicator bacteria. Strauch (1989) found that *Salmonella* spp. possess a fairly pronounced resistance against high temperatures and pH values concluding that *Salmonella* investigation are important for evaluation of hygienic status.

Ascaris lumbricoides eggs were used for the sludge study 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.

Ayres (1992) suggested that there is a need for an indicator for the helminths, and the use of *Ascaris spp.* for sludge treatment is appropriate. 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 system achieved 100%

removal of *Ascaris* eggs then all other helminth eggs had also been removed (Ayres, 1992).

2.8 THE EFFECTIVENESS OF SEWAGE SLUDGE TREATMENT PROCESSES

2.8.1 Introduction

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 accelerated 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 2.19 and 2.20 was originally developed by Stern 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 2.21 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 stabilisation 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 stabilisation, 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 utilising imported equipment and complex operational procedures are not likely to be adopted until development affects the cost and proficiency of labour.

TABLE 2.19. Effectiveness of standard sludge disinfection processes (Reimers *et al.*, 1989)

Disinfection process	Removal or inactivation		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°C	F+	E	-	P	P
Laboratory batch test at 20°C	E	E	-	E	P
Temperature (heat)-time processes					
Anaerobic digestion (35°C)	F	F	-	P+	P
Anaerobic digestion (52°C)	G	E	-	G	P
Anaerobic digestion (60°C)	G	E	-	E (estimated)	E (estimated)
Aerobic digestion	F	P+	-	P	P
Composting (>60°C)	G+	E	R	G+	E
Pasteurisation (70°C, 0.5-1hr)	G	E	R	E	E
Pasteurisation (70°C, 1-2hr)	E	E	R	E	E
Heat treatment (195°C)	E	E	R	E	E
Heat drying	E	E	R	E	E

[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 2.20. Effectiveness of innovative sludge disinfection processes
(Reimers *et al.*, 1989)

Disinfection process	Removal or inactivation		Removal 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)
Ammonisation	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°C)	E	E	R	E	E
High energy electron irradiation (1000 krad)	E	E	R	G+	E

[a] Indicator organisms: total and faecal coliforms, faecal streptococci
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 2.21. The influence of sludge treatment processes on parasite eggs (Reimers *et al.*, 1989)

Unit operation	Effectiveness remarks
	Stabilisation 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°C 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°C and 30 minutes or in less time at higher temperatures. Effectiveness depends on temperature and exposure time, but temperatures below 45°C appear to have no effect
Lagoon storage	50-100% (depends on time and temperature)
Lime stabilisation	80-100% (depends on digestion, time, and temperature)
Ammonification	Up to 95% (depends on dosage and pH)

2.8.2 Sludge Treatment Processes

2.8.2.1 Pasteurisation

Pasteurisation at low temperatures (47-50°C) had been investigated by Carrington (1985) and the results showed that there was little effect on *Ascaris* eggs viability, but apparently the eggs were rendered sensitive to digestion. Pasteurisation at higher temperatures (51-53°C) affects egg viability, but subsequent digestion had no further effect. The system must be operated so that all elements of the sludge are subjected to the heat treatment, so that a continuous system, for example, must include plug-flow characteristics.

2.8.2.2 Aerobic and anaerobic digestion

Varied results have been reported as to the effectiveness of conventional anaerobic and aerobic digestion on parasite inactivation. In most situations, aerobic digestion induces embryonation of parasite eggs (Silverman & Griffiths, 1955; Reyes *et al.*, 1963; Hays, 1977).

Clearly, mesophilic anaerobic digestion fails to achieve complete elimination of parasite eggs present in the original raw sewage (Hamer, 1989). Aerobic thermophilic stabilisation carried out with air can give temperatures of over 60°C, up to as high as 80°C, depending on the sludge solids content, retention time and aeration efficiency. Over three hours at 50°C are required to attain effective inactivation of *Ascaris suum* eggs. In full-scale plants, short circuiting results in a significant proportion of the sludge being inadequately heat treated, and the mixing characteristics of the reactor appear to be as important as its temperature-time characteristics.

The effective destruction of pathogens by anaerobic digestion has been shown to be fallacious (Reyes *et al.*, 1963; Ward, 1977). Even though most microorganisms are thought to be destroyed during anaerobic sludge digestion, a number of microbes survive this process. These include bacteria (*Salmonella typhi*), parasites (various helminth eggs), and viruses (Poliovirus) (Reyes *et al.*, 1963; Ward *et al.*, 1976; Ward, 1977;

Sagik & Sorber, 1978). Survival in the aforementioned categories has caused many health authorities to remain sceptical about the practice of land disposal, as viable pathogens remaining in the waste may become dispersed in the environment. In recent work by Carrington *et al.* (1991) confirmed that the disinfecting ability of anaerobic mesophilic process reduced bacterial counts by 90% and enteroviruses by 99%, but had no effect upon viability of *Ascaris* eggs.

The comparative effect of aerobic and anaerobic digestion on parasite eggs has been reported. Reyes *et al.* (1963) subjected *Ascaris suum* eggs to 20 days of aerobic digestion in nightsoil at temperatures under 15°C and observed that none of the eggs developed further than the one-cell stage. When the temperature of aerobic digestion was raised from 15°C to 30°C, eggs development resumed, with about 6% of viable eggs reaching an embryonated stage. At 30°C and 40°C, 30% and 1% of samples respectively reached an embryonated stage after 20 days. In aerobic digestion at temperatures above 50°C, all the eggs were destroyed within a few hours.

Anaerobic digestion studies conducted by Reyes *et al.* showed that at 30°C, a 16-day detention time did not retard *Ascaris* eggs development, while after 45 days exposure at 25 to 30°C, up to 15% of the eggs remained viable and healthy. At a temperature of 38°C and exposure time of 15 and 45 days, few remaining eggs were capable of development, while after 45 days exposure to anaerobic digestion, the egg destruction was 85% at 25°C, 85% at 30°C and 92% at 38°C.

Killing *Taenia saginata* eggs in anaerobic digestion was found to be more effective than aerobic digestion and lagooning. In all processes, temperature was the most important factor, with the rate of egg-kill increasing with increasing temperature, and at 55°C, eggs both free and within proglottids, were killed within a few hours. Comparison with other reported results is difficult because of the change in resistance of eggs to adverse conditions as they mature, within or free of the proglottid. Earlier results indicated destruction of *Taenia saginata* eggs after 56 days at 26 to 28°C, or 5 days at 35°C, or that *Taenia saginata* eggs survived up to 26 days digestion at 30°C with death rates of 0.24% per day at 39°C in saline conditions, and very rapid deaths at temperatures greater than 50°C.

No evidence exists that proper land spreading of digested or otherwise conventionally stabilised sludges has caused disease in man or animals. Nevertheless, concern exists that the pathogens left in digested sludges may contribute to human and animal diseases. Survival studies of pathogens in stabilised sludges indicate that the parasites, particularly the digester-resistant round worms, will survive the longest in soils under most environmental conditions. Therefore, once these sludges are disposed of on the land, the viable parasite eggs that have survived digestion may develop into an infective stage and pose a potential source of infection to humans and animals (Bond, 1958).

Fitzgerald (1982) tested for the transmission of ascarid helminth from anaerobically digested sludge; his experiments involved the exposure of pathogen-free pigs to anaerobically digested municipal sludge. Sixty out of 215 pigs were subsequently found to have ascarids, indicating that the sludge contained viable eggs.

2.8.2.3 Sludge air drying beds

Sludge treatment and disposal is one of the major problems at wastewater treatment facilities, from a technical and economic point of view. For sludge processing there are alternatives; the ultimate disposal can be less costly by reducing the volume, i.e. the moisture content. Through dewatering, a volume reduction can be achieved which is greater than that achieved by thickening. Sludge drying beds are the simplest and cheapest form of treatment; when land is available dewatering by nature can be an attractive process, and it has been recommended by Pescod (1971) as the most efficient way in tropical regions for concentrating wastewater sludge before disposal.

In the early years of this century, the use of drying beds was quite widespread as a means of dewatering sludge. When liquid sludge is applied to a drying bed, dewatering takes place partly by drainage, decantation and evaporation. It appears that little, if any, research has been carried out into the drying sewage sludges by evaporation and into the mechanisms involved in this process.

(i) Mechanism of drying processes

Climatic conditions are most important. Factors such as the amount and rate of precipitation, percentage of sunshine, air temperature, relative humidity, and wind velocity determine the effectiveness of air drying.

Air drying involves the vaporisation of water and the removal of the vapour from the vicinity of drying solids. The mechanics of the drying process are important because they will indicate whether or not any improvement may be made in the process.

The rate of removal of moisture from a solid is dependent on two distinct groups of factors. The first group comprises the external factors: these are temperature, relative humidity, air velocity and relative geometrical arrangement of the drying substance and the airstream. The second group consists of internal factors, such as the chemical and physical nature of the material being dried and the changes which occur in these properties during the drying process (Coackley & Allos, 1962).

Sherwood (1929) has differentiated between three types of drying, namely: (a) evaporation of liquid at the solids surface, where resistance to the internal diffusion of the liquid is small when compared with resistance to the removal of vapour from the surface, (b) evaporation at the solids surface, where resistance to internal diffusion of the liquid is great when compared with resistance to removal of vapour at the surface,. and (c) evaporation in the interior of solids, where resistance to the internal diffusion of the liquid is great when compared with the total resistance to removal of vapour. Drying of any particular material need not be restricted to one of the classes listed above. Changing the drying conditions or thickness of material may change the mechanism of a specific drying rate period (Coackley & Allos, 1962).

Sludge may be classed as a granular material, and in granular materials the movement of moisture is due to capillary forces. In the case of drying by evaporation the water surface is depressed into the branched passages among the particles, creating a slight curvature. The curvature of the menisci exerts sufficient pull to draw water to the surface. Water will continue to rise in the passage until the curvature of the lower end meniscus of water column is the same as the curvature of surface meniscus. At this stage the surface

meniscus retreats owing to the further evaporation of moisture. This in turn decreases the surface curvature of the water and causes the lift of additional moisture. This process continues until all the menisci of all the passages are about the same, when the water cannot be drawn to the surface and evaporation results in a continuous retreat of the menisci. At this stage the resistance to drying will increase as vapour will have to diffuse through the stagnant column of air in the voids before reaching the surface (Coackley & Allos, 1962).

At least four different conditions of water exist in sewage sludges, these are: (a) free or drainable water, the limit of this being the first critical moisture content, (b) capillary-held water, *i.e.*, that water held between the first and second critical moisture contents, (c) floc or particle moisture, i.e., water held within the individual sludge particles represented by the moisture below the second critical moisture content value, and (d) chemically bound water, this being water held at moisture contents somewhere below the equilibrium moisture content. Moisture in the form of (b) and (c) is removed solely by evaporation (Coackley & Allos, 1962).

(ii) Design considerations

Conditions that may vary in drying beds are the type of sludge, the method used to stabilise the sludge before applying it to drying beds, the temperature to which the sludge is exposed, whether the beds are covered or uncovered, how moisture is removed from the settled sludge, and the length of time allowed for dewatering. All of these factors will influence the levels of pathogenic and indicator organisms after air drying (Water Pollution Control Federation, 1984). The total drying time required depends on the desired final moisture content, and also relates to the methods of removal and subsequent use.

(a) Climatic effects

Regional climatic conditions greatly affect sludge dewatering on drying beds; the drying time is shorter in regions of greater sunshine, low rainfall, and low humidity. The prevalence and velocity of wind also affect evaporation rates from sludge beds. Consequently, climatic conditions may warrant some modifications of design criteria.

Rainfall may affect drastically the length of time a drying sludge must remain on the sand bed. In general, aerobically digested activated sludge will retain 25% or less of the rain that falls on it. However, the percent retained may be higher since it can vary considerably depending on the time during the drying period when rain occurs, and on the nature of the sludge itself (Randall & Koch, 1969).

(b) Effect of digestion

Beds of digested sludges contain many small cracks, which allow more surface exposure to the drying air, greater drainage of water, and easier passage of rainwater directly into the underlying sand bed drains compared to typical raw sludges. Table 2.22 illustrates the beneficial effects of sludge digestion on drying bed performance. The percent of drainable water in aerobically digested activated sludge is considerably greater than that reported for anaerobically digested sludge (Quon & Johnson, 1966).

TABLE 2.22. Effect of digestion on sand bed dewatering*

Removal mechanism	Raw sludge removed (%)	% digested sludge removed on Poor drainage	Good drainage
Drainage	48-52	28	72
Decantation	4-9	22	2
Evaporation	43-44	50	27

*(ASCE Manual and Report on Engineering Practice No. 76, 1991)

According to Randall and Koch (1969), the dewatering properties of aerobically digested activated sludge are related closely to the oxygen use characteristics of the sludge.

Sludges obtained from digesters where the dissolved oxygen concentration is maintained at a low level (less than 1 mg/l) dewater poorly. The drainage and drying properties of the sludges are improved by extending the solids retention time. Some of the sludges studied reach a point, however, where additional digestion does more harm than good.

Digestion also increases friability of the air-dried sludge cake, thereby resulting in easier removal from sand beds and better application or mixing of the sludge cake with soil. Also, odour problems are minimised and grease build-up in the soil is reduced.

Another advantage of digestion is the destruction of pathogenic bacteria. EPA guidelines for disinfection of sludges suggest that anaerobic digestion followed by dewatering on sand drying beds, may provide enough destruction of pathogenic organisms to allow the unrestricted use of the dried sludge, assuming that the end use is not restricted by concentrations of heavy metals or other regulated parameters. Neither anaerobic nor aerobic digestion alone, destroys pathogens as effectively as either would in combination with dewatering on sand drying beds. The inactivation of viable parasite eggs in raw sludges increases with decreasing moisture content of the drying bed sludges, as discussed by Reimers *et al.* (1981).

(c) Effect of depth of application

Quon and Johnson (1966) reported that the depth of applied sludge affects the drainage rate; they conclude that the depth should not exceed 200mm. Haseltine (1951) reports an optimum depth of 230mm, depending on drying time and removal method; his suggested depths of application range from 200 to 400 mm. The applied depth should result in an optimum loading of 0 to 15kg/m^2.

Randal and Koch (1969) found that for a given sludge solids concentration and sludge depth, the drainage rate was constant after 8 hours. In addition, a typical applied sludge depth of 200mm had been reduced to a total depth less than 25mm when it was ready to be removed from the bed. The thickness of the dried cake is primarily a function of the solids concentration and depth applied. In general, the lower the required final moisture content, the longer the drying time.

(iii) Advantages and disadvantages of using drying beds

Compared to mechanical dewatering, sand drying is a more labour intensive process that requires more land. The frequent downtime and the high capital costs of mechanical systems have caused designers to take a second look at sand drying where adequate land is available and environmental conditions are suitable (Table, 2.23).

Dewatering is a physical unit operation used to reduce the moisture content of sludge for one or more of the following reasons: Metcalf and Eddy (1991)

1. The costs of transporting sludge to the "ultimate disposal" site become substantially lower when sludge volume is reduced by dewatering,

2. Easy to handle, in most cases; dewatered sludge may be shovelled, moved about with tractors fitted with buckets and blades, and transported by belt conveyors,

3. Required normally prior to the incineration of sludge, to increase the energy content by removal of excess moisture,

4. Dewatering required before composting, to reduce the requirements for supplemental bulking agents or amendments,

5. Sludge dewatering required prior to landfilling, to reduce leachate production at the landfill site.

Details on the effect of drying beds on parasitic eggs have been described in Section 2.1.1.3.

2.8.2.4 Composting

The basic process requirements for composting of sludge are well established and described in the literature. The major potential advantage of the process that it is exothermic, and temperatures of up to 75°C may be obtained within the composting material. The conditions should effect complete destruction of pathogens.

The destruction of pathogens in sewage sludge by composting has been reviewed by many researchers (Keller, 1951; Hays, 1977; Burge *et al.*, 1978; Passman, 1979; Thevenot *et al.*, 1985). They conclude that composting is the only process that greatly reduces pathogen levels and stabilises the sludge so that it can be applied to land. They also

consider that the destruction of pathogens by forced air composting is much faster than windrow composting and, furthermore, is not adversely affected by rainfall.

TABLE 2.23. Advantages and disadvantages of using sludge-drying beds

Advantages	Disadvantages
Where elaborate lining and leachate control is not necessary and where land is available, capital cost is low for small plants	Lack of rational design approach for sound economic analysis
Lower requirement for operator attention and skill	Large land requirement
Low electric power consumption	Stabilised sludge requirement
Low sensitivity to sludge variability	Impact of climatic effects on design
Low chemical consumption	High visibility to general public
High dry cake solids contents	Labour - intensive sludge removal
	Permitting and groundwater contamination concerns
	Fuel and equipment costs for bed cleaning
	Real or perceived odour and visual nuisances

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2.8.2.5 Sludge storage in lagoons

Lagoon storage of domestic waste sludges as a mean of inactivating pathogens has received little attention. Although some observations on the recovery of pathogens from sludge stored in lagoons have been reported, there have been essentially no accompanying control data.

Although sludge lagooning is not considered to be the best method of handling wastewater sludge (Pescod, 1971), it is accepted that it will continue to be used in

developing countries. Pescod (1971) suggested that the efficiency of lagooning can be improved by incorporating decantation of separated supernatant into the operating procedure. He concluded that the lagooning process will be less efficient than sand-bed drying because it relies solely on evaporation for removal of water.

The differences between cold anaerobic digestion and lagooning relate more to the design of plant and its operation, than to microbiology. Both systems operate at ambient temperatures. The microbial activity is anaerobic and the retention periods are usually many months, so that methanogenesis and microbial breakdown of the dry matter occur slowly, but ultimately to the same extent as in mesophilic anaerobic digestion. The normal period of retention is often two years or longer in lagoons.

In general, sludge storage at ambient temperatures, even for prolonged periods, will not destroy parasitic helminth eggs. Cram (1943) and Bhaskaran *et al.* (1956) have shown that *Ascaris lumbricoides* eggs will survive at least 4 months in domestic sludge at ambient temperatures and much longer times are reported elsewhere; more than 9 months in South Africa (Murray, 1960) and up to 2 years in the USSR (Vassilkova, 1936). Strauss (1985) mentioned that complete inactivation of *Ascaris* eggs would occur upon storage of excreta for 12 months or more. The eggs of the nematodes *Ascaris*, *Trichuris*, and, to some extent, *Toxocara* were found to survive in stored sludge for over three years (Schwartzbrod *et al.*, 1987). There are some other data that suggest the possibility that longer storage of sludge could also inactivate parasites. Schatzle (1969) reported a 10 percent reduction of *Ascaris* egg viability after 7 month's storage at 0 to 20°C. Veerannan (1977) reported a 50 percent reduction in *Ascaris* eggs viability after one year storage and practically no survival after three years.

It was found that when small aliquots of sludge containing parasite eggs were stored at 25°C, destruction of the *Ascaris* eggs occurred after 10 to 16 months (O'Donnel *et al.*, 1984). However, when the sludge was stored at 4°C, some *Toxocara canis* and *Ascaris suum* eggs were still viable after 25 months. Though storage temperature and storage time were the most important factors in the inactivation of these eggs, minor effects were also associated with other factors: types of sludge digestion, timing of egg addition, type of storage (in sludges versus soil), pH, and species of egg. They suggest that sludge

lagooning can be an effective method for eliminating parasite eggs, particularly in warmer geographic locations.

Reimers *et al.* (1989) examine pathogen inactivation in lagoon stored municipal sludge, in the semi-tropical area of the United States. Anaerobic digested sludges artificially seeded with *Salmonella livingstone* and *Ascaris suum* eggs were stored in lagoon. The field and laboratory data have shown that fifteen months of storage was required for pathogen inactivation to meet Processes to Further Reduce Pathogens (PFRP) criteria in a semitropical climate.

2.9 HEALTH RISK ASSOCIATED WITH SLUDGE REUSE

2.9.1 Definitions of Hazard and Risk

A hazard is a set of circumstances which could lead to harm. Harm means disease in man and animals. The toxic effects of sludge may be due to soil organisms and plant life and pollution of surface water courses and ground water. Risk is the probability that disease will occur as a result of exposure to the hazard.

Reviews of available credible epidemiological studies of wastewater use (Shuval *et al.*, 1986a) and excreta and sludge use (Blum & Feachem, 1985) led to an assessment of health risks associated with the use of untreated wastewater and excreta in agriculture and aquaculture (Table 2.24) (Blumenthal *et al.*, 1989), which was also consistent with theoretical considerations (Shuval *et al.*, 1986; Strauss, 1985). As shown in Table 2.24, the intestinal nematodes pose the highest risk in sludge and wastewater reuse schemes: compared to bacterial and viral organisms their infective stages are more persistent in the environment, they have low minimum infective doses and their host immunity is weak, so that repeated infection readily occurs.

The finding of a particular pathogen in sludge does not mean that there is a risk of infection. There are many difficulties in proving a causal relationship between disease and an event, such as use of sludge in agriculture (Pike, 1990). But a risk exists only if it can be established that disease is caused under field conditions. The existence of risk will depend upon a great variety of local factors and individual circumstances, and whether pathogens were ingested in sufficient numbers to cause infection.

TABLE 2.24. Relative health risks from the use of untreated excreta and wastewater in agriculture and aquaculture (Blumenthal *et al.*, 1989)

Class of pathogen	Relative amount of excess frequency of infection or disease
Intestinal nematodes: *Ascaris* *Trichuris* Hookworm	High
Bacterial infections: bacterial diarrhoeas (e.g. cholera) typhoid	Lower
Viral infections: viral diarrhoeas hepatitis A	Least
Trematode and cestode infections: shistosomiasis clonorchiasis taeniasis	From high to nil, depending upon the particular excreta use practice and local circumstances

Potential risk occurs when pathogenic microorganisms are detected in wastewater, sludge, or on crops, even if no cases of disease to due to these microorganisms are detected. It is possible that a potential risk may not become an "actual risk" due to factors including pathogen survival, minimum infective dose, human behaviour, and host immunity. In addition, a particular infection may have other routes of transmission in the community, so that some of the diseases observed may not be associated with waste use. Therefore, the most useful measure is that of "attributable risk", which is a measure of the amount of disease associated with a particular transmission route within a population (Blumenthal *et. al.*, 1989).

The question of the virulence of microorganisms applied to land in sewage sludge has been little studied. Three factors have received particular attention: the survival times of pathogenic organisms applied in sludge to soil or onto growing vegetables; the possible routes by which surviving organisms could infect man; and the availability of epidemiological evidence that human ill-health from a particular pathogen has been caused by the practice of sewage sludge disposal (Alderslade, 1981). He suggested that the risk to human health posed by the application of sewage sludge to land is small, and manageable by adherence to guidelines of good practice which are based on sound scientific evidence. Further advances in understanding and controlling this hazard are more likely to follow an extension of epidemiological surveillance and investigation, than the increased microbiological monitoring of sludge.

Control of parasitic infestations: There are essentially three control mechanisms which can be adopted; 1) to block the transmission of helminth eggs to the environment by the use of drugs which kill the worms in the human body, 2) to control the parasite level within the environment, and 3) to block the transmission of eggs and cysts back to humans. 2 and 3 are the subject of the present investigation.

2.9.2 Prevalence of helminth infections

Several studies have shown that wastewater reuse for irrigation and sludge application to land as fertiliser increases the prevalence rate of parasitic infection. In some countries where water resources are scarce, wastewater is considered as a main water source that cannot be neglected in the water budget, and may be the only water available for irrigation as, for example , in Jordan.

Tapeworms, classed as cestodes, are one of the pathogens of major concern in the UK concerning the agricultural utilisation of sewage sludge. The two tapeworms of importance in Europe are the beef tapeworm, *Taenia saginata*, and the pork tapeworm, *Taenia solium*, which occur wherever pork or beef are eaten in an undercooked condition. In the UK, the incidence of cysticercosis amounts to about 0.04% of slaughtering, although light infections may be missed (Bruce *et al.*, 1990).

The pathogen *Ascaris* is not an important parasite in Europe, with infections by *Ascaris* amounting to about 1300 cases a year in Britain, although as much as 50% of the population may be infected in tropical regions (Feachem *et al.*, 1983). About 12% of pet dogs in the United Kingdom are estimated to be infected with *Toxocara. Toxocara* infection has been estimated to affect the sight of 150-200 children a year in the UK (Lewis-Jones & Winkler, 1991).

The relationship of soil-transmitted nematode infections to the availability of latrines was studied by Sorensen *et al.* (1994) among children living on plantations in Sri-Lanka. They conclude that congested living conditions seem to be a major determinant for ascariasis and trichuriasis, and the provision of latrines and safe water does not substantially change that situation. However, improvement of sanitary facilities will probably have a more immediate effect on the prevalence of hookworm infection. In the long term, improvements in housing conditions with proper sanitary facilities and a better, less congested living environment would be the solution to the control of soil-transmitted nematode infections.

Hawkins and Feachem (1978a) emphasise the paramount importance of sanitation and hygiene in preventing helminth infections. Evison and James (1977) report 10% of the world's population are infected with amoebiasis: In developing countries with poor sanitation a 30% infection rate is common. Roundworm infections (*Ascaris*) usually average 50% of the population in high incidence areas and according to Evison and James (1977) are very frequently transmitted in irrigation agriculture.

However, sanitation alone cannot eliminate the transmission of intestinal parasites because ideal sanitation is seldom achieved. The most frequent shortcomings are the following:(WHO, 1987)

- lack of strict hygienic practices (hand washing after defecating and before eating), resulting in auto-infection and transmission through person-to-person contact;

- use of contaminated water for personal hygiene and, more important, for dishwashing and for cleaning, refreshing and irrigating vegetables eaten raw;

- indiscriminate defecation (rather than using latrines) by certain groups, especially young children, which spreads parasite cysts and eggs in the immediate environment (soil, courtyard); and

- lack of personal hygiene among those who have close contact with domestic animals.

The prevalence of parasitic infestations in Aleppo (Syria) was reported by Hadidy *et al.* (1980) who showed that 60% of the population were infected with intestinal parasites. The most common parasite was *Ascaris lumbricoides*, representing almost 70% of the total infestation. The numbers of parasites in sewage were related to the infection rate of the population producing the sewage.

Abdel-Hafez *et al.* (1984) found the most important species encountered in Jordan Valley for 332 stool sample (males and females' ages 1-25 years) were *Ent. coli, Giardia intestinalis*, *Ascaris lumbricoides*, and *Hymenolepis nana.* The rates or infection with these organisms in all age groups in the various localities were 41, 20, 49 and 24%, respectively. *Taenia* eggs were detected only in a few cases (2%) and this may be correlated with the fact that people in surveyed localities cook their meat well before consumption.

2.10 SUMMARY

Generally, the influence of sludge handling, treatment and disposal along with the effect of wastewater treatment processes on parasite survival is very complex and influenced by many parameters. Such factors include the type of parasite, temperature, moisture content, light, etc. Developing of *Ascaris* eggs are obligate aerobes, heat and desiccation are the most antagonistic parameter to their viability.

There are numerous reports in the literature that indicate that parasitic forms survive in sludge treatment processes and after application to land from several weeks to several years. The majority of the studies cited, however, are lab-scale with artificially seeded pathogens added in large numbers to raw or digested sludge. Very few of these studies are field investigations wherein the survival of indigenous parasitic forms is determined, which makes it difficult to extrapolate from the information in these experiments to actual sludge application sites.

From a review of the literature, there exists a need for the investigation of wastewater pond sludge accumulation, characterisation, and ultimate disposal, especially when considering the Mediterranean (semi-arid) region.

CHAPTER THREE

PREVALENCE AND SEASONAL FLUCTUATIONS OF INTESTINAL PARASITIC INFECTIONS IN AMMAN, JORDAN

3.1 Introduction

Intestinal parasitic infections are distributed virtually throughout the world, with high prevalence rates in many regions. Amoebiasis, ascariasis, hookworm infection, and trichuriasis are among the ten most common infections in the world. Although mortality from these infections is relatively low, complications are not uncommon and many cases need hospital care. In many countries, malabsorption, diarrhoea, blood loss, impaired work capacity, and reduced growth rate due to intestinal parasitic infections constitute important health and social problems (WHO, 1981).

The amount of harm caused by intestinal parasitic infections to the health and welfare of individuals and communities depend on: (WHO, 1987),

- the parasite species;
- the intensity and course of the infection;
- the nature of the interactions between the parasite species and concurrent infections;
- the nutritional and immunological status of the population; and
- numerous socio-economic factors.

All the above factors may in turn be modulated by seasonal and climatic conditions.

There are limitations in using stool examination to measure infection status, since some infected individuals have no patent infection. Guyatt and Bundy (1993) demonstrates that stool examination under-estimates the actual infection prevalence, and that the degree of under-estimation is dependent on the level of infection, the nematode species and the

parasite sex ratio. These findings have implications for the validity of epidemiology surveys and the evaluation of control programmes.

There are a number of reasons why infected individuals are diagnosed negative by stool examination. Firstly, there are the technical problems involved in sampling stools for eggs. Coprological techniques vary in their sensitivity (Pawlowski & Arfaa, 1984), and the probability of detecting an egg will depend on the number of samples examined. The number of eggs in a stool sample is influenced by many factors including the amount of stool passed by the host, the distribution of the eggs within the stool and the daily egg output by the female worms. These factors show daily fluctuations and individual variations (Sinniah, 1982). Egg output is also affected by density-dependent constraints on worm fecundity (Bundy, 1986; Anderson & Schad, 1985). An additional problem, which has received less research attention, is that some infected individuals may harbour non-patent infections.

The effect of mating probability on the estimation of fecundity has been investigated (Anderson & May, 1991). In the case of *Trichuris trichiura* and hookworm, a female worm needs to be fertilised by a male in order to produce eggs; male only, or female only infections will both result in a false negative. Female *A. lumbricoides* worms, on the other hand, produce eggs even in the absence of males (Feachem *et al.*, 1983). Therefore only the absence of female worms will result in the absence of eggs.

Booth and Bundy (1992) studied the comparative distribution of the three geohelminths in different geographical areas and showed that *A. lumbricoides* and *T. trichiura* have closely related distributions, while hookworm infection is largely independent of the other two. These results indicate that many communities are at risk of disease from infection by more than one species of helminth. The similar distributions and epidemiological characteristics of *A. lumbricoides* and *T. trichiura* suggest that simultaneous control of these two parasites by the same strategy would be feasible and highly beneficial to communities.

Intestinal parasites have received a great deal of attention in recent years (WHO, 1981, 1985, and 1987; Al-Ballaa *et al.*, 1993; Kappus *et al.*, 1994). The wide occurrence of *Ascaris lumbricoides* and *Trichuris trichiura* in Jordan has been described by Alicata and Dajani (1955).

Recent and published information on the prevalence and seasonal occurrence of intestinal parasites in Jordan is lacking. This study was therefore aimed at determining the intestinal parasites found in this part of the world, their seasonal occurrence, and their prevalence amongst the Amman population. Data for the years 1990 to 1994 based on examinations done at the Central Laboratories in the Health Ministry have been analysed.

The study was undertaken to evaluate the present status of intestinal parasitic infections, especially the incidence of *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia saginata* in the Jordanian population and their relationship to the availability of sanitary facilities. The aim was to evaluate the potential risk to public health posed by these parasites, in using sludge or wastewater for agriculture. The information should also helpful to decide which will be the best and cheapest treatment for sludge to eliminate pathogens, and the type of crops that can be planted to be sure of no harm to public health.

3.2 Methods

Data has been collected from the Health Ministry Central Laboratory and was statistically analysed by the author. Fresh stool specimens from patients attending the Health Ministry Central Laboratory in Amman (capital of Jordan) were examined for intestinal parasites during the period 1990-1994. These specimens were first examined macroscopically to detect any parasitic worms, and each specimen was then sedimented in normal saline and centrifuged at 2000 rpm for three minutes. The supernatant was discarded and the sediment was examined under the microscope. The pertinent findings in this study are based upon a single faecal specimen from each individual.

The results were tabulated as monthly and annual counts, mean seasonal counts, and percentage of prevalence of infection for each intestinal parasite was calculated.

3.3 Results

A total of 22,214 patients were examined in the period from 1990-1994. Of these 3,352 (15%) were found positive for intestinal parasites (Table 3.1). Nine common parasites were identified and are listed in Table 3.1. Eight helminths were identified, five nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Enterobius vermicularis*, and *Strongyloides stercoralis*; one trematode (*Schistosoma mansoni*); and two cestodes (*Hymenolepis nana*, and *Taenia saginata*).

Entamoeba spp. had the highest percentage of positive cases (39%), followed by *Giardia lamblia* (31%) and *Hymenolepis nana* (7%). Other parasites were much less prevalent (Table 3.1). The total percentage of positive cases increased from 10% in 1990 to 17% in 1994 (Table 3.1). Protozoan infections occurred in 81% of all positive cases, helminthic and intestinal nematodes infections in 19% and 7% respectively (Table 3.2).

Referring to Table 3.2, there was a 55% and 37% reduction in the prevalence of intestinal nematodes and intestinal helminth infections respectively from 1990 until 1994 [specifically, the reduction for *Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia saginata* and *Hymenolepis nana* was 60%, 65%, 64%, and 30% respectively (Table 3.1)]. In contrast, for the protozoa infections there was an increase of 14% in the prevalence of this group in the same period (from 1990 to 1994).

Figures 3.1, 3.2, 3.3, 3.4, and 3.5 show the seasonal occurrence of the five main intestinal parasites (*Entamoeba* spp., *G. lamblia*, *Hymenolepis nana*, *Enterobius vermicularis* and *A. lumbricoides*). Highest incidence of protozoa infections occurred generally in spring and summer; lowest incidence occurred in autumn and winter. Figure 3.6 also shows the gradual increase in protozoan infections between 1990 and 1994. *Hymenolepis nana* and *Enterobius vermicularis* did not show any seasonal variation.

TABLE 3.1. Percentages of incidence of different intestinal parasites in Amman city in the period 1990-1994

Positive cases of individual parasite % (numbers)	Year					
	1990	1991	1992	1993	1994	**Mean % (Total)**
Ascaris lumbricoides	2.5 (9)	1.6 (12)	2 (15)	3 (21)	1 (6)	**2 (63)**
Hookworm spp.*	0.8 (3)	0.6 (5)	0 (0)	1 (6)	0.5 (4)	**0.6 (18)**
Enterobius vermicularis	5.7 (21)	3 (22)	3 (21)	2 (16)	2 (12)	**3 (92)**
Trichuris trichiura	0.3 (1)	0 (0)	0.3 (2)	0.3 (2)	0.6 (5)	**0.3 (10)**
Strongyloides stercoralis	1.1 (4)	3 (22)	1 (7)	1 (7)	1 (9)	**1.4 (49)**
Taenia saginata	1.1 (4)	0.4 (3)	0.4 (3)	0.6 (4)	0.4 (3)	**0.6 (17)**
Hymenolepis nana	10.2 (37)	7 (52)	5 (39)	7 (50)	7 (55)	**7 (233)**
Giardia lamblia	52 (188)	28 (210)	30 (223)	23 (163)	24 (191)	**31 (975)**
Entamoeba spp.♣	2.2 (8)	42 (320)	48 (352)	51 (359)	53 (423)	**39 (1462)**
Others	24 (88)	14 (103)	11 (79)	10 (73)	11 (90)	**13 (433)**
Number of positive cases	363	749	741	701	798	**3352**
Total number examined	3673	4924	4435	4488	4694	**22214**
%	10	15	17	16	17	**15**

* *Ancylostoma duodenale*

♣ *Ent. histolytica* and *Ent. coli*.

The prevalence of various intestinal parasitic infestations among all specimens is shown in Table 3.3. The overall prevalence was 15%. The most common parasites encountered during the period of study were *Entamoeba* spp. with a prevalence rate of 6.6%. *Giardia lamblia* ranked second with a prevalence rate of 4.4% followed by *Hymenolepis nana* (1%). The least common parasite found was *Trichuris trichiura* 0.045%. *Entamoeba* species infection was more common during 1991-1994, while *Giardia* infection was more common during the period between 1990-1992 (Fig. 3.6). Slight variations could be detected during the study period for the common helminthic diseases (Table 3.1 and Fig. 3.6).

TABLE 3.2. Results of microscopic stool examination with respect to the infective parasite groups in the period 1990-1994

Year	*Number of positive cases	Total positive cases of groups % (Numbers)		
		Protozoa	Helminth	Nematode♠
1990	364	70 (255)	30 (109)	10 (38)
1991	748	81 (605)	19 (143)	8 (61)
1992	741	86 (637)	14 (104)	6 (45)
1993	701	80 (558)	20 (143)	7 (52)
1994	798	81 (648)	19 (150)	4.5 (36)
Total	**3352**	**81 (2703)**	**19 (649)**	**7 (232)**

* number of positive cases = total positive cases of protozoa + total positive cases of helminths
♠% incidence of nematode group = incidence of nematode infections/total no. of positive cases for that year

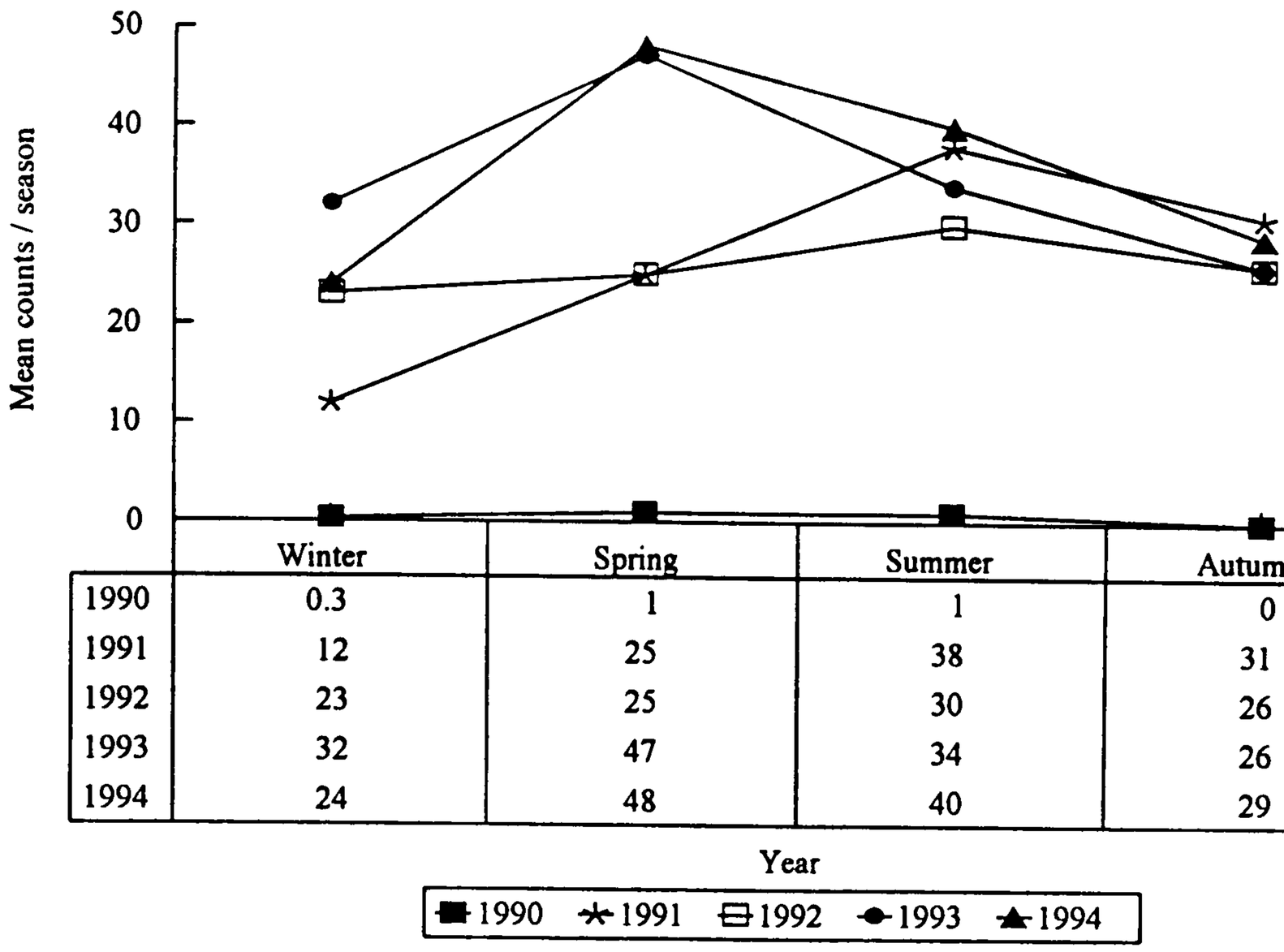


FIG. 3.1. Variations in the means of seasonal counts of *Entamoeba* spp. in the period 1990-1994

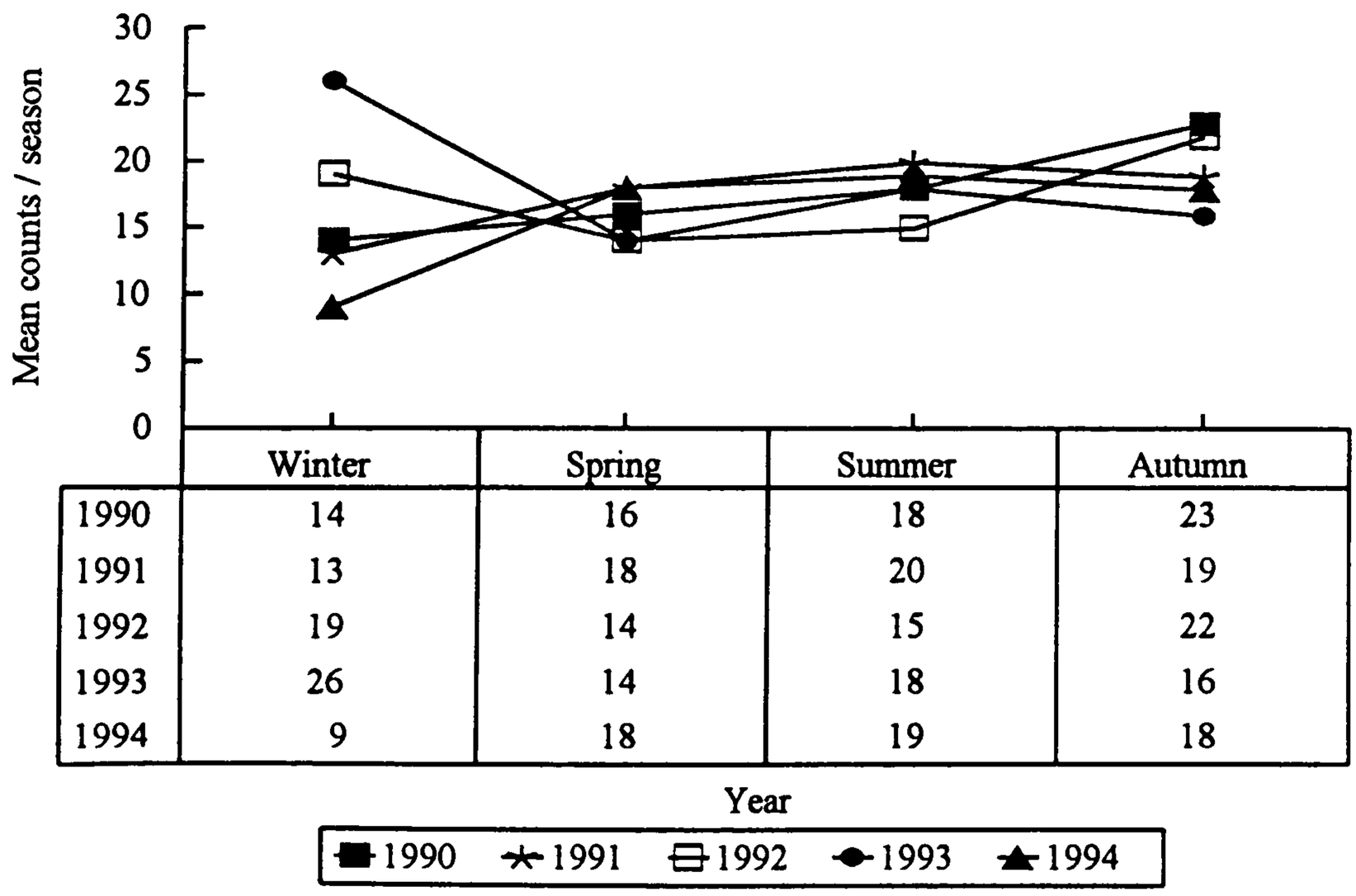
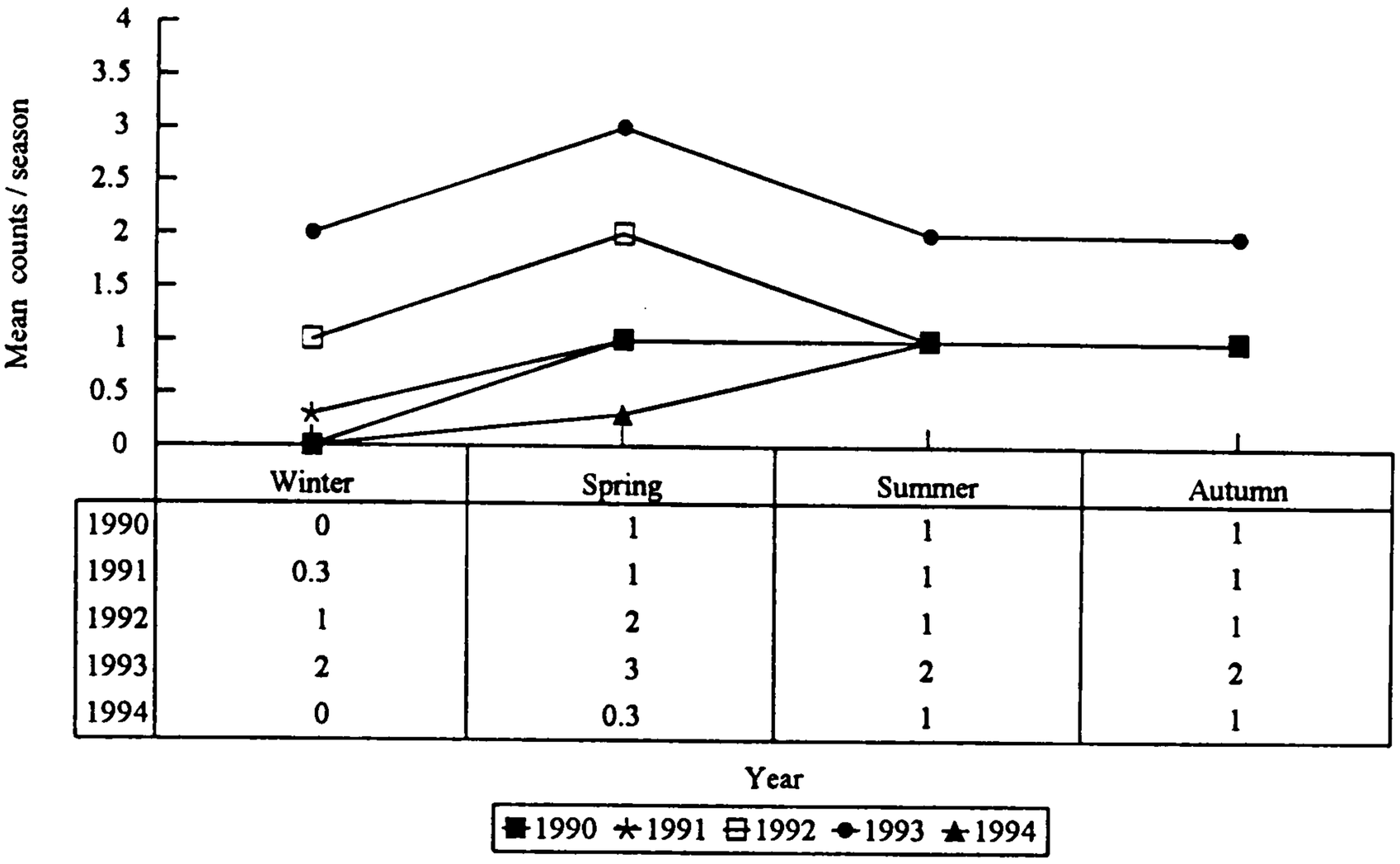
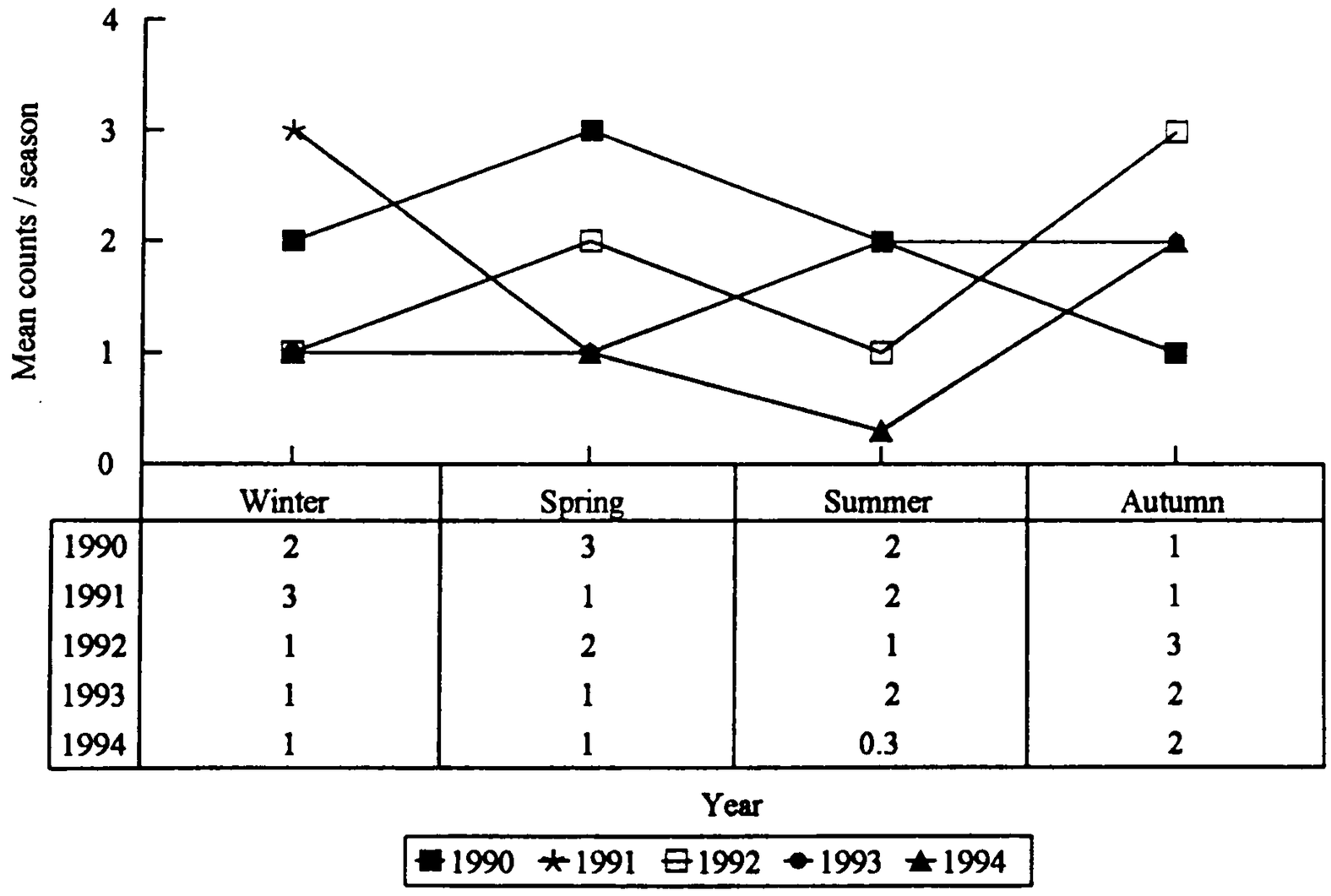


FIG. 3.2 Variations in the means of seasonal counts of *Giardia lamblia* from the period 1990-1994



	Winter	Spring	Summer	Autumn
1990	0	1	1	1
1991	0.3	1	1	1
1992	1	2	1	1
1993	2	3	2	2
1994	0	0.3	1	1

FIG. 3.3. Variations in the means of seasonal counts of *Ascaris lumbricoides* from the period 1990-1994



	Winter	Spring	Summer	Autumn
1990	2	3	2	1
1991	3	1	2	1
1992	1	2	1	3
1993	1	1	2	2
1994	1	1	0.3	2

FIG. 3.4 Variations in the means of seasonal counts of *Enterobius vermicularis* in the period 1990-1994

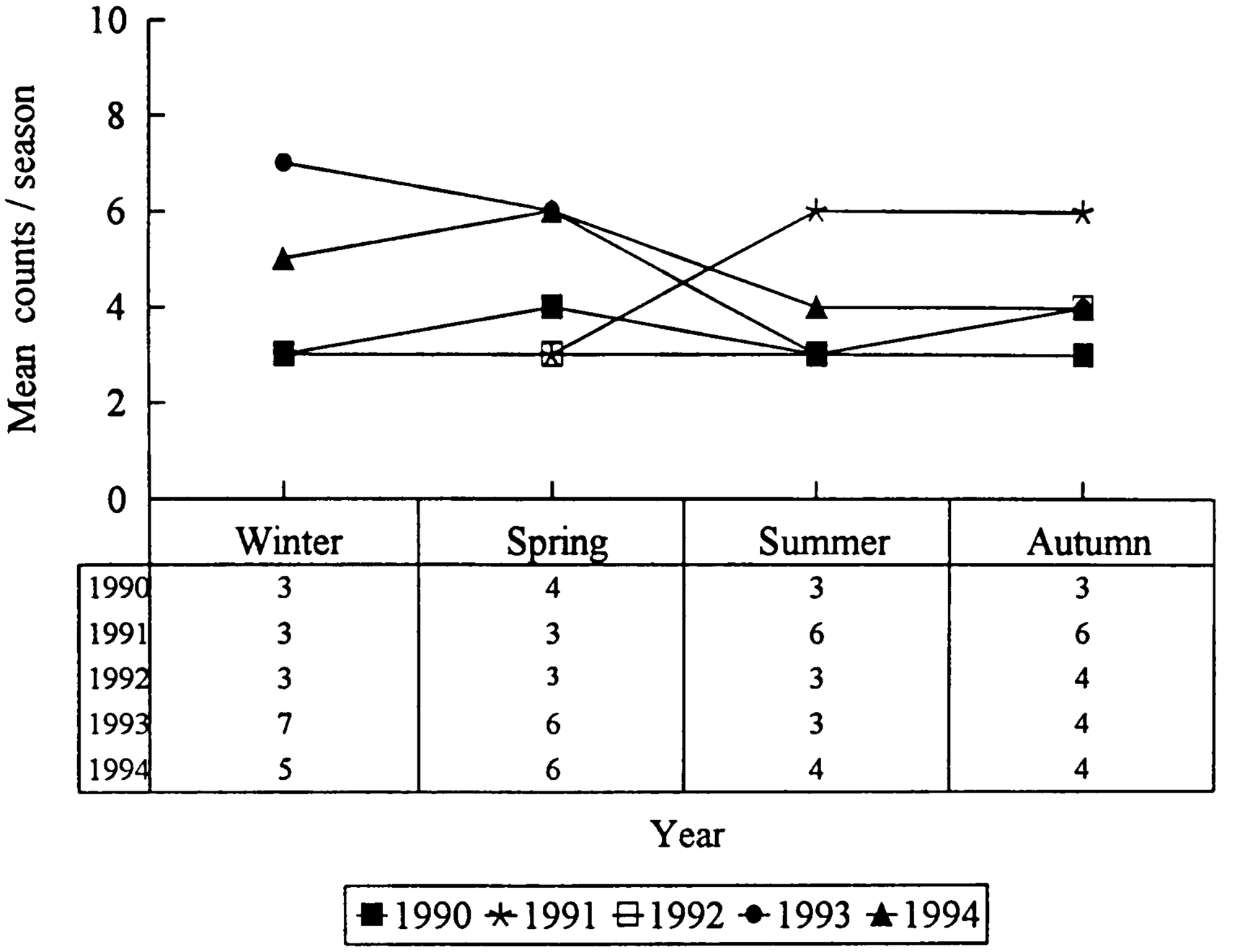


FIG. 3.5 Variations in the means of seasonal counts of *Hymenolepis nana* in the period 1990-1994

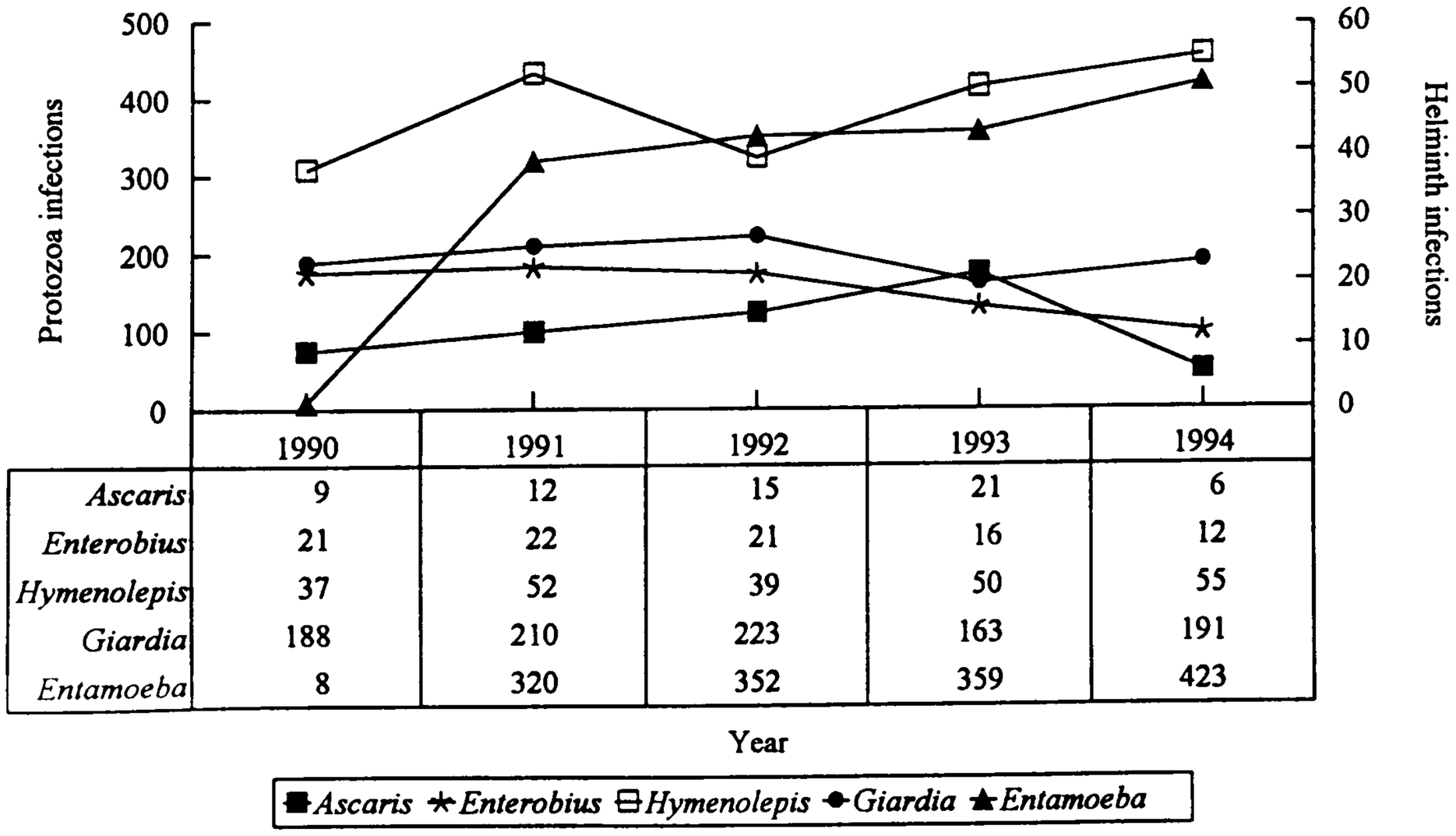


FIG. 3.6. Total number of positive cases for the commonest intestinal parasites in Amman during the period 1990-1994

3.4 Discussion

The production of eggs and cysts by infected persons will be transported by wastewater to the treatment plants and then to the treated wastewater and hence the potential risk encountered in reusing treatment plant effluents or sludges cannot be overlooked. For this reason it was important to know the prevalence and incidence of intestinal parasites in the Jordanian population, to allow some assessment of the risk of the transmission of communicable diseases through wastewater and sludge use.

For a more comprehensive picture of the intestinal parasites, as well as to have an idea about the spread of intestinal parasites among the population in Jordan, Table 3.1 and 3.2 shows the variety of these that may be present in domestic sewage treatment plants. The commonest protozoa infections were amoebiasis followed by giardiasis with highest positivity rate among the examined group. Other protozoa spp. were also observed with low infection rate i.e. *Iodamoeba buetschlii*, *Trichomonas hominis* and *Chilomastix mesnili*. Furthermore, the commonest helminth infections were *Hymenolepis nana.* Other pathogenic helminths were also detected in this study (i.e. *Schistosoma mansoni*), which were only found in non-Jordanian stool samples, mainly dominant in Egypt expatriate workers. Because the snail intermediate host of *S. mansoni* is not found in Jordan, Schistosomiasis most probably will not become endemic. In the late seventies, Saliba *et al.* (1976) recorded the existence of *Bulinus truncatus* snails (the intermediate host for *Schistosoma haematobium*) at a site in the Jordan valley. Hookworms (*Ancylostoma duodenale*) were only found in Asian expatriate workers.

In the present study the detection of protozoa was hindered by practical problems. A lack of the necessary financial support for epidemiological studies for protozoa prevalence; shortfall in both the number and technical expertise of the trained technicians. It was subsequently found that no differentiation was made by the technicians between *Entamoeba coli* and *Ent. histolytica* and furthermore *Ent. histolytica* was sometimes confused with macrophages, thus giving false positive results. To avoid confusion *Ent. histolytica* and *Ent. coli* are thus reported together as *Entamoeba* species, although, from independent observation it is thought that *Ent. coli* predominants

(personal communication, Assaf, 1995). The results collected in the present study show *G. lamblia* to be by for the most prevalent intestinal parasite in 1990, however during the period of 1992 to 1994 the prevalence of *Giardia* slightly decreased (Table 3.1, Fig. 3.2). In 1991 to 1994 there was a sudden increase of amoebiasis. For the reasons stated above it is unwise to rely on this protozoa data, it can however give a general and preliminary idea about protozoa prevalence in Amman.

From Tables 3.1 and 3.2, it can be predicted that the wastewater in Jordan may involve a wide range of intestinal parasites and the potential risk of transmission is very high, because some of these parasites (especially intestinal nematodes) have long persistence in the environment; long latent period or development stage; low infective dose; weak host immunity; minimal concurrent transmission through other routes, such as food, water and poor personal or domestic hygiene that can be expected among those posing the greatest actual risk from sludge application and wastewater reuse.

Feachem *et al.* (1983) have divided infections caused by excreted pathogens into five categories according to their environmental transmission characteristics. In this study, the dominant intestinal parasites are the protozoa (81%) (*Entamoeba* and *Giardia* spp.), followed by the helminths *Hymenolepis nana* (7%) and *Enterobius vermicularis* (3%) are all among Category I which are infective immediately on excretion (non-latent), have a low median infective dose but cannot multiply in the environment. 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 times of excreted protozoa (25 days, estimated maximum life of infective stage at 20-30°C) may be long enough for them to pose a health risk in schemes for the use excreta and wastewater (WHO, 1989).

From a survey of the literature on the distribution of *Trichuris* and *Ascaris*, it was observed in general the *Ascaris* incidence was considerably higher than that of *Trichuris* in countries having a light rainfall. On the other hand in those with a heavy rainfall, the *Trichuris* incidence was nearly always high, frequently exceeding that of *Ascaris*. Spindler (1929) showed that *Trichuris* eggs require more moisture for development, and consequently die under moisture conditions favourable for the development of *Ascaris*

eggs. These findings explain the low prevalence of *Trichuris* (0.045%) compared with *Ascaris* prevalence (0.3%) in Jordan. Similar results are found in Syria, Saudi Arabia, West bank of Jordan but not in the USA (Table 3.3).

It should be noted that *Taenia spp.* is not highly prevalent in Jordan. *Taenia saginata* eggs were detected in few cases, only 0.08%, with a noticeable reduction, about 64%, from 1990 to 1994. This may be related to the fact that people have traditional food and cooking habits in this country, cooking their meat well before consumption.

The modes of transmission of beef tapeworm in Jordan were not applicable. The link from cow to man is readily explained by the eating of well cooked beef; the link from man to cow, might be explained by the fact that there is no application of sewage sludge to pasture land in Jordan, thus preventing the opportunities for cattle to ingest faecal material of human origin.

Long-term reduction in transmission of *Taenia* depends on improved sanitation and sanitary education. Disposal of faeces in a way that prevents any contact between the infective eggs and the intermediate host will break the life cycle. The use of nightsoil or sludges as a fertiliser on pasture, or disposal of effluents into rivers that are a source of drinking water for cattle, necessitates adequate treatment. Educational programs should cover sanitary education, prevention of illegal slaughter and unsupervised meat distribution, meat inspection, and cooking habits (Feachem *et al.*, 1983).

The incidence of intestinal parasites does not shown seasonal variation, especially for helminth infections such as *Hymenolepis nana* and *Enterobius vermicularis* (Figures 3.1-3.5). In contrast, protozoa and *Ascaris lumbricoides* prevalence rate showed a slight increase in the spring or summer season. In general the lowest prevalence rates occurred in the winter months, and this coincides with low temperatures, the rainy season, and less use of wastewater in irrigation. The rate of incidence increases gradually in spring with the rise in temperatures, and continues to increase in the summer months. The peak incidence in the spring and summer months coincides with high temperatures and the intensive use of treated wastewater in irrigation.

TABLE 3.3. Comparison of prevalences of the main intestinal parasites infections between different countries♦

Parasite	Total percentages of positive cases of individual parasite				
	Jordan	USA	West bank of Jordan	Saudi Arabia	Syria
City	**Amman**	49 states	Nablus	Riyadh district	Aleppo
Ascaris lumbricoides	**0.3**	0.8	5.7	3	69
Hookworm spp.*	**0.08**	1.5	-	0.4	-
Enterobius vermicularis	**0.41**	0.4	0.13	0.2	-
Trichuris trichiura	**0.045**	1.2	0.4	2.5	21
Strongyloides stercoralis	**0.22**	0.4	0.004	0.1	-
Taenia saginata	**0.08**	<0.1	0.2	0.2	-
Hymenolepis nana	**1**	0.4	0.8	0.8	0.8
Giardia lamblia	**4.4**	7.2	7.3	6.3	10
Entamoeba spp.	**6.6♣**	5.1	22.9	8.8	11
Number of positive cases	**3352**	43,150	7412	5737	1321
Total number examined	**22,214**	216,275	22,970	23,514	2220
%	**15**	20	32.3	24.4	60
Reference	present study	Kappus *et al.* (1994)	Ali-Shtayeh *et al.* (1989)	Abdel-Hafez *et al.* (1986)	Bradley& Hadidy (1981)

♦Health Ministry Central Laboratory patient stool samples

* *Ancylostoma duodenale*

♣ *Ent. histolytica* and *Ent. coli.*

The incidence of intestinal infections in Amman city is relatively low compared to the surrounding countries. For example, in a study conducted in Yemen Arab Republic by Rudenko (1980), nematodes were found in 69%, protozoal infection in 87%, schistosomiasis in 22% and tapeworms in 8% of the population examined. The incidence of intestinal infections in Saudi Arabia is relatively low; Abdel-Hafez *et al.* (1986) found 24.4% total positive examination for intestinal parasites in 23516 stool specimens in Riyadh (Saudi Arabia). *Ent. histolytica* (8.8%) and *Giardia lamblia* (6.3%) were the commonest parasites found.

Furthermore, in another study on the Saudi population, the prevalence rate was found to be comparatively low at 9.3% (Abu Al-Saud, 1983), and prevailing parasites were *Giardia lamblia*, *Schistosoma mansoni*, and *Ent. histolytica* (Bolbol & Mahmoud, 1984). In the western region of Saudi Arabia, *Ent. histolytica* was found in 9.6% of the stool samples examined, followed by *Giardia lamblia* at 4.5% (Siddiqui *et al.*, 1982).

A study conducted by Ali *et al.* (1992) in Al-Medina City (Saudi Arabia) demonstrated that approximately 14% of 13,2216 individuals harboured potentially pathogenic parasites in which the most common parasite was *Giardia lamblia* (33%). In the present study, of the 22,214 stool specimens studied during the period 1990-1994 in Amman City, 3,352 (15%) overall were positive for intestinal parasites; 11% of all samples were protozoan and 2% were intestinal helminth.

High prevalence rates of intestinal parasites in Bangladesh, India, and Pakistan are well documented. In southeast Asia (Alor Island, southeast Indonesia), the infestation rate of intestinal parasites was found to be 80.1% in the coastal areas and reached 94.1% in the highland regions (Joesoef & Dennis, 1980).

A comparison of the prevalence of the main intestinal parasites between five different countries, (USA, West Bank of Jordan, Saudi Arabia, Syria, and Jordan) are shown in Table 3.3. The prevalence of intestinal infections in Jordan (Amman City) was lower than that in some other Arab countries. In a similar study conducted in the West Bank of Jordan (Nablus city), Ali-Shtayeh *et al.* (1989) found 32.3% of patients were positive for intestinal parasites.

In Saudi Arabia (Abdel-Hafez *et al.*, 1986) reported 24.4% of patients were positive for intestinal parasites, while in this study only 15% of the patients found to have positive parasitic infections in Amman City. Only for *Enterobius* and *Strongyloides* the infection rate was higher in Amman City compared with Nablus City and Saudi Arabia; all the other parasite infections showed lower rates of infection in Amman City. Species of *Dicrocoelium*, *Trichostrongylus* and *Fasciola* were among the intestinal parasites present in Saudi Arabia but absent in Amman and Nablus City. *Trichomonas hominis*, on the other hand, was found in 0.14% of the stool specimens examined in this study; it was also present on the WestBank of Jordan at 0.5%, but was not found in Saudi Arabia stool specimens (Table 3.3).

In general, Aleppo City (Syria) had much higher total rate intestinal parasitic infections within the population (60%) compared with the prevalence rate in Jordan (15%) (Table 3.3). This is due to the extensive use of Aleppo untreated domestic sewage effluents for the irrigation of salad crops such as lettuce, radish, cucumber, onion, aubergine, tomato and mint (Bradley & Hadidy, 1981).

Table 3.3 shows that the Jordanian population had a higher prevalence of amoebiasis and hymenolepiasis than the USA population. This is can be explained by the fact that the mode of transmission of these pathogens occurs predominantly through direct transmission from person to person in the immediate domestic environment, possibly reflecting the crowding and low standards of personal hygiene that prevail in Jordan when compared with USA. In contrast, for the remaining intestinal parasite infections, prevalences were lower in the Jordan population than the USA population. This reflects the development and feasibility of schemes for wastewater treatment and reuse of treated wastewater in agriculture in Jordan, which has increased the degree of health protection.

Study of the epidemiology of amoebiasis and giardiasis suffers from several weaknesses, including (WHO, 1987):

- lack of simple and reliable diagnostic techniques;
- variations in clinical diagnostic criteria;
- shortage of trained personnel; and
- reluctance of institutions and governments to provide the necessary support.

The transmission of most of the intestinal parasites reflects the local level of sanitation and the availability and quality of wastewater and sludge. A high level of Ascariasis is a good indicator of improper faecal disposal and the need to monitor the effectiveness of sanitation projects in the area. A high level of Giardiasis reflects the lack of water or its poor quality.

The low prevalence of *Ascaris lumbricoides* (0.3%), *Taenia saginata* (0.08%), and *Trichuris trichiura* (0.045%) in this study population is noteworthy. This may be related to environmental conditions including hot summers and dry weather, little surface water and presence of waste stabilisation ponds for domestic sewage treatment, with its high efficiency to remove helminth eggs, and the improvement in the sanitary services in this country. All these factors may be detrimental to the development and survival of the infective forms of these parasites.

In summer 1979, Abdel-Hafez and Abdel-Hafez (1984) found the most common human intestinal parasites in the Jordan Valley and the Hosn refugee camp (near Irbid) were *Entamoeba coli*, *Giardia intestinalis*, *Ascaris lumbricoides*, and *Hymenolepis nana*. The rates of infection with these organisms were 42%, 19%, 39%, and 22% respectively (Table 3.4).

Comparing the results of this study with the earlier surveys for the prevalence of intestinal parasites in Jordanian population in 1954 and 1979 (Alicata & Dajani, 1955; Abdel-Hafez & Abdel-Hafez, 1984), referring to Table 3.4, there was an obvious decrease in the prevalence of protozoan infections among the Jordanian population from 1954 to 1994 and from 1979 to 1994(≥47% and ≥77% reduction, respectively). For *Ascaris*

lumbricoides and *Trichuris trichiura,* the reduction for both was more than 99% in both surveys compared with this study.

This clear reduction in the prevalence of intestinal parasitic infections between 1954 and the present day, can be explained as follows:- much development is taking place in Jordan which might have an effect on public health such as better living conditions, a clean water supply, proper sewage disposal and health education, which will certainly reduce the prevalence of many of the communicable diseases.

TABLE 3.4. Percent occurrence of common intestinal parasites in Jordan during different periods

Parasite	Percentage of positive cases during		
	1990-1994	1979	1954
Entamoeba spp.	**6.6**	42	58.3
Giardia	**4.4**	19	8.3
Ascaris	**0.3**	39	51.3
Hymenolepis nana	**1**	22	1.7
Trichuris trichiura	**0.045**	-	44.3
Strongyloides stercoralis	**0.22**	-	0.3
Taenia saginata	**0.08**	1	1.3
Reference	**present study**	**Abdel-Hafez & Abdel-Hafez (1984).**	**Alicata & Dajani (1955)**

The percentage of the Jordanian population connected to sewerage system was increased from 31% in 1986 to 52% in 1990. Furthermore, the new version of Material Law No. 2 for using wastewater for agriculture was issued in 1989, which is considered to be more advanced than the old version (1982); it allows the use of treated wastewater for

irrigation of cereals and vegetables eaten cooked, under certain conditions, and does not allow irrigation of crops eaten uncooked by any type of effluent, regardless of its microbiological quality. The law also prevents irrigation of industrial crops and trees by raw wastewater. Al-Samra waste stabilisation ponds, commissioned in 1985, treated about 81% of the total reclaimed water generated in Jordan in 1989, and had high efficiency in removal of helminth eggs (Saqqar & Pescod, 1992). All these issues may help to explain the reduction in prevalence of parasitic infections in Jordan.

Considering the 5 years of data summarised in this study (1990-1994), it was evident that the prevalence of soil-transmitted helminths such *A. lumbricoides* and *T. trichiura* has decreased remarkably, while that of intestinal protozoans has not. These results indicate that the potential risk of intestinal parasitic infections in Jordan is relatively high. Preventive measures must include further health education of the public regarding the transmission of these diseases, hygienic waste disposal, and improved levels of personal hygiene. Improvement of sanitation has failed to affect the prevalence of protozoan infections, but has caused a marked decrease in the prevalence of infection of helminth diseases.

The contamination of water supply plays an important role in transmitting protozoan diseases. *Entamoeba* spp. and *Giardia lamblia* were the most frequent parasite identified in this study, 11% of the total stool specimen (81% of all positive cases). This parasite has emerged in recent years as an important cause of diarrhoea and has a worldwide distribution; Table 3.3 shows the highest rates of protozoan infection in the West Bank of Jordan, Saudi Arabia and the USA. Consumption of faecally contaminated water is the main mode of transmission. This is followed by direct person-to-person contact, which occurs in groups with poor faecal-oral hygiene. On a few occasions, food has been implicated as a source (Peterson *et al.*, 1988). Though the mode of transmission of this parasite in Amman City is probably similar to that found in other countries, no local data on this issue is available.

None of the protozoa of public health significance (for example *Ent. histolytica*, and *Giardia lamblia*) are mentioned in the guidelines of the reuse of treated wastewater for irrigation (WHO, 1989). However, it is suggested that the intestinal nematodes should serve as indicators for all the large settleable pathogens, and it is implied that all protozoan cysts and helminth eggs are removed to the same extent during wastewater treatment (WHO, 1989). This may be an oversimplification as protozoan cysts and trophozoites are lighter than helminth eggs and therefore take longer to settle (Hays, 1977).

The implications of using the nematode guideline for wastewater reuse for agriculture, for the protection of consumers and workers from protozoan infections, are even less known. Lack of good methodology for the enumeration of protozoa in wastewater and sewage treatment effluents is currently inhibiting research and this is an area which merits further attention.

Pathogenic protozoa have been recorded in the effluents of ponds (Yanez, 1986) and conventional sewage treatment works (Kott & Kott, 1967) where helminth eggs have been removed. Grimason *et al.* (1993), suggest a minimum retention time of 37.3 days to ensure the complete removal of *Giardia* cysts in waste stabilisation ponds. Further research and investigation is needed to cover the gaps in the knowledge of this area.

As the Al-Samra pond system has been in operation since 1985, sludge has accumulated in the anaerobic ponds and decreased the retention time. This has the effect of further reducing the likelihood of settlement of protozoa cysts, hence significant numbers of these will remain in the final effluent. This may explain the high prevalence of protozoan infections in Jordanian populations.

In conclusion, the present investigation demonstrates that the infestation rate remains a compromising health problem, especially when it has been noted that some of the detected types of parasites are transmissible and could easily be transmitted without the need of the vectors of transmission, animal reservoir hosts, or animate or mobile intermediate hosts. *Giardia lamblia* , *Ent. histolytica* and *Hymenolepis nana* are known to be infective

through the faecal-oral route, so the greatest potential risk may be of spreading such diseases throughout the community because of poor personal hygiene.

Also it can be concluded from this study that the prevalence of intestinal infections in Jordan is lower than in other Arab countries. However, health measures should still be taken in Jordan in order to further reduce these infections. These measures may include proper treatment and disposal of wastewater and sludges, a mass campaign for the treatment of these infections, health education and the supply of clean water.

Further research is needed to clarify many aspects of intestinal parasitic infections in Jordan including the mode of transmission, role of animal reservoirs, immunology and its role in child morbidity and mortality. No data are available on the parasite content of vegetables irrigated with raw and treated wastewater from different types of treatment plants.

CHAPTER FOUR

BASIC VITAL STAINS EXCLUSION AS A METHOD FOR DETERMINING THE VIABILITY OF *ASCARIS* EGGS

4.1 Introduction

In recent years increasing attention has been given to the problem of sludge disposal. Monitoring studies should be conducted to determine the effectiveness of the various sludge treatments in destroying pathogens. This must be done to the point where the great majority of the human and livestock population is protected.

Ascaris eggs are environmentally persistent, with survival times usually in excess of one year; the minimum infective dose is only one egg. Schemes for the use of excreta and wastewater are important mechanisms of transmission of this disease, and a major environmental measure for their control is therefore the effective treatment of excreta, wastewater and wastewater-derived sludges prior to use. Since the use of wastewater and sludge in agriculture is necessary, of importance around the world and is a rapidly developing field, research and further investigations are required to facilitate the determination of viability of *Ascaris* eggs in order to develop analytical methods which will be suitable for routine application (WHO, 1989).

The public health significance of dead eggs is minimal; however, when organisms are infective or viable, the risk to public health can be enormous. Hence, information about the viability of *Ascaris* eggs is of considerable importance. When *Ascaris* eggs are isolated from the environment, whether from soil, sludge or effluent, it is important to know whether they are viable. Techniques used for assessing the viability include the incubation technique (Hass and Todd, 1962; Meyer *et al.*, 1978; Cacares *et al.*, 1987), and exclusion or inclusion of vital dyes (Shepherd, 1962; Kagei, 1982; Kaneshiro & Stern, 1985; Zohu *et al.*, 1985) (Table 4.1). Other published methods include differential flotation to

separate fertile from non-fertile eggs (Stien & Schwartzbrod, 1988); Keller (1951) and Smith (1991) induced larval motility using sodium hypochlorite as a possible viability test for embryonated *Ascaris* eggs; and other researchers used infectivity in animal models (Reimers *et al.*, 1989) as a method for viability. The cost, time and large numbers of eggs required to perform animal infectivity studies make the method impractical for routine use. Hughes *et al.* (1985) found that viability tests carried out *in vitro* did not correlate with infectivity.

The technique described by Hass (1962), Meyer *et al.* (1978), as modified by Carrington and Harman (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 0.1N 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.

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 morphologic criteria method was not as accurate as the culturing method. The direct morphological microscopic method is simple, but it lacks objective standards, requires skill and experience; and it 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. 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 refractile granules within the cell.

TABLE 4.1. 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* eggs	Ogata (1925) Kagei (1982)
Iodine and potassium iodide	0.025 g 1%	*Heterodera* spp. larvae ♣*	Boyd (1941)
Chrysoidin	50 mg/l	*Heterodera* spp. larvae	Doliwo (1956)♦
Phloxine B	5%	*Meloidogyne* eggs*	Fenner (1962)
New blue R	0.05%	*Heterodera* eggs	Shepherd (1962)
Eosin Y	0.67%	Free-living nematodes	Chaudhuri *et al.* (1966)
Methylene blue-eosin-borax	♠	*Ascaris* eggs	Zhou *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)

♣ Potato root eelworm larvae
* Plant parasitic nematode spp.
♦ cited by Chaudhuri *et al.* (1966)
♠ details in the materials and methods section in this chapter.

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).

Keller (1951) 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 (Table 4.2) 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. Nevertheless, according to their results, summarised in Table 4.2, 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 and the methanol, ethanol, 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.

TABLE 4.2. List of incorporation and exclusion of vital stains into different types of helminth eggs

Stains name	*Ascaris*		*Toxocara*		*Trichuris*		*Hymenolepis*	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
Trypan Blue	-	-	-	-	-	-	-	-
Nile Blue Sulfate	+	-	-	+	-	-	-	-
Methylene Blue	+	+	+	+	+	+	+	+
Methyl Violet	+	+	+	+	+	+	+	+
Crystal Violet	+	-	+	-	+	+	+	+
Janus Green	-	-	-	-	-	-	-	-
Brilliant Red	-	-	-	-	-	-	-	-
Benzopurpurin	-	-	+	-	+	-	-	-
Saphranin O	+	+	+	+	+	-	+	-
Carmin Alum Lake	+	+	-	-	-	-	-	-
Br. Cresyl Blue	+	+	+	+	+	+	+	-
Neutral Red	+	+	+	+	+	-	+	-
NILE BLUE SULFATE FRACTIONS:								
Acetone	-	-	+	+	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
Methanol	+	+	+	+	+	-	+	+
Ethanol	+	+	+	+	+	-	+	+
Acetone:ethanol	+	+	+	+	+	-	+	+

+ = stain incorporated into the egg
- = stain excluded by the egg
Source: Kaneshiro & Stern (1985).

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 range of tylenchid genera and species, Shepherd (1962) soaked nematodes in 0.05% aqueous solution of the

new blue R basic vital stain for varying times, for up to 24 hours. *Heterodera* eggs were soaked for up to 7 days; after the requisite time, dead specimens are darkly stained but live ones are 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 salts 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.

4.1.1 Objectives

One of the major difficulties in the work of helminthologists has been to differentiate between living and dead nematodes. Little work has been done in this area. There is little knowledge or comprehensive research about the efficiency of using vital stains to detect the viability of *Ascaris* eggs, although several authors report the use of vital stains for the determination of nematode viability. The development of a reproducible, sensitive, user-friendly viability assay which could be used to determine the viability of *Ascaris* detected in environmental samples would be extremely valuable in helminthology. It should be applicable to small numbers of eggs.

The objective of this study was to develop a rapid, simple and reliable microscopic method for determining the viability of *Ascaris* eggs which, when compared to the incubation method (embryonation), would provide a more practical method for estimating egg viability, and ultimately, to evaluate the versatility of the staining method and the advantages and disadvantages inherent in the method. The specific aims of this research are:

1. To determine the effect of the uptake of four different stains by different stages of corticated and decorticated viable *Ascaris* eggs.

2. To determine the effect of the washing method on uptake of each stain by *Ascaris* eggs at different pH and temperatures, to select the best method for the application of stain to the eggs.

3. To compare inclusion or exclusion of four different basic vital stains with the Incubation method;

4. To assess the impact of known triggers such as different contact time, pH values and temperatures on the uptake of the stains by viable and dead eggs;

5. To determine the best stain and optimum conditions for its use.

Systematic experiments were set up to compare the optimum conditions for using different vital stains (Crystal violet; Meldola's blue stain; Methylene blue-eosin-borax; and Nile blue sulphate) to detect the viability of *Ascaris* eggs. The impact of some biochemical and physical triggers on the uptake of the stain by eggs for detection of viability of *Ascaris* eggs was also investigated.

4.2 Materials and Methods

4.2.1 Preparation of *Ascaris suum* as experimental model

Eggs were removed from the lower part of the uteri of mature female *Ascaris suum* worms from infected pigs obtained from a local slaughterhouse. The uteri were hand homogenised, the resulting egg suspension was centrifuged and washed three times in distilled water before storage at 4°C. To decorticate the shell from the egg, 2% sodium hypochlorite was mixed with corticated *Ascaris* eggs, and allowed to stand for 30 minutes with frequent shaking; the eggs were then washed at least five times with distilled water until neutral pH was achieved. The n-Butanol method was used for separation of unfertilised and dead eggs from the egg suspension (Stien & Schwartzbrod, 1988) (Appendix 4.1).

4.2.2 Preparation of working solutions of vital stains

Crystal Violet stain: 2g crystal violet (Sigma Co.), 20ml 95% ethanol, mixed with 80ml 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 another 10 minutes; after cooling, mix 0.1g eosin; after dissolving, filter, pH 10-11 (Zhou *et al.*, 1985).

Meldola's Blue stain: 0.05g of meldola's blue stain (Sigma Co.) in 100ml of distilled water (Shepherd, 1962).

Nile Blue Sulphate stain: 0.05g nile blue sulphate (Sigma Co.) in 100 ml of distilled water (Lillie, 1977).

Incubation method: Eggs of *Ascaris suum* were placed in 0.1 N H_2SO_4 and incubated at 30°C for periods up to 3-4 weeks. The percentage of developed and undeveloped *Ascaris* eggs was then estimated by microscopic examination.

Microscopy: Ten-microliter aliquots of egg suspension were viewed under phase contrast with an Olympus BH2 microscope (magnification 400x). In each test the percentage of stained viable eggs was compared with the percentage of the viable eggs of the control.

Enumeration of percentage of viable eggs : For each enumeration, at least 100 eggs were counted. The percentage viability of eggs using vital stains was calculated as follows:

$$\frac{\text{Number of unstained eggs (viable)}}{\text{Total (no. of unstained + stained eggs)}} \times 100 = \% \text{ viability}$$

The percentage viability of eggs by using the Incubation method was calculated as follows:

$$\frac{\text{Number of developed eggs (viable)}}{\text{Total no. of } \textit{Ascaris} \text{ eggs counted}} \times 100 = \% \text{ viability}$$

4.2.3 Temperature and pH treatments

Incubation of *Ascaris* eggs with each type of stain (i) at 4°C, 21°C±2, 37°C, 48°C, and 64°C, with different contact times (5 min., 10 min., 30 min., one hour, two hours, three hours, six hours, twelve hours) at pH 5.5 ± 0.5; (ii) boiling for 15 minutes to kill *Ascaris* eggs completely; (iii) using 0.1N H_2SO_4 (pH 1.5); acetic acid buffer (pH 4.5);

phosphate buffer (pH 7.2); sodium bicarbonate (pH 10); 0.1N NaOH (pH 12); (Standard Methods, 1989) (Appendix, 4.2), with different contact times (5min., 10 min., 30 min., one hour, two hours, three hours, six hours, and twelve hours) at 21°C ± 2. Three replicates were run for each experiment.

4.2.4 Effect of washing on the uptake of these different vital stains by *Ascaris* eggs

To detect the viability of *Ascaris* eggs by staining method (Crystal violet; Meldola's blue stain; Methylene blue-eosin-borax; and Nile blue sulphate), by adding one drop of each stain to the test tube with 3ml suspension of decorticated viable eggs at the tested pH or temperatures; after 10 minutes the eggs were then washed twice with distilled water, centrifuged and the viability of the eggs checked under the microscope. The viability of the eggs were compared with and without the washing step.

Experiments were run at different temperatures of 4°C, 21°C±2, 37°C, 48°C, and 64°C, with different contact times (10 min., 30 min., one hour, two hours, three hours, six hours, twelve hours) at pH 5.5 ± 0.5; also different pH using acetic acid buffer (pH 4.5); phosphate buffer (pH 7.2); sodium bicarbonate (pH 10); 0.1N NaOH (pH 12), with different contact times (10 min., 30 min, one hour, two hours, three hours, six hours, and 12 hours) at 21°C±2. Three replicates were run for each experiment.

4.2.5 Statistical analysis

Calculation of correlation coefficients, linear regression analysis and analysis of variance (ANOVA) tests were performed using MICROSOFT EXCEL and CRICKET GRAPH software.

4.3 Results

4.3.1 Exclusion or inclusion of four different vital stains on corticated and decorticated different stages of *Ascaris* eggs

The results of the use of basic vital stains Crystal violet, Methylene blue eosin-borax, and Nile blue sulphate in determining viability of corticated fertilised *Ascaris suum* eggs were inefficient, both dead and live eggs at different stages showed uptake of the blue colour. This was because the shell took up the stain and did not allow the stain to enter inside the eggs (Table 4.3). Only Meldola's blue seem to be excluded from live eggs and absorbed by dead corticated *Ascaris* eggs. The results in Table 4.3 showing decorticated fertilised *Ascaris* eggs viability at different stages, all four stains were capable of distinguishing between viable and non-viable *Ascaris suum* eggs. The internal structure of the dead eggs are stained blue and the viable eggs remained unstained, as can be seen in Plate 4.1.

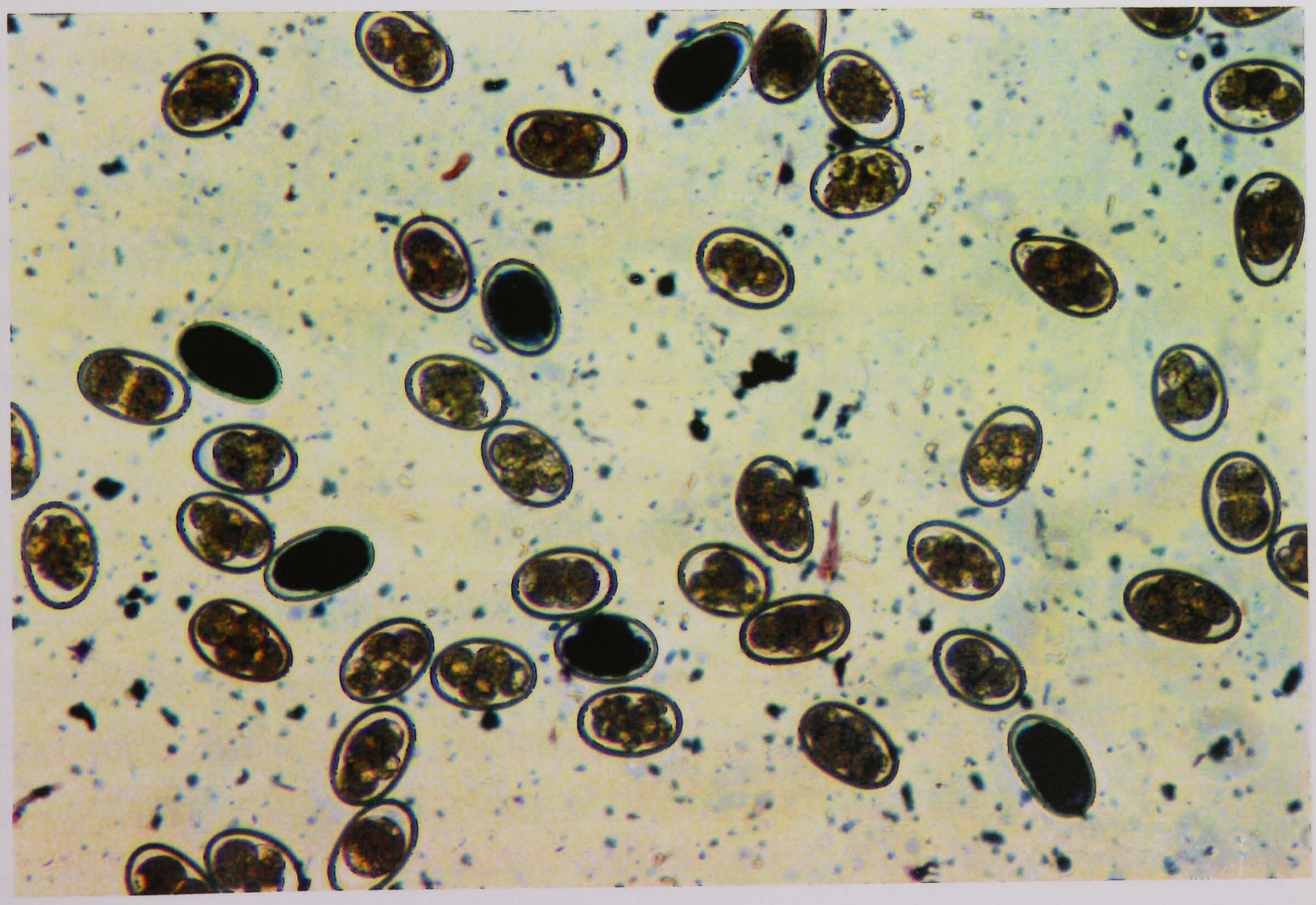


Plate. 4.1. Internal structure of the dead *Ascaris* eggs are stained blue and the viable eggs remained unstained, 100x, Phase Contrast Microsocope

TABLE 4.3. Results of uptake of basic vital stains (Crystal violet, Meldola's blue, MBEB, Nile blue sulphate) by different cell-stage development of *Ascaris suum* eggs.

Cell-stage development	Basic vital stains	Without shell		With shell	
		Live	Dead	Live	Dead
One-cell stage	Crystal violet	-	+	+	+
	Meldola's blue	-	+	-	+
	MBEB	-	+	+	+
	Nile blue sulphate	-	+	+	+
Multi-cell stage	Crystal violet	-	+	+	+
	Meldola's blue	-	+	-	+
	MBEB	-	+	+	+
	Nile blue sulphate	-	+	+	+
Larval-stage	Crystal violet	-	+	+	+
	Meldola's blue	-	+	-	+
	MBEB	-	+	+	+
	Nile blue sulphate	-	+	+	+

- : stain excluded by the egg.
+ : stain incorporated by the egg.
MBEB : Methylene blue eosin-borax.

4.3.2 Effect of the washing experiment on prediction of *Ascaris* eggs viability

To choose the method for application of the vital stain to test the viability of decorticated *Ascaris* eggs, the impact of a washing procedure after application of different vital stains with different pH and temperatures on decorticated *Ascaris* eggs has been assessed. The viability of the eggs were statistically compared with and without the washing step.

A significant increase in predicted egg viability was observed at pH 4.5 and 7.2 (Table 4.4; $P < 0.15$ and F test > 11.1) after washing the eggs with distilled water compared with the uptake of Crystal violet stain by non-washed eggs. This means that the uptake of Crystal violet stain is not efficient at pH 4.5 and 7.2 and it will give unstained dead eggs after washing. Uptake of Crystal violet stain by dead eggs was not affected by washing the eggs with distilled water at a range of temperatures (4°C, 21°C, 37°C, 48°C), and with high pH values (10 and 12), (Table 4.4; $P \geq 0.08$ and F test < 4.6); the results were similar to the non-washing experiments.

TABLE 4.4. Summary of analysis of variance results for the difference between without washing experiment and with washing experiment of Crystal violet, Meldola's blue, MBEB, and Nile blue stains from *Ascaris* eggs at different pH and temperatures

Treatment	Anova♠	Crystal violet	Meldola's blue	MBEB	Nile blue
pH 4.5	P-value	0.007	2.3E-05	3.6E-05	0.8
	F-value*	15.7	137	118	0.07
pH 7.2	P-value	0.15	0.009	0.33	0.8
	F-value*	11.1	14	1.1	0.04
pH 10	P-value	0.11	0.95	0.45	0.69
	F-value*	3.6	0.004	0.64	0.17
pH 12	P-value	0.08	0.06	0.39	0.29
	F-value*	4.6	5.2	0.86	1.33
4°C	P-value	0.66	2.5E-06	0.6	0.6
	F-value*	0.20	293	0.3	0.3
21°C	P-value	0.13	0.005	0.16	0.85
	F-value*	3.11	19	2.6	0.04
37°C	P-value	0.26	0.05	0.03	0.6
	F-value*	1.55	5.6	7.8	0.3
48°C	P-value	0.28	0.02	0.66	0.95
	F-value*	1.42	9.9	0.2	0.003
64°C	P-value	0.15	0.13	0.94	0.83
	F-value*	2.7	3.2	0.005	0.05

Two-Way ANOVA Without Replication.
* Experimental F-value.
Critical F value = 5.99
Significant at the $\alpha = 0.05$ level.
(Note: Running the pH experiments at 21 ± 2; while for temperature experiments at pH 5.5 ± 0.5).
♠Experimental F-value, if it is > than F-critical this mean there is significant difference in treatment; if P > 0.1 this mean there is no significant difference.

Washing *Ascaris* eggs with distilled water had a significant impact on the uptake of the Meldola's blue stain by the dead eggs and affected the prediction of viability. For pH 4.5 and 7.2 (Table 4.4; $P < 0.1$ and F test ≥ 14), or temperatures 4°C, 21°C and 48°C ($P \leq 0.02$ and F test ≥ 9.9) there was significant difference between the two experiments at these conditions. Uptake of Meldola's blue stain by dead *Ascaris* eggs was not affected with washing at pH 10 and 12, and temperatures of 37°C and 64°C ($P \geq 0.05$ and F test ≤ 5.6).

Comparing the results of washing and non-washing experiments for uptake of MBEB by *Ascaris* eggs to detect the viability at different pH's and temperatures, the data show in Table 4.4 that at pH 7.2, 10 and 12, and at temperatures of 4°C, 21°C, 48°C and 64°C the detection of the viability of *Ascaris* eggs did not demonstrate any significant difference (Table 4.4, $P > 0.15$ and F test ≤ 2.6). For washing and non-washing experiments the only difference appeared at pH 4.5 and temperature 37°C ($P \leq 0.031$ and F test > 7.7).

The statistical analysis results in Table 4.4 showed that washing with distilled water after applying Nile blue sulphate stain on to *Ascaris* eggs to detect the percentage viability was similar to the experiment without washing the Nile blue sulphate stain. So uptake of the stain of Nile blue sulphate was not affected by washing with distilled water at different pH values (4.5, 7.2, 10 and 12) and temperatures (4°C, 21°C, 37°C, 48°C and 64°C) (Table 4.4, $P > 0.29$ and F test ≤ 1.33).

4.3.3 Without washing experiments: Impact of different pH and temperatures on the correlation of observed and predicted viability

Table 4.5, summarises the correlation coefficient between each stain viability value (predicted viability) and the Incubation method (observed viability) at pH 4.5, 7.2, and 10; and with temperatures 4°C, 21°C and 37°C values together. A good correlation between Incubation and Crystal violet staining method viabilities was observed (Fig. 4.1), with a correlation coefficient of 0.927. The same treatments performed as above on eggs had a significant impact on the uptake of Meldola's blue or Nile blue sulphate stain by the eggs and affected the prediction of viability. A weak correlation was observed between

observed and predicted viabilities for each stain, with a correlation coefficient of 0.31 and 0.35 respectively (Table 4.5). The uptake of the MBEB stain by *Ascaris* eggs to detect viability will give imprecise detection under these conditions, with a correlation coefficient -0.117 (Table 4.5).

TABLE 4.5. Pearson correlation coefficient between observed and predicted method after treatment with pH (4.5, 7.2, and 10) and temperature (4°C, 21°C, and 37°C)

Basic vital stains	Correlation coefficient
Crystal violet	0.927
Meldola's blue	0.31
MBEB	-0.117
Nile blue sulphate	0.35

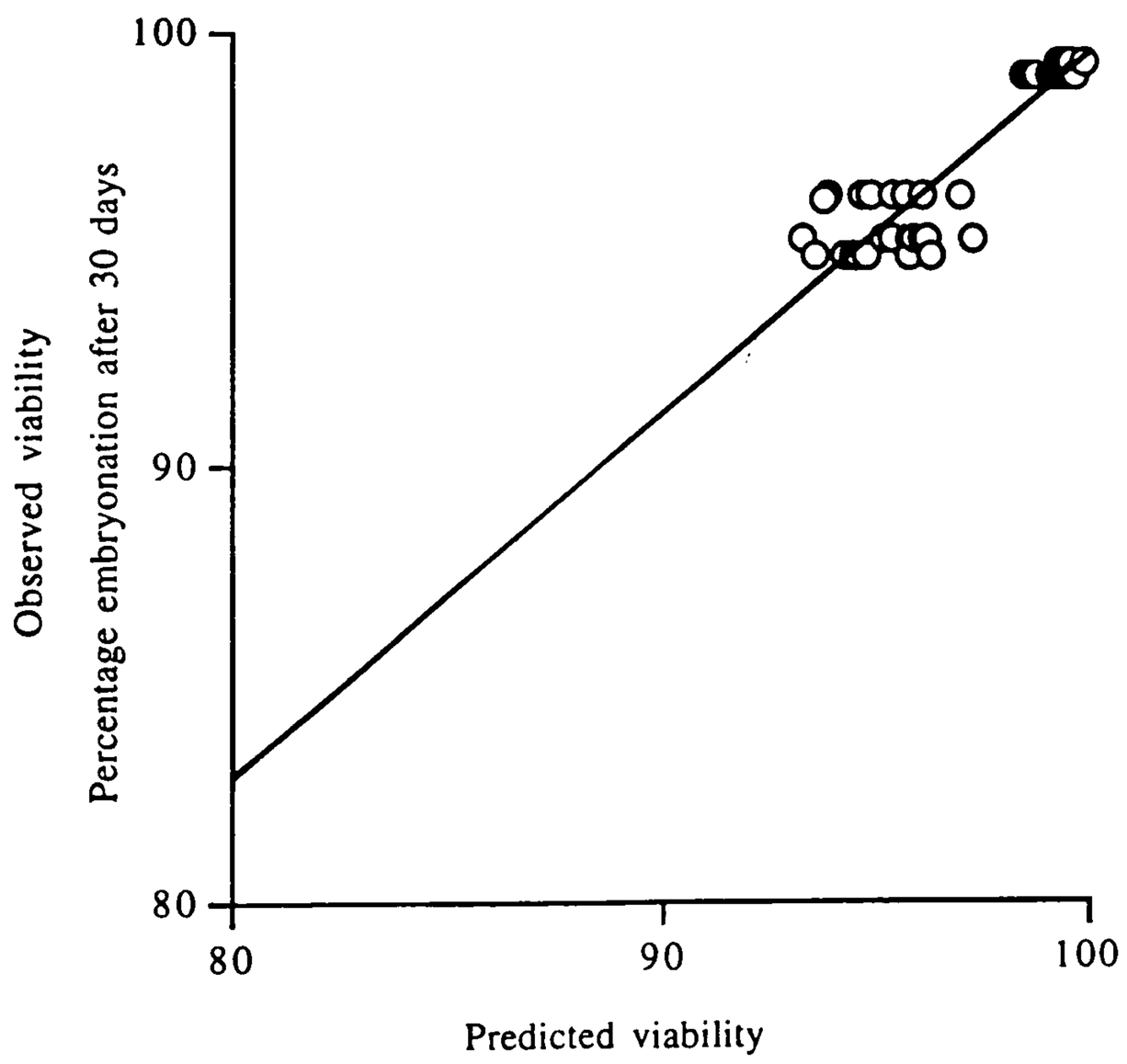


FIG. 4.1. Correlation of predicted (Crystal violet stain) and observed viabilities after treatment with pH (4.5, 7.2 and 10) and temperature (4, 21 and 37°C)

4.3.4 Prediction of maximal embryonation efficiency with different types of vital stains

It was hypothesised that inclusion or exclusion of each vital stain after 5-10 min at 21°C ± 2 could be used to predict the viability of *Ascaris suum* eggs. The proportion of exclusion of vital stain by eggs was the predicted viability and the observed or actual viability was calculated from maximal embryonation using the Incubation method. Eggs were considered viable if the cells inside the eggs was developed at 30°C after 3-4 weeks.

The predicted viabilities (after 5-10 min applying the stain at 21°C ± 2) for each stain (Crystal violet, Meldola's blue, MBEB, and Nile blue sulphate stains) were comparable to the maximum observed embryonation efficiencies (Fig. 4.2, 4.3, 4.4, and 4.5, respectively), and the correlation coefficient and mathematical relationship from linear regression analysis for each stain are summarised in Table 4.6.

TABLE 4.6. Summary for the prediction of eggs viability by four different stains after 5-10 min applying the stain at 21°C ± 2 compared with maximal embryonation viability

Stain	Correlation coefficient	Mathematical relationship	R squared
Crystal violet	0.979	y = 6.034 + 0.937x	0.959
Meldola's blue	0.945	y = 1.479 + 0.988x	0.892
MBEB	0.978	y = 4.083 + 0.957x	0.957
Nile blue sulphate	0.963	y = 13.497 + 0.865x	0.928

Correlation of the Incubation method with predicted viability (exclusion of Crystal violet) gave a correlation coefficient of 0.979. Linear regression analysis provided the following mathematical relationship between predicted viability (x) and observed viability (y): $y = 6.034 + 0.937x$ ($r^2 = 0.959$). Analysis of the regression coefficient demonstrated no significant difference between this relationship ($y = 6.034 + 0.937x$) and the simpler

mathematical relationship y = x (intercept = 0, and slope = 0.9996) (Fig. 4.2, and Table 4.6).

Also the Incubation method gave a correlation coefficient of 0.945 with predicted viability (exclusion of Meldola's blue). Linear regression analysis provided the following mathematical relationship between predicted viability (x) and observed viability (y): y = 1.479 + 0.988x (r^2 = 0.892). Analysis of the regression coefficient demonstrated no significant difference between this relationship (y = 1.479 + 0.988x) and the simpler mathematical relationship y = x (intercept =0, and slope = 1.00) (Fig. 4.3 and Table 4.6).

Correlation of the Incubation method with predicted viability (exclusion of MBEB) gave a correlation coefficient of 0.978. Linear regression analysis provided the following mathematical relationship between predicted viability (x) and observed viability (y): y = 4.083 + 0.957x (r^2 = 0.957). Analysis of the regression coefficient demonstrated no significant difference between this relationship (y = 4.083 + 0.957x) and the simpler mathematical relationship y = x (intercept =0, and slope = 0.999) (Fig. 4.4, Table 4.6).

Correlation of the Incubation method with predicted viability (exclusion of Nile blue) gave a correlation coefficient of 0.963. Linear regression analysis provided the following mathematical relationship between predicted viability (x) and observed viability (y): y = 13.497 + 0.865x (r^2 = 0.928). Analysis of the regression coefficient demonstrated no significant difference between this relationship (y = 13.497 + 0.865x) and the simpler mathematical relationship y = x (intercept = 0, and slope = 1.00) (Fig. 4.5, Table 4.6).

Since a simple correlation between predicted viability and observed viability had been derived, it was suggested that those eggs which embryonate at 30°C after 3-4 weeks were those which excluded the Crystal violet, Meldola's blue, MBEB, and Nile blue sulphate stain after 5-10 min at 21°C±2.

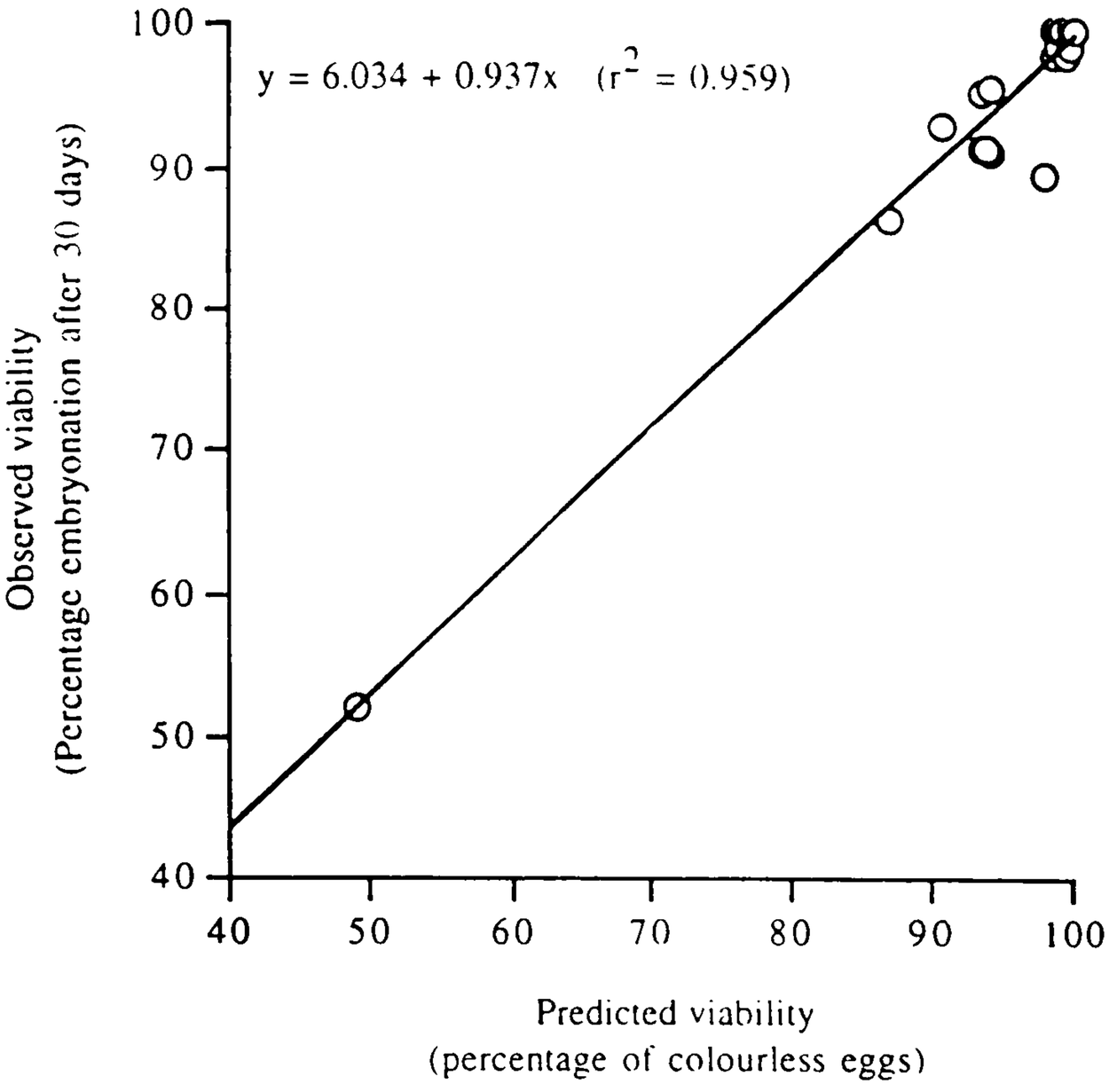


FIG. 4.2. Correlation of predicted (Crystal violet stain) and observed viabilities for *Ascaris suum* eggs, for 5 mins at 21 ± 2°C

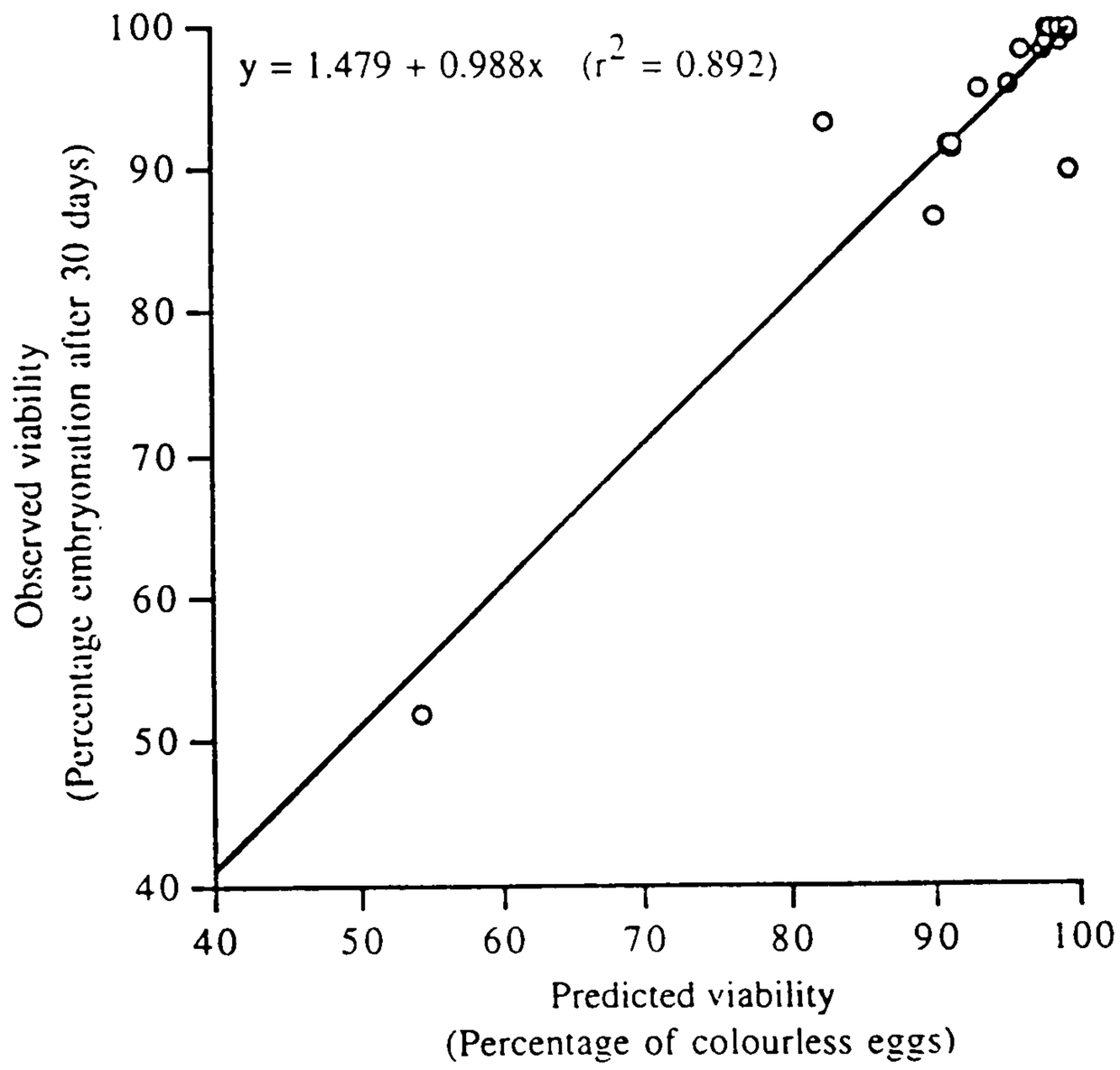


FIG. 4.3. Correlation of predicted (Meldola's blue stain) and observed viabilities for *Ascaris suum* eggs, for 5 mins at 21 ± 2°C

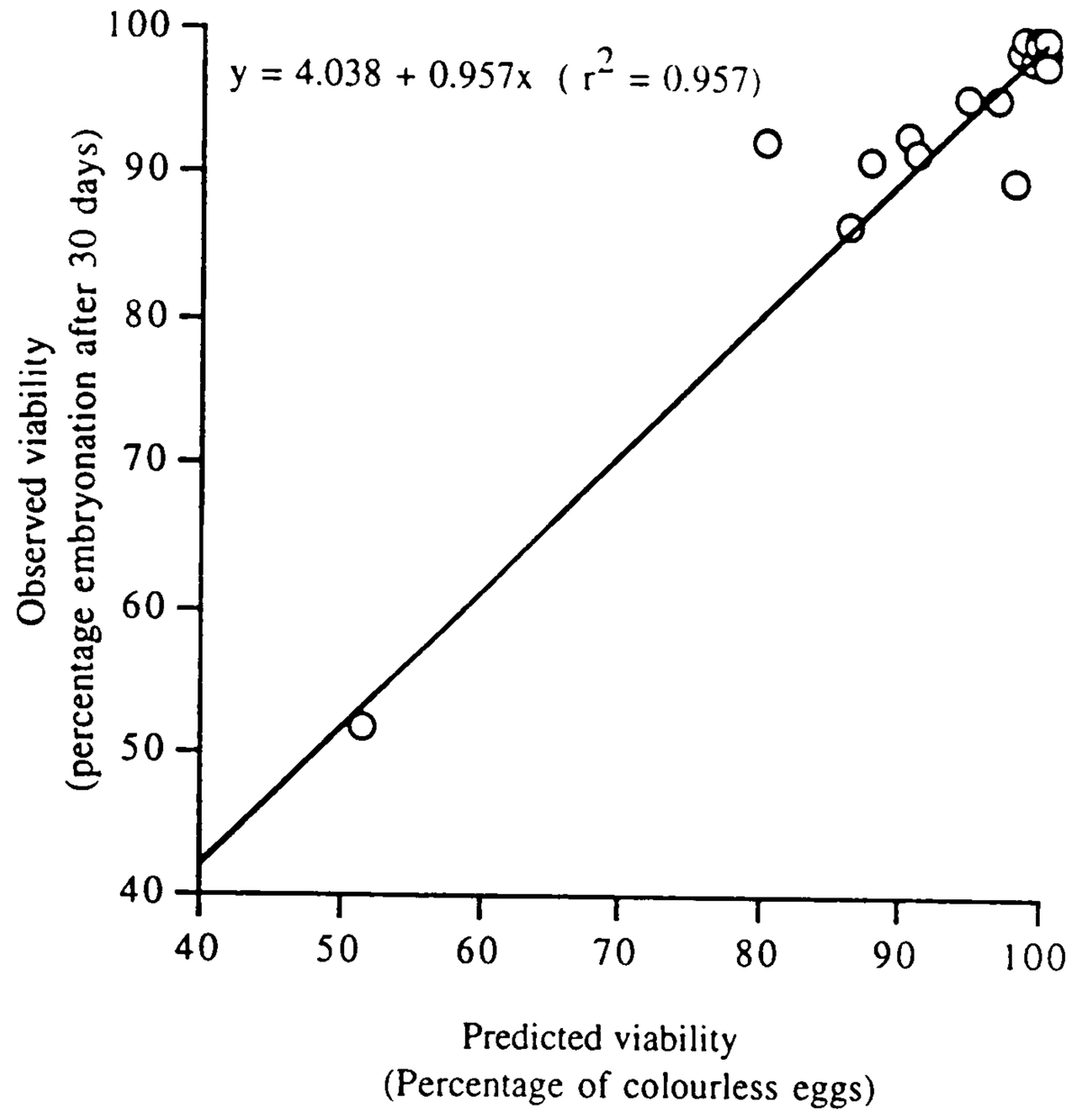


FIG. 4.4. Correlation of predicted (Methylene blue stain) and observed viabilities for *Ascaris suum* eggs, for 5 mins at 21 ± 2°C

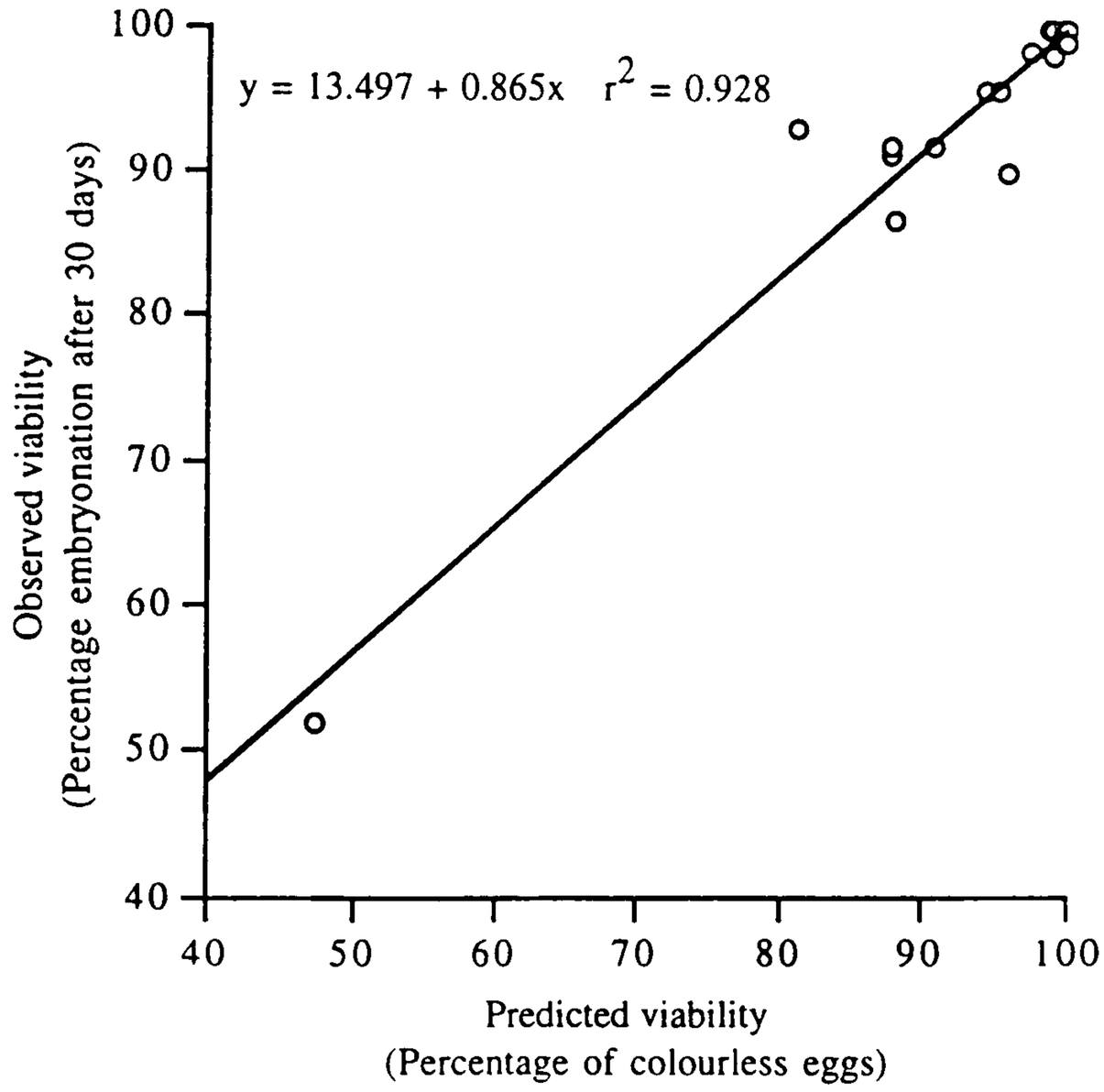


FIG. 4.5. Correlation of predicted (Nile blue stain) and observed viabilities for *Ascaris suum* eggs, for 5 mins at 21 ± 2°C

4.3.4.1 Uptake of Crystal violet stain

Uptake of Crystal violet by *Ascaris* eggs was maximal 5 minutes after applying the stain to the eggs. The proportion of eggs which took up the stain did not vary with time, (Tables 4.7 and 4.8; Figs. 4.6 and 4.7; $P > 0.96$ and F test < 2.3) at pH 4.5, 7.2, and 10; or with temperatures 4°C, 21°C and 37°C. Also pH 1.5, 12; or temperature 48°C ($P = 0.3$; 0.6 and 0.8 respectively) did not show any significant difference with time. Correlation of the Incubation method and exclusion of Crystal violet stain to determine viability of *Ascaris* eggs after 5-10 min contact time for these variables gave a correlation coefficient of 1.

Comparing the Incubation method and the Crystal violet staining method at different pH and temperature values, the data show (Tables 4.7 and 4.8) that at pH 4.5, 7.2, and 10; and with temperatures 4°C, 21°C and 37°C the detection of *Ascaris* eggs viability by Crystal violet was very similar to the Incubation method ($P \geq 0.3$, F test < 4.15) with a correlation coefficient of 0.927 (Fig. 4.1 and Table 4.5). However at pH 12 and pH 1.5 ($P \leq 0.01$) there was difference between the methods, which means that the uptake of Crystal violet stain by *Ascaris* eggs to detect viability will give imprecise detection at pH 12 and 1.5.

4.3.4.2 Uptake of Meldola's blue stain

Uptake of Meldola's blue by *Ascaris* eggs was maximal 5 minutes after applying the stain to the eggs; the proportion of eggs which took up the stain did not vary with time, (Table 4.8 and 4.9; Figs. 4.8 and 4.9; $P > 0.92$, F test < 2.3) at pH 4.5, 7.2, and 10; and with temperatures 4°C, 21°C, 37°C and 48°C. A similar pattern was observed with the treatment of eggs with 0.1N H_2SO_4 at room temperature (Fig 4.9, $P = 0.6$). Correlation of the Incubation method and exclusion of Meldola's blue stain to determine viability of *Ascaris* eggs after 5-10 min contact time for these variables gave a correlation of 1.

TABLE 4.7. Effect of pH, temperature and contact time on the uptake of Crystal violet stain by viable eggs, compared with the Incubation method (embryonation) (% mean ± SD, n = 3)

Treatment	Methods	Time after staining							
		5 min	10 min	30 min	60 min	120 min	180 min	360 min	720 min
pH 1.5	Crystal violet	98.5 ± 0.4	98.3 ± 1.1	98.6 ± 30.3	98.6 ± 1.1	98.9 ± 0.3	98.5 ± 0.5	99.1 ± 0.3	99.3 ± 0.6
	Incubation	99.1 ± 0.0							
pH 4.5	Crystal violet	99.3 ± 1.1	99.4 ± 1.0	99.3 ± 1.2	99.2 ± 1.4	99.1 ± 1.6	99.2 ± 1.4	99.2 ± 1.5	99.5 ± 0.9
	Incubation	99.1 ± 0.9							
pH 7.2	Crystal violet	99.7 ± 0.5	99.6 ± 0.8	98.7 ± 1.5	99.5 ± 0.9	98.8 ± 1.4	98.5 ± 1.3	98.7 ± 2.3	98.8 ± 2.1
	Incubation	99.1 ± 0.9							
pH 10	Crystal violet	99.4 ± 0.7	99.9± 0.2	99.4 ± 0.3	99.6 ± 0.3	99.5 ± 0.4	99.9 ± 0.2	99.6 ± 0.7	99.7 ± 0.6
	Incubation	99.4 ± 1.0							
pH 12	Crystal violet	98.3 ± 0.6	98.6 ± 1.2	99.4 ± 0.6	98.5 ± 1.2	98.4 ± 1.4	97.4 ± 1.9	97.8 ± 2.0	96.5 ± 1.1
	Incubation	99.7 ± 0.5							
4°C	Crystal violet	95.3 ± 2.1	97.4 ± 0.8	95.8 ± 2.0	95.5 ± 2.8	96.3 ± 1.9	96.2 ± 2.4	95.94 ± 2.7	93.4 ± 4.6
	Incubation	95.2 ± 2.7							
21°C	Crystal violet	97.1 ± 2.1	95.8 ± 3.8	93.9 ± 4.5	94.9 ± 3.5	95.0 ± 3.5	95.5 ± 2.5	94.8 ± 4.2	96.1 ± 2.7
	Incubation	96.2 ± 1.8							
37°C	Crystal violet	96.4 ± 2.2	94.7 ± 3.2	95.8 ± 2.2	94.3 ± 4.7	94.6 ± 3.7	93.7 ± 4.1	94.8 ± 3.1	94.7 ± 3.3
	Incubation	94.9 ± 3.0							
48°C	Crystal violet	94.8 ± 2.4	92.6 ± 5.1	95.3 ± 1.8	95.1 ± 3.5	94.2 ± 3.6	93.0 ± 4.0	92.6 ± 4.9	89.2 ± 4.6
	Incubation	95.2 ± 2.7							
64°C	Crystal violet	97.3 ± 2.8	96.4 ± 2.1	95.6 ± 3.1	93.4 ± 5.1	92.6 ± 6.2	92.8 ± 2.9	55.1 ± 12.0	0.0 ± 0.0
	Incubation	94.9 ± 3.1							

TABLE 4.8. Summary of analysis of variance results for Crystal violet stain and Meldola's blue with Incubation method, time, temperature and pH variables

Treatment	ANOVA ♠	Crystal violet		Meldola's blue	
		Methods	Contact time	Methods	Contact time
pH 1.5	P-value	0.01	0.62	6.00E-04	0.61
	F-value*	6.00	0.76	14.00	0.80
pH 4.5	P-value	0.59	0.99	3.00E-04	0.93
	F-value*	0.30	0.02	16.00	0.34
pH 7.2	P-value	0.83	0.98	0.11	0.99
	F-value*	0.04	0.20	2.72	0.03
pH 10	P-value	0.35	0.99	0.68	0.99
	F-value*	0.90	0.09	0.17	0.07
pH 12	P-value	7.93E-06	0.40	0.09	0.99
	F-value*	28.00	1.10	2.87	0.09
4°C	P-value	0.55	0.96	0.78	0.99
	F-value*	0.37	0.28	0.08	0.06
21°C	P-value	0.29	0.99	0.56	0.99
	F-value*	1.17	0.18	0.35	0.08
37°C	P-value	0.99	0.99	0.68	0.99
	F-value*	2.45	0.10	0.18	0.09
48°C	P-value	0.06	0.81	0.32	0.99
	F-value*	3.90	0.50	1.04	0.13
64°C	P-value	1.5E-14	3.1E-19	7.6E-10	4.06E-11
	F-value*	176.00	90.00	74.20	24.00

ANOVA: Two-Way ANOVA With Replication.
* Experimental F-value.
Critical F value (Methods) = 4.15.
Critical F value (Contact time)) = 2.31.
Significant at the $\alpha = 0.05$ level.
♠Experimental F-value, if it is > than F-critical this mean there is significant difference in treatment; if P > 0.1 this mean there is no significant difference.

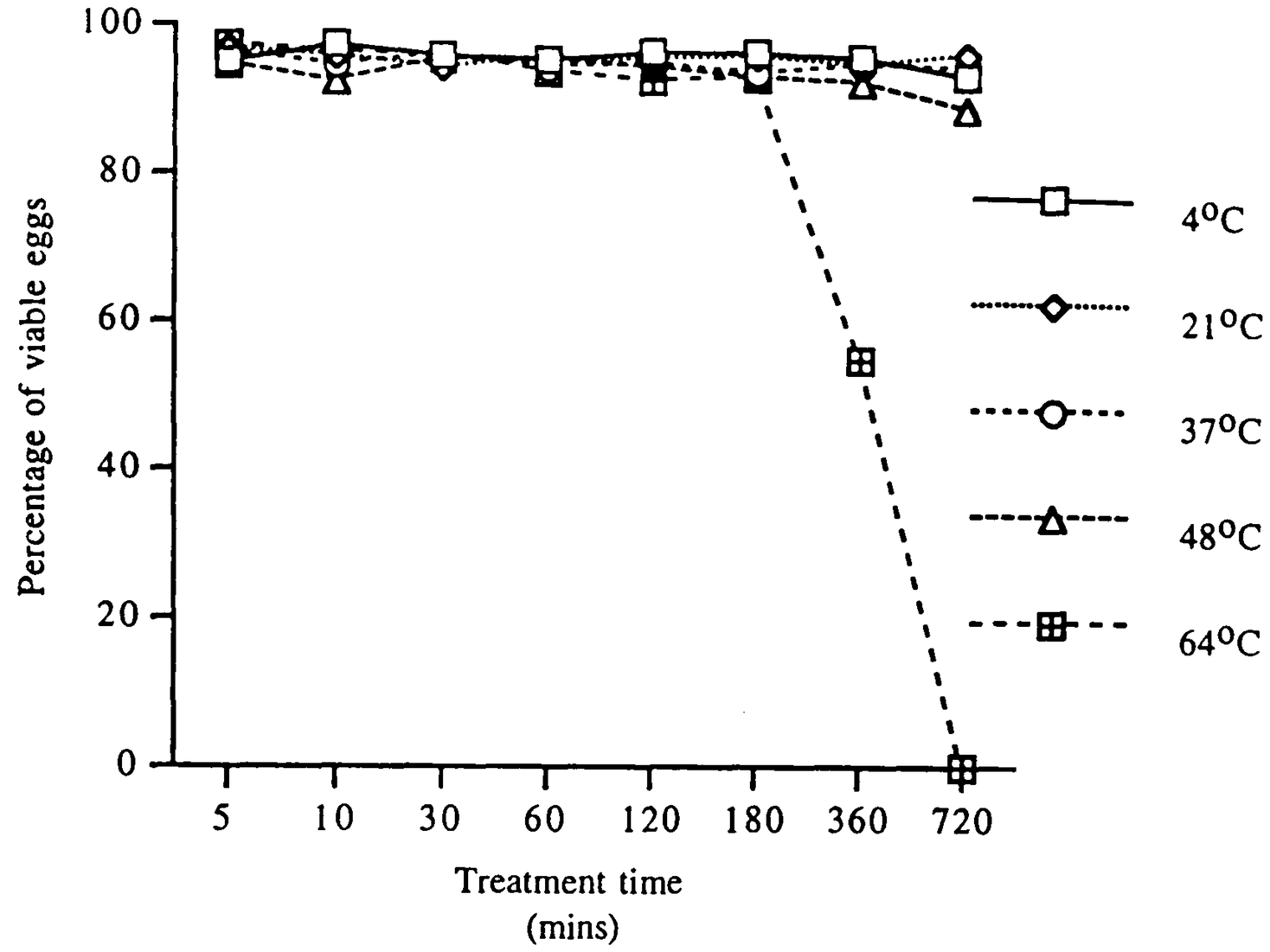


FIG. 4.6. Effect of temperature and contact time on the uptake of Crystal violet stain by viable *Ascaris suum* eggs

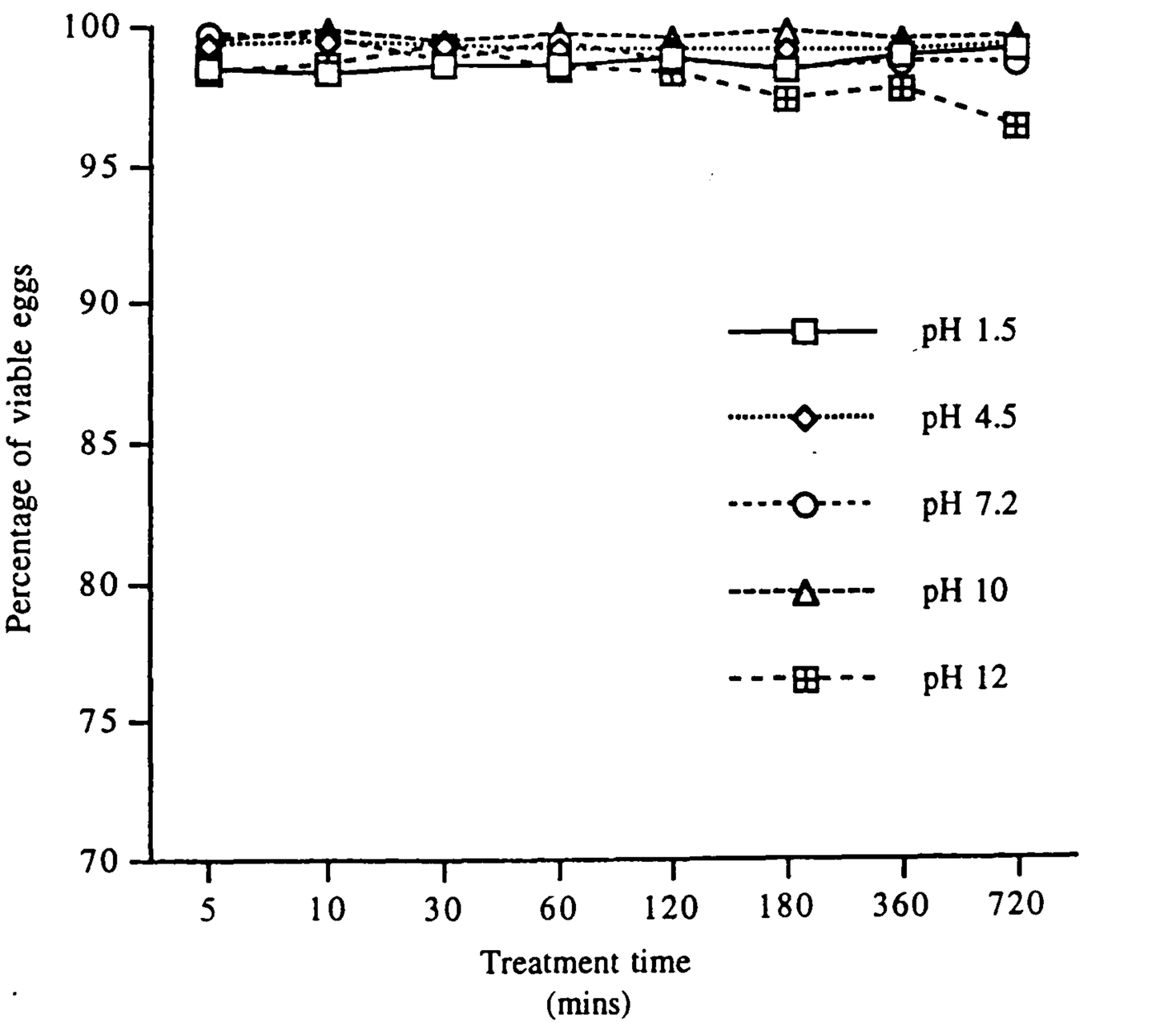


FIG. 4.7. Effect of pH value and contact time on the uptake of Crystal violet stain by viable *Ascaris suum* eggs

Treatments performed on eggs had a significant impact on the uptake of the Meldola's stain by the eggs and affected the prediction of viability. For treatments at pH 4.5, 7.2, and 10, or temperatures 4°C, 21°C, 37°C, a weak correlation was observed between observed and predicted viabilities, with a correlation coefficient of 0.31 (Table 4.5).

Comparing the Incubation method and Meldola's blue staining method at different pH's and temperatures, the data show (Tables 4.8 and 4.9) that at pH 7.2, 10, and 12 the detection of the viability of *Ascaris* eggs did not demonstrate any significant difference with the Incubation method ($P \geq 0.1$); also with temperatures 4°C, 21°C, 37°C and 48°C ($P < 0.78$) the results were similar to the Incubation method. A significant increase in predicted egg viability was observed at pH 1.5 and 4.5 ($P < 0.001$) compared with the Incubation method, which means that the uptake of Meldola's blue stain by dead eggs is not efficient at pH 4.5 and 1.5.

4.3.4.3 Uptake of Methylene blue eosin-borax stain (MBEB)

Uptake of MBEB by *Ascaris* eggs was maximal 5 minutes after applying the stain to the eggs. The proportion of eggs which took up the stain did not vary with time. Uptake of the stain by *Ascaris* eggs was not affected with time at different pH values (1.5, 4.5, 7.2, 10 and 12) and temperatures (4°C, 21°C, 37°C, 48°C) (Figs. 4.10 and 4.11; Table 4.10, $P > 0.9$; F test < 2.3). Correlation of the Incubation method and exclusion of MBEB stain to determine viability of *Ascaris* eggs after 5-10 min. contact time for these variables gave a correlation coefficient of 1.

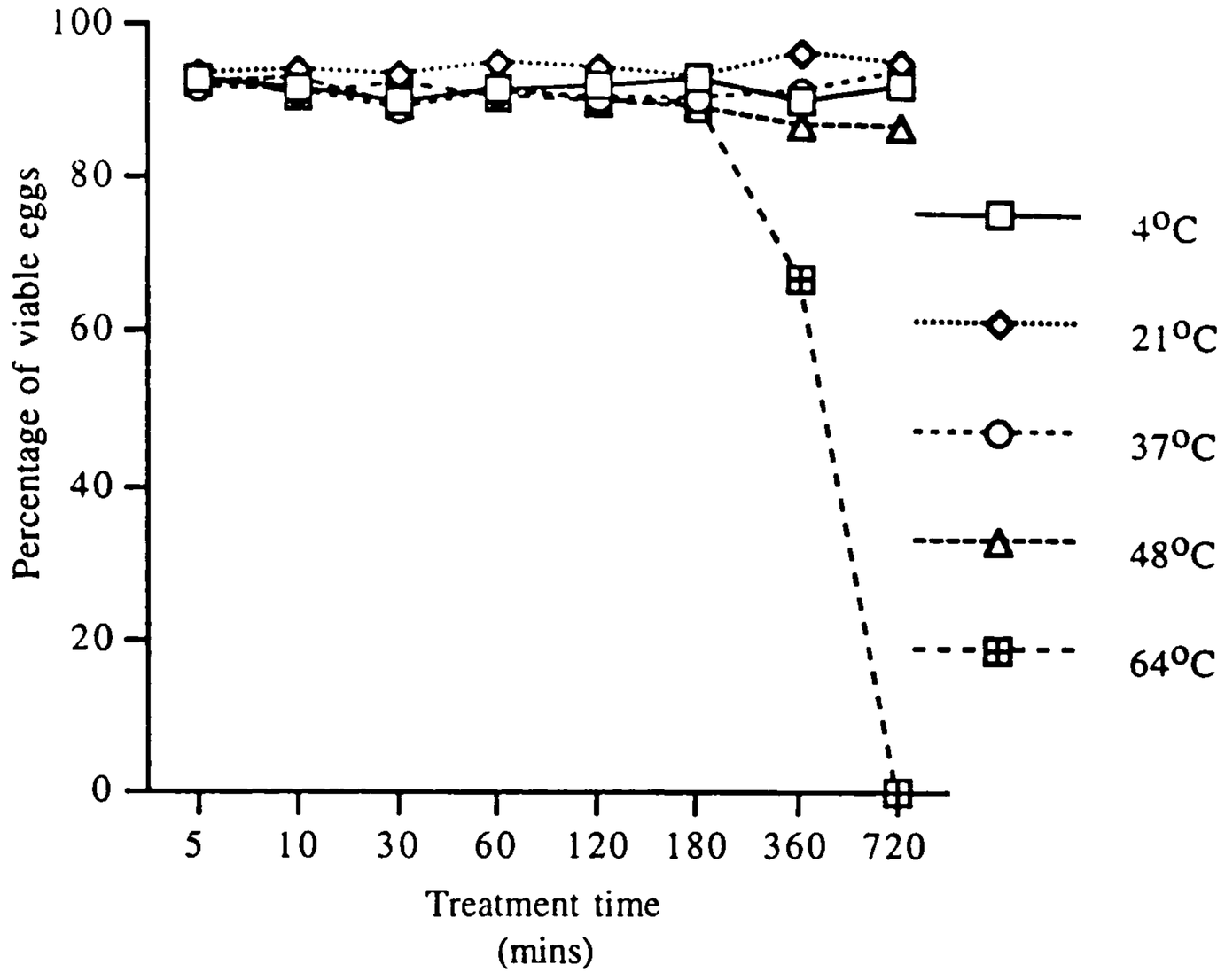


FIG. 4.8. Effect of temperature and contact time on the uptake of Meldola's blue stain by viable *Ascaris suum* eggs

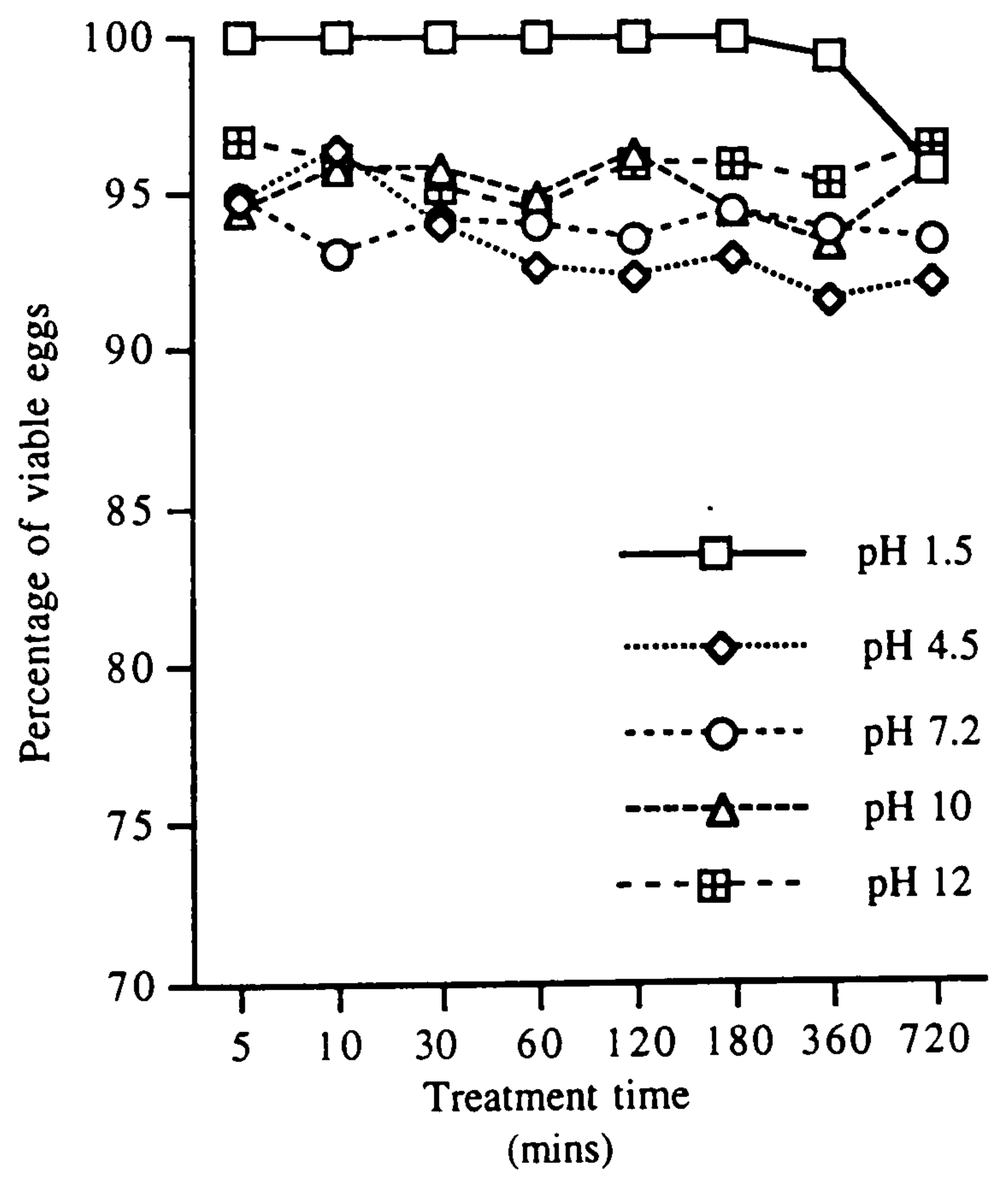


FIG. 4.9. Effect of pH value and contact time on the uptake of Meldola's blue stain by viable *Ascaris suum* eggs

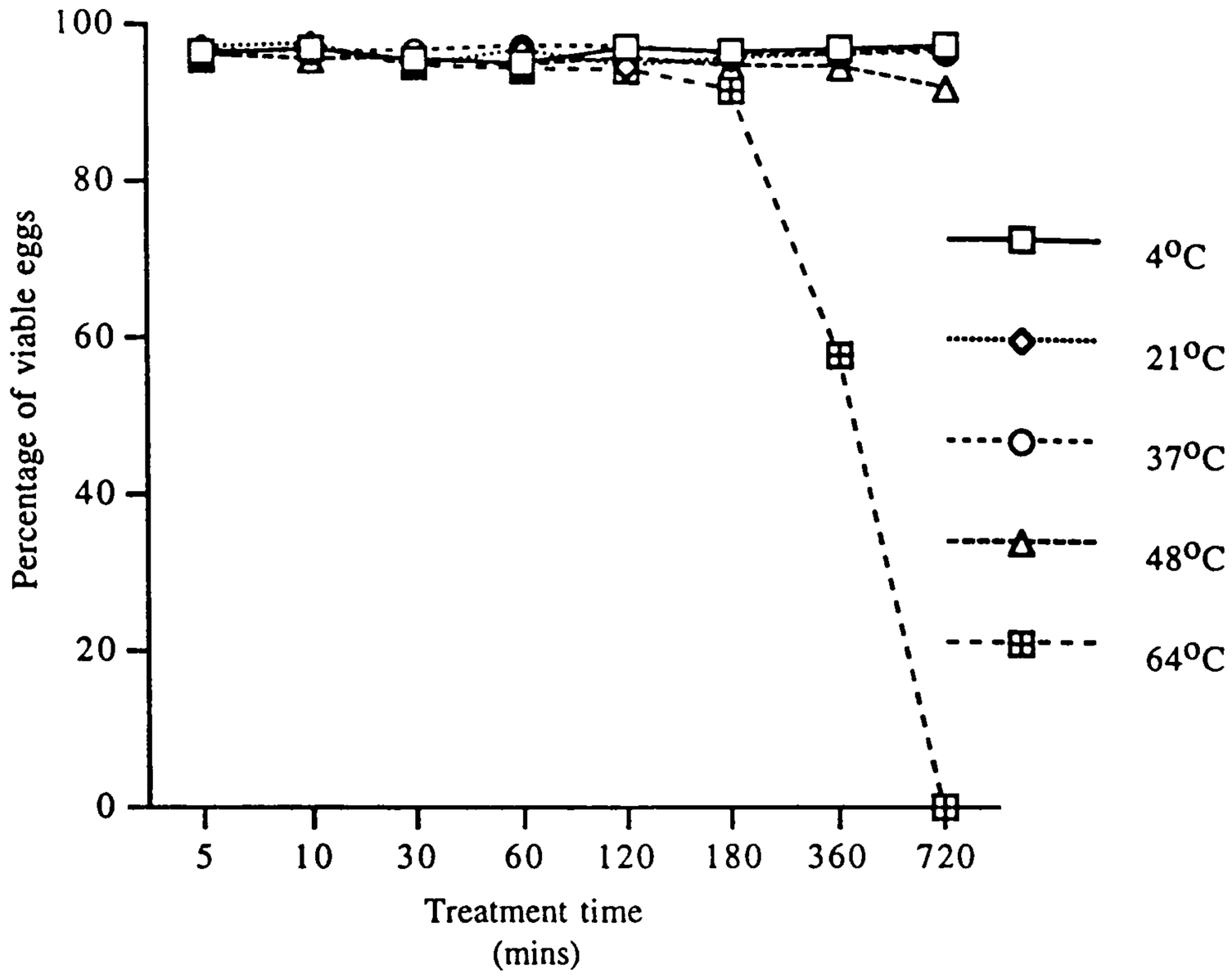


FIG. 4.10. Effect of temperature and contact time on the uptake of MBEB stain by viable *Ascaris suum* eggs

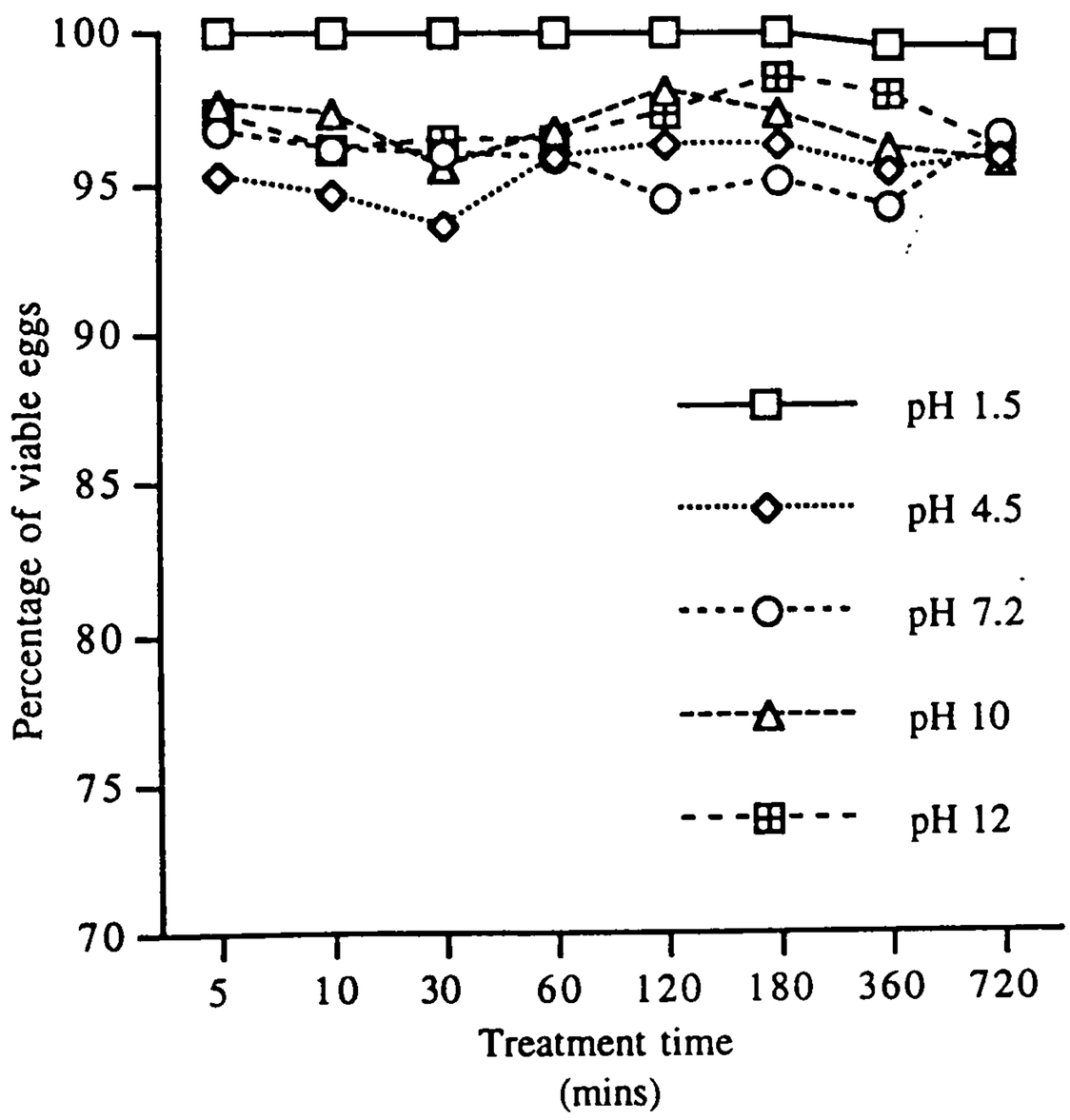


FIG. 4.11. Effect of pH value and contact time on the uptake of MBEB stain by viable *Ascaris suum* eggs

TABLE 4.9. Effect of pH, temperature and contact time on the uptake of Meldola's blue stain by viable eggs, compared with the Incubation method (embryonation) (% mean ± SD, n = 3).

Treatment	Methods	Time after staining							
		5 min	10 min	30 min	60 min	120 min	180 min	360 min	720 min
pH 1.5	Meldola's blue	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.3 ± 1.2	95.7 ± 4.1
	Incubation	97.1 ± 2.5							
pH 4.5	Meldola's blue	94.6 ± 4.7	96.2 ± 3.6	93.9 ± 2.2	92.5 ± 3.3	92.4 ± 5.3	92.9 ± 4.5	91.55 ± 3.2	92.1 ± 2.5
	Incubation	97.1 ± 2.5							
pH 7.2	Meldola's blue	94.8 ± 3.3	93.0 ± 4.5	94.0 ± 4.3	93.9 ± 3.8	93.5 ± 4.5	94.4 ± 4.2	93.8 ± 2.6	93.5 ± 2.8
	Incubation	95.8 ± 4.1							
pH 10	Meldola's blue	94.4 ± 4.7	95.7 ± 4.8	95.7 ± 3.4	94.8 ± 4.6	96.2 ± 4.1	94.3 ± 3.8	93.3 ± 5.6	95.8 ± 3.8
	Incubation	94.5 ± 4.8							
pH 12	Meldola's blue	96.7 ± 2.9	96.0 ± 3.1	95.1 ± 4.7	94.4 ± 5.1	95.8 ± 3.4	95.8 ± 3.0	95.3 ± 4.8	96.4 ± 1.1
	Incubation	97.2 ± 2.4							
4°C	Meldola's blue	92.7 ± 8.4	91.5 ± 6.6	90.0 ± 6.6	91.5 ± 6.4	92.2 ± 5.7	93.6 ± 4.6	90.7 ± 7.0	93.1 ± 4.2
	Incubation	92.4 ± 5.8							
21°C	Meldola's blue	93.4 ± 6.0	93.7 ± 5.2	93.5 ± 5.6	94.8 ± 4.6	94.3 ± 4.3	93.4 ± 4.7	96.5 ± 2.6	95.5 ± 3.7
	Incubation	93.6 ± 5.1							
37°C	Meldola's blue	91.6 ± 7.3	91.0 ± 8.0	89.2 ± 8.5	91.4 ± 6.6	90.9 ± 5.8	90.5 ± 4.6	91.9 ± 5.7	94.3 ± 4.0
	Incubation	92.1 ± 5.2							
48°C	Meldola's blue	92.2 ± 6.8	92.7 ± 5.5	89.6 ± 7.9	91.5 ± 6.76	90.2 ± 8.45	89.6 ± 8.7	87.4 ± 10.4	87.4 ± 10.1
	Incubation	92.1 ± 5.3							
64°C	Meldola's blue	92.8 ± 6.0	90.8 ± 6.4	92.0 ± 4.7	90.8 ± 6.6	90.3 ± 8.2	89.8 ± 7.3	67.5 ± 27.0	0.0 ± 0.0
	Incubation	96.6 ± 1.7							

TABLE 4.10. Summary of analysis of variance results for MBEB stain and Nile blue with Incubation method, time, temperature and pH variables

Treatment	ANOVA	MBEB		Nile blue	
		Methods	Contact time	Methods	Contact time
pH 1.5	P-value	2.14E-11	0.99	0.16	0.99
	F-value*	100.00	0.15	2.12	0.10
pH 4.5	P-value	6.60E-03	0.99	0.82	0.99
	F-value*	8.45	0.16	0.05	0.13
pH 7.2	P-value	0.01	0.99	0.37	0.99
	F-value*	7.02	0.18	0.83	0.12
pH 10	P-value	0.96	0.99	0.52	0.99
	F-value*	2.00E-03	0.19	0.42	0.15
pH 12	P-value	0.02	0.96	2.23E-06	0.57
	F-value*	5.90	0.27	33.00	0.83
4°C	P-value	0.01	0.91	0.59	0.99
	F-value*	7.10	0.38	0.30	0.09
21°C	P-value	0.91	0.93	0.29	0.99
	F-value*	0.01	0.30	1.15	0.16
37°C	P-value	0.03	0.99	0.89	0.99
	F-value*	5.30	0.09	0.02	0.06
48°C	P-value	0.19	0.98	0.02	0.77
	F-value*	1.79	0.20	6.50	0.57
64°C	P-value	3.64E-11	4E-15	1.02E-09	6.12E-14
	F-value*	96.00	47.00	72.00	39.00

Two-Way ANOVA With Replication.

* Experimental F-value.

Critical F value (Methods) = 4.15.

Critical F value (Contact time)) = 2.31.

Significant at the $\alpha = 0.05$ level.

♠Experimental F-value, if it is > than F-critical this mean there is significant difference in treatment; if P > 0.1 this mean there is no significant difference.

TABLE 4.11. Effect of pH, temperature and contact time on the uptake of MBEB stain by viable eggs, compared with the Incubation method (embryonation) (% mean ± SD, n = 3)

Treatment	Methods	Time after staining							
		5 min	10 min	30 min	60 min	120 min	180 min	360 min	720 min
pH 1.5	MBEB	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.6 ± 0.6	99.6 ± 0.8
	Incubation	98.2 ± 0.8							
pH 4.5	MBEB	95.2 ± 4.3	94.6 ± 5.9	93.6 ± 2.2	95.8 ± 1.4	96.2 ± 1.4	96.2 ± 2.5	95.4 ± 2.0	95.9 ±1.3
	Incubation	97.7 ± 2.4							
pH 7.2	MBEB	96.7 ± 1.1	96.1 ± 2.4	96.0 ± 2.1	95.8 ± 2.4	94.5 ± 4.9	95.1 ± 3.9	94.1 ± 2.5	96.5 ± 2.6
	Incubation	97.7 ± 2.4							
pH 10	MBEB	97.6 ±1.5	97.4 ± 2.0	95.6 ± 3.3	96.8±2.4	98.0 ±1.4	97.4 ± 1.4	96.1 ± 3.2	95.8 ± 3.2
	Incubation	96.8 ± 2.8							
pH 12	MBEB	97.3 ± 2.3	96.2 ± 3.7	96.4 ± 3.0	96.4 ± 3.6	97.4 ± 2.4	98.6 ± 1.0	97.9 ±1.9	96.2 ± 2.6
	Incubation	98.5 ± 1.3							
4°C	MBEB	96.7 ± 2.9	97.1 ± 1.3	95.4 ± 3.2	95.0 ± 4.2	97.1 ± 1.9	96.7 ± 1.9	96.9 ± 1.1	97.4 ± 0.6
	Incubation	97.9 ± 0.2							
21°C	MBEB	97.2 ± 2.2	97.6 ± 1.6	94.8 ± 4.3	96.7 ± 2.7	94.5 ± 4.1	95.7 ± 3.8	96.1 ± 3.0	97.2 ± 2.0
	Incubation	96.2 ± 1.6							
37°C	MBEB	96.8 ± 2.5	96.8 ± 2.6	96.5 ± 2.6	97.1 ± 2.1	97.2 ± 2.0	96.0 ± 2.7	96.0 ± 3.2	96.8 ± 2.6
	Incubation	97.9 ± 0.2							
48°C	MBEB	96.2 ± 3.3	95.7 ± 3.6	95.8 ± 3.7	94.8 ± 5.6	95.5 ± 3.4	94.7 ± 3.2	94.7 ± 4.6	92.2 ± 6.2
	Incubation	96.2 ± 1.7							
64°C	MBEB	95.4 ± 3.7	97.1 ± 1.8	94.7 ± 4.2	94.1 ± 4.5	94.2 ± 3.2	91.5 ± 3.0	58.2 ± 20.2	0.0 ± 0.0
	Incubation	95.2 ± 3.7							

MBEB : Methylene Blue Eosin-Borax stain

Comparing the Incubation method and MBEB staining method at different pH's or temperatures, the data show (Tables 4.10 and 4.11) that at pH 10 or at 21°C the detection of the viability of *Ascaris* eggs by MBEB was closely similar to the Incubation method ($P \geq 0.9$; F test < 4.15), while at pH 1.5, 4.5, 7.2 and 12 ($P \leq 0.02$) and at 4°C and 37°C ($P < 0.05$) there was difference between the two methods under these conditions, which means the uptake of the MBEB stain by *Ascaris* eggs to detect viability will give imprecise detection under these conditions, with a correlation coefficient -0.117 (Table 4.5).

4.3.4.4 Uptake of Nile blue stain

Uptake of Nile blue by *Ascaris* eggs was maximal 5 minutes after applying the stain to the eggs. The proportion of eggs which took up the stain did not vary with time, (Tables 4.10 and 4.12; Figs. 4.12 and 4.13; $P > 0.99$) at different pH values (1.5, 4.5, 7.2, and pH 10) and temperatures (4, 21, and 37°C) (F test < 2.3). Also at pH 12 and temperature 48°C ($P = 0.6$ and 0.8 respectively) there is no effect of time on the uptake of Nile blue stain. Correlation of the Incubation method and exclusion of Nile blue stain to determine the viability of *Ascaris* eggs after 5-10 min contact time for these variables gave a correlation coefficient of 1. Treatments performed on eggs had a significant impact on the uptake of the stain by the eggs and had an effect on the prediction of viability by using the Nile blue staining method. For pH 4.5, 7.2, and 10, or temperatures 4°C, 21°C or 37°C, a weak correlation was observed between observed and predicted viabilities, with a correlation coefficient of 0.35 (Table 4.5).

Comparing the Incubation method and the Nile blue staining method at different pH's or temperatures, the data show (Tables 4.10 and 4.12) that at pH 1.5, 4.5, 7.2, and 10; and with temperatures 4°C, 21°C and 37°C the detection of viability of *Ascaris* eggs by Nile blue was very similar to the Incubation method ($P \geq 0.1$, F test < 4.15), while at pH 12 ($P \leq 0.001$) there was significant difference between the two methods at these conditions, which means the uptake of the Nile blue stain by *Ascaris* eggs to detect viability will give imprecise detection at pH 12.

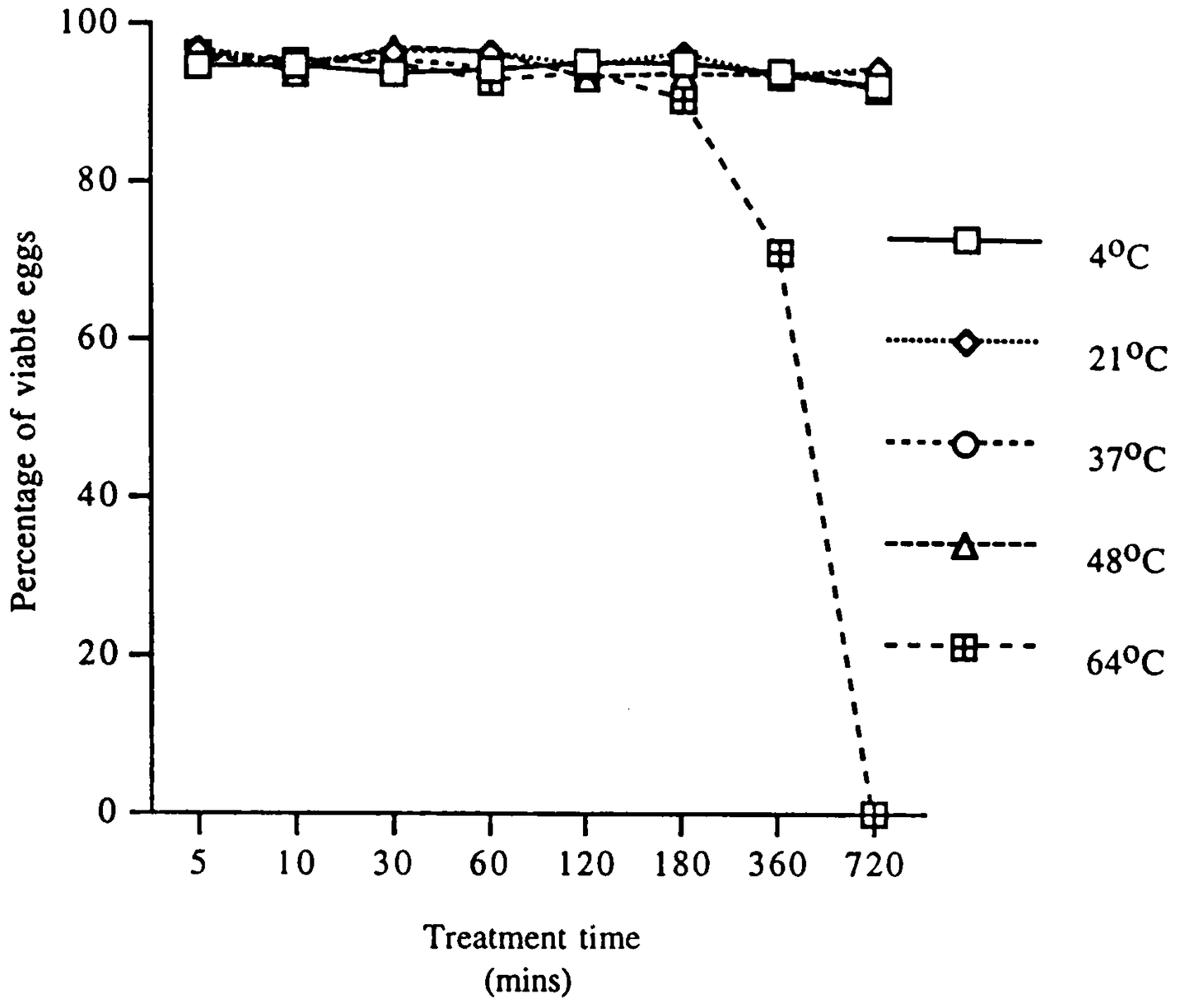


FIG. 4.12. Effect of temperature and contact time on the uptake of Nile blue stain by viable *Ascaris suum* eggs

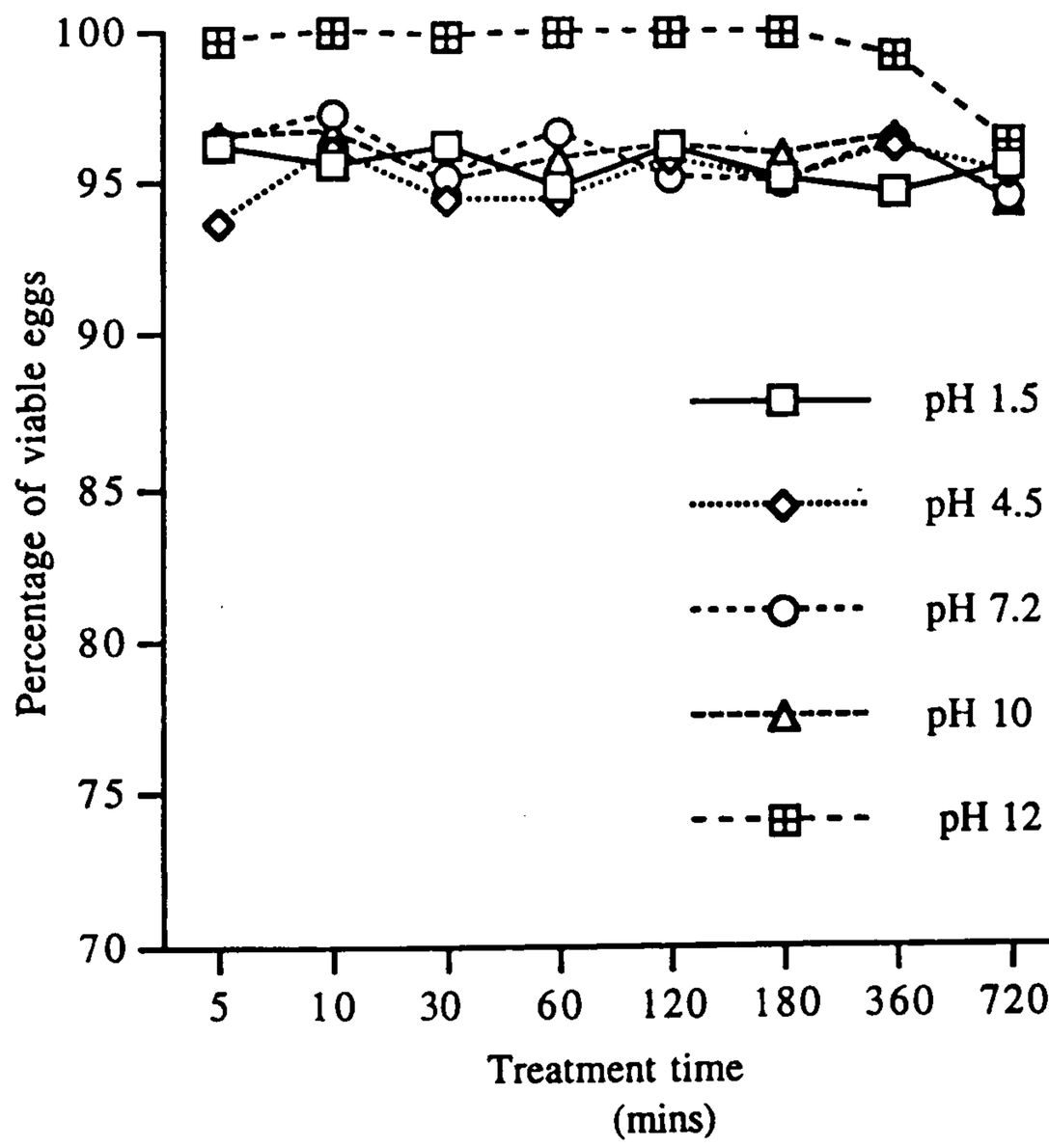


FIG. 4.13. Effect of pH value and contact time on the uptake of Nile blue stain by viable *Ascaris suum* eggs

TABLE 4.12. Effect of pH, temperature and contact time on the uptake of Nile blue stain by viable eggs, compared with the Incubation method (embryonation) (% mean ± SD, n = 3).

Treatment	Methods	Time after staining							
		5 min	10 min	30 min	60 min	120 min	180 min	360 min	720 min
pH 1.5	Nile blue	96.3 ± 2.3	95.6 ± 3.4	96.2 ± 2.9	94.8 ± 2.9	96.2 ± 1.3	95.2 ± 4.0	94.7 ± 2.8	95.6 ± 3.1
	Incubation	96.6 ± 2.1							
pH 4.5	Nile blue	93.7 ± 1.86	96.1 ± 1.4	94.4 ± 3.8	94.4 ± 1.3	95.8 ± 1.2	94.9 ± 1.6	96.2 ± 2.1	95.3 ± 2.3
	Incubation	94.9 ± 3.6							
pH 7.2	Nile blue	96.3 ± 2.6	97.2 ± 2.2	95.2 ± 3.9	96.7 ± 2.4	95.2 ± 3.6	94.9 ± 3.4	96.3 ± 3.2	94.6 ± 3.19
	Incubation	94.9 ± 3.6							
pH 10	Nile blue	96.6 ± 3.1	96.7 ± 2.4	95.0 ± 4.8	95.8 ± 3.8	96.3 ± 2.9	95.9 ± 3.4	96.5 ± 2.7	94.4 ± 2.2
	Incubation	96.4 ±1.6							
pH 12	Nile blue	99.6 ± 0.6	100 ± 0.0	99.8 ± 0.3	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.2 ± 0.3	96.4 ± 2.8
	Incubation	96.6 ± 2.1							
4°C	Nile blue	94.6 ± 4.0	94.7 ± 3.7	93.5 ± 3.1	94.1 ± 3.4	95.3 ± 3.5	95.0 ± 2.5	94.2 ± 2.3	92.9 ± 3.5
	Incubation	94.8 ± 3.1							
21°C	Nile blue	96.5 ± 2.6	94.7 ± 4.5	96.0 ± 3.6	96.2 ± 2.8	94.8 ± 3.6	95.9 ± 2.8	93.9 ± 5.0	94.8 ± 4.2
	Incubation	96.3 ± 1.8							
37°C	Nile blue	95.1 ± 3.6	95.1 ± 3.6	95.3 ± 3.2	94.4 ± 4.0	94.8 ± 3.7	95.2 ± 3.5	93.4 ± 4.6	94.0 ± 3.1
	Incubation	94.8 ± 3.1							
48°C	Nile blue	96.2 ± 3.0	93.9 ± 4.8	96.8 ± 1.4	95.9 ± 3.11	93.3 ± 4.5	93.6 ± 3.8	93.8 ± 2.7	92.2 ± 1.1
	Incubation	96.4 ± 1.7							
64°C	Nile blue	96.0 ± 2.9	95.3 ± 2.7	94.5 ± 3.6	92.8 ± 4.8	93.9 ± 4.3	90.4 ± 4.8	71.8 ± 21.5	0.0 ± 0.0
	Incubation	95.2 ± 3.7							

4.3.5 Comparison between four stains to determine the death rate of *Ascaris* eggs incubated at 64°C

The results from this experiment show the relationship of high temperature and times of contact in the killing effect on *Ascaris* eggs (Fig. 4.14). The eggs of *Ascaris* can withstand heating up to 180 min at 64°C. If they are held at this temperature for 360 min reduction in viability starts and reaches zero (100% dead eggs) after 720 min. Also the results showed only a slight effect on the eggs of *Ascaris* at 48°C after 720 min compared with complete destruction at 64°C (Figs. 4.6, 4.8, 4.10, 4.12, and 4.14). It is interesting to note the susceptibility of *Ascaris* eggs to high temperatures; demonstration that the stain uptake by dead eggs increased at 64°C after 720 min means that it can spontaneously detect any changes in the viability of the eggs.

In an attempt to compare the behaviour of these four stains with gradual killing of eggs with time at 64°C we observed clearly (Fig. 4.14) that Crystal violet and MBEB are more precise for detection of viability of the eggs than Meldola's blue and Nile blue which were less easily taken up by the eggs after death.

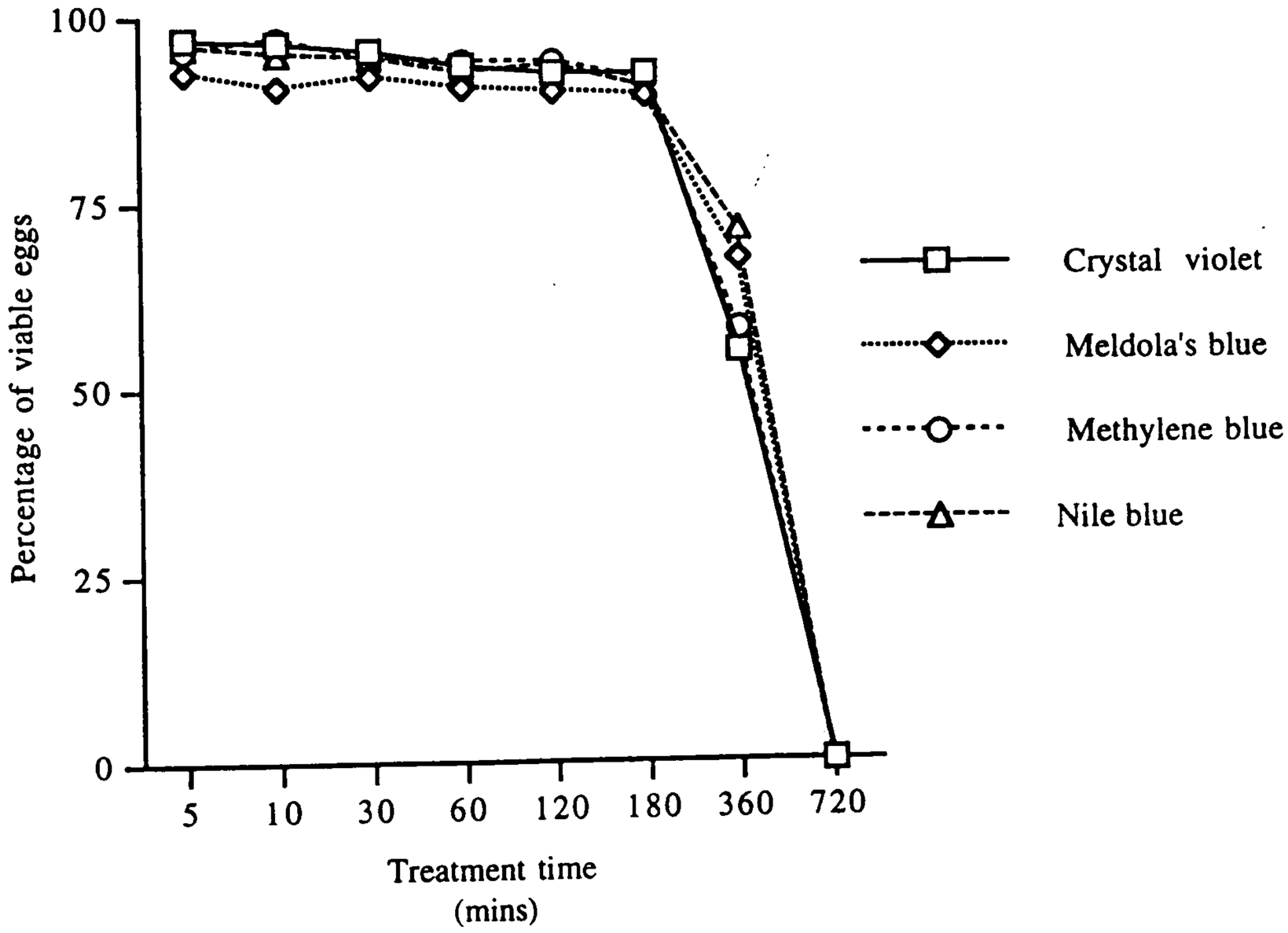


FIG. 4.14. Comparison of four different stains, Crystal violet, Meldola's blue, Methylene blue, and Nile blue to determine the death rate of *Ascaris suum* eggs incubated at 64°C

4.3.6 Dye-toxicity study

Crystal violet, Meldola's blue, Methylene blue eosin borax, and Nile blue sulphate, were found to be non toxic in the concentration used in these experiments. They acted as good culture media, and all viable eggs embryonated in stained culture after 3-4 weeks. In fact, cells inside eggs in stained cultures continued to develop with no apparent inhibition, which suggests a possible use for these stains in determining the effects of various agents (e.g., chemicals) upon the viability of eggs in culture, without sacrificing the cultures.

4.4 Discussion

The results of this study demonstrate that no differentiation was possible between dead and live *Ascaris* eggs which have shells. The outer layer of the *Ascaris* egg shell consists of lipoprotein substances which are stained by basic vital stains, so it is necessary to remove the shell first before staining.

The egg-shell of *Ascaris* consists of an outer uterine layer which is lipoprotein; the second layer is vitelline, which is also lipoprotein; this is followed by the thickest layer of the egg shell called the chitinous layer, which consists of protein and chitin providing structural strength. Finally there is the lipid layer, which is responsible for the extreme impermeability of egg shells; it has a unique chemical nature, consisting of 25% protein and 75% lipid. The lipid fraction contains a mixture of ∝-glycosides called ascarosides (Wharton, 1980).

After removing the shell from the eggs (decortication), the differentiation between stained and unstained cells inside eggs could be easily seen using Crystal violet, Meldola's blue, Methylene blue eosin-borax, and Nile blue stain. The internal structure of the dead eggs are stained blue and the viable eggs remained unstained. From the results in Table 4.3, it is very obvious that all four stains were able to distinguish between viable and non-viable eggs, whatever the stage of the *Ascaris* eggs.

In the literature there were differences in the application of the vital stain on eggs. Zhou *et al.* (1985) suggested to use one of two methods for the application of the Methylene eosin borax stain on eggs, to detect the viability of *Ascaris* eggs; either, one drop of egg suspension was placed on a glass slide, and mixed with one drop of the stain, or mix one drop of egg suspension in the test tube with the stain, after 30 minutes wash it two to three times with distilled water. 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. Unfortunately he only mentioned the staining method briefly, and no reference was cited.

To choose the method for application in this study, the staining procedure before microscopy has been studied. The effect of the washing method on uptake of each stain by eggs at different pH and temperature were assessed. The results showed that it is not advisable to add a drop of basic vital stains to the suspension of *Ascaris* eggs in a tube and then wash the eggs with distilled water, before taking a drop from the suspension to examine microscopically. Statistical analysis of the washing experiment showed that it had a negative impact on the prediction of the viability of *Ascaris* eggs at different pH values and temperatures for Crystal violet, Meldola's blue and MBEB stain.

It seems that these stains had weak interaction with the dead cells inside the eggs, and the stain is easily removed by washing with distilled water. Also Meldola's blue stains pale blue and after washing the colour was even lighter, so the washed and unwashed experiments had the weakest correlation. Nile blue sulphate stain had a good correlation between the two experiments, the dark blue colour being clear and there was no impact of pH or temperature on the detection of the viability of *Ascaris* eggs.

So in this study, the method for application of the stain to eggs to detect viability was as follows: one drop from the decorticated egg suspension was placed on a clean glass slide; the end of a wire loop touched a drop of vital stain and was mixed thoroughly with the

eggs; after 5-10 minutes stained and unstained eggs were counted using a light microscope.

This staining method showed striking differences between non-viable and viable eggs, and showed good correlation (Crystal violet =0.979; Meldola's blue = 0.945, Methylene blue eosin-borax = 0.978, and Nile blue = 0.963) with embryonation (the Incubation method) as a method for determining the viability of *Ascaris* eggs, 5-10 min after applying the stain at 21°C ± 2. This leads to the conclusion that, all the vital stains used in this experiment with these conditions had similar good correlation with incubation method. Similar results were found by Zhou *et al.* (1986), who used the methylene blue eosin borax method to detect the viability of *Ascaris* eggs in the same conditions as above; they also found 0.994 correlation coefficient with the incubation method, and they recommended this should be used as a simple and rapid method to detect the viablity of *Ascaris* eggs, although they did not do any comprehensive testing of this stain.

The dye exclusion test for egg viability depends upon the fact that viable eggs have cells which 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 (Phillips, 1973), and cannot decolourize basic dyes.

Each dye, however, has staining characteristics which preclude its use under certain conditions. One of the factors affecting the accuracy of the viability test, the degree to which cells take up dye, is pH dependent (Phillips, 1973). Crystal violet uptake of stain occurs maximally at pH 7.2, and has good correlation with the Incubation method within a range of pH 4.5 to 10. Whereas eggs treated with 0.1N NaOH or 0.1N H_2SO_4 at 21°C exhibited reduced reproducibility of the staining method to detect viability. Uptake of Meldola's blue stain occurs maximally at pH 10 and only has good correlation with the Incubation method within the pH range 7.2 to 10. Uptake of Methylene blue eosin borax stain occurs maximally at pH 10, and did not match the Incubation method at other pH ranges. Uptake of Nile blue sulphate stain occurs maximally at pH 4.5 and has good correlation with the Incubation method within the pH range of 1.5 to 10.

The degree to which cells take up dye is not temperature dependent for Crystal violet, Meldola's blue and Nile blue sulphate stain at 4°C, 21°C and 37°C. Whereas Methylene blue eosin borax gave maximal correlation with the Incubation method at 21°C.

In general, this study showed that there was no impact of contact time on the uptake of the stain by viable eggs over the range of conditions investigated. In contrast, Hudson and Hay (1980) have used Trypan blue to detect cell viability and found the stain inaccurate in the identification of dead cells; cells must be counted within 3-5 min because the number of blue-staining cells increase with time. While Chaudhuri *et al.* (1966) found that the length of exposure to the stain did not affect the percentage of stained free living-nematodes by using Eosin-Y vital stain. The percentage stained increased with the concentration of stain. Also they concluded that 0.67 percent Eosin-Y was toxic to nematodes when the exposure time exceeded one hour.

This study demonstrated that temperature and pH treatment of eggs had a significant impact on the uptake of stain and affected the prediction of viability using Meldola's blue, MBEB, and Nile blue staining method, with correlation coefficients of 0.31, -0.117, and 0.35 respectively. However for Crystal violet, there was no impact of temperature and pH treatment on the uptake of the stain, and there was a high correlation coefficient (0.927) with the Incubation method.

The basic vital staining method was simple to perform, rapid (5-10 min), and could be readily interpreted microscopically. This method is also less subjective than embryonation (the Incubation method). The major reason is that the vital staining method relies on the observation of stained and unstained eggs as a measure of viability. The Incubation method is long (3-4 weeks), tedious and requires control of a number of chemical and physical variables (temperature, pH, aerobic conditions, etc.) as optimal conditions are required for maximal embryonation efficiency, which may be difficult for untrained investigators to achieve consistently.

The two main disadvantages of the staining process are: (i) some of the stains used may have a toxic effect, i.e. Eosin and acridine orange were tested by Tennant (1964) but were found to be relatively toxic to the viable cells and (ii) staining characteristic may not change immediately after death, as found by Boyd (1941) who used Iodine and Potassium Iodide solution to detect the viability of Potato Root Eelworm larvae. In our experiments the four stains tested shows no evidence of toxicity, and proved excellent as culture media after 30 days incubation, whereas non-viable eggs which stained blue never proliferate. Viable *Ascaris* eggs suspended in these stains embryonate and give very healthy and active larvae that also hatch and swim in the stain suspension.

Furthermore the staining methods (in particular Crystal violet) seems initially to reflect almost immediately a change in viability due to incubation of eggs at 64°C for 720 minutes. In the next chapter more details will be given about gradual killing of *Ascaris* eggs by different temperatures and ultraviolet light, for a more comprehensive comparison between incubation and the staining method, to evaluate the versatility of the staining procedure.

Shepherd (1962) used water-soluble, non toxic New blue R (Meldola's blue) stain to detect the viability of Tylenchida (*Meloidogyne*, *Ditylenchus*, *Aphelenchoides*, *Aphelenchus* and *Anguina*), New blue R stains the body contents of dead nematodes from pale mauve to deep purple while living ones remain unstained. With all these genera, any nematodes which take up the stain, either partially or completely, are considered to be dead or dying. The stain appears to enter the body first through the oral and anal openings.

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, reliable, inexpensive, rapid, and practical for routine use. Second, when using Crystal violet the 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 needs to be paid to

the proper 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 simple light microscope, which is available in most laboratories. The sixth advantage is that these stains are not toxic, and act 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.

In conclusion, it is suggested that Crystal violet as a vital staining method has potential for use in the rapid assessment of viability of *Ascaris* eggs. The data indicate in the experimental conditions investigated, a close agreement between Crystal violet viability values and embryonation of eggs using the Incubation method, with a correlation coefficient of 0.979, after 5-10 min applying the stain at 21°C ± 2.

Furthermore, at pH 4.5, 7.2, and 10; and with temperatures 4°C, 21°C and 37°C values all together, a good correlation between Incubation and Crystal violet staining method viabilities was observed (Fig. 4.1), with a correlation coefficient of 0.927. Also, after *Ascaris* eggs treated with high temperature (64°C) for different contact times, Crystal violet shows the best spontaneous detection of changes in egg viability, although the differences between stains are slight. This means that Crystal violet stain had the highest correlation with incubation method, and was more precise than for the other stains tested.

Based on the above results it is concluded that Crystal violet apparently indicates similar egg viability to the Incubation method but further investigations are needed to establish this unequivocally. It acts over a wide range of pH values and temperatures, but more research is needed to investigate the applicability of the staining method to the detection of viability of *Ascaris* eggs under different conditions in soil, wastewater and sludge samples in the environment.

Further studies should investigate the effects of environmental conditions on the reliability of viability detection using the staining method. Also further work should investigate the selection of vital stains that can work on corticated *Ascaris* eggs. Furthermore, selection of vital stains which can differentiate between viable and dead *Trichuris* eggs, would be extremely useful.

4.5 SUMMARY

Exclusion of basic vital stains (Crystal violet, Meldola's blue, Methylene blue eosin-borax, and Nile blue) from decorticated *Ascaris* eggs was compared with the Incubation method as a measure of egg viability. Non-viable *Ascaris* eggs accumulated the stain (blue), whereas viable eggs excluded the dye and were colourless. The effect of different pH values, temperatures and contact time on the uptake of the stain by viable eggs compared with the Incubation method, were studied to determine the best stain and optimum conditions for routine use.

Correlation and regression analyses indicate a high degree of association between the two methods. The data indicate that the Crystal violet staining method has the greatest potential for use as an alternative method to Incubation as a measure of egg viability. In the staining method, Crystal violet stain is added directly to an egg preparation. Observations are made immediately using a light microscope. The results are available in only 10 minutes, compared to the 30 days required for incubation. Since only stained or unstained eggs were observed, the method is less subjective than the Incubation method.

The data presented shows that Crystal violet is more reproducible and more precise than other vital stains, with a correlation coefficient of 0.979 with the Incubation method. Crystal violet as a vital staining method has potential for use as rapid screening of the viability of *Ascaris* eggs, and for rapid assessment of the effectiveness of chemical and physical agents on viability. Preliminary evidence suggests that the Crystal violet stain has great potential for use in assessing the viability of *Ascaris* eggs.

CHAPTER FIVE

ASCARIS SUUM: EFFECT OF TEMPERATURE AND ULTRAVIOLET RADIATION ON UNEMBRYONATED EGGS

5.1 Introduction

A staining technique has been developed in conjunction with research studies of *Ascaris* eggs in sludge. A series of 4 vital stains have been assessed to determine whether exclusion or inclusion of them correlated with viability as assessed by the Incubation method. Of these stains, a Crystal violet vital stain showed significant promise, staining dead eggs, while live eggs remain unstained. A short staining period is employed to facilitate routine examination and to minimise changes in the samples. Two representative lethal agents have been employed, temperature and ultraviolet radiation to evaluate the versatility of the staining procedure using Crystal violet stain.

In order to obtain more complete knowledge of the effects of upper-range temperatures on the *Ascaris* eggs viability, a series of experiments were performed in which eggs were exposed to temperatures ranging from 37°C to 105°C for varying amount of time. To assess the effectiveness of ultraviolet light on *Ascaris suum* eggs viability, a second series of experiments were performed in which eggs were exposed to UV for between 5 seconds and 3 days. These temperatures and ultraviolet light were chosen to give comprehensive knowledge about the applicability of using vital stains as an alternative to the conventional Incubation method; the differences inherent between staining and conventional method could then be systematically evaluated.

5.2 Methods

5.2.1 Experimental procedure

Two different methods of killing *Ascaris* eggs were studied for their effects on the Crystal violet staining reaction:

(i) Temperature

Eggs prepared as described in section 4.2.1 in chapter four, were placed in distilled water in test tubes and subjected to a series of constant temperatures and 100% relative humidity. The effect of temperature was evaluated by incubating one-cell stage eggs in various temperatures for different times, 37°C for 3, 4, 5, 6, 7, 10, 11, 13, 15, 21, and 30 days; 50°C for 10, 30 minutes, 1, 2, 4, 6, 48, 72 hours, and 7, 14 days; 60°C for 5, 10, 20, 30 minutes, and 1, 2, 24, 54 and 72 hours; 70°C for 5, 10, 20, 30, 45, 60, and 120 minutes; 80°C for 5, 10, 20, 30 minutes; 90°C for 5, 10, 20 minutes; 105°C for 5 minutes. These times are the total lengths of time in the incubator but not the actual length of time the eggs were at these temperatures. After each exposure time the tubes were removed from the incubator at the tested temperature and placed in an incubator at 30°C. Three replicates of each batch of eggs were prepared at each incubation temperature. Three control tubes for each temperature experiment were treated in the same manner as the experimental tubes, but were not subjected to heat.

(ii) Ultraviolet light

A PURAQ ultraviolet light (UV) unit model 15/3p was used, consisting of a light resistant plastic cylinder totally enclosing a 15 watt germicidal lamp surrounded by a quartz sleeve. The UV dose used was 10-15 mW/cm²/sec. Variations in intensity of lamp output were minimised by using a standard warm up period of 10 minutes before beginning irradiation of the eggs.

In a schematic diagram of the experimental setup is presented in Figure 5.1; the plastic cylinder length was 41.5 cm with a diameter of 8.5 cm. At the top of the plastic

cylinder were 15 holes of 1.5 cm diameter. The distance between the UV lamp and the upper part of the plastic cylinder was 3.5 cm. The length of the silica glass tube was 3 cm, with internal and external diameter of 1 cm and 1.5 cm respectively, and a volume of 2 ml. The base of the silica tube was 0.5 cm from the radiation source.

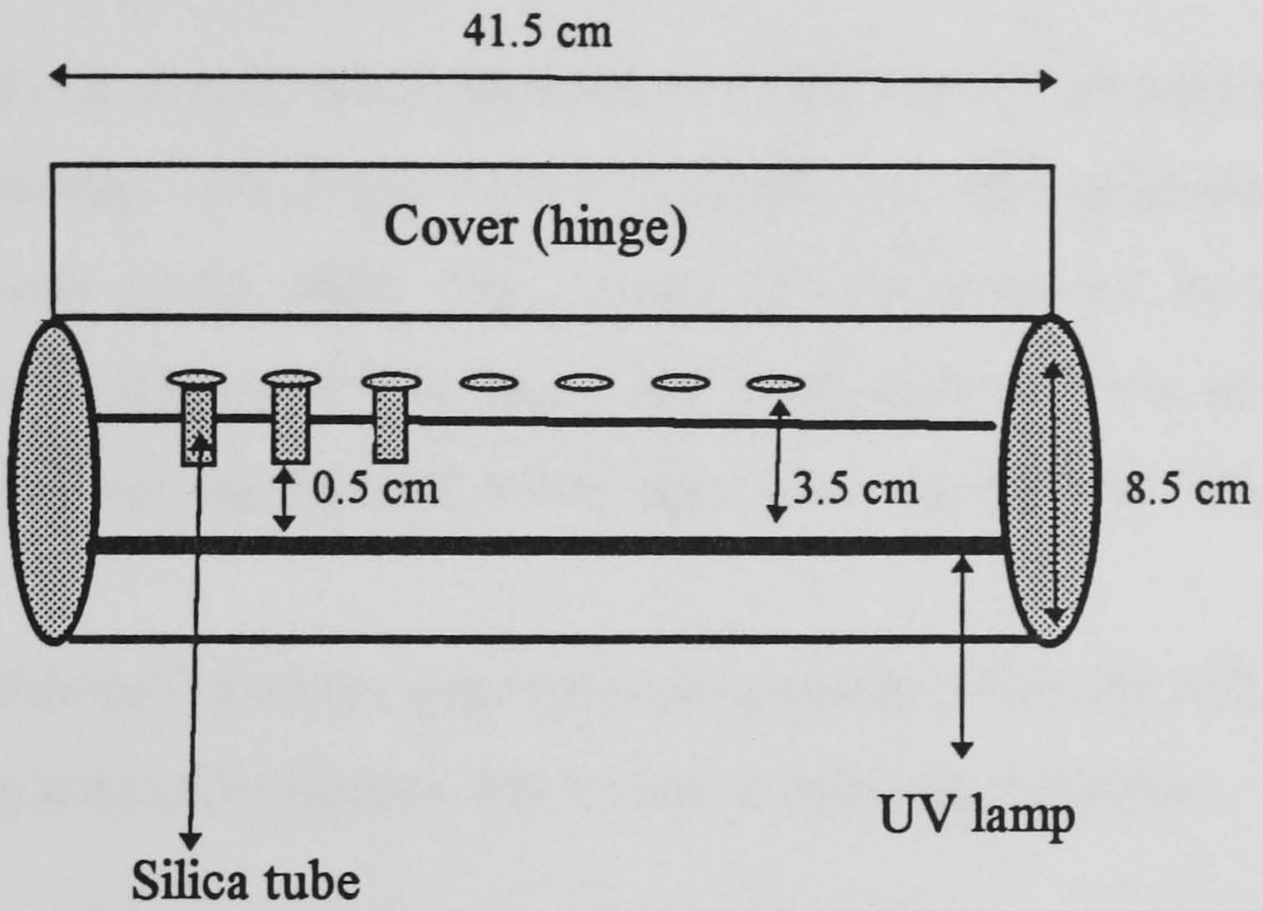


FIG. 5.1. Schematic diagram for PURAQ ultraviolet light unit

One millilitre of distilled water containing viable one-celled stage *Ascaris* eggs were placed in a silica glass tube. Three replicates of each batch of eggs were prepared and the effect of radiation exposure time for 5, 15, 30, and 45 seconds, 1, 3, 5, 15, 30, 60, 360, and 600 minutes, 2 and 3 days were tested. All eggs were irradiated at room temperature ($18 \pm 2^{\circ}C$) and the temperature of the egg suspension remained below that which is optimum for the eggs development. After each exposure time the tubes were removed from the UV light unit and placed in an incubator at 30°C. Three control tubes were treated in the same manner as the experimental tubes but were not subjected to UV light, and then incubated at 30°C for 20-30 days.

The effect of radiation for different exposure times was evaluated by determining the percentage of viability (partial and/or full development) of *Ascaris* eggs by the

incubation method at 30°C and comparing it with the percentage of the viability by the staining method with Crystal violet.

5.2.2 Assessment of *Ascaris* eggs viability

(i) Incubation method (Observed viability)

The experimental and control tubes remained at 30°C±1 for 20-30 days as a check on their ability to develop:- Six stages were recognised; one cell (undeveloped fertilised eggs); early morula (2-16 cells); late morula (16 to complete morula); tadpole (incurved morula to early vermiform stage); and motile embryo (from early vermiform stage to completely formed larvae); other eggs were regarded as "degenerates" or "damaged
(in the process of dying)" showing large refractile granules within the cell, formation of vacuoles disintegration of cytoplasm, and broken or split egg membrane.

Ascaris eggs were pipetted onto a clean microscope slide and the eggs were categorised microscopically as: (a) undeveloped fertilised eggs (one cell stage); (b) developed eggs: summation of the eggs that started to develop but for some reason did not reach larval stage (from 2-celled to tadpole) plus the eggs that have developed to motile larval stage; (c) Complete development (motile larval stage): from early vermiform stage to L2 stage.

Definitions of these categories were as follows:

Developed (or embryonated) eggs = partial development and/or complete development.

Partial development = egg cells that proliferate but do not differentiate.

Complete development = egg cells able to complete proliferation phase and morphogenesis phase.

(ii) Staining method (Predicted viability)

The assessment of *Ascaris* eggs viability using Crystal violet stain is dependent on inclusion of the stain inside the decorticated dead eggs and staining the internal structure of dead eggs blue, while the viable eggs remain unstained. One drop from the decorticated egg suspension was placed on a clean glass slide; the end of a wire loop touched a drop of Crystal violet stain and was mixed thoroughly with the eggs; after 5-10 minutes stained and unstained eggs were counted using a light microscope (100x magnification).

5.3 Results of Temperature Experiments

By treating *Ascaris* eggs of known initial viabilities with a variety of environmental pressures, such as different temperatures and ultraviolet radiation, the effectiveness of these treatments on *Ascaris* survival can be assessed. The relationship of high temperature and time in its effects on development to the motile-larval stage of *Ascaris* eggs is shown in Figure 5.2, 5.3, 5.4, 5.5, 5.6, and 5.7. It demonstrate that all five temperatures

(50, 60, 70, 80, and 90°C) produced complete inhibition of normal egg development. The percentage of damaged eggs increased in relation to increased temperature and time of exposure at a selected temperature. Five minutes exposure to 105°C resulted in obvious damage and abnormal appearance to *Ascaris* eggs. Those eggs subjected to 90°C and 80°C for 5 minutes exhibited a similar degree of damage. Obviously the percentage of degenerated eggs was directly proportional to the time spent at the selected temperature. The controls demonstrated that over 80% of *Ascaris suum* eggs developed to the larval stage when placed in a 30°C incubator for 20-30 days.

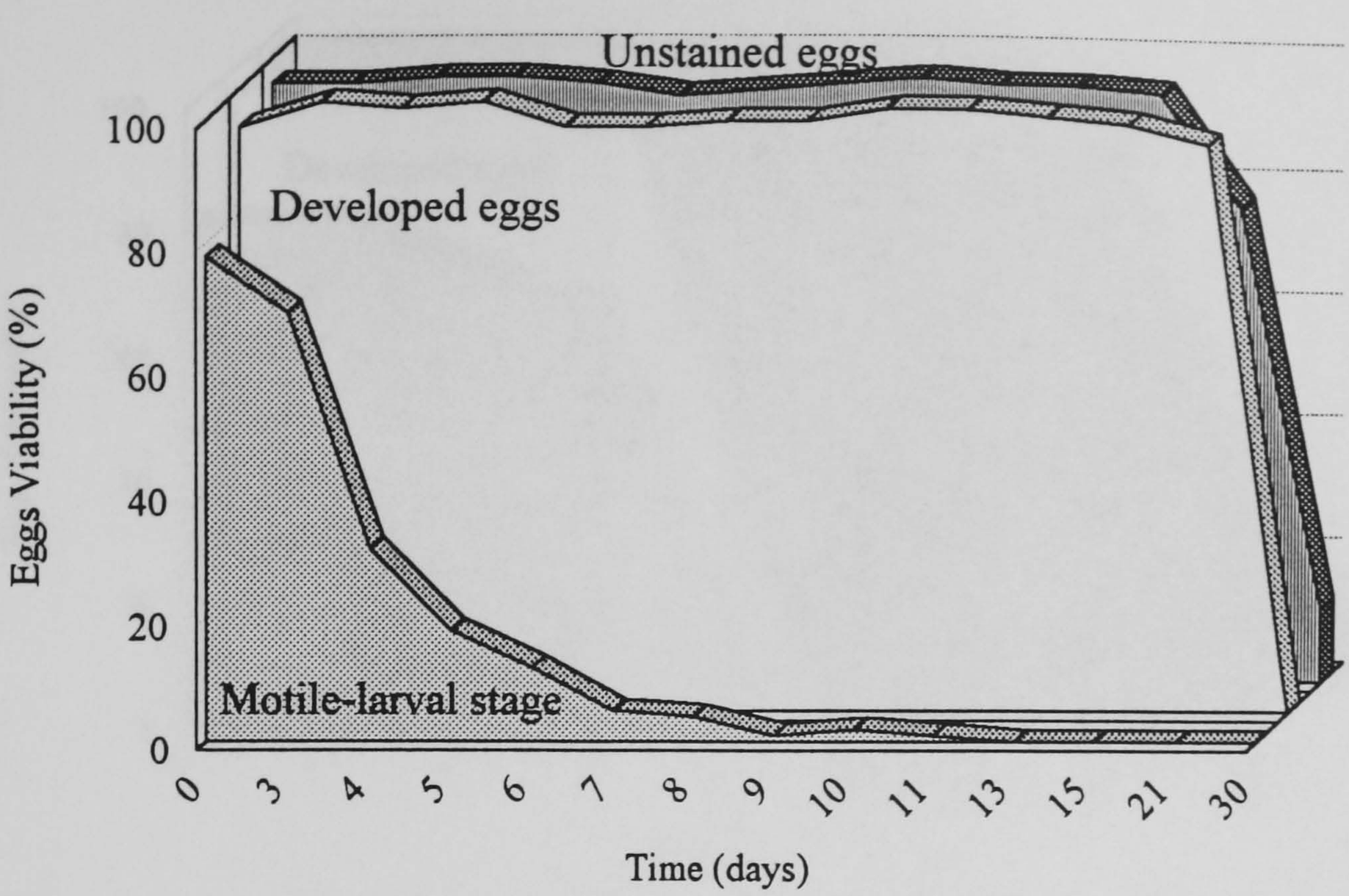


FIG. 5.2. The effect of 37°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

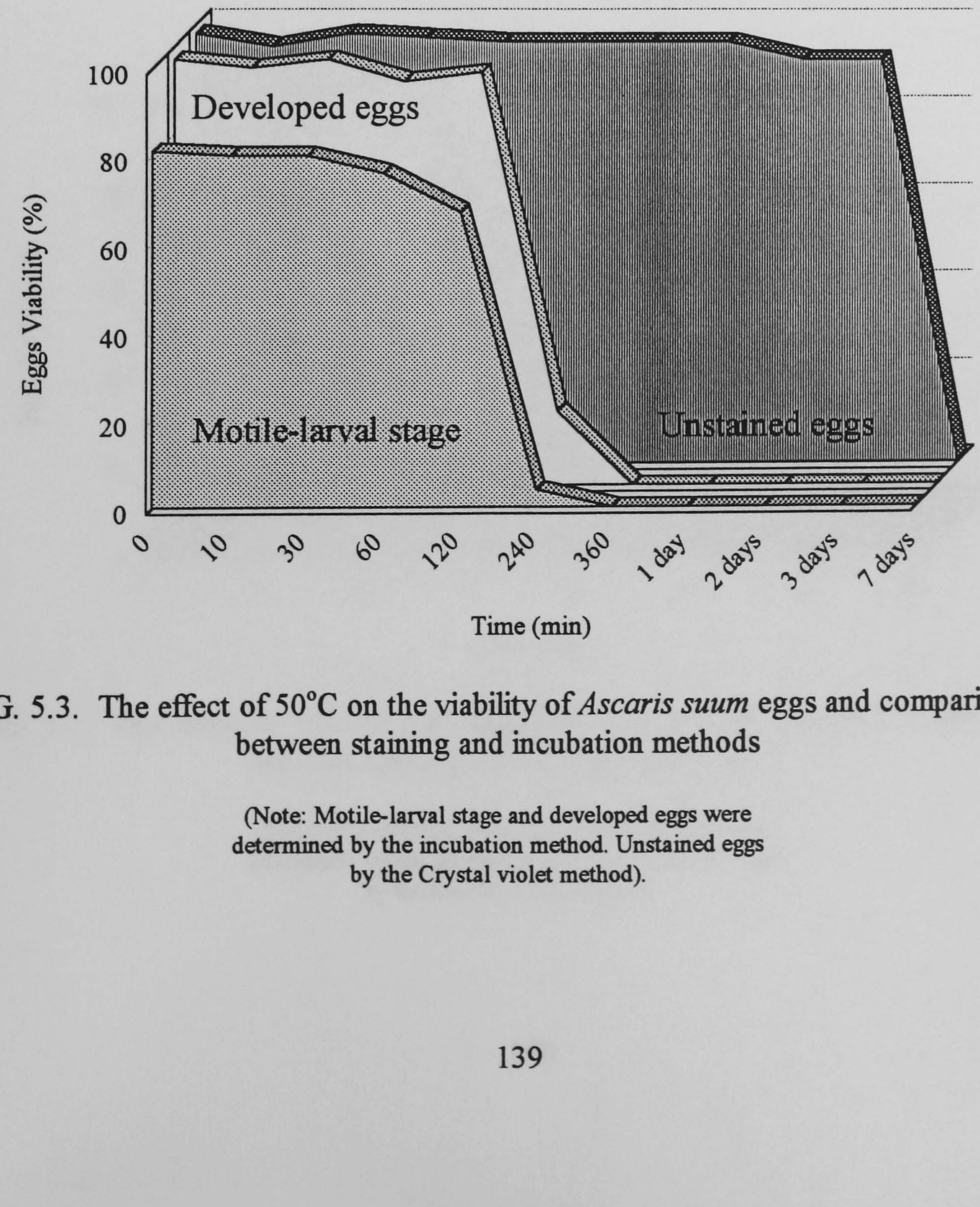


FIG. 5.3. The effect of 50°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

(Note: Motile-larval stage and developed eggs were determined by the incubation method. Unstained eggs by the Crystal violet method).

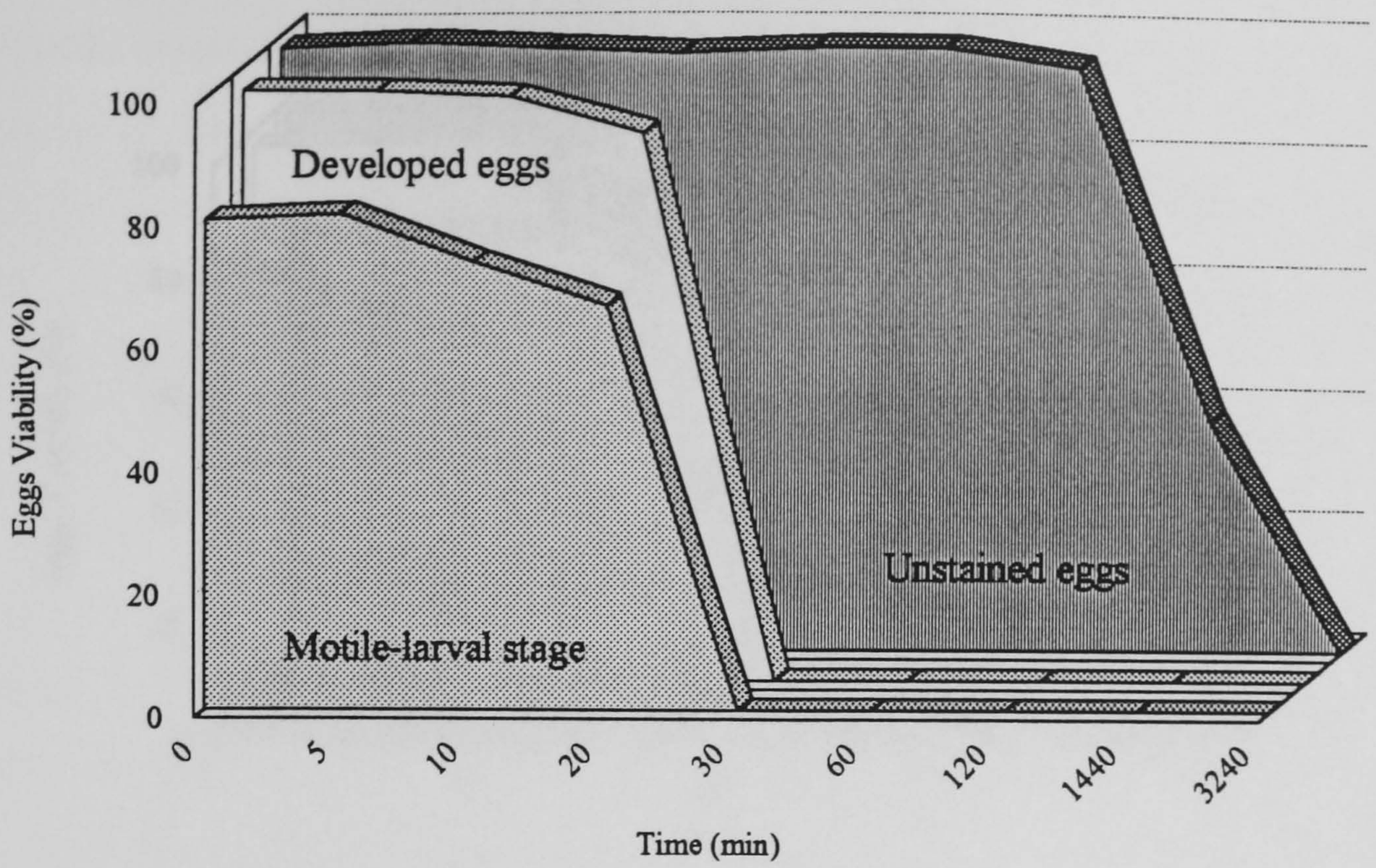


FIG. 5.4. The effect of 60°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

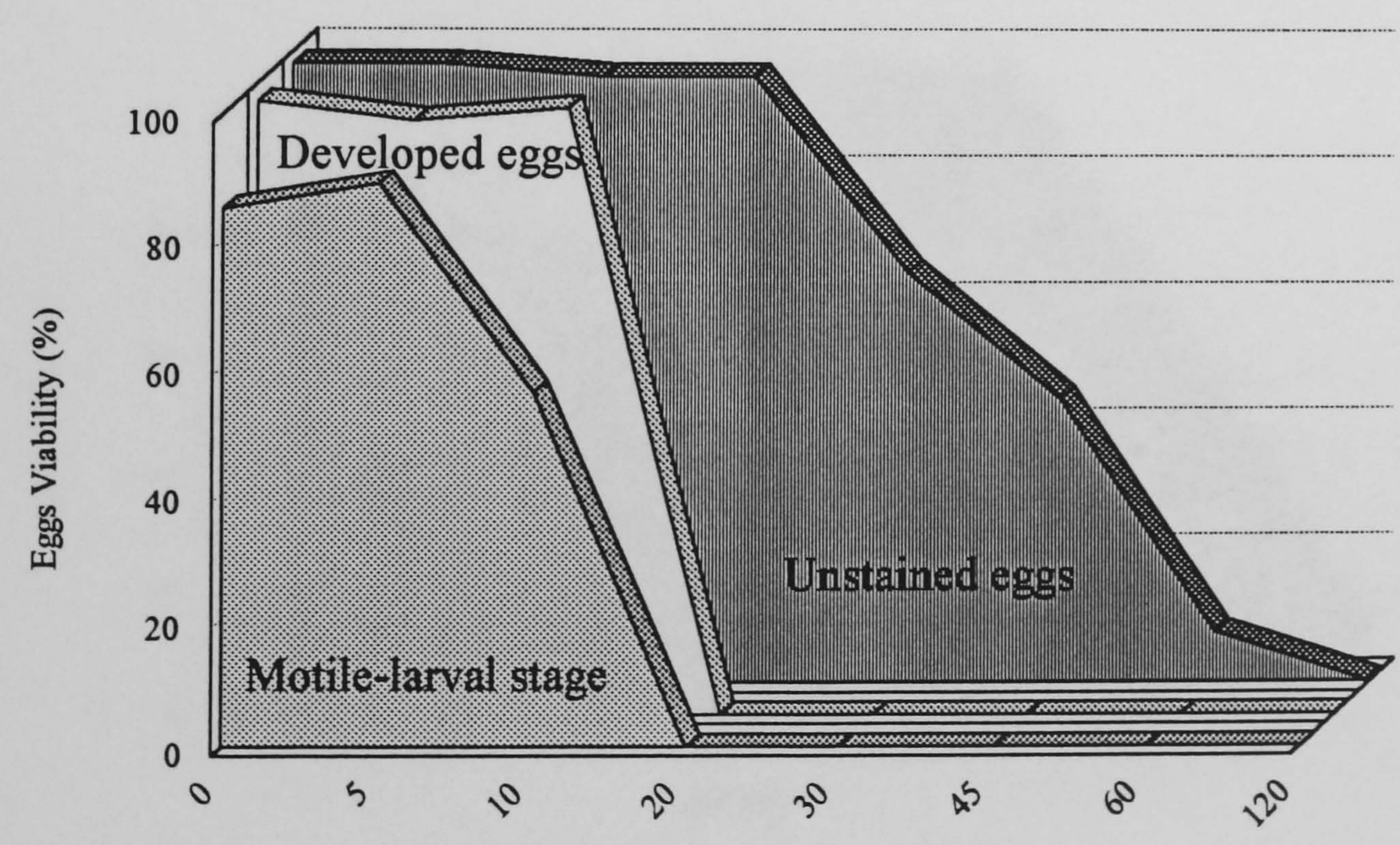


FIG. 5.5. The effect of 70°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

(Note: Motile-larval stage and developed eggs were determined by the incubation method. Unstained eggs by the Crystal violet method).

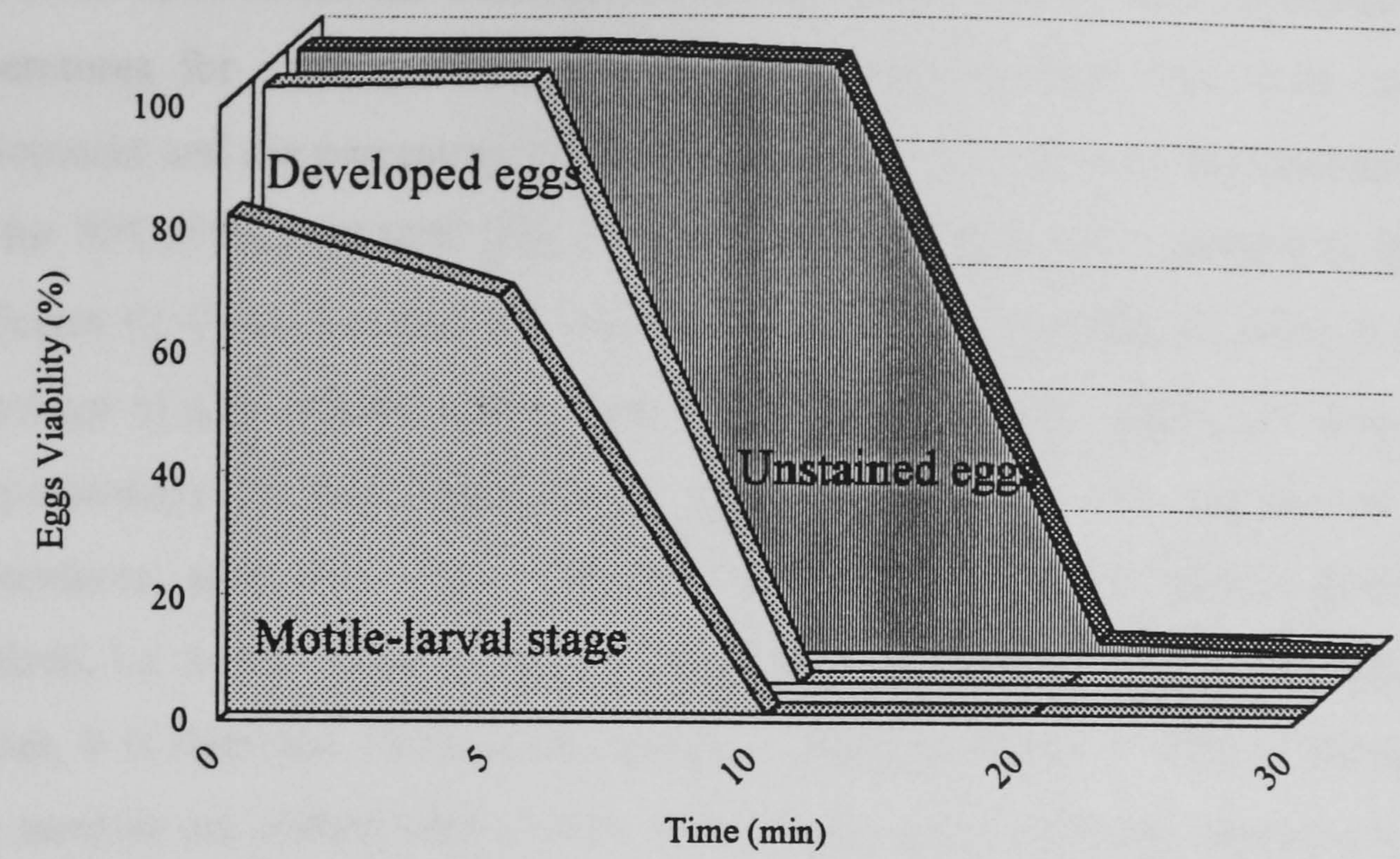


FIG. 5.6. The effect of 80°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

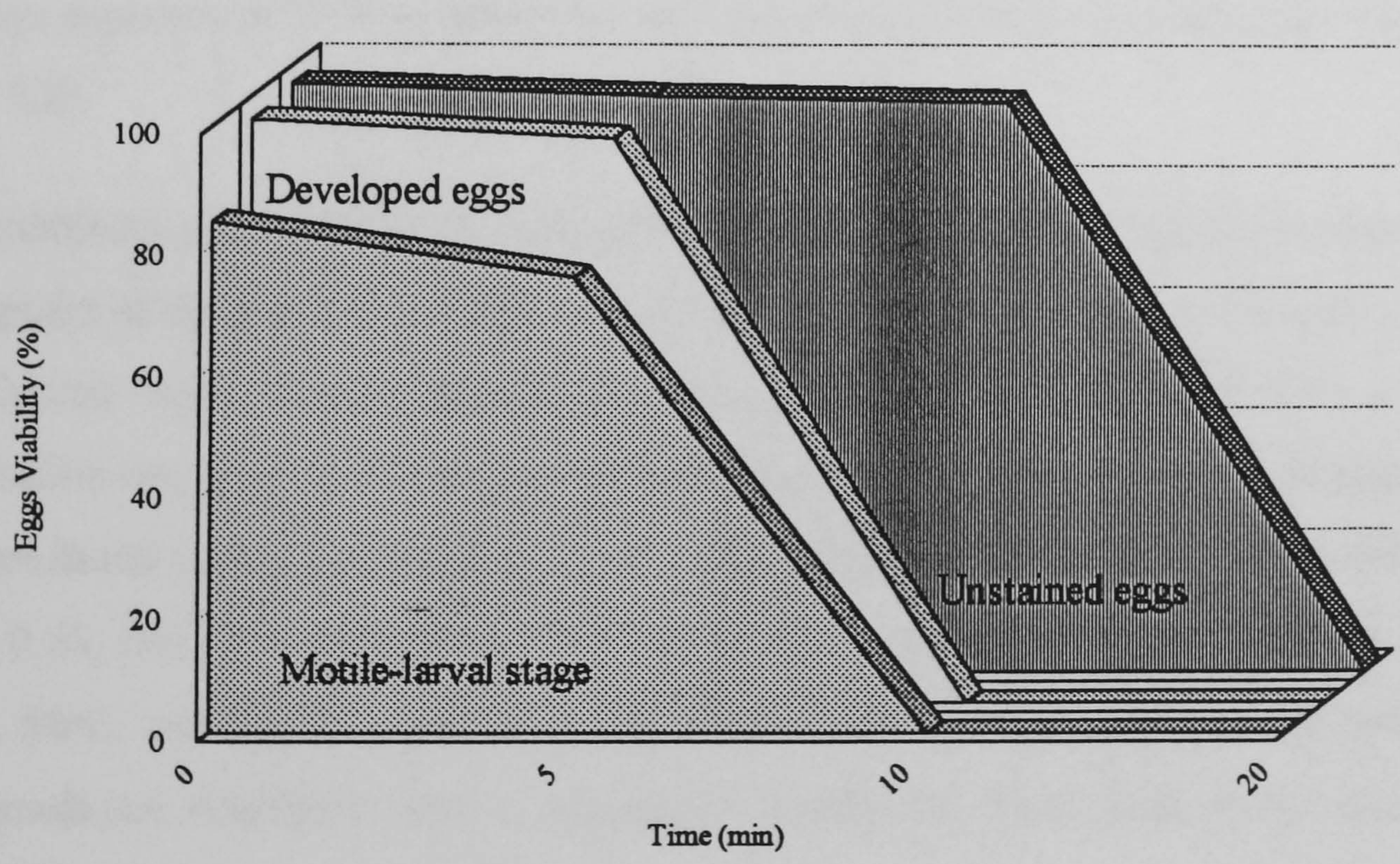


FIG. 5.7. The effect of 90°C on the viability of *Ascaris suum* eggs and comparison between staining and incubation methods

(Note: Motile-larval stage and developed eggs were determined by the incubation method. Unstained eggs by the Crystal violet method).

The results showed for the treatment of one-cell stage *Ascaris* eggs at different high temperatures for various times, that the percentage of eggs that show retarded development and the percentage of stained eggs with Crystal violet both increase with time for 70°C, 80°C, and 90°C (Fig. 5.5, 5.6, and 5.7). There was a positive correlation coefficient (≥ 0.60) between the two methods at these conditions (Table 5.1), the percentage of eggs stained with Crystal violet (presumed non viable) was lower than the percentage of non embryonated eggs, especially at 10 minutes at these temperatures; after that, when only the data for the longest exposure period are examined, i.e. at 90°C after 20 minutes, 80°C after 30 minutes, and at 70°C after 120 minutes, it is seen that 100% of the eggs are degenerated and > 99% of the eggs in these samples are stained with Crystal violet. Thus, given sufficient exposure to heat, the correlation between the observed and predictive methods is almost perfect. However at a lower temperature such as 37°C, it took more than 30 days exposure time to stain 80% of the eggs with Crystal violet stain, while on contrast it needed only 13 days exposure at 37°C to retard the morphogenesis phase in the cells inside the eggs (Fig. 5.2).

The treatment performed on decorticated undeveloped eggs had a significant impact on the uptake of the stain by the eggs and had an effect on the prediction of viability using the Crystal violet staining method. For temperatures 37°C, 50°C, and 60°C a weak correlation was observed between observed (incubation method, % of motile embryos) and predicted (using Crystal violet vital stain) viabilities with a correlation coefficient, 0.26, 0.33, and 0.47 respectively (Table 5.1). But as the temperatures increased, 70°C, 80°C, 90°C, and 105°C, good to strong correlation was observed between observed and predicted viabilities with a correlation coefficient 0.67, 0.68, 0.59, and 0.99 respectively.

TABLE 5.1. Pearson Correlation coefficient between the two methods at different temperatures and UV radiation.

Treatment	Correlation coefficient between staining* and Incubation method	
	% motile-embryo	% development eggs
37°C	0.255	0.998
50°C	0.333	0.347
60°C	0.471	0.472
70°C	0.667	0.682
80°C	0.674	0.678
90°C	0.593	0.593
105°C (stained)	0.99 (no development)	
UV light	0.260	0.352

*% unstained eggs

Using the analysis of variance (ANOVA) statistical test to compare between the predicted and observed methods at temperature of 80°C, 90°C, and 105°C, the detection of viability of *Ascaris* eggs by Crystal violet stain was not significantly different to the incubation method forming the motile-larval stage ($P > 0.1$, and F test < 5.4), while at temperatures of 37°C, 50°C, 60°C, and 70°C there was a significant difference between the two methods at these conditions ($P < 0.05$, and $F > 4.84$), from which we can conclude that the uptake of the Crystal violet stain by *Ascaris* eggs to detect the fully-developed stage and the motile-larval stage will give imprecise detection under these conditions.

At 37°C, the detection of viability of *Ascaris* eggs by Crystal violet was similar to the incubation method from the point of view of only partial development of the cells inside eggs without considering full development ($P > 0.1$, F test < 4.84), with correlation coefficient 0.998. This is due to the capability of the cells inside eggs to partially develop although the formation of the motile-larval stage was retarded (Table 5.1). As can be seen from Table 5.2 there was a strong correlation coefficient (more than 0.90) between the percentage of eggs that developed and the percentage of

eggs that fully developed at temperatures of 50°C, 60°C, 70°C, 80°C, and 90°C. At 37°C a weak correlation coefficient (0.25) was observed between the developed and fully developed eggs, because the cells inside the eggs proliferate at 37°C, but do not completely develop to a motile embryo.

TABLE 5.2. Showing Pearson correlation coefficient between the % of development eggs and the % of eggs that developed to motile embryos at different temperatures or UV radiation.

Treatment	Correlation coefficient between developed eggs and motile-larva stage
37°C	0.255
50°C	0.994
60°C	0.997
70°C	0.970
80°C	0.993
90°C	0.998
UV light	0.927

Cleavage of cells inside *Ascaris* eggs at 30°C incubation starts at 3 to 5 days, reaching the morula stage in 6 to 8 days, and motile larvae developed in 14 to 25 days. At 37°C development was more rapid than at the optimum temperature (30°C). Thus, increase in incubation temperature (from 30 to 37°C) increased the rate of development of the eggs, thus reducing the time taken to develop to the various stages. The development of *Ascaris* eggs terminated and permanently suppressed at the morula stage after 13 days incubation at a temperature of 37°C; cells inside the eggs lost the capability to reach the morphogenesis phase, and no motile-larval stage formed (Fig. 5.2). Similar results were reported by Keller (1951), Arene (1986) and Lewis-Jones and Winkler

(1991) who found that the development of *Ascaris* eggs terminated at the morula stage at a temperature of 36°C, and at the 2-celled stage at 38°C.

Upon removal of the eggs exposed at 37°C for 11 days to 30°C, approximately 1% motile-larval stage were formed in an additional 19 days, but the embryos of these were very sluggish (Table 5.3). These observations indicate that initially, rather than inactivating the eggs, the action of 37°C inhibits the mechanism controlling the development (differentiation) of organs or larvae within the egg, without inhibiting initial cell division.

The effect of the various temperatures on the development of the eggs is shown in Tables 5.3 and 5.4. Table 5.4 shows the percentages of eggs that developed to the motile-larval stage after exposure to high temperatures for different lengths of time. There was no partial or full development of eggs at temperatures of 50°C, 60°C, 70°C, 80°C, 90°C, and 105°C after 360, 30, 20, 10, 10, and 5 minutes respectively. Most undeveloped eggs are degenerated (abnormal morphological changes in the appearance of the eggs) and can easily be detected microscopically after 7 days incubation at 50°C; at the same time eggs stained blue after 7 days incubation at 50°C with Crystal violet (Fig. 5.3). At 60, 70, 80 and 90°C all undeveloped eggs were degenerated after 3240 minutes (54 hours), 20, 10, and 10, respectively (Fig. 5.4, 5.5, 5.6, 5.7). While 88% of undeveloped eggs were degenerated after 30 days incubation at 37°C, all degenerated eggs easily take up Crystal violet stain.

More than 60% of eggs developed motile larvae inside, after exposure to 50°C and 60°C for 120 and 20 minutes respectively, while more than 60% developed to motile larvae at 70°C, 80°C and 90°C after 5 minutes exposure (Table 5.4). As shown in Table 5.4, no larvae developed after incubating the eggs at 105°C for 5 minutes, and all of them degenerated and easily took up the stain.

TABLE 5.3. A summary of the time and percentages for development or killing at various constant temperatures of *Ascaris suum* eggs

Temperature (°C)	Time (minutes)	stage of development
105	5	*egg dead
90	5	75% motile larvae
90	10	egg dead
80	5	68% motile larvae
80	10	egg dead
70	10	56% motile larvae
70	20	egg dead
60	20	66% motile larvae
60	30	egg dead
50	240	4% motile larvae
50	360	egg dead
37	11 days	1% motile larvae
37	13 days	♣ morula-stage

*egg dead: i.e. no cell cleavage and no motile larvae formation

♣egg cells proliferate but do not differentiate.

TABLE 5.4. Showing percentage of *Ascaris* eggs that developed to the motile-larval stage after exposure to high temperatures for different lengths of time

Time	Temperature						
Minutes	50°C	60°C	70°C	80°C	90°C	105°C	37°C*
0	81	80	85	81	84	80	78
5	82	81	89	68	75	0	69
10	80	73	56	0	0		31
20	80	66	0				18
30	80	0					12
45	79						5
60	76						2
120	67						0
240	4						0
360	0						

*Exposure time per day at 37°C = 0, 3, 4, 5, 6, 7, 10, and 13 days

5.4 Discussion of Temperature Experiment

The robust nature of *Ascaris* eggs has long been recognised. They are well known to be resistant to many forms of environmental stress that would prove lethal to other species of infectious agents. It is well known that temperature and desiccation are the greatest lethal factors to the development of *Ascaris* eggs.

The fact that all physiological processes are inhibited by temperatures above their optima indicates that a similar inhibition would be found in the developing egg exposed to high temperatures. Such observations have been previously reported for the eggs of nematodes by several authors (Barnard *et al.*, 1987; Arene, 1986; Arfaa, 1978; Seamster, 1950; and Spindler, 1929). Details of the nature of the biochemical changes

which occur in the eggs during the course of their development have not yet been fully elucidated (Fairbairn, 1957; Costello & Smith, 1964).

Generally speaking, the ideal to be aimed at in disinfection by heating is simplicity of method and rapidity of action. *Ascaris* eggs are instantly killed by very high temperatures; while at lower temperatures (50, 60, 70, 80, and 90°C) they are merely deprived of their ability to develop; and after being incubated at 37°C the eggs cells develop but lose their capability to differentiate to the motile-larval stage. This leads to the conclusion that the destruction of *Ascaris* eggs by high temperatures can be effected also at lower temperatures such as 37°C, but it is exposure time dependent. This is in agreement with Ogata's (1925) results, who found that *Ascaris* eggs were instantly killed after immersing them in hot water, and with lower temperatures, the development of the eggs was inhibited. Furthermore, immersion of the eggs in water at temperatures below 45°C for one hour showed that the eggs developed in the usual manner. However, this is in contrast to the results of Arfaa (1978) who found partial development with a temperature of 70°C for 10 minutes, and at 60°C for 15 minutes. Many studies have been conducted on the heat resistance of *A. lumbricoides* eggs. Barnard *et al.*(1987) studied the thermal death time of unembryonated eggs in sealed tubes within a silicone bath, but his methods are not comparable to those used in this study.

The direct morphological microscopic method to detect the viability of *Ascaris* eggs proved to be not as accurate as the culturing method, as many eggs appeared in this study as healthy eggs, with no change in the morphology of the eggs but never-the-less losing their ability to develop. Only when degeneration is apparent can death be confirmed, and this may take many weeks or months. So living and dead eggs cannot be distinguished by direct observation under a microscope. This confirms the observations of Ayres (1992), but not Reimers *et al.* (1989), who found a good correlation between the estimation of the viability of *Ascaris* eggs using morphologic criteria and the method of culturing the eggs.

Ascaris eggs which have lost their power of development can be divided into two groups, one stainable with Crystal violet, the other unstainable with that dye (Fig. 5.8).

The former show a morphological change (degeneration and distortion of the eggs); the latter do not differ morphologically from healthy eggs i.e. "active viable undeveloped eggs", and yet are in a state of halted development (physiologically viable) and they will be "inactive viable undeveloped eggs". This condition of halted development may continue for 30 days or more, but if left they are likely to perish gradually. It is important to emphasise that in the context of this discussion the word **viable** does not imply successful motile embryo production, but simply that the egg has not died. **Active** mean the cells inside the eggs had the **capability** to complete development up to motile-larval stage and indicates potential infectivity.

Three modes of action thus result by using different degrees of temperature on *Ascaris* eggs viability (Fig. 5.8): i.e.

(a) Dead eggs: degenerated and abnormal morphological appearance, for example after incubation at high temperature (105°C) for 5 minutes.

(b) Inactive viable undeveloped eggs: The eggs at one-celled stage have lost the power of proliferation and morphogenisis, but are still physiologically viable; for example after exposure of the eggs to 60°C for 30 minutes.

(c) Inactive viable developed eggs: One-celled stage eggs which proliferate but have lost the ability to differentiate, although they are still physiologically viable; for example after exposure of the one-celled stage eggs to 37°C for 13 days.

An indirect test serving as an indicator of death is subject to the question of whether it can indicate precisely the border line between dead and live forms. This question may be restated: Is the dividing line between stained and unstained specimens coincident with the border line between dead and live *Ascaris* eggs?.

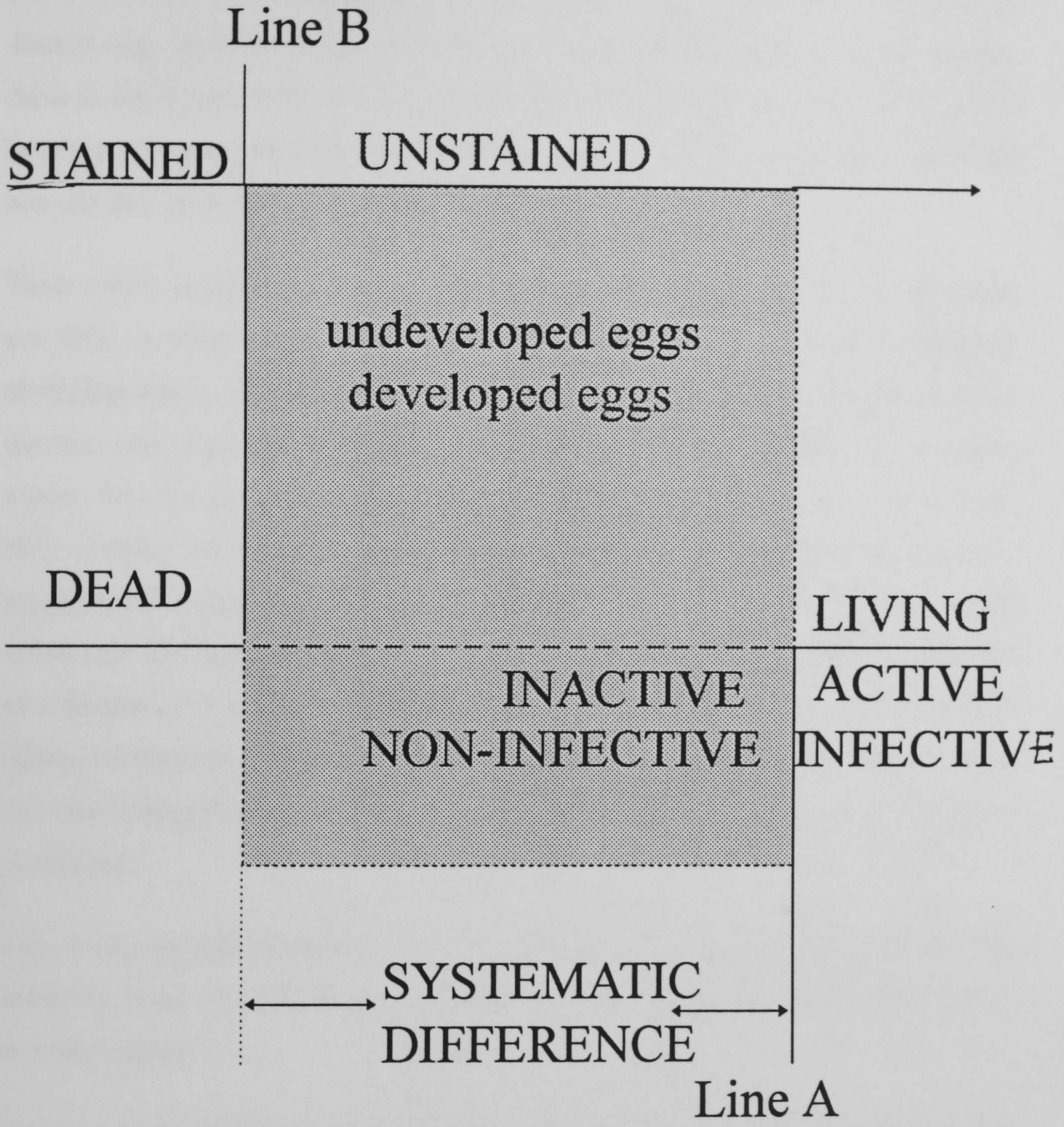


FIG. 5.8. A diagrammatic representation of staining as a criterion for death of *Ascaris* eggs

A diagrammatic representation, as shown in Figure 5.8, may assist in explaining the situation. Let line A be the actual border line between the dead and living *Ascaris* eggs. Let line B indicate the border line between the stained and unstained *Ascaris* eggs. *Ascaris* eggs which remained unstained in this study can be divided into two groups; those in which their cells can fully develop and produce infective larvae; those which have lost the power of partial and/or full development these eggs are found within the two dividing lines, A and B (shaded area).

These results suggest that the eggs cells which are neither stained with Crystal violet nor fully developed were either (1) probably dead but not sufficiently damaged physiologically to allow entry of the stain or (2) alive but incapable of development. In the first case, eggs containing dead cells may still maintain an intact dye-excluding barrier. The fact that at 240 minutes at 50°C staining exclusion indicated greater than 90% viability while the incubation method indicated only 4% motile-larval stage, supports first theory. The second case may apply in those instances where eggs contain viable cells which developed but have lost the capability to differentiate, possibly due to a diminution of energy reserves, as occurs at 37°C. The *Ascaris* eggs in the shaded square are inactive viable non infective eggs (at least they have lost the power to reach the morphogenesis phase during development; they failed to recover under favourable conditions).

Line B may be either coincident with, or to the left of line A. The difference between these two lines, A and B, may be considered as a systematic difference inherent in the staining method.

The size of the shaded square between lines A and B for unstained eggs may change depending on the mode of action and exposure time. For example in extremely adverse conditions such as exposure of eggs to high temperatures (> 105°C for 5 minutes) the size of the shaded square for unstained eggs will be minimal and the correlation coefficient between stained and dead eggs will be greater than 0.9. Decreasing temperature from 90°C to 50°C, will increase the size of the shaded square.

UV radiation results in a larger shaded square than temperature. Thus, the staining method will give a conservative indication of the number of viable eggs present within certain conditions.

However all these intermediate stages are considered to be harmless and non-infective. Which means that the viability of the eggs did not always predict infectivity and just indicates potential infectivity.

The possible explanation for these three modes of action is that the triglyceride, which is normally abundant at the beginning of embryonation of the eggs, is extensively used throughout the period of development (Passey & Fairbairn, 1955). It is possible that at high temperatures the rate of utilisation of the development lipids may be accelerated or the metabolic pathways otherwise modified, resulting in no proliferation and/or morphogenesis phases.

Chaudhuri *et al.* (1966) developed a staining method to detect the viability of free-living nematodes (*Diplogasteroides* spp. and *Diplogasteritus nudicapitatus*) from wastewater treatment plant effluents or polluted surface waters by using Eosin-Y dye which stains dead nematodes, while living nematodes remain unstained. The procedure was also found to be of assistance in the counting of nematodes and did not interfere with subsequent basic taxonomic identification, as well as the short staining period which facilitate routine examinations and to minimise changes in the samples. Similar to this study results he found also that the Eosin-Y vital stain method gave a conservative indication of the number of dead nematodes present, due to his finding unstained nonmotile nematodes failing to revive under favourable conditions (assumed to be dead), when they were killed by less severe conditions. The same pattern of results were also found by Ogata (1925), after using Sudan III vital stain to distinguish between live and dead *Ascaris* eggs.

The results in this research confirms those by previous investigators (Arene, 1986; Spindler, 1940; Cram & Hinks, 1944) who have shown that under optimum conditions of aeration and moisture, the rate of development of *Ascaris* eggs increased with increasing temperature. Seamster's (1950) results showed that exposure of *Ascaris* eggs at 37.8°C for 8 days was lethal but that exposure at 37.2°C permited the development to the 2 and 4 cell stages in 8 days. It had been suggested from early studies that the reason that *Ascaris* eggs do not develop at 37°C might be due to lack of sufficient oxygen, but Brown's (1928) results proved that it is not lack of oxygen that prevents their development at 37°C.

Arene (1986) showed that the temperature at which *A. suum* eggs embryonated has a marked effect on the viability of the resultant infective larvae. Also he explained why it has often proved difficult to obtain heavy experimental infections in pigs, while heavy natural infections occur commonly (Schartz, 1959), as being due to the different optimal temperatures for rate of development and larval survival. The reason for this reduction in larval viability following embryonation of the eggs at higher temperatures is not obvious.

In conclusion, the Crystal violet staining method is not an absolute measure of fully development of *Ascaris* eggs; it is however within certain parameters a strong indicator of the state of the eggs. A better estimate of egg viability might be determined as a presumptive test by using Crystal violet stain (as a quick method to be used for routine purposes), if the method employed depended on the identification of dead eggs rather than of live eggs, since it only stained dead eggs. From the experimental results it can be deduced that if greater than 60% of eggs take up the stain, approximately 100% of the eggs under examination are dead; in this case no confirmatory test (Incubation method) is needed. If less than 60% of the eggs take up the stain the confirmation of the eggs mortality must be obtained by using the Incubation method.

5.5 Results and Discussion of UV Radiation Experiment

The results of treating *Ascaris* eggs with UV radiation are shown in Figure 5.9, and Table 5.5. Results similar to those found with heat were obtained. Treatments performed on *Ascaris* eggs with UV radiation had a significant impact on the uptake of the stain by the eggs and had an effect on the prediction of viability by using the Crystal violet staining method. There appeared to be only a slight correlation (Pearson correlation coefficient 0.26) between the percentage of motile-embryo formation determined by the incubation method and percentage of eggs that were unstained with the Crystal violet method.

Statistical analysis confirmed that this difference between the observed and predicted viability method was significant ($P < 0.05$). Examination of Figure 5.9 reveals, with the time exposure, the percentage of eggs stained blue (assumed dead eggs) tended to be lower than the percentage of not developed (dead) eggs determined by the incubation method. However, this difference disappeared once the point at which all of the eggs degenerated was reached after 3 days of UV exposure. It was noted, however, that although many eggs were able to develop after such exposures, others showed evidence of damage, such as exploding internal cellular material and damage to the outside membrane of *Ascaris* eggs

In the limited experiments made, undeveloped viable fertilised eggs exhibited no great resistance to the dose of ultraviolet radiation used. Treating undeveloped eggs with UV radiation for various times, the percentage of eggs that show retarded development and the percentage of stained eggs with Crystal violet both increase with time (Fig. 5.9, Table 5.5). It took more than 3 days exposure time to stain 99% of the eggs with Crystal violet, while only 60 seconds exposure were needed to retard the development processes in the cells inside the eggs (Fig. 5.9). After 5 seconds exposure time, 32% of the undeveloped eggs survived and completed their development to motile larval-stage, some of them morphologically abnormal; at the same time little response for the reduction in the viability appeared when the staining method was used to detect the viability of *Ascaris*. In this experiment, continued irradiation at room

temperature with sufficient energy effectively reduced the percentage survival of *Ascaris* eggs.

In the case of eggs in control cultures, an average of 80% of those examined at completion of the tests were fully developed. This fact demonstrates that death of the vast majority of eggs in the exposed cultures was due to the inimical effects of UV only.

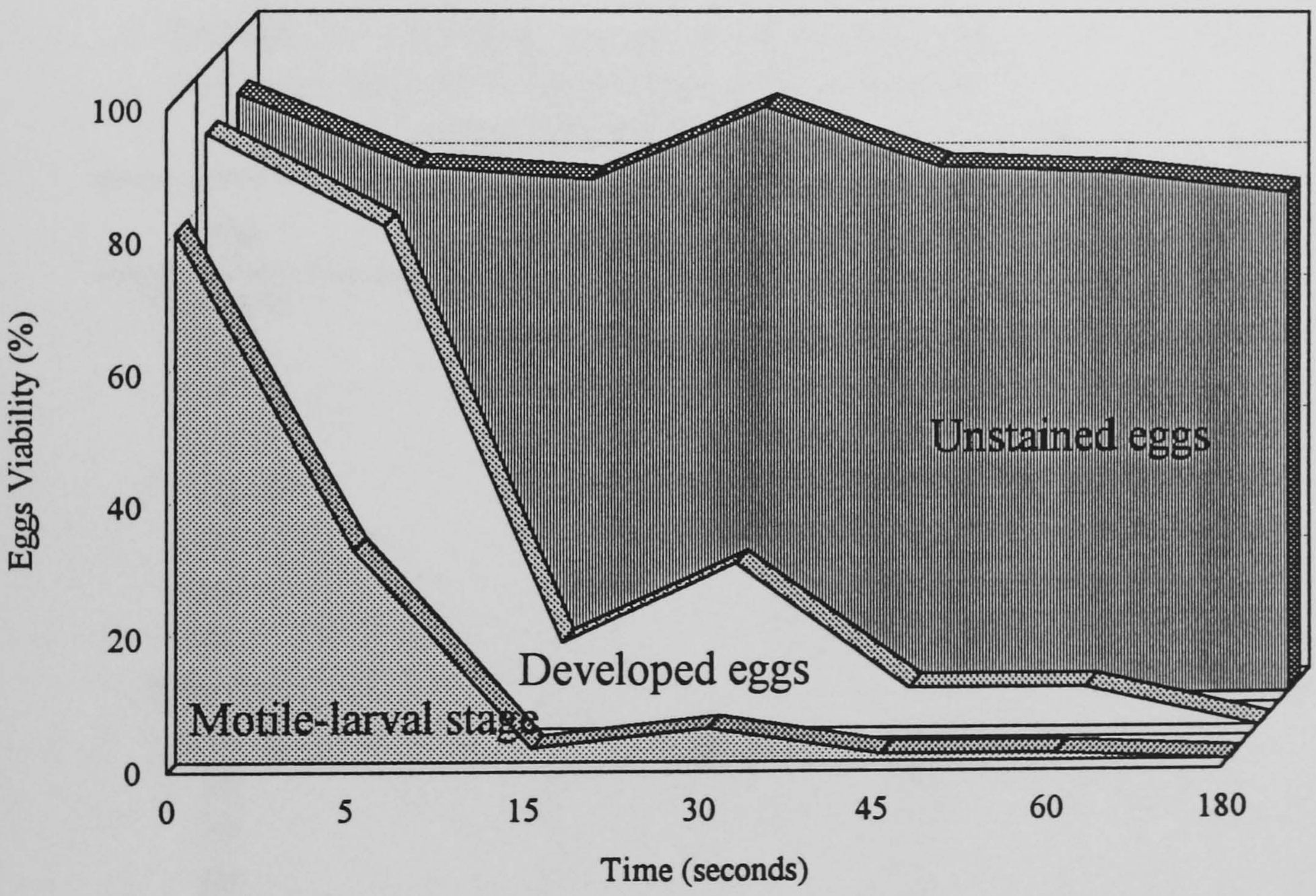


FIG. 5.9. The effect of UV radiation (10-15 mW/cm^2/sec) on the viability of *Ascaris suum* eggs and comparison between staining and Incubation methods

Discussion of the results demonstrate that the proliferation and morphogenesis phases of *Ascaris* eggs were inhibited after 60 seconds exposure to UV radiation. After 3 days or more, complete degeneration of eggs was observed using the Crystal violet stain. The energy necessary to produce a damaging effect with radiation of 15 watts germicidal lamp (with UV light dose used 10-15 mW/cm²/sec) is very great. For *Ascaris* eggs, the exposure time necessary to halt development is 60 seconds.

TABLE 5.5. Showing percentages of *Ascaris* eggs reaching complete embryonation after exposure to ultraviolet radiation

Time	% motile-embryo	stain method*
Seconds		
0	80	91
5	32	80
15	2	78
30	5	89
45	0	80
60	0	79
180	0	76
Minutes		
5	0	76
15	0	76
30	0	77
60	0	71
360	0	63
600	0	58
Days		
1	0	19
2	0	5
3	0	1

* percentage of unstained eggs

The radiation energy of UV rays can be used to destroy microorganisms. In order to kill, the electromagnetic waves of UV radiation must actually strike the organism. In this process some of the radiation energy is absorbed by the organism and other constituents in the medium surrounding the organism. Energy dissipation by excitation, causing disruptions of unsaturated bonds, particularly of the purine and pyrimidine components of nucleoproteins, appears to produce a progressive lethal biochemical change (White, 1986). The 1% of *Ascaris* eggs that developed after 45 seconds exposure were probably protected from the direct rays by being massed together; similar observations were made by Nolf (1932). Hollaender *et al.* (1940) noted that a number of factors may complicate assigning a limiting value to the effects of UV radiation. Specimens may clump together or be situated in the containing vessel in such fashion that some are protected from the full effects of the radiation. Furthermore, there may be a natural range of resistance in the organism itself. Another factor, noted by Stowens (1942), was that *T. spiralis* larvae suspended in a broth/gelatin mixture required much higher levels of radiation for observed effects than those exposed in thin films of water. It was apparent that ultraviolet radiation could not be relied upon to kill eggs under all circumstances. Finally, the wavelength of the UV radiation is most important.

Extensive studies have been made on factors affecting the development of the eggs of *Ascaris* spp. However, little work has been done on the effect of ultraviolet light on the development and viability of *Ascaris* eggs that can be compared with this experiment.

Few references dealing with the effect of ultraviolet radiation on nematodes eggs have been published. Stevens (1909) showed that ultraviolet radiation caused abnormal embryogenesis in developing eggs of *Ascaris megalocephala*. The same observation are shown in this research (Table 5.5), i.e. that the exposure of *Ascaris* eggs to ultraviolet radiation for 5 seconds permitted 76% of the eggs to develop, 32% of the developed eggs reach the motile embryo stage, although some had abnormal morphology; the remaining 44% lost their capability to differentiate, most of them

developed to the 2 and 4 celled-stage only. White (1986) commented that viruses, bacteria, plant and animal cells show a loss of reproductive ability following radiation. Radiation energy is deposited in living matter as random ionisation and excitation events. The target theory states that cells contain one or more critical sites, "targets", (i.e., DNA and membranes) within which an ionization event would be fatal to the cell. Some of the side effects of UV on plants and animals include retardation of cell division, increase in the rate of mutation, chromosomal aberration, and changes in cellular viscosity.

Results observed in this research are to some extent comparable with the observations of Dognon and Tsang (1928) cited by Nolf (1932), who reported that the effects of exposure of *Ascaris megalocephala* to ultraviolet rays for 10 to 60 seconds at both 16 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.

Similar results obtained by Spindler (1940), showed that *Ascaris* eggs in cultures exposed to UV radiation were not all inhibited from development as a result of the same period of exposure, but the number of inactive undeveloped eggs in the cultures increased rapidly as the exposures were lengthened. In general, small numbers of eggs were able to survive exposures that destroyed the activity of the majority of eggs in the cultures. A similar phenomenon was observed by Wright (1936) cited by Spindler (1940) in connection with tests of the effect of UV light on eggs of *Toxascaris leonina* and *Toxocara canis*. Wright found that while the vast majority of eggs in cultures exposed were killed, apparently by the "dosage" of UV light used, a few eggs were able to survive and complete development.

Its clearly shown by Nolf (1932) that *Ascaris* eggs are more susceptible to the lethal action of UV radiation than are those of *Trichuris*. He also demonstrated that a very short exposure is sufficient to prevent a large percentage of the *Ascaris* eggs from reaching motile-larval stage and that slightly larger exposure is completely lethal to them.

Eggs of the nematodes *Enterobius vermicularis* and *Ascaris lumbricoides* were subjected to long ultraviolet and near visible radiation in the 3500 to 4900A range, exclusive of short ultraviolet and infrared radiation. Lethal effects were observed after radiation with sufficient energy. A lowered resistance of the eggs of *E. vermicularis* to radiation at higher temperature was observed by Jones and Hollaender (1944). Studies have been made by Coggle (1971) into both the physico-chemical and the biochemical effects of radiation on enzymes. The physico-chemical criteria of damage include a decrease in molecular weight due to fragmentation of polypeptide chains; changes in solubility; disorders of the secondary and tertiary structure; cross linkage and the formation of aggregates, as well as the destruction of amino acids in the chain. The biochemical criterion of damage is the loss of the ability of the enzyme to carry out its reaction.

CHAPTER SIX

MATERIALS AND METHODS FOR FIELD STUDIES

6.1 Introduction

The major purpose of this study was to provide solutions and added insight into sludge characterisation, accumulation and resulting treatment and disposal problems associated with wastewater sludges from waste stabilisation ponds and oxidation-ditches in Jordan. Sludge samples were collected from two waste stabilisation ponds (Al-Samra and Madaba); and from Jerash oxidation-ditch sewage treatment plant (JTP), for pathogen characterisation. The sludge samples collected from Jerash sludge thickener and Al-Samra primary anaerobic ponds were used in field and pilot scale respectively, on sand or gravel drying beds studies directed at characterising the sludge dewatering and the effect of desiccation on the reduction of bacterial pathogens and intestinal nematodes (mainly *Ascaris* eggs).

Because accumulation of sludges in the primary anaerobic ponds in Al-Samra WSP's reduces pond volume, thereby shortening the hydraulic residence time, it may be necessary to determine the volume of sludge in these ponds and the level of pathogen and heavy metals contamination. Also it will be necessary to remove the partially digested sludge from primary anaerobic ponds and investigate open sand or gravel drying beds as a treatment alternative for these partially digested sludges to reduce pathogens.

6.2 Field Studies

The bases for selecting the wastewater treatment plants to study were summarised in Table 6.1: (1) method of treatment (2) region served (3) expected high prevalence of helminth eggs (4) size of the plant. Table 6.1 presents characteristics and background data for the three waste treatment plant (Jerash, Al-Samra and Madaba) sites studied during this research.

6.3 Treatment Plant Description and Sampling Locations

The study is concerned with three wastewater treatment plants, Al-Samra and Madaba waste stabilisation ponds, which represent the non-conventional wastewater processes (natural treatment). While Jerash represents the conventional extended aeration (oxidation-ditch, as an activated sludge process) wastewater treatment plant. Figure 6.1 shows the locations of the three wastewater treatment plants with the direction of flow of treated wastewater from the plants.

Al-Samra WSP was designed to serve Amman and Zarqa city (population served 1352000); Madaba WSP serves Madaba city (population served 19000); Jerash extended aeration system serves 16000 (Table 6.1).

TABLE 6.1. Criteria for selecting wastewater treatment plants, site description, and mean values of common operational parameters.

Plant name	Al-Samra	Madaba	Jerash
Location	North-East	South-West	North
commissioned	1985	1988	1983
Plant type	WSP	WSP	Oxidation-ditch
Climate	dry/arid	dry/arid	dry/arid
Population served	1352000	19000	16000
Influent flow (m^3/d)	120000	1700	1500
Influent BOD_5 (mg/l)	504	1439	1012
Influent TS (mg/l)	1516	2625	1927
Influent TVS (mg/l)	-	1248	903
Influent TSS (mg/l)	387	1051	793
TSS reduction (%)	64	75.3	95.4
BOD_5 reduction (%)	76	80.8	98.4

Based on 1992 year data.

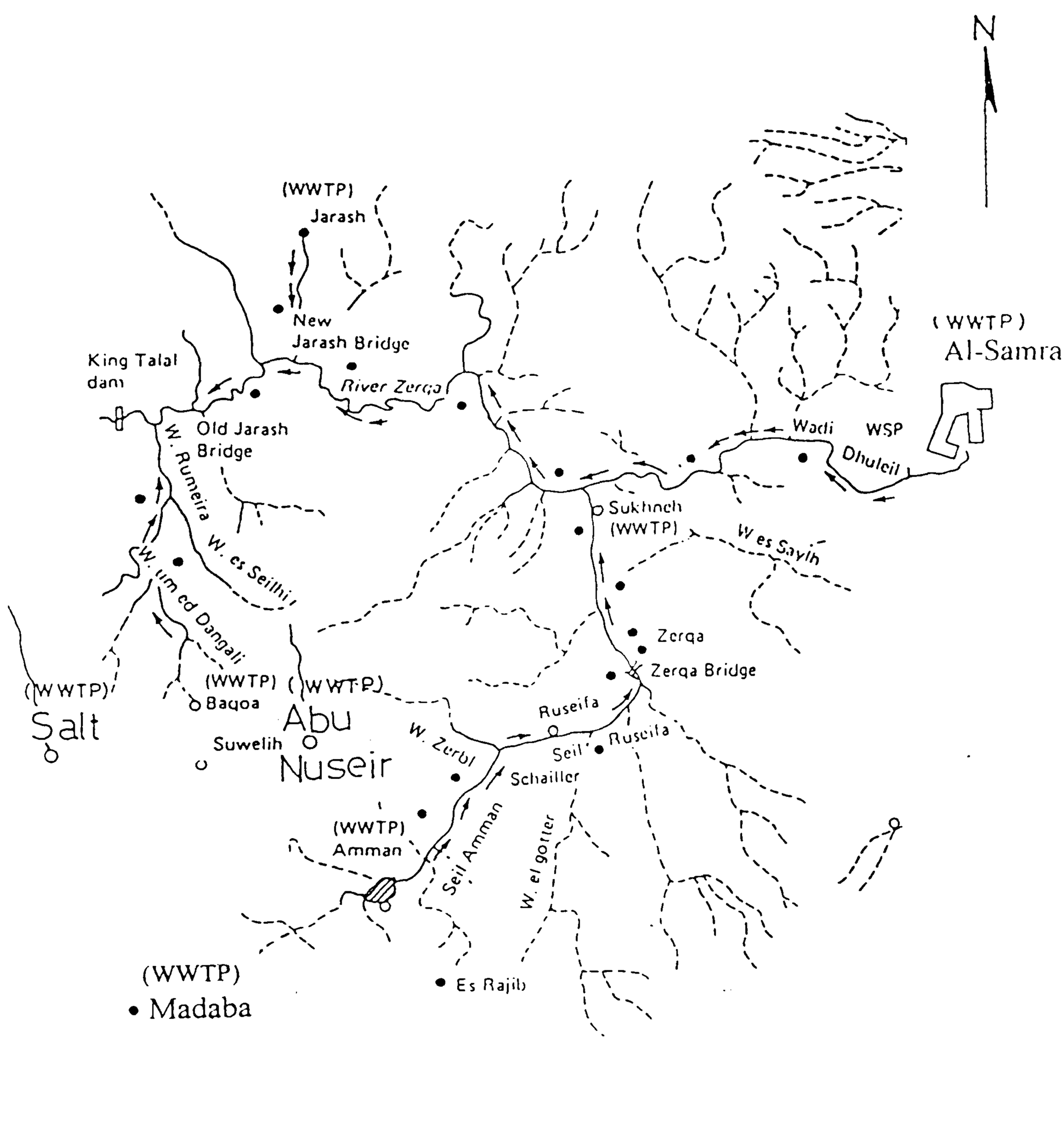


FIG. 6.1. Locations of wastewater treatment plants (Tarazi, 1989)

6.3.1 Al-Samra wastewater stabilisation ponds

The Al-Samra wastewater treatment plant utilises a waste stabilisation pond system (WSP), commissioned in May 1985, and received about 57000 m^3 of domestic wastewater and septage per day discharged from the Metropolitan Area of Greater Amman, Jordan. Recently, it serves also Zarqa and Rusaifa city.

The wastewater, prior to entering the stabilisation ponds, passes through a large screening and grit removal facility at Ain-Ghazal. At Al-Samra, there are a total of 32 ponds. The general lay out of the pond system for Al-Samra is shown in Figure 6.2.

The ponds cover a mid depth area of 181.4 hectares designed for an average capacity of 68,000 m^3/d although the 1994 average hydraulic loading is 110,000 m^3/d. The ponds are divided into three trains each of which can operate independently of the others. Each train is subdivided into ten ponds: two anaerobic (A1, A2) followed by four facultative (F1, F2, F3, and F4) and finally four maturation (M1, M2, M3, and M4) as designated in the original design. The site of the ponds is about 40 Km North East of Amman. Table 6.2 gives the pond sizes in the Al-Samra WSP, and Table 6.5 and 6.6 shows the sampling points for the sludge assessment which was made.

The three primary anaerobic ponds in operation were almost identical in size, experienced identical climatic conditions, and received influents identical in quality. The only difference for A1-1 pond was that it was brought into operation from the beginning of March, 1987.

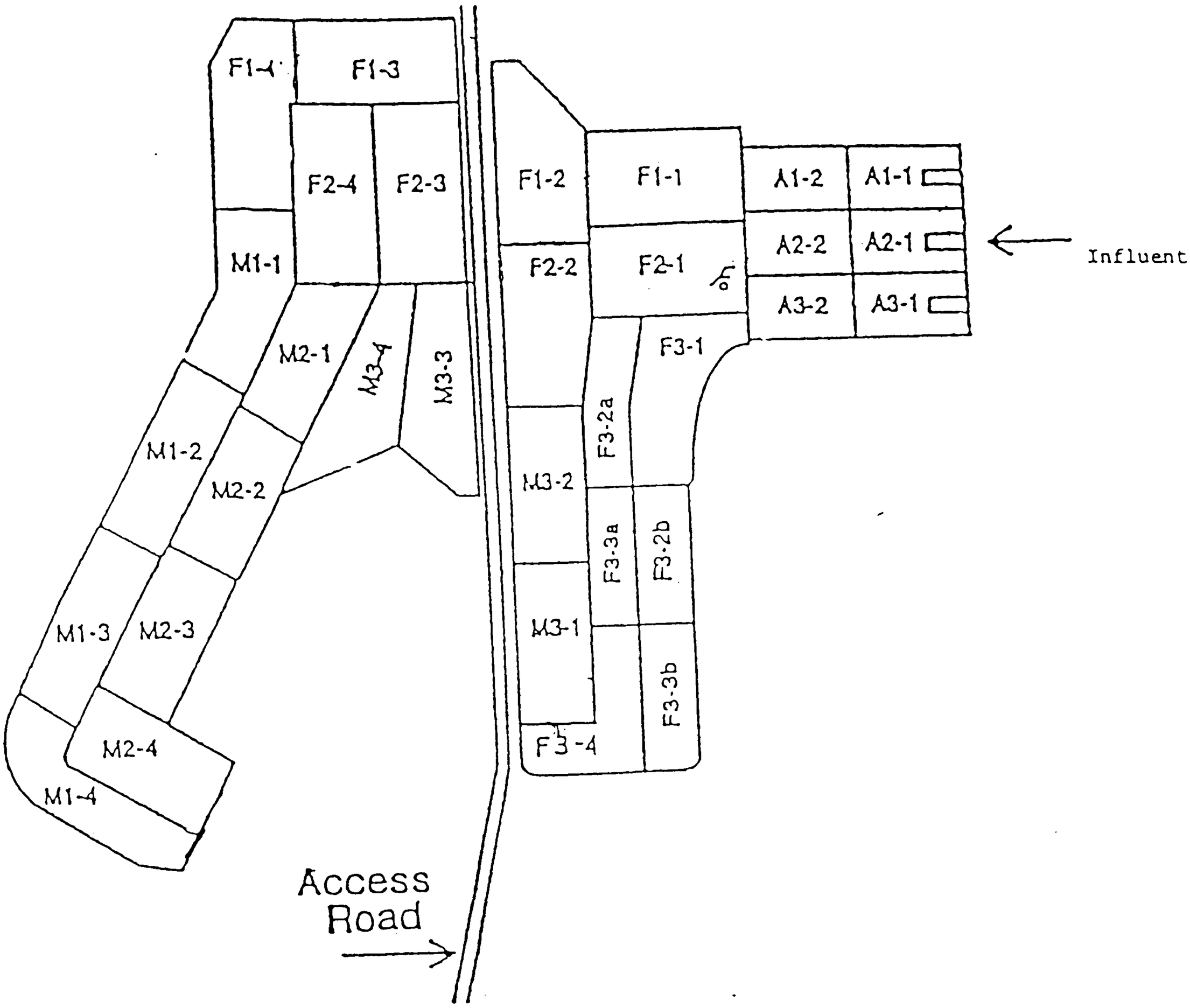


FIG. 6.2. Al-Samra wastewater stabilisation pond system

The first and second anaerobic ponds, which are the subject of this study, are 140 x 260m (measured on the surface). The depth from the surface of the wastewater in the pond to the bottom is 5.1 m with a mid depth area of 3.16 ha (Table 6.2). The raw wastewater is discharged into the pond at 35.5m from the water edge measured along the longitudinal direction. The point of raw wastewater discharge is at the middle of the pond's width. The wastewater is carried to the point of discharge, in the pond, through a 1270 mm pipe over a concrete weir supporting the pipe. At the point of wastewater delivery in the pond, a grit storage sump was constructed (60m x 34m x 0.75m). The pond has one inlet and one outlet both located in the middle of the width. The pipelines from the inlet structure to the three first anaerobic ponds have an estimated capacity of 103,500 m^3/d, 156,900 m^3/d and 100,600 m^3/d to trains 1, 2, and 3, respectively. At greater rates of flow, the inlet structure downstream of the gates will overflow to the ground.

TABLE 6.2. Effective pond sizes and detention time in Al-Samra WSP's

Pond	Total depth (m)	Effective depth (m)	One train		Total three trains		
			Area (ha)	Volume ($m^3 \times 10^4$)	Area (ha)	Volume ($m^3 \times 10^5$)	*Detention time (days)
A1	5.0	3.0	3.17	9.5	9.5	2.85	4.2
A2	5.0	3.0	3.17	9.5	9.5	2.85	4.2
F1	2.25	1.5	7.25	10.9	21.75	3.26	4.8
F2	2.0	1.5	7.25	10.9	21.75	3.26	4.8
F3	1.5	1.5	7.25	10.9	21.75	3.26	4.8
F4	1.5	1.5	7.25	10.9	21.75	3.26	4.8
M1	1.25	1.25	6.25	7.8	18.75	2.34	3.4
M2	1.25	1.25	6.25	7.8	18.75	2.34	3.4
M3	1.25	1.25	6.25	7.8	18.75	2.34	3.4
M4	1.25	1.25	6.25	7.8	18.75	2.34	3.4
Total			60.33	93.7	181.0	28.1	41.2

* Based on a flow of 68,000 m^3/day
(Source: Design Report (1983), Water Authority-Amman)

6.3.2 Jerash wastewater treatment plant

The Jerash wastewater treatment plant, about 50 km north Amman, utilises an extended aeration (oxidation-ditch) treatment process; it was commissioned in 1983. An oxidation-ditch is similar to an aerated lagoon in that the wastewater is oxidised by bacteria in flocculent suspension and that the oxygen required for bio-oxidation is supplied by mechanical aeration.

Currently, the plant receives approximately 1,500m^3/d domestic sewage. Prior to entering the oxidation-ditch, the wastewater flows through the bar rack and grit chamber to remove gravel, sand, and heavy particulate matter. Within the oxidation-ditch, the screened sewage is aerated in and circulated around a continuous oval ditch by special aerators, called rotors, placed across the ditch.

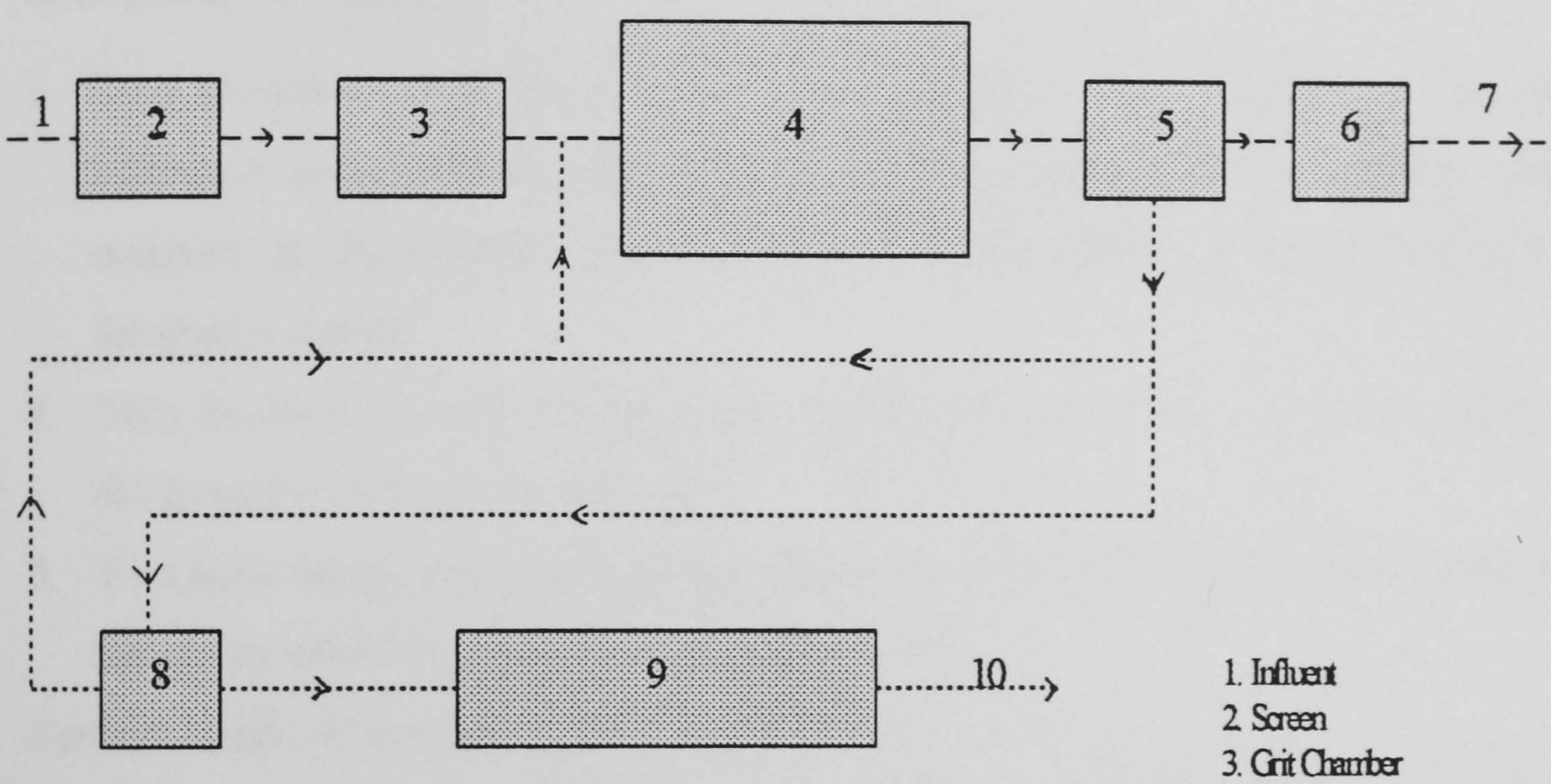
The ditch effluent is settled in a conventional secondary sedimentation tank and most of the sludge is returned to the oxidation-ditch. The small quantity of excess sludge goes to sludge thickener before being piped to sludge drying beds. The supernatant from the sludge thickener is recycled back to the oxidation ditch. The effluent from the sedimentation tank is disinfected by chlorination before being discharged to the valley. Table 6.3 describes Jerash treatment plant unit capacities.

A flow schematic and sampling points are presented in Figure 6.3. Three sampling points were examined for assessment of pathogens especially nematode eggs in sludge samples.

TABLE 6.3. Jerash wastewater treatment plant unit capacities

Unit	Capacity (m^3)	Detention time
Oxidation-ditch	2300	26 hours
Sedimentation tank	360	5 hours
Chlorination tank	24	30 min
Sludge thickener	14	1.3 days
Drying bed	1000	-

(Source: Design report/Water Authority-Amman)



1. Influent
2. Screen
3. Grit Chamber
4. Aeration tank-Oxidation ditc
5. Settling Tank
6. Chlorination Tank
7. Effluent
8. Sludge Thickener
9. Drying Beds
10. To Sanitary Landfill

FIG. 6.3. Jerash wastewater treatment plant

Key:

..... sludge flow

--------- wastewater flow

* sampling points: from site 5, 8 and 9

6.3.3 Madaba wastewater stabilisation ponds

The wastewater treatment plant is situated in Madaba city south-west Amman; when the treatment plant was constructed, it was designed to treat an average daily flow of 2000 m^3. The raw wastewater passes through screening and grit removal to get rid of large objects, sand and silt. The plant consists of two trains in parallel, each train consist of three ponds, as follows:

1. Two anaerobic ponds: the anaerobic ponds dimension 38 x 67m provide first stage biological decomposition and sludge digestion and storage of the organic material received at the Madaba plant. Anaerobic ponds effluent is discharged to two facultative ponds;
2. Two facultative ponds: the facultative ponds dimension 147 x 76m. The effluent is discharged to two maturation ponds
3. Two maturation ponds: the maturation ponds dimension 110 x 57m. The effluent treat further by chlorine contact basin prior to discharge.

Further details are summarised on Table 6.4.

It was, unfortunately, not possible to measure the sludge depth from Madaba WSP due to the lack of a platform or a boat; so sludge samples from anaerobic, facultative and maturation ponds were taken from the outlet edge site of each pond at several points and gently mixed. Sludge samples for most locations were analysed for heavy metals, total solids (TS), total volatile solids (TVS), pH, moisture, total coliforms, total faecal coliforms, faecal streptococci and presumptive *Salmonella* counts, total helminth egg counts.

TABLE 6.4. Effective pond sizes and detention time in Madaba WSP's

Pond	Total depth (m)	Effective depth (m)	Two Trains		
			Area (ha)	Volume (m^3)	Detention time (days)
Anaerobic	5.0	4.0	0.51	11440	5.72
Facultative	2.8	1.8	2.21	35600	17.8
Maturation	2.3	1.3	1.25	14565	7.3
Total			3.97	61605	30.82

(Source: Design Report (1986). Water Authority-Amman)

6.4 Sampling for Sludge Volumes in the Al-Samra Anaerobic Ponds

The primary and secondary anaerobic ponds, at Al-Samra, have not been emptied of sludge since they were commissioned in May 1985. For Al-Samra WSP there is an urgent need for desludging the anaerobic ponds due to the build up of sludge. First there is a need to measure the quantity of sludge, to characterise it and select a suitable and economical method to reduce pathogens.

The sludge layers in the six anaerobic ponds in Al-Samra were measured for thickness to determine sludge distribution, volume, the physico-chemical and biological characteristic of sludge. SURFER programme had been used for drawing sludge contours and accumulation at the bottom of the ponds.

To determine suitable sampling points, as well as to map the accumulated sludge depth, a grid pattern was established by marking the lagoon berm at regular intervals. Samples were collected from a boat that was positioned at the appropriate points between opposite berm markings. Table 6.5 and 6.6 shows the sampling point locations and dimensions of the grids. As much uniformity as possible was exercised in collecting, handling, and storing the samples.

A program of depth soundings was carried out to determine the volume of sludge in the six anaerobic ponds. Three different instruments were tested for the purpose of determining the depth to which sludge had accumulated in these ponds, including:

1. Sludge Judge: a graduated tube with a check valve at its lower end, is designed for sampling light to moderately compacted sludges; manufactured by NASCO, Inc., of Ft. Atkinson, WI (USA). However, the sludge in the anaerobic ponds proved to be well-compacted and contained a high amount of hair, fibre, vegetable and fruit seeds and other debris, which frequently prevented the check valve from admitting an accurate sludge sample or closing upon withdrawal. This device was not used beyond the equipment testing stage.

2. Photometric Probe: used to measure light transmissivity ("Series 22 Sludge Blanket Detector", pHOX Systems Ltd, Eaton Socon UK); the instrument is composed of a meter and detector, works by signalling zero light transmissivity as the probe enters the compacted sludge layer. The probe was found to be accurate in determining the depth to the top of the sludge layer, although it proved difficult to use.

3. A small, perforated metal plate assembly: fabricated from slotted 5-cm steel angle stock and measured 30cm × 35cm. This device was suspended in a horizontal orientation from a graduated rope and bridle arrangement. The weight of the metal plate, in combination with the slotted perforations, helped it to quickly sink to the level of the accumulated sludge, while its surface area allowed it to be supported by the sludge rather than sink into it.

The photometric probe and the plate assembly gave essentially identical sludge depth results. The Sludge Judge, when successfully operated after repeated attempts, confirmed the results of the plate assembly system. The plate assembly system was ultimately chosen for use because it required no calibration, put no expensive equipment at risk, and was quicker and easier to use.

Depth sampling was performed at nodes on a pre-established grid (Tables 6.5 and 6.6). Grid tick marks were spray-painted at intervals along the four shores of each pond to help in sighting each node. Depth sampling was performed from a rowing boat provided by Water Authority of Jordan.

However, due to the heavy layers of scum and high level of sludge depth in some places on the first set of anaerobic ponds (A1-1, A2-1, and A3-1) through which it was impractical to row (Fig. 6.4 and 6.5), ropes were tied to the bow and stern of the boat so that on-shore crews (or a vehicle) could help in hauling it to each sampling location. This made sampling of scum-covered areas more feasible than would otherwise have been possible through conventional rowing; even so, not all areas of ponds A2-1 and A3-1 could be covered without waiting several days for a change in wind direction to push the floating scum masses away from areas not reachable earlier. The second row of anaerobic ponds, A1-2, A2-2 and A3-2, had no accumulated scum.

FIG. 6.4. Photograph showing the sludge sampler and the rowing boat during sludge sampling at primary anaerobic ponds in the Al-Samra system

FIG. 6.5. Part of primary anaerobic ponds of Al-Samra WSP's system, showing the heavy floating scum on the surface of the pond

6.5 Sampling Procedure for Sludge Characterisation from Al-Samra Anaerobic Ponds

Sludge samples were taken at least from six points in each of the six anaerobic ponds. These samples were taken at the top of the accumulated sludge layer using a clam-style grab sampler (Van Veen Grab Sampler, Duncan & Associates, UK). The Van Veer Grab Sampler is a simple sampling device which does not require any messenger which closes when it hits against a solid object (Fig. 6.4) (details in Appendix 6.1).

In addition, a seventh sample was taken from the bottom of the sludge layer in each pond. These samples were obtained with 75 mm diameter, 6m long stiff plastic pipe using the pipetting technique: the pipe, with both ends open, was driven vertically downward through the water and the underlying sludge until the bottom of the pond was reached. Sludge would flow into the pipe in a uniform manner as the pipe was driven through the sludge deposit. The sludge at the bottom of the pipe, therefore, was assumed to be representative of the sludge at the bottom of the pond. The top of the pipe would then be closed with a screw cap, and the pipe pulled out of the sludge deposit and the pond.

In all but one pond (A1-2), the sludge was sufficiently thick and viscous that it remained in the lower, open end of the pipe until a sample of sludge could be removed to a sample bottle. In pond A1-2, however, the sludge was insufficiently viscous to stay in the pipe as the pipe was withdrawn from the pond.

Sludge samples were collected in the winter 1992-1993. A total of forty three samples of sludge have been taken from the six anaerobic ponds from different points, the sample points are shown in Tables 6.5 and 6.6.

The following analyses were performed on sludge samples from all six anaerobic ponds: total solids (TS), total volatile solids (TVS), pH, oxidation-reduction potential (only for two samples due to the difficulty to measuring in the ponds), total coliform (TC), total faecal coliform (FC), faecal streptococci (FS), presumptive *Salmonella* spp. counts (SC); counts, type of helminth eggs and stage of development of *Ascaris* eggs, and finally the heavy metal concentrations.

Only for Al-Samra anaerobic ponds wastewater and sludge samples, heavy metal analyses were determined by Atomic Absorption Spectrometer (PU9400X, Philips) at the Royal Scientific Society, through Camp Dresser & Mckee International, Inc. (CDM) USA, in association with Sigma Consulting Engineers, Jordan.

TABLE 6.5. Sludge sampling points in first set of Al-Samra anaerobic ponds

INFLOW											
A1-1				A2-1				A3-1			
				*HM							*HM
			*								
	*	*			*HM	*HM			*HM	*HM	
*HM											
		*							*HM	*HM	
*		D/HM			*	*D/HM			D/HM		
							*	*			
OUTFLOW											

TABLE 6.6. Sludge sampling points in second set of Al-Samra anaerobic ponds

INFLOW											
A1-2				A2-2				A3-2			
				*							*
	*		*		*HM	*			*	*HM	
					D/HM						
*		*HM			*	*			*	*	
								D/HM			
	*		*				*	*			
OUTFLOW											

Notes, for tables 6.5 and 6.6

1. * Positions in grid are the sampling points for physical and biological analysis in the pond.
2. "D" denotes a deep sample; all others are from the top of the sludge deposit.
3. HM referred to the surface sludge sampling points for metals analysis.

6.6 Experimental Procedures

6.6.1 Sample collection

Sludge samples for bacterial and parasitological enumeration were aseptically collected in sterile 200 ml screw-capped bottles. For chemical analysis, sludge samples were collected in polyethylene bottles. All samples were packed in an ice-box during transportation to the laboratory. Bacteriological analysis were carried out within 2-4 hours of sampling.

6.6.2 Analysis of physical, chemical and biological characteristics of sludge

Sludge samples for most locations were analysed for heavy metals, total solids (TS), total volatile solids (TVS), temperature, oxidation-reduction potential, pH, moisture, total coliforms, total faecal coliforms, faecal streptococci and presumptive *Salmonella* counts, total helminth egg counts and viability tests. Recommended analytical methods are shown in Table 6.7.

6.6.2.1 Physical and chemical analysis

(i) Total solids (TS) and Total volatile solids (TVS) analysis

Total solids, Volatile solids, moisture were analysed according to Standard Methods of APHA, (1989).

(ii) Temperature and oxidation-reduction potential (ORP)

Sludge samples temperature and ORP were scored directly in the field. For ORP measurements a Redox Potential Tester was used, between range -900 to +900 mV, resolution 1 mV with operating temperature range from 0 to +50°C, manufactured by Hanna Instruments, Italy.

(iii) Hydrogen ion concentration

The pH values of the sludge samples were measured in the lab directly within 2-4 hours of sampling, using a Corning pH meter, M115 UK. For dry sludge samples the following pH procedure was followed according to (HMSO, 1992): weigh 10 ± 0.1g of the air dried

sample, crush to pass a 2mm sieve; add 25 ± 1ml of water, shake for 15 min, then measure the pH value by the pH meter.

TABLE 6.7. Methods of analysis of sludge samples that have been carried out in this study

Test	Unit	Method no.	Test method
Chemical			
Total Solids	mg/l	2540-G	Gravimetric
Metals	mg/l mg/kg	3110	Atomic Absorption
Physical			
ORP	mV	-	-
pH	SU	4500-A	pH-Electrode
Temperature	°C	2550	Mercury Thermometer
Biological			
Total coliform counts (TC)	MPN/100ml MPN/100g	9221-B	Multiple Tubes
Faecal coliform counts (FC)	MPN/100ml MPN/100g	9221-C	Multiple Tubes
Faecal streptococci	CFU/ml CFU/g	9230, 9215-C (Dudley *et al.*, 1980)	Spread Plate Method (Dudley *et al.*, 1980)
Salmonella spp.	CFU/ml CFU/g	9260	Spread Plate Method (Dudley *et al.*, 1980)
Intestinal nematode eggs			
Sludge (quantity)	Eggs/g	Appendix 6.2	Flotation Sedimentation Method
Sludge (viability)	Eggs/g	Appendix 6.3	

ORP = Oxidation-Reduction Potential
(Standard Methods for the Examination of Water and Wastewater, 1989, 17th ed.)

6.6.2.2 Analysis of metals in sludge

Grab samples of wastewater and sludge from the Al-Samra WSP's and only sludge samples from Jerash and Madaba treatment plant, were analysed at the RSS and at Water Authoriy laboratories. The atomic absorption technique was used for analysis. Grab sludge samples from the sites shown in Tables 6.5 and 6.6 within each anaerobic pond in Al-Samra were collected and analysed for the following metals: aluminium (Al), arsenic (As), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silicon (Si), silver (Ag), tin (Sn), titanium (Ti), vanadium (V) and zinc (Zn).

For Madaba and Jerash treatment plant sludge samples only the following metals were analysed: copper (Cu), chromium (Cr), cadmium (Cd), iron (Fe), manganese (Mn), and zinc (Zn) using Atomic Absorption Spectrometer (SpectrAA-30/40 and SpectrAA-300/400 Systems manufactured by Varian Techtron Pty. Limited, at WAJ laboratories). All analyses were performed in accordance with standard procedures (APHA, 1989). Conversion factors based on concentrations of sludge dry solids were used to report concentrations on a dry weight basis.

6.6.2.3 Bacteriological analysis

Counting faecal streptococci and *Salmonella* spp. in sludge samples were estimated by the spread plate method. The sample was mixed for 3-5 minutes. A 0.1ml aliquot of sample and/or sample dilution was transferred to duplicate pre-dried agar plates. This was spread evenly on the surface using a sterile glass spreader.

For detection of faecal streptococcus Slantez and Bartley medium (Oxoid) used for sludge samples; the petri dishes were incubated at 37°C for 4 hours then transfered to 44°C for 44 hours. Red or maroon colonies were counted (Slantez & Bartley, 1957). For *Salmonella* counts, Bismuth Sulphite agar (modified by Oxoid) and XLD agar (Oxoid) was used. Incubation was at 35°C for 40- 48 hours, then enumeration of black metallic

sheen and red black centre colonies, respectively. The results are expressed as $\log_{10}$ colony forming unit per gram total solids (dry weight basis).

6.6.2.4 Parasitological analysis

The methods adopted for use in the parasitological examination of sludges from municipal plants selected for study in this research are given in Appendix 6.2 and 6.3. For enumeration the method of Satchwell (1986) and for viability and enumeration of *Ascaris* eggs the method of Meyer *et al.* (1978) was used.

The efficiency of Meyer *et al.* (1978) method was estimated by examining sludges which had known numbers (around 10 eggs/ml of wet sludge, with 14 replicates) of *Ascaris* eggs added to sludge prior the examination. The technique recovered 15% to 80% (average 43%) of the *Ascaris* eggs expected to be present in the samples. However, the *Ascaris* eggs added to these test samples were eggs that had been obtained from the uteri of worms *Ascaris suum* that were recovered from the intestines of naturally infected pigs.

The standard deviations for the recovery and viability of the test eggs points to the need for further investigation on improving the analytical efficiencies. Small physical changes in sludge characteristics, pH, particle charges, etc. may play a significant role in affecting the efficiency of recovery of parasite eggs from sludges. Eggs were identified on the basis of morphology, size, and stage of cell development.

6.7 Sampling Procedure for Pathogen Accumulation and Distribution in the Sludge of A1-1 pond

The pond A1-1 was operated at the beginning of March 1987, 21.5 months later than the operation of pond A2-1, A3-1. The raw wastewater is discharged into the pond at 35.5m from the waters edge measured along the longitudinal direction. The point of raw wastewater discharge is at the middle of the pond's width.

For sludge sampling from A1-1 anaerobic pond in Al-Samra WSP's, was divided the pond into 5 sections crosswise (A-E) and 3 lengthwise (1-3) sections, resulting in fifteen plots

70m x 27m plots (Fig. 6.6). In each plot triplicate sludge sample taken using a Van Veer Grab Sampler.

A homogeneous sludge sample was analysed for water content, temperature, ORP, and solids (total and volatile), using the methods described in APHA (1989). Total coliform, total faecal coliform count, faecal streptococci and presumptive *Salmonella* spp. counts were detected (Table 6.7). Human parasitic nematode eggs were enumerated from each plot using the method of Meyer *et al.* (1978) as described in Appendix 6.3. When the eggs were counted their stage of development was recorded. The percentage of eggs at each stage of embryonation in each plot was then calculated. After enumeration, the recovered eggs were washed twice in tap water and incubated in 0.1 N H_2SO_4 at 30°C for 28 days. Eggs which were fully embryonated after 28 days were considered viable. Under optimum conditions all viable eggs will have fully embryonated after 21-28 days (Fairbairn, 1961). Eggs per gram dry weight were calculated for each plot using the mean percentage of water content.

Analysis of variance was used to test the differences between the number of eggs and logarithmic bacterial counts per g total solids (dry weight basis) of sludge throughout the pond. The distribution pattern was plotted graphically to determine the regions of maximum egg and bacterial cells sedimentation in relation to the pond inlet and outlet. Differences in the percentage viability of *A. lumbricoides* eggs along the pond were determined by Generalised Linear Interactive Modelling (Glim) program, because number of eggs counts were different in each sludge sample. All analyses of data expressed as percentages values were carried out on Generalised Linear Interactive Modelling (Glim) program (Healy, 1988).

The geometric mean is actually the preferred method of recording central tendency since it minimizes the effect of inordinately high or low values, e.g. those frequently reported using MPN procedures.

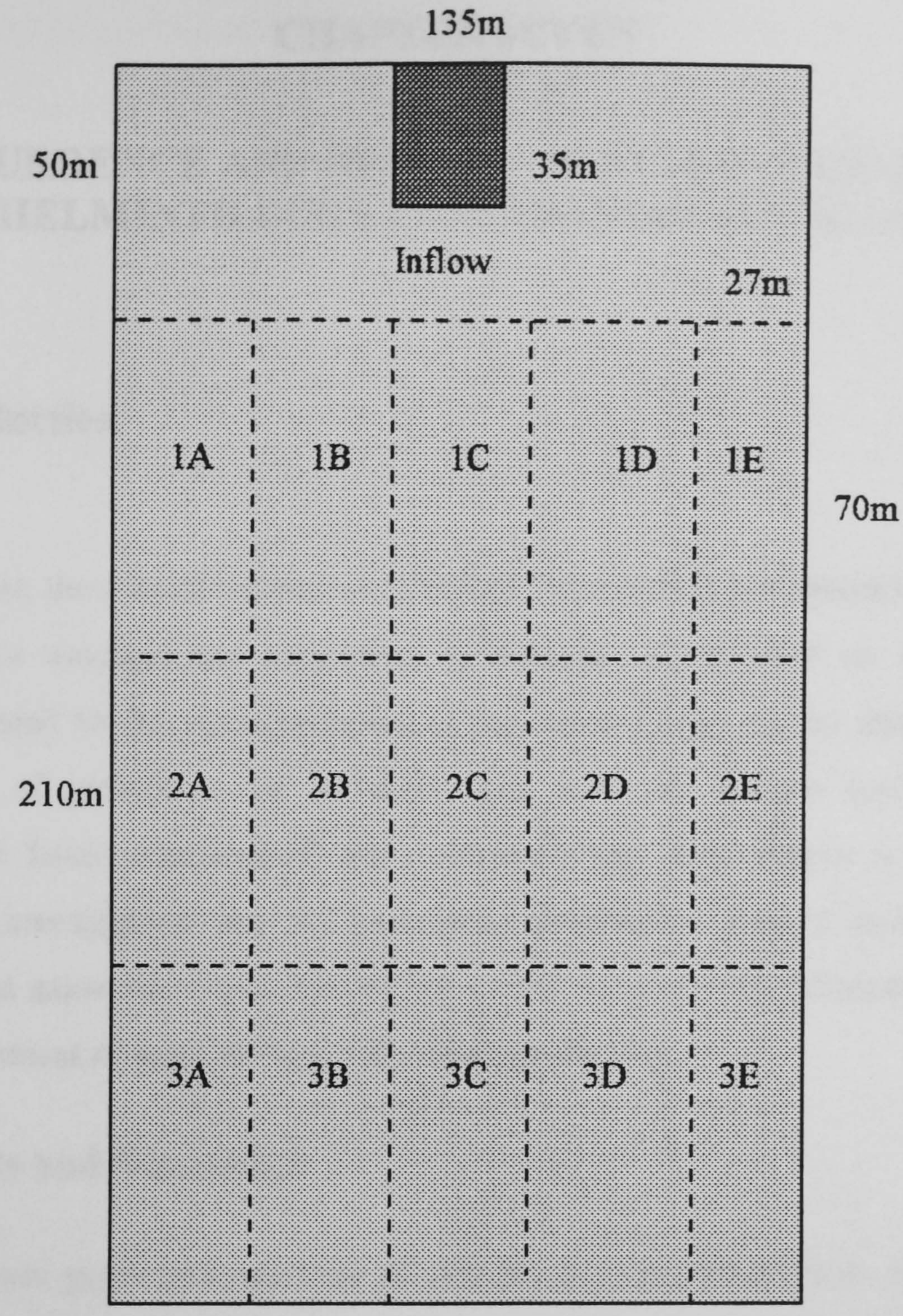


FIG. 6.6. Scheme for sludge sampling in pond A1-1

6.8 Meteorological Measurements

The Water Authority of Jordan has installed a complete meteorological station on Al-Samra wastewater treatment plant site, which is located at the treatment plant site at 32° 12'N Latitude and 560 m above sea level. The main measured parameters are air temperature, sunshine hour and solar radiation, and rainfall and evaporation.

CHAPTER SEVEN

OCCURRENCE AND DENSITY OF HUMAN PARASITIC HELMINTH EGGS IN JORDANIAN SLUDGES

7.1.1 Introduction

In chapter three, the parasitic intestinal nematode infestation is considered to be relatively low prevalence amongst the population in Amman. There were no data recorded previously related to the characterisation of Jordanian sludge on the identification and quantification of pathogens, i.e. helminth eggs, indicator bacteria (coliforms, faecal coliforms, and faecal streptococci) and *Salmonella* spp. This chapter is based on the results of an investigation into the pathogenic potential of sewage sludge in Jordan, especially from anaerobic waste stabilisation ponds, to give some information about the necessary treatment of pond sludges before land application.

7.1.2 Results and Discussion

Table 7.1 shows genus and numbers of parasite eggs recovered from the 3 types of wastewater sludge tested. Nematode eggs include *Ascaris lumbricoides*, *Trichuris trichiura*, and *Enterobius* spp.; the Cestodes include *Taenia saginata*, and *Hymenolepis nana*. Eggs of *Taenia saginata* and *Enterobius* were rarely found, and the eggs of hookworm were not detected at all.

The predominance of Nematode eggs was marked in comparison with Cestodes; from 0-787 nematode eggs, while only 0-166 cestode eggs were found per g dry weight basis in sludge samples from the Al-Samra anaerobic ponds. Generally, the average total helminth eggs counts were found to be highest in Jerash sludges (313 eggs/g), followed by Al-Samra sludges (303 eggs/g), with the lowest counts observed at Madaba WSP (64 eggs/g); all expressed on dry weight basis (Table 7.1).

TABLE 7.1. Mean and range of parasite eggs counts found in different wastewater treatment plants sludges

Plant	Total helminth eggs	Nematodes			Cestodes	
Mean (Range)		*Ascaris lumbricoides*	*Enterobius* spp.	*Trichuris* spp.	*Hymenolepis nana*	*Taenia saginata*
Al-Samra	**303** (45-1015)	262 (25-787)	0	5 (0-25)	27 (0-166)	9 (0-62)
Madaba	**64** (0-150)	52 (0-117)	0	0	12 (0-46)	0
Jerash	**313** (288-619)	157 (38-357)	3 (0-27)	1 (0-13)	147 (125-208)	5 (0-35)

Unit = eggs/g dry weight basis

Sludge samples from Al-Samra anaerobic ponds were found to have helminth eggs counts varying from 5.0 to 131 eggs per ml of wet sludge, or from 45 to approximately 1015 eggs per g of dry solids. Keller and Hide (1951) studied the incidence of *Ascaris* eggs in sludge samples in the Johannesburg area. Their counts of *Ascaris* eggs varied from
104 eggs/ml to 403 eggs per ml of the liquid sludge, or from 5745 to 27000 eggs per g of dry solids, which is much higher than counts recorded in this study.

In Al-Samra anaerobic ponds sludges, out of 21 samples, 100% were positive for nematode eggs (*Ascaris lumbricoides* eggs were found in all samples, and *Trichuris trichiura* eggs were found in 29% of samples). Overall, nematode eggs were present in the highest numbers: in Al-Samra anaerobic ponds the counts ranged from 0 - 787 eggs/g dry weight. Cestodes eggs were found in much lower numbers 0 - 166 eggs/g dry weight. *Hymenolepis* made up average of 75% of the cestode eggs identified.

The incidence of *Ascaris* eggs in sewage sludges at the three Jordanian sewage treatment plant has been investigated. The results indicate that the low infestation of the Jordanian

population with intestinal nematodes (*A. lumbricoides*) is responsible for the low incidence of intestinal nematodes (*Ascaris* eggs) in sewage sludge.

On the other hand, of the helminth species reported from sludge samples, *Ascaris lumbricoides* were the most dominant. This is attributed to several reasons. The high reproductive potential of *Ascaris lumbricoides* (200,000 eggs/day from each female), probably contributed to the recovery of a considerable number of eggs in Al-Samra sludge samples (up to 1015eggs/g dry weight); the prolonged accumulation of eggs in the pond itself; the presence of a shell around *Ascaris* eggs giving it high resistance to the anaerobic environment compared with other type of eggs.

Schwartzbrod *et al.* (1987) found that the highest egg counts were in the first pond rather than the second pond at Marrakech treatment plant, this is in agreement with Madaba results (total helminth counts in anaerobic ponds = 128 eggs/g dry weight, 62 eggs/g dry weight in facultative pond, and no eggs were found in the maturation pond). While this is differs from Al-Samra helminth eggs (except *Trichuris* eggs), where twice the number of helminth eggs were found in the secondary than in the primary anaerobic ponds (Table 7.2).

The higher counts of eggs in the secondary anaerobic ponds at Al-Samra might be related to the following reasons: (1) as a result of hydraulic short circuiting, known to have occurred in the pond (Saqqar & Pescod, 1993); (2) overloading and high sludge depth in the primary anaerobic ponds, especially train 2, (more detail in the chapter nine). (3) resuspension of the eggs due to gas production, which caused a considerable amount of sludge mass to float to the surface. (4) mixing due to the wind might only occur in the Al-Samra anaerobic ponds but no mixing due to thermal stratification; from the literature, no thermal stratification was found to develop within ponds < than 5m (Moreno, 1983; adapted from Llorens *et al.*, 1992); Llorens *et al.* (1992) confirmed this phenomenon in deep ponds (> 5m) only. (5) Schwartzbrod *et al.* (1987b) did not find continuous accumulation of helminth eggs in stabilisation pond sediments with time, and her results may support the idea that the low counts of eggs in primary anaerobic ponds at Al-Samra may be due to highly anaerobic conditions prevailing in the primary anaerobic ponds sludge, which accelerates the damage of parasitic eggs, and reduces the accumulation of

eggs with time. (6) the adhesive properties of the shell around *Ascaris* eggs makes them very sticky, so they easily pass with particles during short circuiting to the secondary anaerobic ponds. In contrast, 89% of *Trichuris* eggs settled in the primary anaerobic ponds, and the rest continued to settle progressively in the secondary anaerobic ponds.

Newton *et al.* (1949) found from their experiments with primary sedimentation that *Taenia saginata* eggs are, under certain conditions, likely to escape with primary effluent. Maybe this can explain why greater number of *Taenia* eggs have been recorded most of the time from secondary anaerobic ponds. *Taenia* eggs have low theoretical settling velocity at 20°C in water (Appendix 7.1), which is one of the reasons why Cestode eggs settle in the secondary and not directly in the primary anaerobic ponds.

Table 7.1 shows that *Enterobius* eggs were only found in Jerash treatment plant but not in the WSP sludges. This is might be explained by the fact that *Enterobius* eggs are not robust, and probably cannot stand conditions in anaerobic ponds, so they are easily destroyed in WSP. Little information is available in the literature on behaviour of *Enterobius* eggs during sewage treatment. *Enterobius* eggs are normally laid on the perianal skin and not usually passed in the faeces, but they have been found in Barcelona, Germany, India, and USSR sewage (Feachem *et al.*, 1983; Schwartzbrod, 1989), and these eggs probably came from egg-filled female worms, which are often passed in the faeces.

Of the two different treatments investigated here, WSP's were the most efficient in destroying and inhibiting the development of the cell inside the eggs in the sludge layer, compared with oxidation ditch treatment. Eggs from Jerash treatment plant sludge samples have been found developed to different cell stages, which is evidence that the viability of the embryo inside the eggs has not been impaired by the sewage treatment processes. Apparently the Jerash sewage treatment plant had favourable conditions for the eggs to develop, in Jerash sludge samples, 63% of the *Ascaris* eggs were in the multi-cell and larval stage, and only 12 % of the eggs were in the one-cell stage, and they look viable by microscopic observations.

TABLE 7.2. Counts of helminth eggs in each anaerobic pond sludge samples at Al-Samra system (dry weight basis)

Type of helminth	Primary anaerobic ponds				Secondary anaerobic ponds			
Mean (eggs/g) (Range)	A1-1	A2-1	A3-1	Grand mean	A1-2	A2-2	A3-2	Grand mean
Total helminth	252 (174-310)	124 (45-200)	235 (51-479)	204 (45-479)	289 (121-583)	669 (202-1015)	243 (88-468)	400 (88-1015)
Ascaris lumbricoides	202 (174-310)	113 (39-200)	195 (25-419)	170 (25-419)	285 (112-583)	579 (124-787)	194 (53-358)	352 (53-787)
Trichuris trichiura	3 (0-11)	7 (0-20)	16 (11-25)	9 (0-25)	0	0	4 (0-17)	1 (0-17)
Hymenolepis nana	29 (0-92)	4 (0-6)	25 (0-48)	19 (0-92)	2 (0-5)	67 (0-166)	36 (0-92)	35 (0-166)
Taenia saginata	18 (0-61)	0	0	6 (0-61)	2 (0-5)	23 (0-62)	8 (0-30)	11 (0-62)

Jerash had higher range values than Madaba sludge samples for all helminth eggs species that were reported. From the results and observations of this study, it is clear that only robust eggs can be detected and withstand the harsh conditions which prevail in the anaerobic pond sludges of waste stabilisation systems; this is also confirmed by the inhibition of cell development of *Ascaris* eggs, only one-cell stage eggs had been observed in the sludge of anaerobic ponds.

Thus it appears that storage of sludge in anaerobic ponds for a period of time can be an effective method for destruction of parasite eggs in warm climates. 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 as found by Lee and Atkinson (1976). This is confirmed by Cram (1943), who tested the viability of *A. lumbricoides* eggs during anaerobic sludge digestion at 20-30°C. The results showed that no development occurred in the sludge and that for the first 3 months the viability was not affected.

Reyes *et al.* (1963) found that the dieaway curve for *Ascaris* eggs at 30°C in anaerobic digestion seems to demonstrate an increase in destruction rate of *Ascaris* eggs after the first week of digestion, so that "oxygen starvation" appears to be one, if not the only, lethal factor. Still there is a gap in knowledge on the effect of gases such as H_2S, NH_3, CO_2, etc. on the viability of *Ascaris* eggs; research needs to be done on this in the future.

Some of the *Ascaris* eggs that were isolated from Al-Samra anaerobic ponds showed a blackened appearance, which make it impossible to evaluate the internal cell stage. It must be assumed that this pigmentation was expressive of a degenerative process leading to the relatively imminent destruction of the egg. The observations from Jerash treatment plant, confirmed that there was no noticeable effect of the process upon the *Ascaris* eggs. None of the examinations revealed any evidence of blackening or any other alteration of the eggs from the normal appearance. The same findings had been recorded by Newton *et al.* (1949) but on artificial seeding of *Taenia* eggs during lab scale anaerobic digestion, and activated sludge experiments.

Most of the eggs found in the sludge were from the families Nematode and Cestode, never Trematode eggs.The range of helminth eggs were from 45-1015 eggs/g dry weight in the anaerobic ponds at Al-Samra, at Jerash the range was from 288-619 eggs/g dry weight, and ranged from 0-150 in Madaba WSP sludges.

Three genera of Nematoda important for public health risk evaluation were identified: *Ascaris*, *Trichuris*, and *Enterobius*. Two Cestoda genera were identified: *Taenia* and *Hymenolepis*. In most European countries (Owen & Crewe, 1982; Schwartzbrod *et al.*, 1983, 1989; Ayres *et al.*, 1993), as in the USA (Arther *et al.*, 1981; Theis *et al.*, 1978), Iran (Sadighian *et al.*, 1976) nematode eggs predominate and *Ascaris* eggs are most frequently identified, followed by *Trichuris*.

It is difficult to compare the levels of helminth eggs found in domestic sewage sludges in Jordan to those found in sludges in foreign countries due to different procedures used in examining the sludges and the way the results are reported. In most cases, the research data give the number of eggs found per volume of liquid waste (litre, millilitre, gallon, etc.) or wet weight (g, Kg, etc.) and do not give the dry weight or amount of solids in the samples. However, it appears that the levels of helminth eggs in Jordan sludges are, in general, intermediate values compared with those that have been reported in literature from other countries.

A comparison of values reported in the literature shows that the number of helminth eggs recovered from sludge varies greatly, from 10 (Kabrick & Jewell, 1982), to 1,440 eggs /100g wet matter (Reimers *et al.*, 1981) with intermediate values of 160-340 eggs/100g wet weight (Schwartzbrod *et al.*, 1989), and 460 (Arther *et al.*, 1981). Sadighian *et al.* (1976), reported finding 14,000 to 25,000 *Ascaris* eggs per gram of processed sludge in the sewage treatment facilities in Isfahan, Iran. In municipal wastes works receiving abattoir wastes of Southern USA, Reimers *et al.*, (1981) found an average of 81,800 *Ascaris* eggs/kg dry weight of sludge. Most studies report the same species recovered from sludge as found in this study, namely *Ascaris*, *Enterobius*, *Trichuris*, *Toxocara*, *Taenia* and *Hymenolepis* (Collomb *et al.*, 1983; Fitzgerald, 1977; Schwartzbrod *et al.*, 1989, 1987a, b).

Arther (1979) examined sludge from lagoons in the Chicago Sanitary District in Illinois. He recovered an average of 203 *Ascaris*, 173 *Toxocara*, 36 *Trichuris*, and 48 *Toxascaris leonina* eggs per 100g dry weight. When these eggs were cultured, the percentage of eggs developing to the infective stage was 24% for *Ascaris* and 6% for *Trichuris*. Unfortunately, no information was provided on the length of time that the sludge had been stored in a lagoon

Watson *et al.* (1983) studied the prevalence of parasitic helminth eggs and cysts in sewage sludges disposed of to agricultural land in UK. The results showed that the eggs of parasitic helminths are present in nearly all sewage sludges applied to land in Wales. Egg numbers appear to be low, the average mean of *Ascaris* eggs /l of sludge from 7 treatment plant sludges(activated-sludge or biological filter plants) equal 17 (with egg counts ranging from 0-95 eggs/l sludge). For *Taenia* eggs the mean count was 0.86 eggs/l sludge (with egg counts range 0-5eggs/l sludge). For *Trichuris* eggs a mean count of 3.4 eggs per liter of sludge (with egg counts ranging from 0-17eggs/l sludge), was found.

A number of factors may have affected the occurrence and concentrations of parasites reported in this study, including the endemicity of disease within the indigenous animal and human population; the size and socio-economic status of the population; the percentage of the population sewered; type and efficiency of sewage treatment (including the presence of predatory organisms); the volume of effluent sampled; the recovery efficiency of the sampling method; and the skill of the investigator.

Any study conducted under controlled laboratory conditions may not precisely reflect the conditions that actually occur. Laboratory experiments by various workers, using columns of raw sewage, support the view that 2 to 3 hours sedimentation is sufficient for the removal of most helminth eggs (Cram, 1943; Jones *et al.*, 1947; Newton *et al.*, 1949).

Provided sufficient time is allowed and turbulence is minimal, sedimentation gives a high rate of removal of helminth eggs. Liebmann (1964) estimated that with a specific gravity of 1.1, helminth eggs will settle at a rate of 2 to 3 feet per hour in static conditions, and thus for most tanks a sedimentation time of 2 hours should be sufficient. Liebman also

noted that *Taenia saginata* eggs were the slowest to settle; in one experiment, 68% had settled after 2 hours and 89% after 3 hours.

In India, Panicker and Krishnamoorthi (1981) found the removal of *Ascaris* eggs in an oxidation ditch ranged from 95 to 100% with 6.94 hours detention time. Also, they found that the parasitic eggs removal from aerated lagoon ranged from 75 to 95%, rather less than for an oxidation ditch.

7.2 DENSITY OF INDICATOR BACTERIA IN JORDANIAN SLUDGES

7.2.1 Introduction

There is not much reliable data available on the density levels of indicator organisms, pathogenic bacteria, viruses, protozoa, or parasites in primary and secondary sludges. Reimers *et al.* (1981) assesed types and densities of parasites in municipal wastewater sludges in the southern USA; and Pedersen (1981) prepared an extensive literature review on the density of pathogenic organisms in municipal waterwater sludges, which covered the pertinent literature from 1940 to 1980.

Tables 7.3 and 7.4 shows the levels of indicator bacteria and pathogens from different type of sludges that are reported in the literature (Dudley *et. al.*, 1980 and Pederson, 1981); Table 7.5 refers to the bacterial removal rates in different wastewater treatment processes (James, 1987).

To ascertain the present health risks that may be posed by the land application of sewage sludges, a scheme was devised in this study to determine the types and numbers of potentially pathogenic bacteria present in sludges, from anaerobic ponds of the Al-Samra system, Madaba WSP's, and Jerash treatment plant.

TABLE 7.3. Levels of indicator bacteria and pathogens in raw primary, secondary, and mixed sludge (Pedersen, 1981)

Agent	Range of levels reported (number/g dry weight)	Average level reported (number/g dry weight)
Total coliforms	1.1×10^1 - 3.4×10^9	6.4×10^8
Faecal coliforms	ND - 6.8×10^8	9.5×10^6
Faecal streptococci	1.4×10^4 - 4.8×10^8	2.1 - 10^6
Salmonella spp.	ND - 1.7×10^7	7.9×10^2
Shigella spp.	ND	ND
Pseudomonas aeruginosa	1.5×10^1 - 9.4×10^4	5.7×10^3
Parasite eggs or cysts	ND - 1.4×10^3	1.3×10^2

ND = not detected

TABLE 7.4. Enumeration of bacterial indicators in primary raw and lagoon sludges

Agent	Enumeration (CFU /g dry weight)	
Total coliforms	6.1×10^7	1.2×10^8
Faecal coliforms	4.7×10^6	2.0×10^7
Faecal streptococci	4.5×10^5	8.9×10^5
Salmonella spp.	2.0	4.1×10^2
References	Dudley *et al.* (1980)*	Pedersen (1981)

* from lagoon sludge after undergoing anaerobic digestion for 11 days, Texas.

TABLE 7.5. Comparison of bacterial removal rates in different wastewater treatment processes (James, 1987)

Process	Retention time	% Removal	Removal rate
Primary sedimentation + Activated sludge	12 hr	95	20%/hr
Primary sedimentation + percolating filter	7 hr	95	25%/hr
WSP	30 days	99.999	20%/day

7.2.2 Results and Discussion

Tables 7.6, 7.7, 7.8, and 7.9 shows the levels of bacterial indicators (coliforms, faecal coliforms, and faecal streptococci) and pathogens (*Salmonella* spp.) isolated from sludge samples at six anaerobic ponds at the Al-Samra system, Madaba WSP's and Jerash treatment plant, during the period Nov. 1992 to Mar. 1993. The numbers shown in Tables 7.6, 7.7, 7.8, and 7.9 are expressed per gram dry weight sludge solids. The levels of indicator bacteria were found to be in the same range for the Madaba and Al-Samra samples, while a difference can be observed between WSP's and Jerash sludge samples; the results show at least 2 $\log_{10}$ higher numbers of indicator bacteria in Jerash sludge samples. This confirmed that the microbiological quality of WSP sludges is better than that from less conventional sewage treatment plants, i.e. oxidation ditch.

Jerash treatment plant had the highest level of indicator bacteria compared with anaerobic ponds sludge results at Al-Samra. The levels of faecal streptococci were higher in both Madaba and Jerash sludge samples, compared with the levels found in the Al-Samra anaerobic ponds. 36% of sludge samples from Al-Samra anaerobic ponds contained no faecal streptococci, while Madaba and Jerash showed positive faecal streptococci in all sludge samples. The explanation for the detection lower levels of faecal streptocooci in the Al-Samra sludges might be due to the toxicity of H_2S on this indicator. This is supported by Almasi and Pescod (1995) findings, who showed that the effect of influent sulphur (sulphate and sulphide) concentration on *E. coli* removal from lab-scale anoxic ponds was not significant, but it influenced *Streptococcus faecalis* removal positively.

The densities of coliforms and faecal coliforms in the anaerobic ponds of the Al-Samra system show lower counts than the average data in the literature, but within the ranges of the data available in the literature on primary and lagooned sludges (Tables 7.3 and 7.4), while faecal streptococci in sludge samples from anaerobic ponds at Al-Samra had very low counts. In contrast *Salmonella* spp. showed higher mean counts than the densities reported in the literature in all treatment plant sludges that have been tested in this study, but within the range levels reported in Table 7.3. In general, the actual species and density of microorganisms present in the sludge produced from a particular municipality,

especially pathogens, depends to a large extent on the health status of the local community.

Lue-Hing *et al.* (1992) reported that the total coliform densities in primary sludges ranges from 1 x 10^6 to 1.2 x 10^8 organisms per gram dry weight. Two pathogenic bacteria, *Salmonella* spp. and the opportunistic pathogen *Pseudomonas aeruginosa* occur in primary sludges at lower densities than the indicator bacteria. The average *Salmonella* and *Pseudomonas aeruginosa* densities were 4.1 x 10^2 and 2.8 x 10^3 per gram dry weight, respectively.

Anaerobic ponds sludges at Al-Samra ranged in solids from 8 to 219g/kg, their bacterial indicators and pathogens (*Salmonella* spp.) were quantified in each primary and secondary anaerobic ponds as well from the surface and deep layers. The results are shown in Tables 7.6 and 7.7.

Negligible difference in the levels of indicator bacteria were found between primary and secondary anaerobic ponds at Al-Samra system (Table 7.6). Almasi and Pescod (1995) found that *E. coli* removal in anaerobic/anoxic ponds was in the range 1.5-1.9 $\log_{10}$ /1000ml, with only a marginal improvement at a lower anoxic organic loading level (30g $BOD_5/m^3.d$). The removal rates for *Streptococcus faecalis* were approximately the same as for *E. coli*. The mean values of removal rates were 92 and 92.6% for *E. coli* and *S. faecalis*, respectively. The removal rates of *E. coli* and *S. faecalis* were significantly affected by volumetric organic loading and pond temperature.

Many researchers have shown that faecal coliform removal is not as good in primary anaerobic ponds as in later facultative or maturation ponds. Polprasert *et al.* (1983) and Saqqar and Pescod (1991) suggested that the faecal coliforms die-off coefficient decreases with increasing organic loading, although the low slope of a regression line between these parameters allowed the suggestion that other environmental factors might be more important than the surface organic loading. Van der Drift *et al.* (1977) reported that the removal of bacteria from wastewater was a biphasic process. Initially, the organisms are sorbed rapidly to the solids biomass, followed by predation by ciliated protozoa.

The concentrations of TC, FC, and *Salmonella* spp. in sludge samples at Al-Samra anaerobic ponds decreased slightly with depth (Table 7.6), with only 1.2 times higher counts in the surface than in the deep sludge layer. With respect to the faecal streptococci, another pattern is observed, since the concentrations appear 4.2 times higher in the surface than in the deep sludge layer. The effect of stabilisation of the sludge on concentrations of the faecal indicator organisms (except faecal streptococci) is a decrease not exceeding 1.5 $\log_{10}$. It is well established that anaerobic digestion of sludge does not completely remove bacterial pathogens (Dudley *et al.*, 1980). Hess and Breer (1975; cited by Dudley *et al.*, 1980) found *Salmonella* spp. in 90% of the sludges they examined, and that neither aerobic nor anaerobic digestion significantly reduced the counts of *Salmonella*.

TABLE 7.6. Average of the geometric mean counts for bacterial indicators in all anaerobic ponds , primary, secondary, surface and deep layer sludge samples of Al-Samra system, expressed on a dry weight basis

Pathogenic indicator	Unit	All anaerobic ponds	Primary	Secondary	Surface layer	Deep layer
TC	MPN/g	3.0×10^6	6.0×10^6	1.8×10^6	1.4×10^7	1.7×10^6
FC	MPN/g	3.2×10^5	5.6×10^5	2.0×10^5	1.7×10^6	1.7×10^5
FS	CFU/g	4.4×10^1	1.3×10^2	5.6×10^1	1.0×10^2	2.9
Salmonella spp.	CFU/g	1.3×10^4	5.6×10^4	3.5×10^4	9.9×10^4	2.1×10^4

TABLE 7.7. Geometric mean counts for bacterial indicators in sludge samples in each anaerobic pond at Al-Samra system, expressed on a dry weight basis

Pathogenic indicator	TC	FC	FS	*Salmonella* spp
Unit	MPN/g	MPN/g	CFU/g	CFU/g
A1-1	6.3×10^6	4.7×10^5	3.3×10^2	3.7×10^4
A2-1	4.6×10^6	8.0×10^5	2.7×10^1	1.3×10^5
A3-1	7.1×10^6	4.2×10^5	3.6×10^1	2.1×10^3
A1-2	1.1×10^6	2.2×10^5	9.1	1.0×10^5
A2-2	9.8×10^5	1.2×10^5	1.4×10^2	1.6×10^3
A3-2	3.3×10^6	2.5×10^5	1.9×10^1	2.6×10^3

TABLE 7.8. Geometric mean counts for bacterial indicators in sludge samples from Jerash treatment plant, expressed on a dry weight basis (n = 3)

Pathogenic indicator	Unit	Settling tank	Thickener	Drying bed
TC	MPN/g	5.6×10^8	5.6×10^8	2.3×10^8
FC	MPN/g	2.0×10^7	6.3×10^7	3.6×10^7
FS	CFU/g	2.0×10^7	5.5×10^6	2.3×10^6
Salmonella spp.	CFU/g	1.3×10^5	8.6×10^3	4.1×10^3

TABLE 7.9. Geometric mean counts for bacterial indicators in sludge samples Madaba WSP's, expressed on a dry weight basis (n =3)

Pathogenic indicator	Unit	Anaerobic	Facultative	Maturation
TC	MPN/g	2.2×10^8	1.1×10^6	2.7×10^6
FC	MPN/g	3.8×10^7	1.5×10^5	8.3×10^5
FS	CFU/g	2.0×10^5	1.7×10^3	3.4×10^3
Salmonella spp.	CFU/g	6.5×10^6	6.9×10^4	3.2×10^4

Watson (1980) noted that most enteric bacteria survive pH values between 5 and 8, and outside this range they die off rapidly. It is likely that the pH of the sludge from most of the pond samples did not adversely affect the organisms present. Only deep samples from A3-1 had pH values as high as 8.4.

None of the sewage treatment plants studied gave absolutely safe sludges. Sludges from waste stabilisation ponds can be probably considered much better from the point of view of pathogens content. The growing practice of disposal to land of these pathogen-containing materials should be coupled with land use limitations for such sites. Pathogens removed through wastewater treatment should not be reintroduced into a population via new reservoirs that may be established by irresponsible land management of application sites.

Total coliforms, faecal coliforms, *E. coli*, faecal streptococci and amoebae have been demonstrated to survive in marine sediments, reported by Gerba and Mcleod (1976) and O'Malley *et al.* (1982). Indeed, bacterial concentrations were found to be much higher in sediments than in the overlying waters (Hendricks, 1971; Van Donsel & Geldreich, 1971; Goyal *et al.*, 1977, 1979). This is probably due, in part, to the settling of bacteria adsorbed to temporarily suspended sediments (Schillinger & Gannon, 1985).

The possibility that indicator bacteria are capable of multiplying in the aquatic sediments has been suggested numerous times in the literature. This is based upon the general finding that indicator bacterial densities in sediments are 2-3 logs greater than in overlying

waters. However, Marino and Gannon (1991) studied this hypothesis, and their results suggested that indicator bacteria (particularly FC) may indeed be multiplying in aquatic sediments, since the populations maintained themselves at high densities in the presence of constant predation, competition/antagonism effects and without significant external supplementation.

Many researchers have investigated the effects of predation, competition, and antagonism (i.e. biotic factors) on bacterial populations in soils and activated sludges, (Curds *et al.*, 1968; Curds & Fey, 1969; Curds, 1973; Ropper & Marshall, 1978). All these studies indicate that protozoan predation is a major biotic factor limiting bacterial survival in soils and sludges. Bacterial predation/parasitism constitutes a lesser factor, because the bacterial agents are themselves subject to protozoan predation, which limits their predatory activity.

CONCLUSIONS

Wastewater sludge may contain beneficial plant nutrients, and have desirable soil conditioning properties. It may also contain bacteria, viruses, protozoa, parasites, and other microorganisms, some of which can cause disease to humans. Land application of sludges thus creates the potential for human exposure to these organisms.

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 (WPCF, 1989) 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.

In this study the indicator and pathogen analysis (i.e. faecal coliforms and streptococci, *Salmonella* spp., and helminth eggs) of sludges show levels in excess of those considered acceptable for sludge applied in bulk to agricultural land, forest, public contact sites, reclamation sites, lawns, or home gardens (USEPA Class A Regulations). The sludge would therefore have to undergo a process to significantly reduce pathogens (PSRP) before they can safely be applied to agricultural land or used as a soil amendment.

CHAPTER EIGHT

THE DISTRIBUTION AND VIABILITY OF PATHOGENS IN PRIMARY ANAEROBIC WASTE STABILISATION POND SLUDGE

8.1 Introduction

Waste stabilisation ponds are often the preferred method of wastewater treatment, particularly in warm climates and where a high quality effluent is required for agricultural reuse. The removal of human intestinal nematode eggs has been measured in WSP's in different countries, by many researchers (Saqqar & Pescod, 1992; Ayres, 1992; Grimason, 1995). No information is available in the literature on the effect of the anaerobic pond stabilisation process on the viability of pathogens (helminth eggs) in pond sludges, or on the potential bacteriological and helminthological health risk from using anaerobic pond sludges for agricultural purposes. The little information in the literature concern survival in primary facultative pond sludges (Schwartzbrod *et al.* 1987; Carre & Baron 1987; Ayres, 1992).

To understand better the mechanism of nematode and bacterial removal in anaerobic ponds, and to determine the factors which affect the rate at which bacteria and parasite eggs settle in anaerobic ponds. The accumulation, distribution pattern, persistence of bacterial indicators (coliforms and streptococci), pathogens (*Salmonella* spp.), helminth eggs (*Ascaris* eggs) viability and stage of development of eggs in anaerobic pond sediments has been determined. This should give information about the necessary treatment of anaerobic ponds sludges before land application.

8.2 Results

8.2.1 Physico-chemical analyses of A1-1 pond sludge

Sludge pH values in anaerobic A1-1 pond at Al-Samra, were within the range 6.8-7.2 (Table 8.1). The higher pH of the sludge near the inlet (section 1) can be explained by the nature of the influent compounds which accumulate there (the yearly average of the pH inflow value equals 7.0, for the year 1993). The average Al-Samra inflow wastewater temperature was 19.1°C, while the pond sludge ranged from 18-22°C which is ideal for anaerobic decomposition. Iwema *et al.* (1987) found the pond sediment temperature varied from 15°C to 17°C in France. Pescod (1995) stated that sediments accumulate in primary anaerobic ponds as a benthos and decompose anaerobically if the temperature is 15°C or more.

Eh values are observed in the sludge samples throughout the ponds with a lower value detected near the middle of the ponds (-228mV) (Table 8.1). The intensification of the anaerobic conditions can result from the accumulation of reduced compounds. Barnes *et al.* (1991) demonstrated that an ORP of -100mV was sufficient for sulphate-reducing bacteria.

The percent of volatile matter increased in section 2 (the middle of the pond) and decreased near the inlet (section 1) and near the outlet (section 3) showing that the suspended load deposited mostly around the middle section of the pond.

TABLE 8.1. Physico-chemical parameters of sludge from A1-1 anaerobic pond

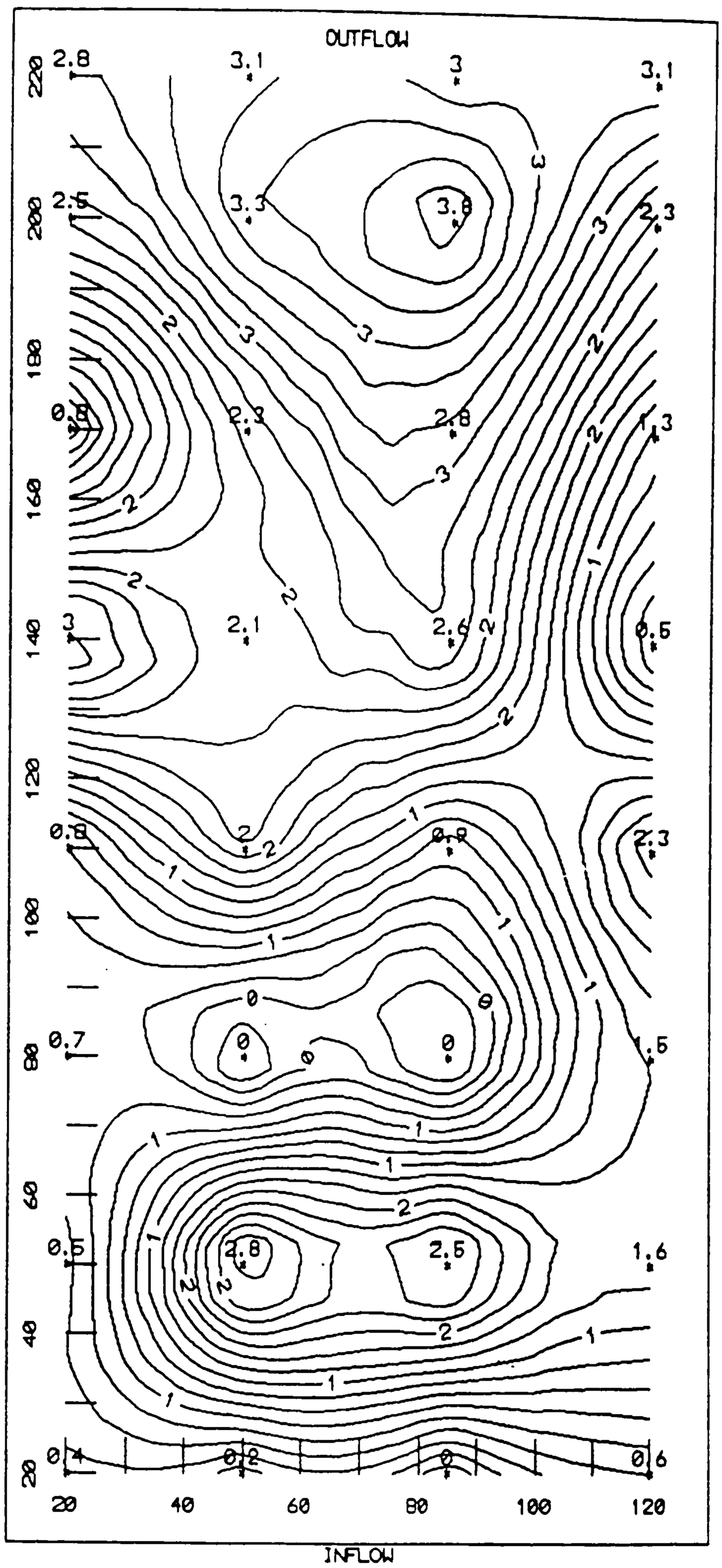
Section	Temperature (°C)	pH	TS (%)	TVS (% of TS)	ORP (mV)
1	20	7.2	8.2	43	-220
2	19	7.1	12	44	-228
3	19	6.8	10	40	-198

Figure 8.1 shows contouring map of the sludge distribution in pond A1-1. The distribution was unexpected and not in agreement with the literature, with low sludge depth near the inlet and increasing from the middle of the pond towards the outlet; the greatest sludge depth was 3.8m recorded near the outlet. This means that the majority of the heavy solids (inorganic matter such as sand and grit), sediment out first in the middle of the pond (section 2), which is explained by the higher proportion of solids (TS% and TVS%), and lower water content. Table 8.1 shows that the middle section had higher average content of total solids (12%) than the inlet (8.2% TS) and outlet (10% TS) sections (Table 8.1).

8.2.2 Enumeration, viability and distribution of helminth eggs in the sludge of A1-1 pond

The total helminth egg counts (*Ascaris lumbricoides*, *Trichuris trichiura*, and *Hymenolepis nana*) per g dry weight of sludge is shown in Table 8.2 with standard deviations.

Enumeration of helminth eggs in the sludge of A1-1 pond showed that 95% of all eggs recovered were *A. lumbricoides* and the remaining 4% were *Trichuris trichiura* and 1% *Hymenolepis nana*, with an average total helminth eggs counts in A1-1 pond were 39 eggs/g dry weight basis. No other species of human parasitic helminth eggs were found by using Meyer *et al.* (1978) technique for enumeration and viability determination of helminth eggs in sewage sludge. The majority of *Ascaris* eggs (84%) were found to be dead (most of them destroyed and had ruptured shells, others coloured yellow-black to coal-black). Of the 16% determined to be viable (cells inside the eggs had the capability to develop if the eggs were incubated at the optimum conditions for development), all *Ascaris* eggs were at the single-cell stage. That means that no development of *Ascaris* eggs had taken place before, during, or after settlement in the A1-1 pond bottom.



Scale 1cm = 20m, contour lines indicate approximate sludge accumulation (m), * = sampling points

FIG. 8.1. Sludge depth distribution over the bottom of A1-1 pond

TABLE 8.2. Total helminth eggs per g dry weight of sludge in A1-1 anaerobic pond, mean and standard deviation, (n = 3)*

Section	A	B	C	D	E	Mean (SD)
1	4	9	0	4	113	26 (48)
2	62	38	25	59	85	54 (23)
3	80	16	25	50	20	38 (27)

* refer to chapter six (section 6.7) for more details about sampling method and diagram.

TABLE 8.3. Analysis of variance of data in Table 8.2*

Source	df	SS	MS	F	F critical	P**
Sections (1-3)	2	1.8	0.91	4.43	4.46	0.05
Sections(A-E)	4	1.1	0.27	1.33	3.84	0.34
Error	8	1.6	0.20			
Total	14	4.53				

* due to the presence of zero eggs counts, all egg counts were changed to $\log_{10}$ (no. of eggs + 1), before applying the ANOVA test.
**Significant at the $\alpha = 0.05$ level

Figure 8.2 and Figure 8.3 shows the distribution of the eggs along the length of the pond in each of the five lengthwise sections. Obviously the trend of the total helminth and *Ascaris* eggs was similar, because 95% of the helminth eggs counts were *Ascaris* eggs. From the average mean of total helminth eggs in Table 8.2 and Figure 8.2, it can be seen that slightly higher egg counts occurred in the middle of the pond, ≈ 155m from the inlet (section 2) than either near the inlet or outlet sections. The settling process occurs in the middle of the pond, it seems there is less disturbance, turbulence and scouring of the sludge. Also the results showed a high positive correlation between the percentage of total solids and the number of eggs detected in the sludge.

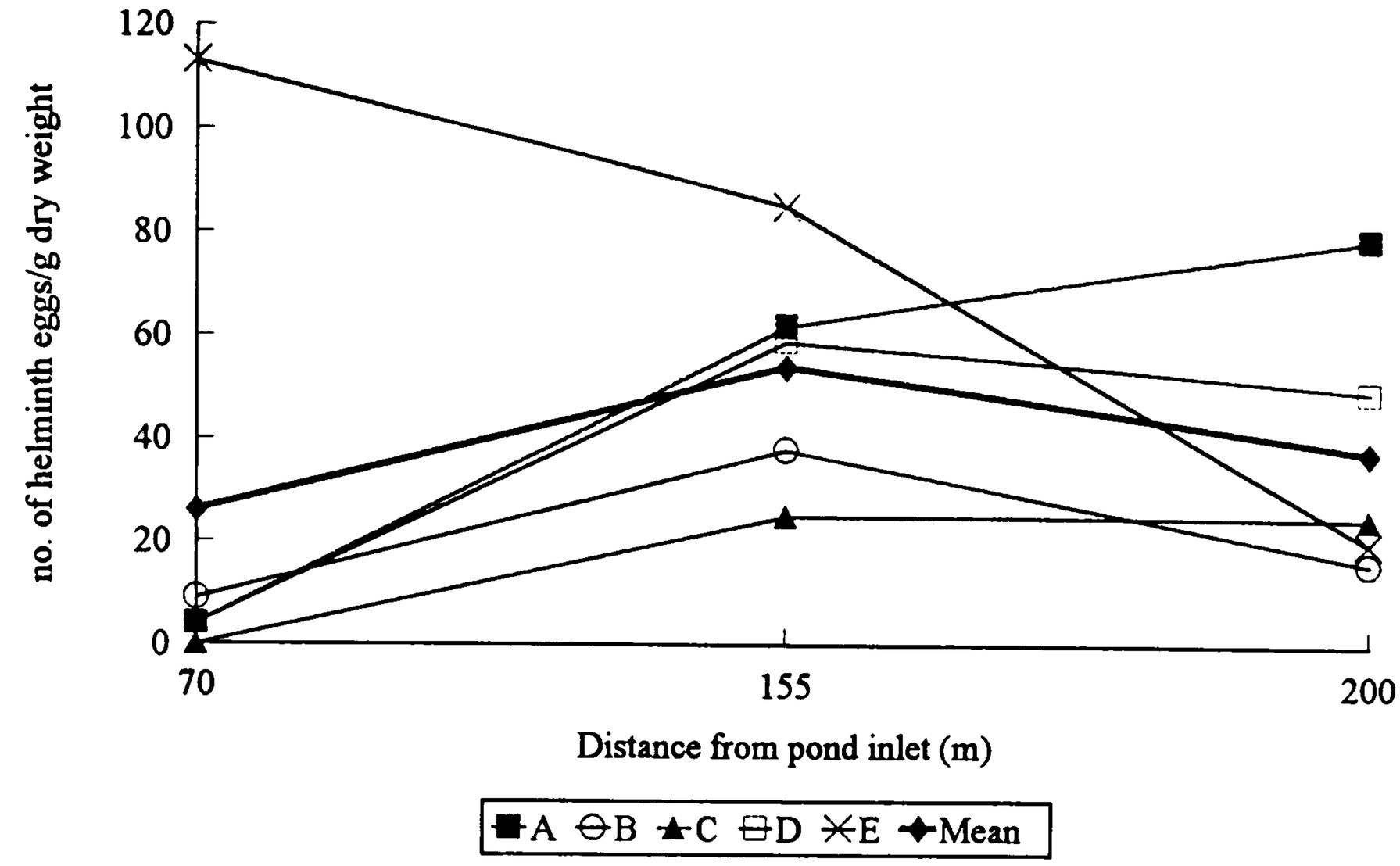


FIG. 8.2. Distribution of helminth eggs in sludge along the length of A1-1 pond in five longitudinal sections (A-E)

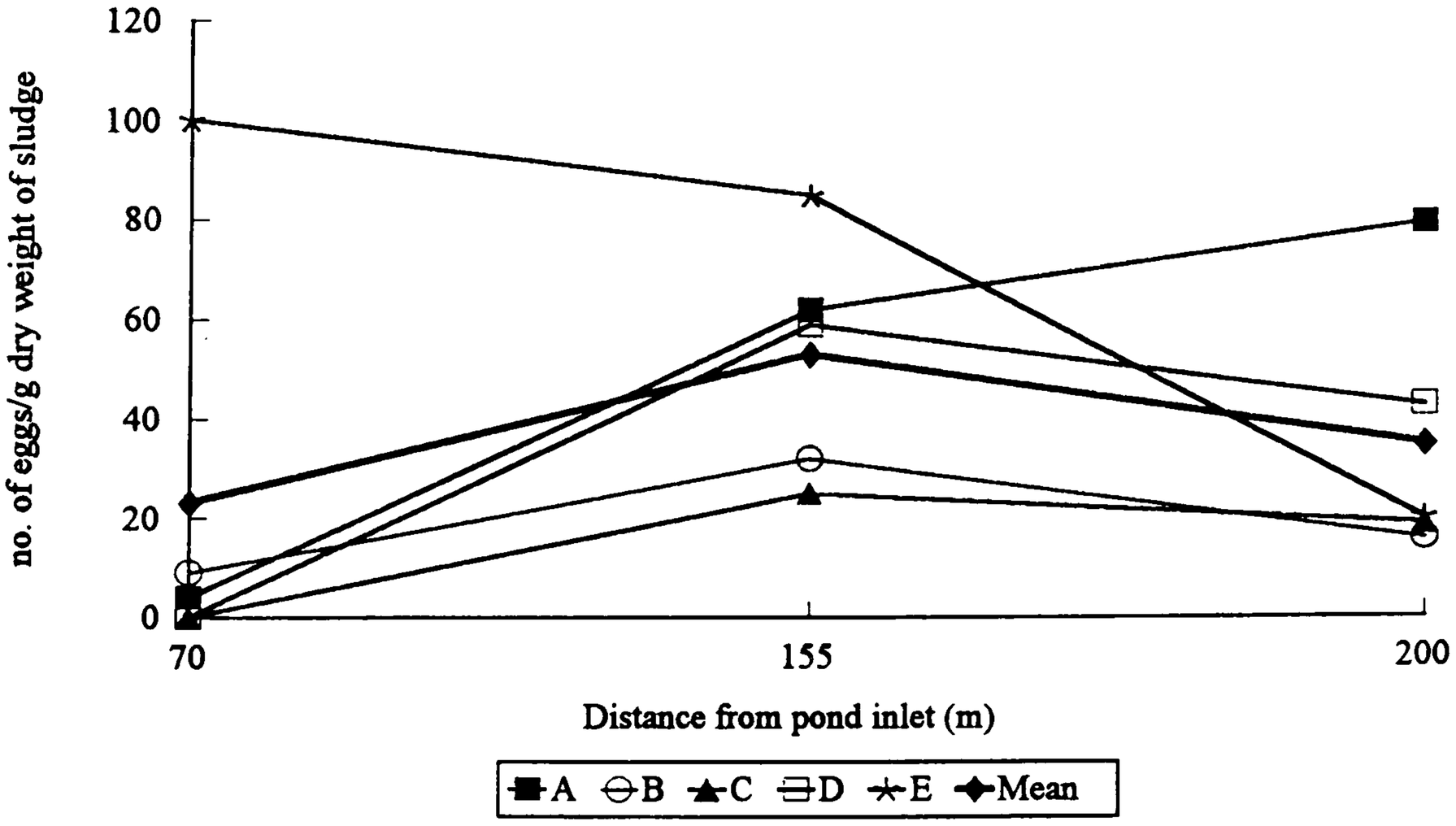


FIG. 8.3. Distribution of *A. lumbricoides* eggs in sludge along the length of A1-1 pond in five longitudinal sections (A-E)

Two way analysis of variance showed similarity in the number of helminth eggs per g dry weight along the length of the pond (sections 1-3), with no difference recorded between the lengthwise sections (A-E) (Table 8.3). This may be attributed to the high strength of wastewater discharge of to the pond, and the fact the pond is overloaded with a maximum sludge depth around 3.8m reducing the efficiency of particle settlement. Also gas production from the anaerobic sediments causes eggs to resuspend and resettle throughout the pond.

Closer examination of the percentage viability of *Ascaris* eggs in sludge along the pond length in Table 8.4 and Figure 8.4, shows that there was a slight decline in the viability of eggs in the middle of the pond. The reason for this may be that the high ORP value and total solids content together with low moisture content, make the eggs and solid particles more compact leading to highly anaerobic conditions which may destroy eggs easily.

The main point of interest is whether the percentage of *Ascaris* egg viability differs along the length (sections 1-3), between the lengthwise sections (A-E), or in the corners of the A1-1 ponds; and if there is good correlation between *Ascaris* egg numbers and viability. The analysis of the data by the GLIM program (Table 8.5) shows, neither the length, nor width of the pond seem to have much effect on the percentage of egg viability in A1-1 pond. Logically, the data analysis by GLIM confirmed that the higher number of eggs detected, the higher is the probability to find viable eggs. Apparently *Ascaris* eggs are mainly accumulated in the corners of the pond. Middlebrooks *et al.* (1965) found that accumulation of sludge in corners is probably due to excessive sludge accumulation around the inlet; then the sludge layer becomes anaerobic and is buoyed up by the gaseous products of anaerobic decomposition, temperature inversion, or other natural phenomena that resuspend the material. These floating masses are then blown into the corners by the wind action. This phenomenon might explain the higher numbers of eggs in the corners of the A1-1 pond in this study.

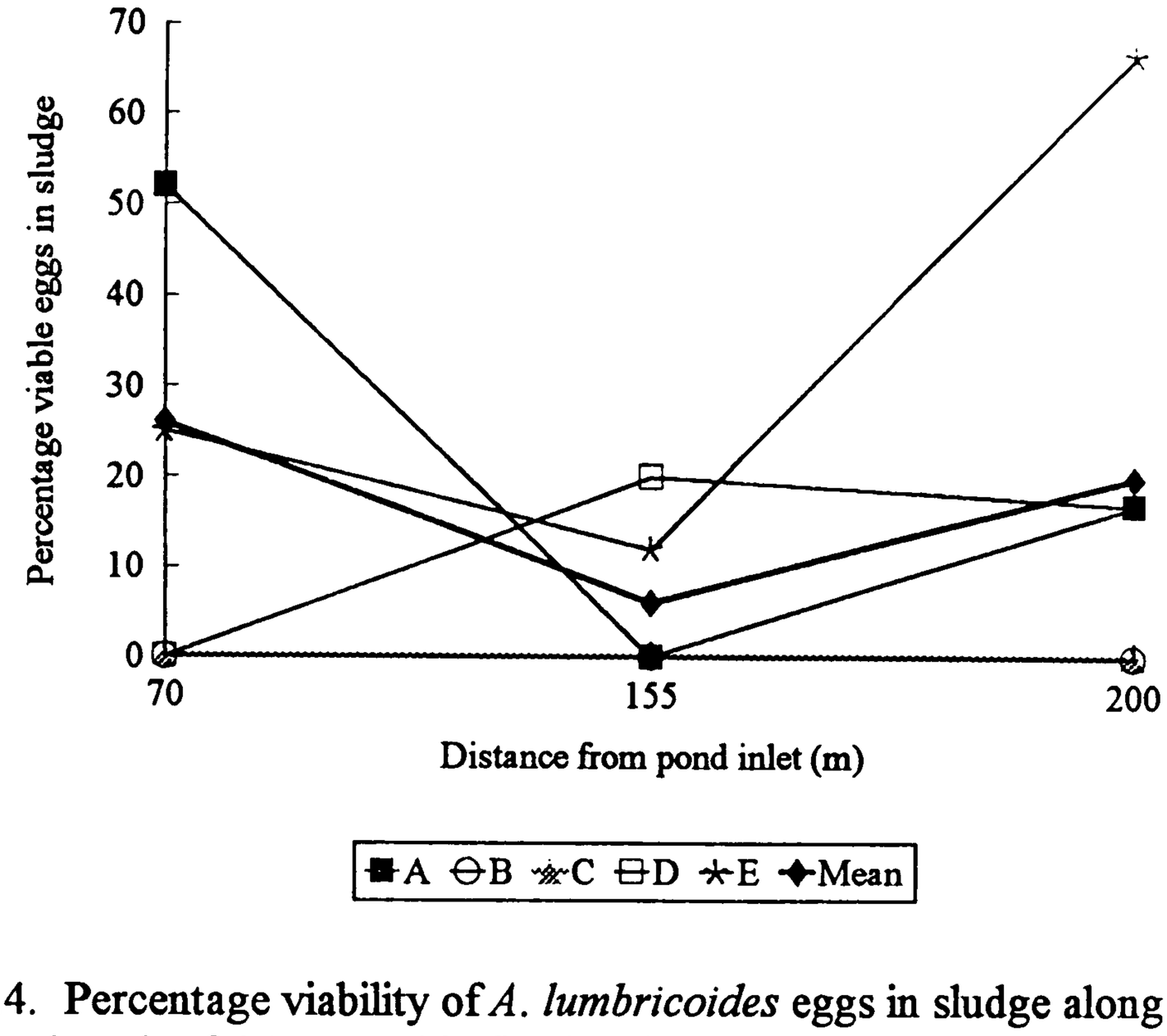


FIG. 8.4. Percentage viability of *A. lumbricoides* eggs in sludge along the length of A1-1 pond in five longitudinal sections (A-E)

TABLE 8.4. Percentage viable *Ascaris lumbricoides* eggs in A1-1 pond sludge

Section	A	B	C	D	E	Mean (SD)
1	52	0	0	0	25	26 (23.15)
2	0	0	0	20	12	6 (9.21)
3	17	0	0	17	67	17 (27.51)

TABLE 8.5. Generalised Linear Interactive Modelling (GLIM) analysis on the distribution of the percentage of viable *Ascaris* eggs through the pond length, width, corners, and numbers of *Ascaris* eggs

Variables	Estimated variance	Standard error	Scaled deviance	Degree of freedom
Section 1	-0.25	0.36	10.5	12
Section 2	-1.5	0.54	10.5	12
Section 3	-1.1	0.55	10.5	12
Corners	1.2	0.84	8.1	11
Ascaris egg numbers	0.04	0.03	8.9	11

Table 8.6 shows the percentage of viable *Ascaris* eggs in the sludge samples, using the incubation (cells had capability to develop) or vital staining methods. It is interesting to note that there was a **moderate** correlation coefficient of **0.57** between the viability staining method using crystal violet and incubation method. The results show that higher eggs counts were predicted to be viable by using the staining method than was found after incubation, throughout the pond (Table 8.6).

TABLE 8.6. Showing percentages of viable *Ascaris* eggs using the incubation method compared with staining method along the length of A1-1 pond

Section	method	
	Incubation (%)	Staining (%)
1	26	52
2	6	42
3	17	36

8.2.3 Distribution of indicator bacteria in the sludge of A1-1 pond

Two way analysis of variance showed no significant difference in the logarithmic number of total coliform, faecal coliform, and faecal streptococci counts per gram dry weight of sludge along the length of the pond (sections 1-3) and the lengthwise sections (A-E) (Tables 8.7, 8.8, and 8.9). Only *Salmonella* spp. showed highly significant differences in the counts along the length of the pond (sections 1-3), but no overall differences between the lengthwise sections of the pond (A-E) (Table 8.10). Figure 8.5 shows the logarithmic bacterial counts, per gram dry weight basis, distribution in sludge along the length of A1-1 pond.

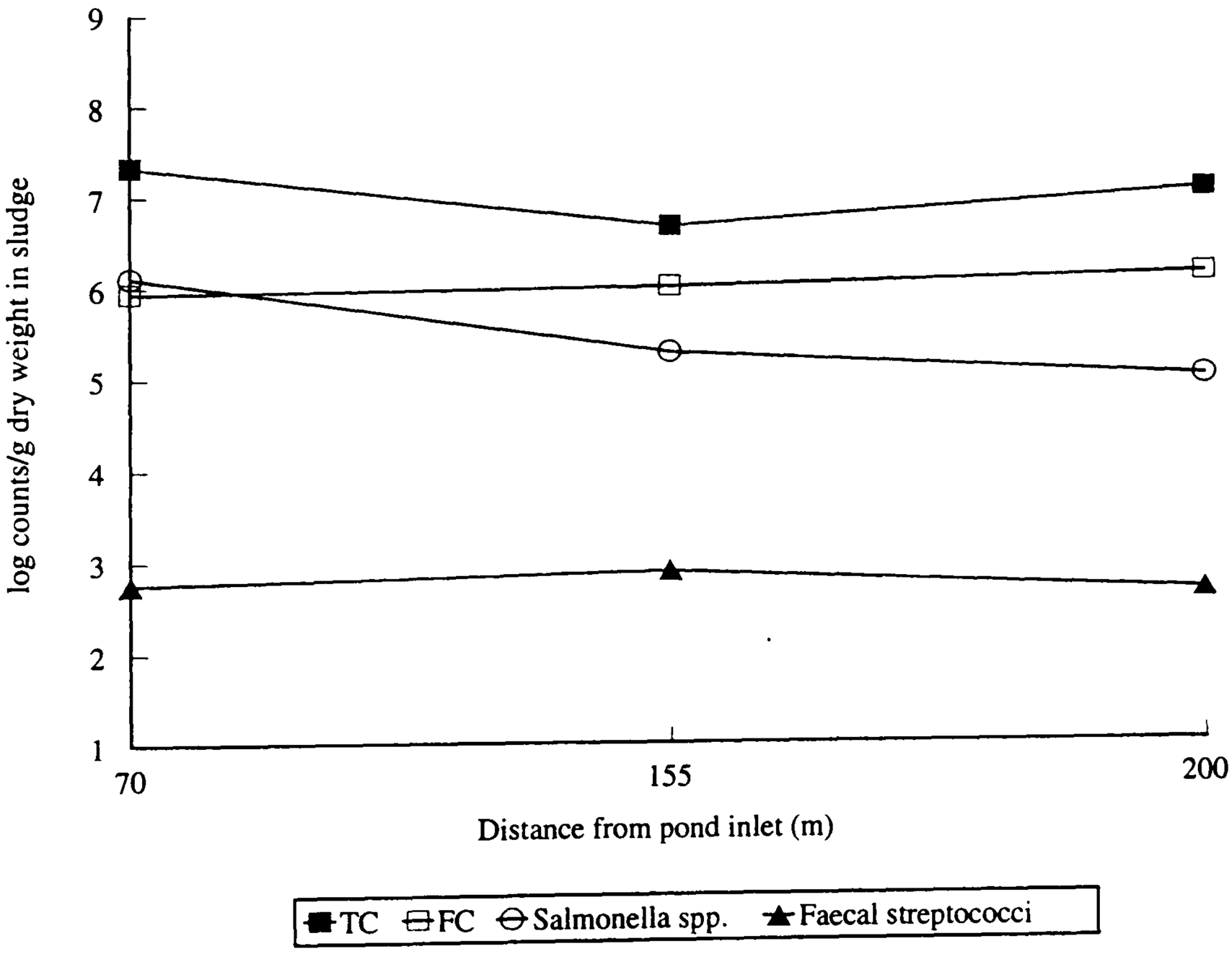


FIG. 8.5. Distribution of bacterial counts in sludge along the length of A1-1 pond

TABLE 8.7. Analysis of variance of the distribution of total coliform counts in sludge along the length and width of A1-1 pond

Source	df	SS	MS	F	F critical	P*
Sections (1-3)	2	0.99	0.49	1.13	4.46	0.37
Sections (A-E)	4	0.22	0.05	0.12	3.84	0.97
Error	8	3.48	0.44			
Total	14	4.69				

*Significant at the $\alpha = 0.05$ level

TABLE 8.8. Analysis of variance of the distribution of total faecal coliform counts in sludge along the length and width of A1-1 pond

Source	df	SS	MS	F	F critical	P*
Sections (1-3)	2	0.16	0.08	0.26	4.46	0.78
Sections (A-E)	4	0.28	0.07	0.23	3.84	0.91
Error	8	2.41	0.3			
Total	14	2.84				

*Significant at the $\alpha = 0.05$ level

TABLE 8.9. Analysis of variance of the distribution of faecal streptococci counts in sludge along the length and width of A1-1 pond

Source	df	SS	MS	F	F critical	P*
Sections (1-3)	2	0.13	0.06	3.72	4.46	0.07
Sections (A-E)	4	0.18	0.04	2.59	3.84	0.12
Error	8	0.14	0.02			
Total	14	0.45				

*Significant at the $\alpha = 0.05$ level

TABLE 8.10. Analysis of variance of the distribution of *Salmonella* spp. counts in sludge along the length and width of A1-1 pond

Source	df	SS	MS	F	F critical	P*
Sections (1-3)	2	3.11	1.55	20.13	4.46	0.00
Sections (A-E)	4	0.04	0.01	0.13	3.84	0.97
Error	8	0.62	0.08			
Total	14	3.76				

*Significant at the $\alpha = 0.05$ level

8.3 Discussion

Certain anomalies and irregularities in all the results probably reflect the heterogeneous nature of the sewage sludge. Samples often contained a lot of debris like hair, sand, seeds amongst the organic matter, and this latter varied greatly in consistency.

The majority of eggs as shown in Figure 8.2 settled out in the middle section (section 2, a mean distance of ≈ 155m from the inlet) as well near the corners. Three factors are suggested to be affecting the distribution of particles through the pond. First, as the inlet to pond A1-1 is below the water surface of the pond, the distribution pattern in Figure 8.1 suggests that the vertical component of the flow initially lifts eggs up through the water column and they are carried along the pond length before they start to settle out. The horizontal component of the flow carries eggs along the pond where they progressively settle and hence were found in the sludge throughout the length of the pond. Ayres *et al.* (1993) found eggs settled at a mean distance of 7.5 m from the inlet of primary facultative ponds in Brazil.

Secondly, sludge settlement perhaps occurs near the inlet, but the particles are buoyed up by the gaseous products of anaerobic decomposition, temperature inversion, or other natural phenomena that resuspend the material. These floating masses are then blown into the corners by the wind action and resettle again.

Thirdly, a high jet velocity of the incoming wastewater can create turbulent conditions unfavourable for the settling process near the inlet, and can also agitate the accumulated sludge and cause scouring. This problem can be reduced by the proper design of the inlet, and proper choice of the inlet and outlet configuration; this point has been discussed in detail by Saqqar and Pescod (1994). It can be concluded that the design of inlet and outlet have a major effect on pond flow patterns and hence on the sedimentation characteristics of human parasite eggs.

The lack of difference in the distribution of indicator bacteria along the pond length requires some explanation. First, the smaller size of bacteria enables them to attach to any size of particle, and when they attach to smaller particles this results in less settlement and wider distribution throughout the pond. Maturation of the sludge in the pond bottom may also liberate fine particles to which bacteria adhere, these may be buoyed to the surface due to gas production which results in redistribution throughout the pond. Secondly, the MPN technique used for indicator bacterial counts probably does not allow the correct evaluation of the exact number of bacteria adsorbed on the particles, since several bacteria on one particle are counted as one bacteria only.

The slight decrease in the *Salmonella* spp. counts along the length of the ponds is not surprising- a steady inactivation with time might reflect the sensitivity of this microorganism to the anaerobic conditions and H_2S gas prevailing in the pond, and the longer retention time of cells near the outlet compared to those near the inlet. Almasi and Pescod (1995) found that the effect of influent sulphur concentration on *E. coli* removal was not significant but it influenced *Streptococcus faecalis* removal positively. May be this can explained the no significant difference of faecal coliforms along the length of the pond, comparing with faecal streptococci and *Salmonella* spp..

In conclusion, the removal of *Ascaris* eggs in anaerobic ponds may be related to the dimensions of the particles which settle towards the middle of the ponds, where high counts of eggs are also found to occur. In contrast, the bacteriological counts appear as a random distribution, with no concentration gradient established along the pond (except for *Salmonella* spp.).

The settling velocity and the sedimentation distance of intestinal helminths was calculated theoretically for the A1-1 anaerobic pond by using a method similar to that used by Ayres (1992) (see Appendix 8.1 for calculations details). The minimum settling velocity found = 6.2×10^{-5} m/s had been calculated assuming that nematode eggs are removed by gravity, using an idealised continuous flow sedimentation tank as a model to predict the removal of helminth eggs in ponds. WSP do not behave as perfect plug flow reactors and their effective volume is always reduced by dead zones, particularly at the corners, so it

was anticipated that the model would need to provide a generous margin of error to account for this.

As the theortical settling velocity for some intestinal helminth eggs in Appendix (7.1) was greater than the calculated minimum settling velocity (6.2×10^{-5} m s^{-1}). That mean the model shows that *A. lumbricoides* (Us = 3.4×10^{-4} m s^{-1}), *T. trichiura* (Us = 2.7×10^{-4} m s^{-1}), *A. duodenale* (Us = 1.1×10^{-4} m s^{-1}), and *Taenia* spp. (Us = 1.9×10^{-4} m s^{-1}), would be removed in one retention time in pond A1-1, even allowing a large margin of error.

The distance (L) at which *A. lumbricoides* eggs may be expected to sediment out equal 22.4m if assumed that the A1-1 pond was 260m long (for calculation details see Appendix 8.1). This underestimates the real sedimentation distance to such an extent that the model seems to have little application to Al-Samra. This is in agreement with Ayres (1992) conclusion for the settling velocity and sedimentation distance of *A. lumbricoides* eggs in primary facultative pond in Brazil.

Clearly other factors may be contributing to the settlement of parasitic eggs, such as temperature, type of wastewater, percentage of solids, type of parasite (i.e. size), the shape of the ponds, the position of the inlet and outlet, short circuiting, and season.

Developing *A. lumbricoides* eggs are obligate aerobes, the undeveloped state of the majority of eggs recovered from the sludge indicates that freshly laid eggs entering the anaerobic ponds with raw sewage had their development halted due to the anaerobic conditions that prevail in this pond. Ayres *et al.* (1993) results showed that very little development of *Ascaris* eggs had taken place in primary facultative ponds sludge in Brazil. Cram (1943) found no development occurred for *Ascaris* eggs in anaerobic sludge digestion at 20-30°C and the viability was not affected for the first 3 months.

WSP's normally function as a continuous process, and the most recently deposited *A. lumbricoides* eggs will always be viable. Even when ponds have been functioning for 8 years and most of the sludge has been well stabilised, its immediate disposal presents a problem in terms of risk from helminth eggs and pathogenic bacteria, particularly *Ascaris* eggs and *Salmonella* spp..

Coliform removal kinetics have been studied in detail by only a few investigators (Marais, 1970, 1974; Klock, 1971; Bowles *et al.*, 1979; Johnson *et al.*, 1979; Polprasert *et al.*, 1983). This is due to the fact that bacterial removal kinetics are extremely complex, and settlement plays a relatively small part in their removal, in contrast to helminth eggs.

CHAPTER NINE

QUANTITY AND PHYSICAL CHARACTERISTICS OF SLUDGE IN AL-SAMRA ANAEROBIC PONDS

9.1 Introduction

The extent of accumulation of solids in an anaerobic ponds will vary considerably depending primarily upon the quantity and characteristics of wastewater, the degree of destruction of the organic material by digestion, and the concentration of solids carried in the effluent (Dornbush, 1970; Pescod, 1995).

Sludge solids reductions is one of the main objectives of anaerobic treatment. It helps to make sludge less putrescible but also reduces the amount of solids for ultimate disposal. It is usually assumed that this reduction takes place only in the volatile portion of sludge solids (EPA, 1979). The degree of volatile solids reduction achieved in any particular application depends on both the operating parameters of the digestion system and the character of the sludge.

Many pond systems have been designed without consideration being given to the need for removal of accumulated sludge (Pescod, 1995). The sludge layer exerts a significant influence on the behaviour of ponds and Marais (1970) suggested that this was probably the most neglected factor in design. It can affect pond performance in different ways:

(1) by reducing the pond volume and shortening the hydraulic retention time as a result of excessive sludge accumulation (Schneiter *et al.*, 1984);

(2) by feed-back of soluble organic matter to the overlying liquid layer at high temperatures (Bryant & Bauer, 1987; Saqqar & Pescod, 1993);

(3) by resuspension of particulate substrate during periods of mixing, increasing the turbidity and suspended solids concentration in the overlying water (Parker *et al.*, 1959).

Ghosh and Kshirsagar (1982) investigated the fertilising constituents at different depths of the sludge bed at a WSP that decomposed slowly over a period of many years. They found an increasing trend of ammonia and decreasing trend of nitrate concentrations in the lower layers. A significant decrease of volatile solids, nitrogen, phosphorus and potassium were recorded in the deeper layers. Ghosh and Kshirsagar (1982) found that the first two layers of the sludge bed were suitable to be used as fertiliser for croplands after proper disinfection, through sludge composting or sun drying for a year, at a reasonable cost. The deposits of the lower layers were useful for conditioning sandy as well as lower grades of solids.

Information describing lagoon sludge removal is limited. Common practice for removing sludge from unlined or earthen-lined lagoons usually involves draining the lagoon and excavating the sludge with a scraper or a front-end loader (Schneiter *et al.*, 1993). The provision of pipework for sludge removal is a debatable point. Since sludge is only removed infrequently it may be cheaper to utilise a submersible sludge pump, particularly since drainage pipework has a tendency to clog (Bradley & DaSilva, 1976).

Removal of sludge solids where it has been practice, has been best effected by pumping the wet sludge to drying areas. In large installations this has been expedited by having a deep transverse sump across the lagoon into which the sludge will flow or can be pushed and from which it can be pumped (Parker, 1970).

To facilitate cleaning, designs should be based on a three pond system employing two active cells and one inactive cell. After each cell is rested, the accumulated sludge can be removed by pumping or excavation (Dawson & Grainge, 1969).

The literature indicates that substantial work has been conducted in the areas of wastewater pond design, operation, and economy of stabilisation ponds. A wastewater pond system can be effectively designed and operated to provide adequate waste treatment. Nevertheless, factors affecting sludge accumulation, methods of accumulated sludge removal, stabilisation of accumulated, and specific sludge characteristics have been generally neglected relative to wastewater ponds.

Also, information regarding the characteristics of sludge from the biological point of view and comparing the pattern of sludge accumulation in different anaerobic ponds in Jordan and the Middle East Region is scarce.

Some data is available in literature (Miqdadi, 1989) about heavy metals concentrations in sludge also he developed two empirical models in his study, to calculate the amount of sludge accumulation in pond; Saqqar (1990) and Saqqar & Pescod (1994b) developed a model to predict accumulated sludge volume based on non-biodegradable solids and BOD_5 in the raw wastewater and a coefficient for the accumulated sludge, which takes account of the biodegredation of the organic fraction of the primary anaerobic pond sludge. Ayres (1992) brief study in Brazil and Schneiter *et al.* (1993) in Alaska, are based on the experiences in other countries and not much information is available under Jordanian conditions.

If excess sludge is accumulating in wastewater ponds, then the sludge volume and characteristics require definition, and techniques for removal, treatment, and disposal need to be investigated.

To investigate the desludging processing and disposal options for sludge and to provide recommendations for sludge handling, it was necessary to investigate (1) the amounts of sludge in these anaerobic ponds, and (2) provide information on the physical, and biological characteristics of the sludge.

9.2 RESULTS

9.2.1 Calculation of sludge layer depth

Sludge layer depths were computed by subtracting the soundings from the presumed pond water depth of 5.0 meter. These depths are listed in Table 9.1 for the first three anaerobic ponds, and in Table 9.2 for the second three anaerobic ponds. These tables are arranged so that they may be viewed as schematic diagrams of the ponds in plan view, with the inlets at the top of the diagram and the direction of flow being down the page. The total

depth minus the depth to the sludge-water interface equals the sludge depth. The sampling grid was four nodes wide by eight nodes long in the first three ponds, but only six nodes long in the second three ponds. The ponds are all the same actual size, however.

TABLE 9.1. Sludge depth (m) distribution primary anaerobic ponds of Al-Samra

Inlet of raw wastewater to the ponds											
Inflow											
A1-1				A2-1				A3-1			
0.4	0.2	0.0	0.6	0.8	0.7	0.7	2.0	0.7	2.6	2.6	2.7
0.5	2.8	2.5	1.6	0.7	4.0	1.8	2.5	1.7	2.5	0.0	2.5
0.7	0.0	0.0	1.5	2.3	3.4	2.6	0.7	0.6	3.7	1.6	0.4
0.8	2.0	0.9	2.3	2.8	2.7	2.6	1.2	1.0	1.5	2.4	2.2
3.0	2.1	2.6	0.5	1.7	3.4	3.2	2.7	2.0	2.0	1.5	2.0
0.8	2.3	2.8	1.3	4.2	3.7	3.1	3.4	2.2	1.5	2.2	1.5
2.5	3.3	3.8	2.3	3.6	3.5	3.7	4.4	3.7	2.8	2.7	1.3
2.8	3.1	3.0	3.1	4.6	4.6	4.5	4.7	0.2	0.2	0.5	1.8
Outflow											

TABLE 9.2. Sludge depth (m) distribution in secondary anaerobic ponds of Al-Samra.

Inlet of wastewater from the first set of anaerobic ponds to the second set of anaerobic ponds											
Inflow											
A1-2				A2-2				A3-2			
1.0	0.7	0.7	0.7	2.0	2.7	2.0	2.7	1.4	1.5	1.7	1.6
0.8	0.6	0.6	0.8	1.7	1.9	2.0	2.7	1.5	1.6	1.7	1.7
0.8	0.7	0.7	0.7	1.7	1.8	2.0	2.7	1.5	1.6	1.7	1.7
0.7	0.7	0.6	0.7	1.7	1.5	2.0	2.1	1.5	1.5	1.7	1.7
0.7	0.6	0.6	0.7	1.7	1.9	2.0	2.2	1.5	1.5	1.5	1.5
0.8	0.6	0.6	0.7	1.7	1.9	2.0	2.0	1.5	1.5	1.6	1.6
Outflow											

9.2.2 Computation of sludge volume

The surface area of the filled pond, 135m wide by 260m long, was divided, conceptually, into the following regions:

- The central region, 105m × 230m, underlain by the original flat, horizontal pond bottom (All depth sampling nodes are in this central region).
- Two side regions, each 15m × 230m, underlain by 1:3 sloping banks of the ends of the ponds.
- Two end regions, each 15m × 105m, underlain by the 1:3 sloping banks of the ends of the ponds.
- Four corner regions, each 15m × 15m, underlain by 1:3 sloping banks of one side and one end as they intersect.

The central region was then further conceptually subdivided into rectangular cells centred on the nodes where depth samples were taken. The sludge volume in each cell is the cell area multiplied by the sludge depth (D). The sludge volume in the central region is the sum of the sludge volumes of all of its cells.

Each of the two side regions was subdivided into cells, each adjacent to a nearby sampling node in the central region. It was assumed that the sludge level in each side region cell was the same as measured at the nearby sampling node. The sludge volume in each side region cell is therefore (see Fig. 9.1):

$$1.5D^2N \qquad \text{(eq. 1)}$$

Where:

D: the sludge depth at the sampling node, or the sludge depth at the toe of the slope under the cell,
N: the length of the cell parallel to the side.

The two end regions were treated similarly.

Each of the four corner regions contains sludge of depth D as measured at the deepest point of the cell, which is at the intersection of the toes of slope of the two sides that meet at that corner. The sludge deposit is in the shape of an inverted pyramid of height D and basal area $(3D)^2$; the volume of a pyramid is 1/3 × basal area × height; hence the volume of each corner cell is $3D^3$. The sludge volume in each pond is the sum of the volumes of all the cells (central, side, end, and corner) of each pond (Fig. 9.1).

A limited effort was made to measure sludge depth in the first facultative pond of the third train, pond F3-1, which is nominally 2.35m deep. The soundings were all in the range of 2.0 to 2.05m indicating a sludge deposit of only about 0.3m. It is therefore assumed that there is negligible sludge deposit in any of the ponds down stream of the six anaerobic ponds.

9.2.3 Quantity of sludge accumulation

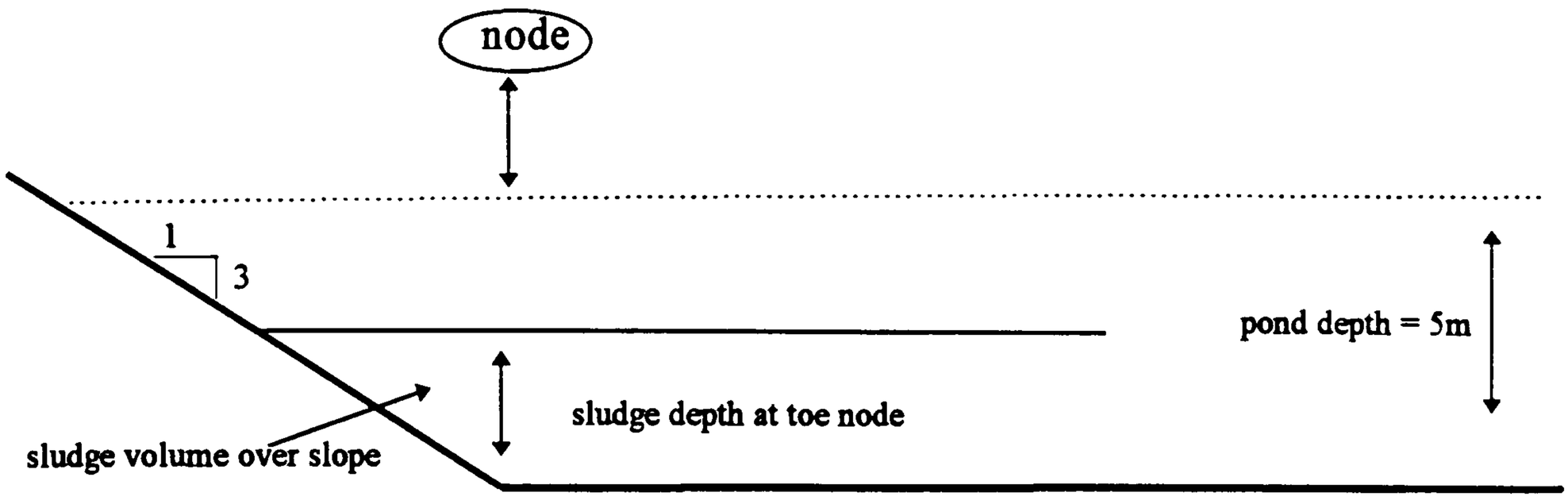
The computed sludge volumes are listed in Table 9.3. Also shown the average values for solids content obtained at the top and the bottom of the sludge deposits in that ponds. Multiplying the sludge volumes by the average value of solids content measured for each pond provides an estimate of sludge mass to be handled on a dry solids basis. In all but one pond (A1-2), the sludge was sufficiently thick and viscous to obtain samples readily.

Anaerobic ponds are noted for their ability to store sludge for a long period; in the case of Al-Samra anaerobic ponds they had the capability to store a huge volume of sludge (36,600 m^3 dry weight basis) from 1985-1993 in six anaerobic ponds.

Surface scum in the first three anaerobic ponds was thick enough to form large floating mats capable of supporting grass and small animals (mice) and birds. The mats were sufficiently thick in places as to render rowing among them infeasible and towing the boat through them by a land-based vehicle was the only option. The mats covered one-third to one half of the area of the first three ponds, particularly pond A2-1. Their location depends on the wind direction on any given day, since they accumulate in the downwind parts of the ponds.

One means of scum mat generation was seen to occur when accumulated methane in the sludge would break free and rise to the surface of the pond, lifting with it portions of fibrous sludge deposits (like fruit and vegetable skin and seeds, hair, and straw with wind-blown sand and soil forming thick floating mats). Floatable matter in the sewage not removed by screens at the Ain Ghazal Treatment Plant, and possibly at Zarqa Wastewater Treatment Plant, would contribute to the mat layer.

Volume at the sides

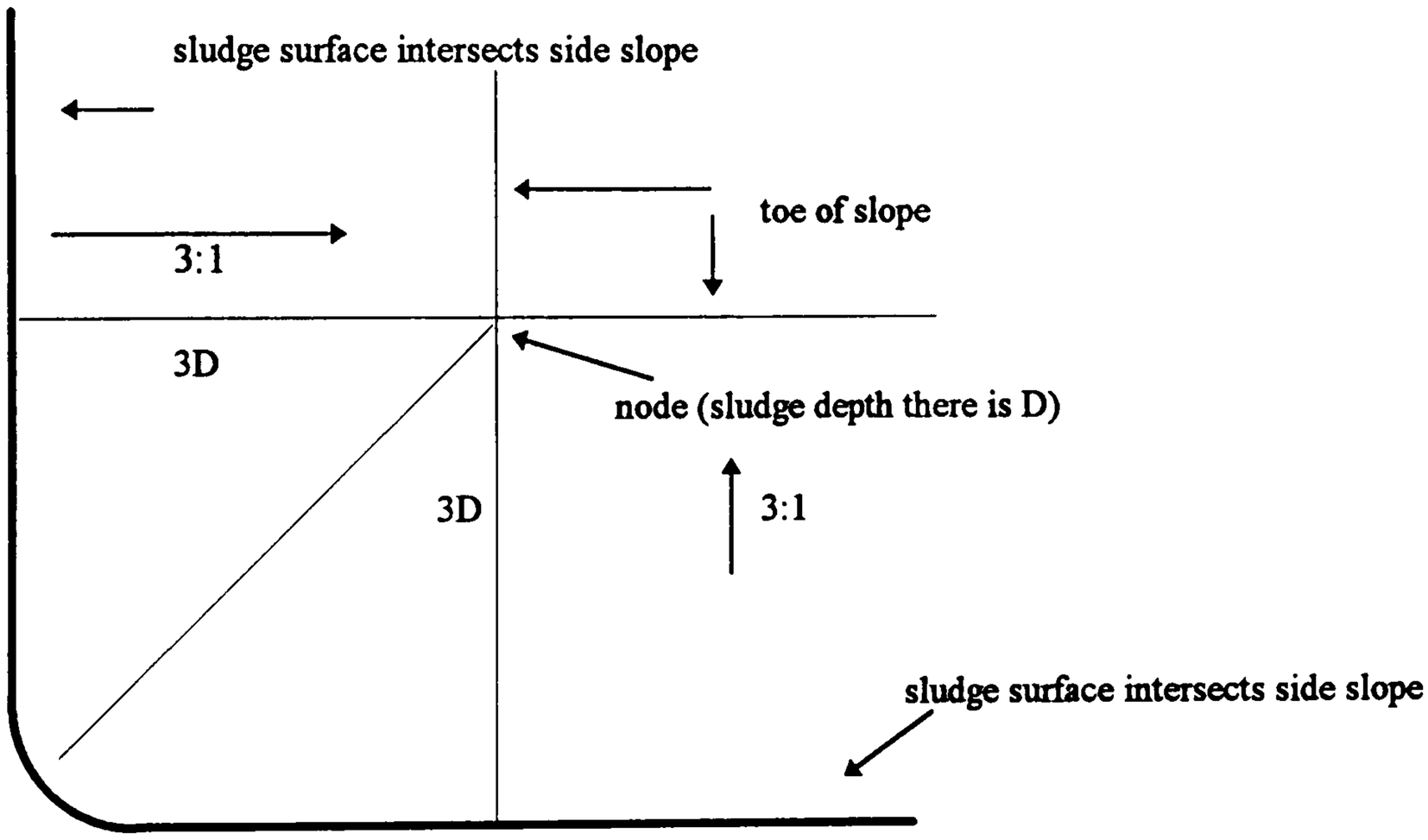


= 1/2 (3D X D) node interval, (normal to plane of figure)

$= 1.5D^2N$

Volume in the corners:

- **Sides and ends**



plan of corner

- **Corners**

Volume of sludge in eccentric corner pyramid
= 1/3 base x height, height = D
base area = 3D x 3D
$\therefore$ Volume = 1/3 $(3D)^2$ D

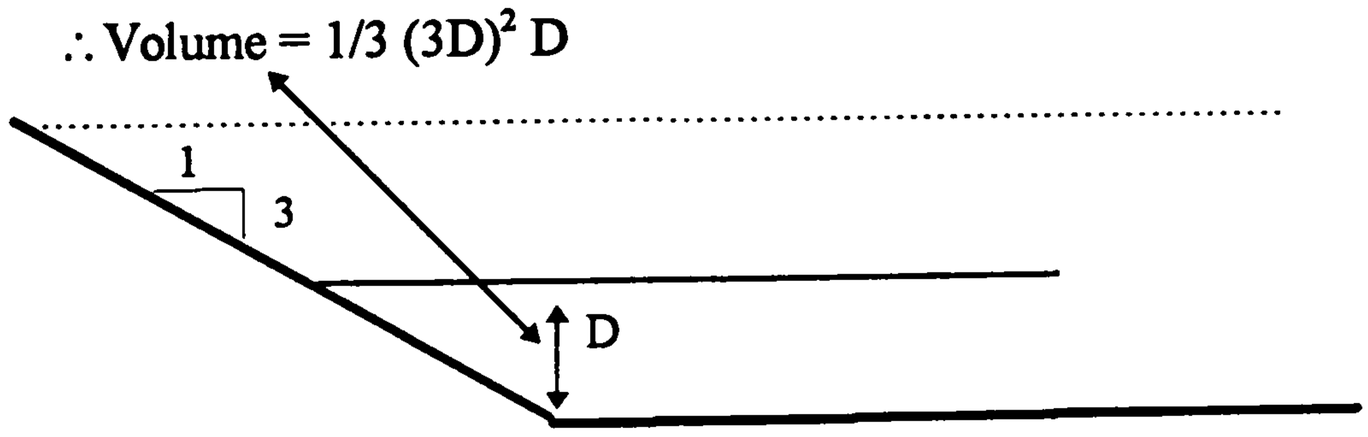


FIG. 9.1. Calculating sludge volume at the edges of anaerobic ponds at Al-Samra system

TABLE 9.3. Sludge volumes and dry-solids mass for each anaerobic pond

Pond	Sludge volume (m^3)		Solids content		
	wet basis	dry basis	Top of sludge (%)	Bottom of sludge (%)	Average (%)
A1-1	46,758	6078	12	20	13
A2-1	79,314	9518	10	22	12
A3-1	59,011	7671	12	19	13
A1-2	18,369	2204	12	-	12
A2-2	50,986	6628	12	18	13
A3-2	40,920	4501	10	14	11
Total	**295,358**	**36,600**	**11**	**19**	**12.4**

- no sample

9.2.4 Anaerobic ponds sludge accumulation and distribution

The pattern of sludge accumulation over each pond bottom of the primary and secondary anaerobic ponds at Al-Samra is shown in Figure 9.2 to 9.7. The depth of sludge layer was determined at different points of the pond in operation.

The sludge depth in the anaerobic ponds of Al-Samra has been examined to determine the accumulation rate, and accumulated sludge characteristics. Table 9.4 contains a summary of the sludge accumulation data collected from both primary and secondary anaerobic ponds from the Al-Samra system. The maximum depth recorded was 4.7m at a point near the outlet (toward the corner) in the primary anaerobic ponds A2-1.

TABLE 9.4. Average and range of the sludge depth and estimated annual sludge accumulation rate in both primary and secondary anaerobic ponds at Al-Samra

Pond	Sludge depth		Estimated annual sludge accumulation rate (cm/year)
	Average (m)	Range (m)	
A1-1	1.7	0.0-3.8	29
A2-1	2.8	0.7-4.7	35
A3-1	1.8	0.0-3.7	22
Average primary anaerobic ponds	2.1	0.0-4.7	-
A1-2	0.7	0.6-1.0	12
A2-2	2.0	1.5-2.7	25
A3-2	1.6	1.4-1.7	20
Average secondary anaerobic ponds	1.4	0.6-2.7	-

The mean sludge depths in the first sets of the ponds are approximately 1.5 times more than the mean sludge depths in second anaerobic ponds. Although these sludges were accumulated over varying periods of time, the primary anaerobic ponds have a mean sludge depth near 2.1m, and the secondary anaerobic ponds average sludge depth near 1.4m (Table 9.4).

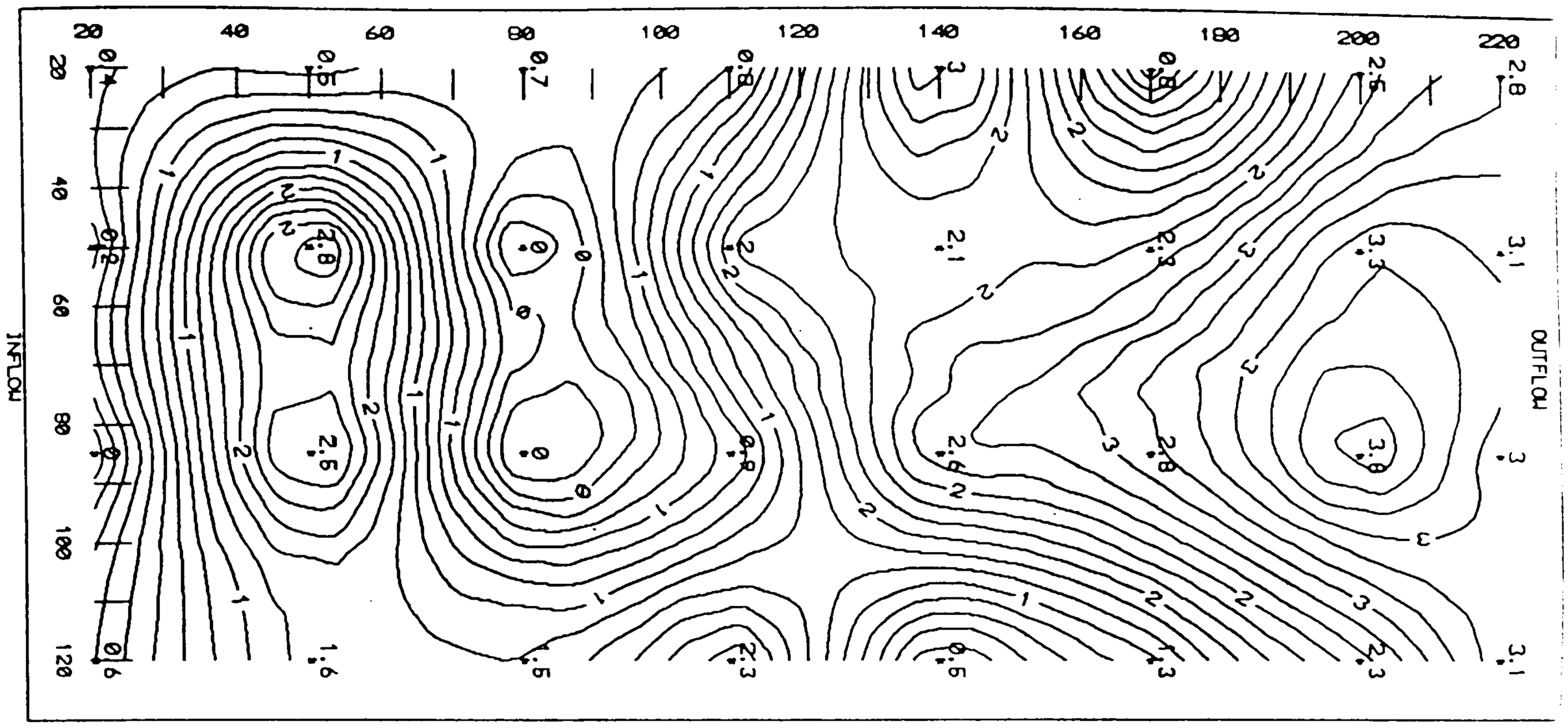
The greatest solids deposition had been expected to occur only near the inlets, however, the results of this study did not confirm this pattern, which is in agreement with the findings of Miadadi (1989) and Saqqar and Pescod (1994). Homogeneous distribution of sludge over the pond bottom was only achieved in the subsequent facultative pond. The two sets of anaerobic ponds were found to have different contours of sludge distributed over the pond bottom, which reflect the hydraulic conditions of the system: accumulation near the inlet, the outlet, in the corners and near the pond walls.

Examination of Al-Samra primary anaerobic pond data (Fig. 9.2 to 9.7; and Tables 9.1 and 9.2) shows that there is a greater accumulation of sludge near the corners of the ponds. Middlebrooks *et al.* (1965) explanation is that excessive sludge accumulation probably occurs around the inlet; then the sludge layer becomes anaerobic and is buoyed

up by the gaseous products of anaerobic decomposition, temperature inversion, or other natural phenomena that resuspend the material. These floating masses are then blown into the corners by the wind action. Ghrabi and Ferchichi (1994) found that most sludge accumulation occurred near the outlet in the maturation ponds; they contributed this to the effect of the dominant winds. In contrast, Ayres *et al.* (1993) found the greatest depth near the inlet of the primary facultative ponds.

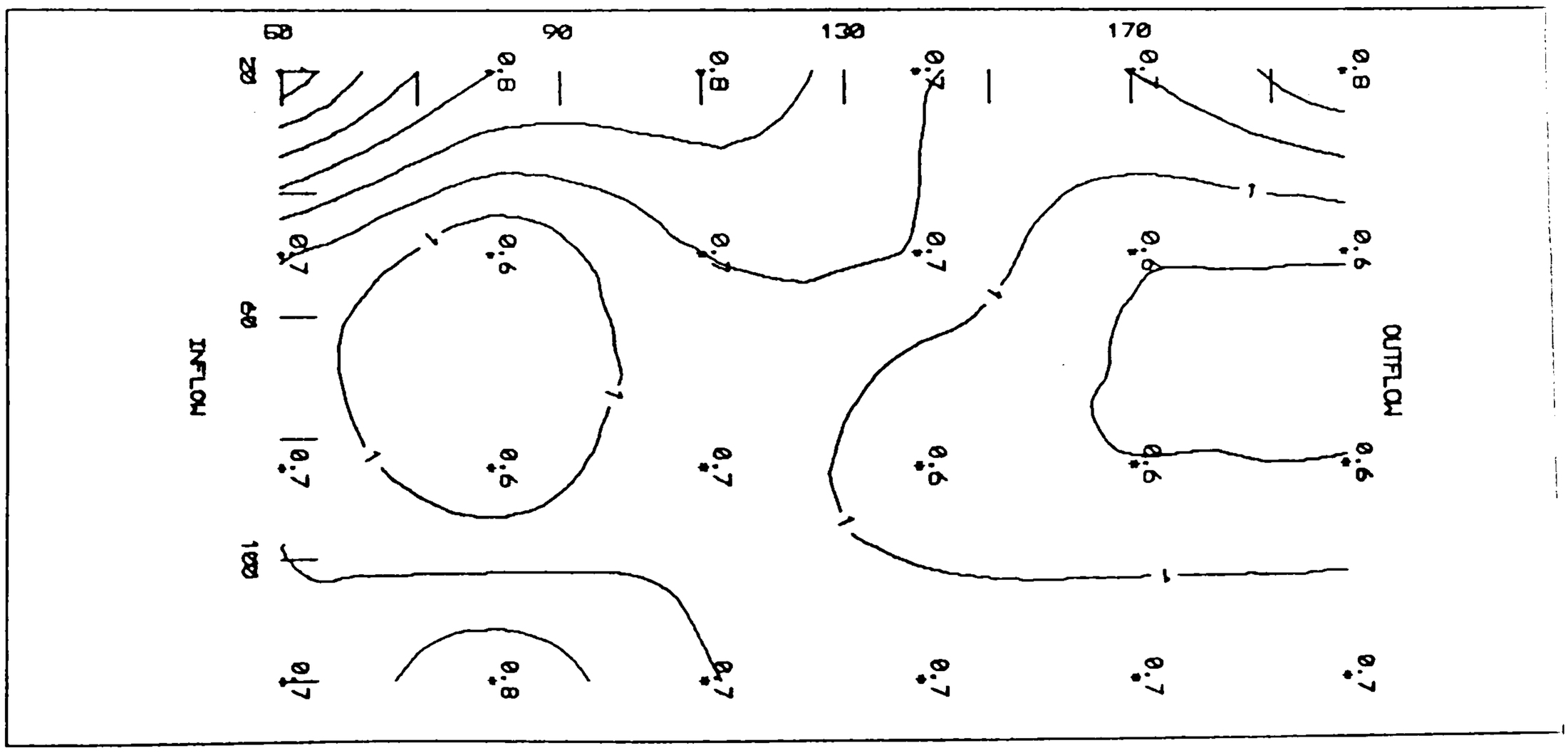
The results showed that approximately equal average sludge depths occurred for ponds A1-1, and A3-1 of the same type and size, operating in a similar climate and conditions even though A3-1 pond had operated for 21.5 months longer than the A1-1 ponds. This was in agreement with the findings of Schneiter *et al.* (1993) that approximately equal sludge depths occurred for lagoons of the same type with similar climate, although the operational periods of the ponds varied by several years. However, this was not found for ponds A2-1 and A3-1, even though, they started operation on the same day.

The greater sludge depth in the A2-1 pond, and the similarities in depth observed among the primary anaerobic ponds A1-1 and A3-1, probably result from differences in the pipeline capacity, from the inlet structure to the three first anaerobic ponds, with an estimated capacity of 103,500 m^3/d, 156,900 m^3/d and 100,600 m^3/d to trains 1, 2, and 3 respectively.



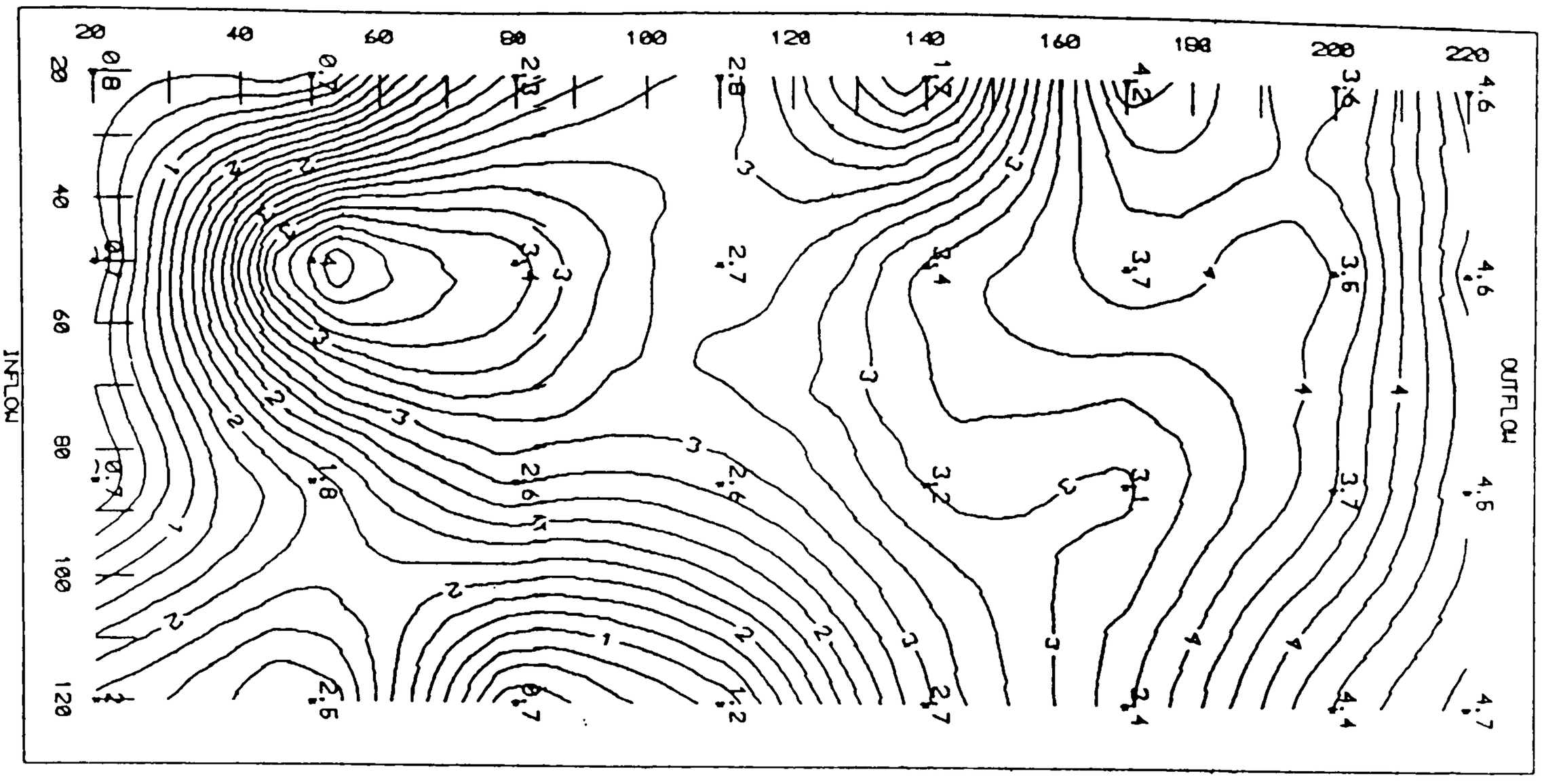
[Note: Contour lines indicate approximate sludge accumulation (m), Scale 1 cm = 20 m; * Sampling points]

FIG. 9.2. Sludge depth distribution over the bottom of A1-1 pond



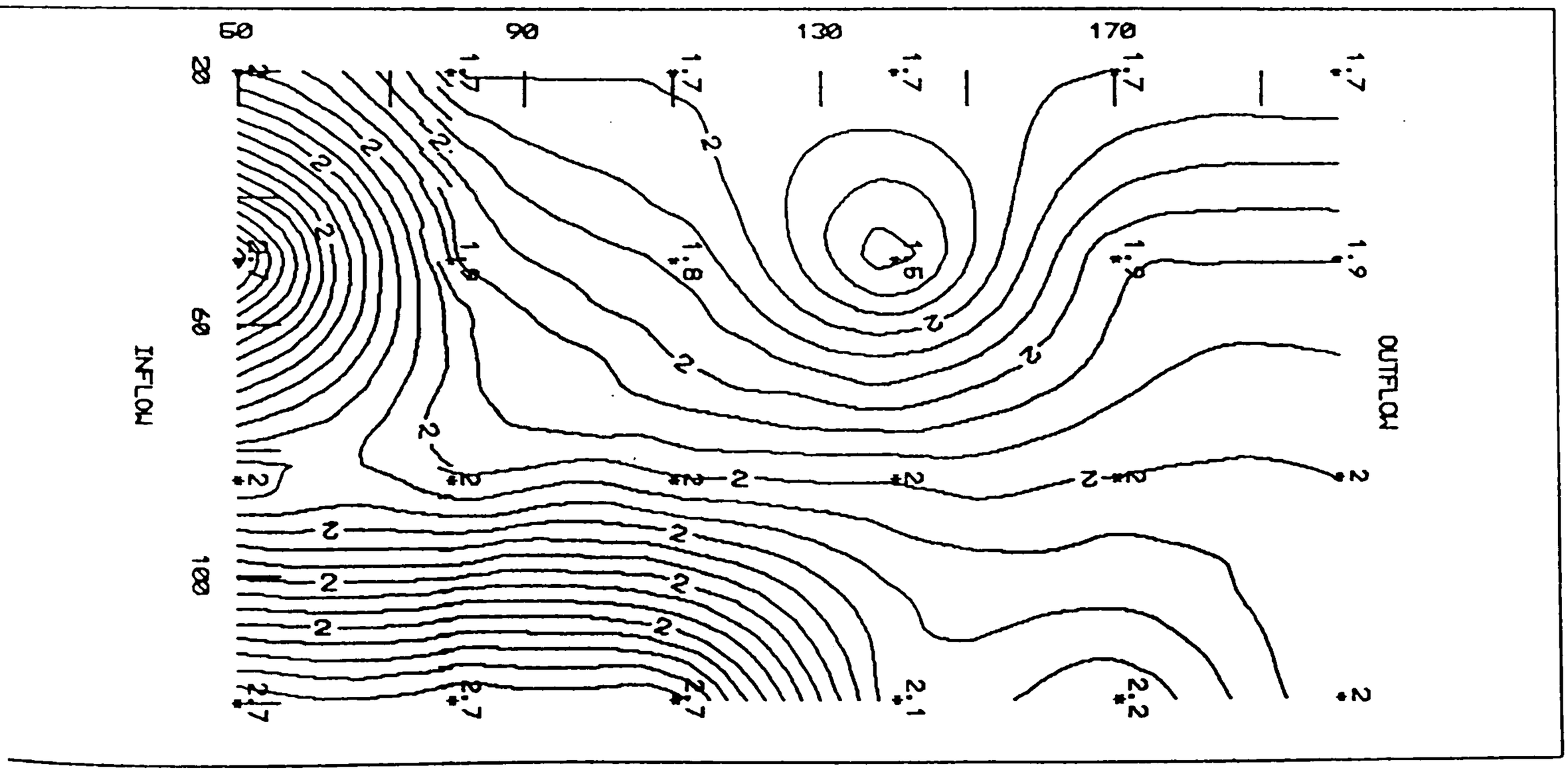
[Note: Contour lines indicate approximate sludge accumulation (m); Scale 1 cm = 21.43 m;* Sampling points]

FIG. 9.3. Sludge depth distribution over the bottom of A1-2 pond



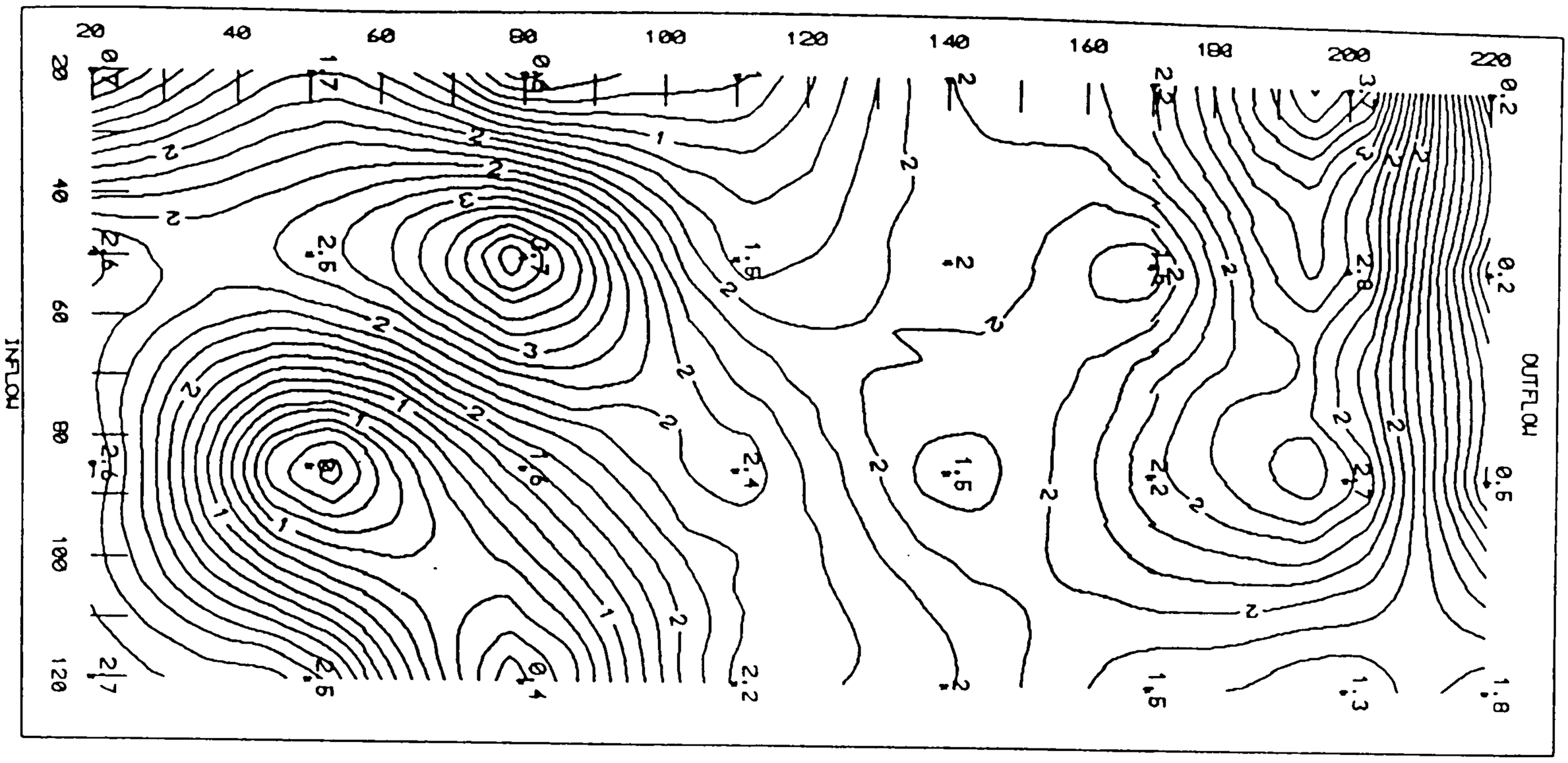
[Note: Contour lines indicate approximate sludge accumulation (m), Scale 1 cm = 20 m; * Sampling points]

FIG. 9.4. Sludge depth distribution over the bottom of A2-1 pond



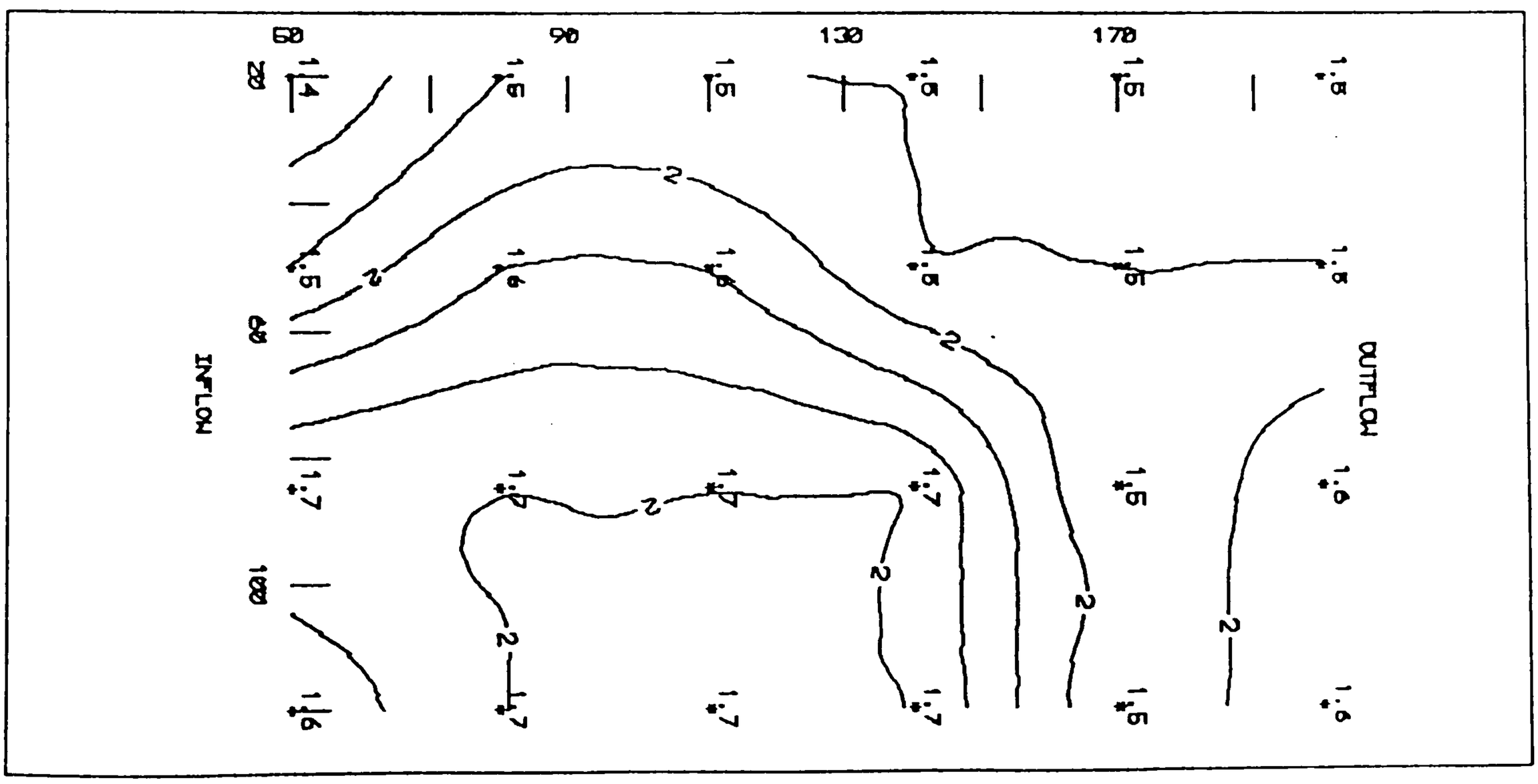
[Note: Contour lines indicate approximate sludge accumulation (m); Scale 1cm = 21.43m;* Sampling points]

FIG. 9.5. Sludge depth distribution over the bottom of A2-2 pond



[Note: Contour lines indicate approximate sludge accumulation (m), Scale 1 cm = 20 m; * Sampling points]

FIG. 9.6. Sludge depth distribution over the bottom of A3-1 pond



[Note: Contour lines indicate approximate sludge accumulation (m); Scale 1cm = 21.43 m;* Sampling points]

FIG. 9.7. Sludge depth distribution over the bottom of A3-2 pond

The results of measurements of the silting in the different ponds is presented in Tables 9.3 and 9.4. The silting is limited mainly to the primary and secondary anaerobic ponds. The annual sludge accumulation rates estimated from mean sludge depth and operation period for the primary anaerobic ponds A1-1, A2-1 and A3-1 ponds were 29, 35, and 22cm/year, respectively. While for the secondary anaerobic ponds A1-2, A2-2, and A3-2, the annual sludge accumulation rate were 12, 25, and 20 cm, respectively. Sludge accumulation rates in secondary anaerobic ponds are lower than those found in primary anaerobic ponds. The differences in these values may be explained by the same factors discussed for differences in accumulated sludge depth.

The rate of deposition in the six anaerobic ponds is high compared to the values given in the literature. In cold region ponds, much lower annual sludge accumulation rates were estimated by Schneiter *et al.* (1983) compared with Al-Samra anaerobic ponds in Jordan. The sludge accumulation rates for Logan and Corinne, Utah, and Palmer and Galena, Alaska, lagoons were 0.68, 0.84, 6.7 and 3.5cm/yr respectively.

Sludge accumulation measurements in the Northway, Alaska, partial-mix aerated lagoon indicated an accumulation rate of greater than 2.54cm/yr, or approximately 0.25m^3/1000 people.d, during 4.5 years (Clark *et al.*, 1970). In Tunisia, Gharbi and Ferchichi (1994), found the sediment depth increase rates were 5 cm/yr for the facultative pilot-scale pond and 1.3, 1.3, and 1.6 cm/yr for first, second and third maturation pilot-scale ponds, respectively.

In some climatic regions sludge accumulates during cold winter months but digests sufficiently during the warmer summer months to maintain the desired balance. Unfortunately, the sludge is not always completely digested and accumulation is a function of the cycles of cold winters and warm summers (Schneiter *et al.*, 1993). The solids that settle and accumulate on the lagoon bottom are typically organic and inorganic solids in the untreated wastewater entering the lagoon, and the biological solids produced by the treatment process (Schneiter *et al.*, 1993).

9.2.5 Physical quality parameters

The physical state of sludge is determined by the nature and amount of solids present. These include different parameters such as moisture content and solids concentration, and specific gravity of sludge. The definition of the physical state helps in deciding whether to transfer the sludge by pumping or by tankers.

The sludge in primary and secondary ponds from WSP's was black in colour, granular in texture, compact and had no objectionable odour. The sludge in all anaerobic ponds were generally in the range between pH 6.5 and 8.4. From the deep sites sludge samples pH values were mostly in the alkaline range 7.0-8.4 (Tables 9.5 and 9.6). The pH increase with depth may be a result of the predominance of methanogenesis accompanied by degradation of the initially produced volatile fatty acids, this is in agreement with Carre and Baron (1987) findings. Also it confirms with Kaneshiro & Stern (1985) results, who found that the pH of soil and sludge samples changed with storage time. There were large initial increases in the pH values of all soil and anaerobically digested sludge samples, whereas aerobically digested sludge samples showed an initial decrease in pH.

The moisture content of the sludge was between 78%-99% in the primary ponds, while in the secondary ponds the range was between 82%-91%. From the deeper sludge sample data, moisture contents were mostly lower than the surface (78%-86%). The characteristics of the deep samples of sludge deposits, with neutral or alkaline pH and low organic and moisture content, show that the sedimented material in the deep layers of anaerobic ponds in the Al-Samra system was at an advanced stage of stabilisation.

The physico-chemical characteristics of the sludge in the ponds are summarised and presented in Tables 9.5 and 9.6. The average solids concentration of the anaerobic ponds sludge is estimated at approximately 12% (with a wide range between 0.8%-22%). The measured total volatile solid content (TVS) of the sludge in the anaerobic ponds ranges between 47 to 73% of the total solids (TS) (Table 9.6). The top layers of the sludge are less digested than the bottom layers.

TABLE 9.5. Physical characteristics of sludge in each anaerobic pond at Al-Samra

Parameter	Unit	Average measured value					
		A1-1	A2-1	A3-1	A1-2	A2-2	A3-2
pH	-	6.9	6.9	7.5	6.8	7.0	6.9
TS	g/kg	131	118	134	124	129	109
TS	%	13	12	13	12	13	11
TVS	g/kg	71	66	73	66	72	59
TVS	(% of TS)	55	58	56	53	56	54

TABLE 9.6. Average of the physical characteristics of sludge in Al-Samra anaerobic ponds

Parameter	Unit	all Anaerobic ponds	Primary ponds	Secondary ponds	Surface samples	Deep samples
pH	- (range)	7.0 (6.5-8.4)	7.1 (6.5-8.4)	6.9 (6.6-7.7)	6.8 (6.5-7.3)	7.6 (7.0-8.4)
TS	g/kg (range)	124 (8-219)	128 (8-219)	121 (87-185)	114 (8-186)	187 (140-219)
TS	% (range)	12 (0.8-22)	13 (0.8-22)	12 (9-18)	11 (0.8-19)	19 (14-22)
TVS	g/kg (range)	68 (5-104))	70 (5-104)	66 (46-94)	64 (5-92)	93 (73-104)
TVS	% of TS (range)	55 (47-73)	56 (47-73)	55 (51-59)	56 (49-73)	50 (47-52)

The volatile solids (TVS) content as a percent of total solids (TS) were 55, 58, 56, 53, 56, and 54% for the primary anaerobic ponds A1-1, A2-1, and A3-1, and for the secondary anaerobic ponds A1-2, A2-2, and A3-2, respectively. These values indicate that organic matter contributes at least as much to the sludge accumulation as does inorganic matter in these ponds. In cold climates, Schneiter *et al.* (1983) found the Logan, Corinne, Palmer and Galena lagoons volatile solids (VS) content were 69, 80, 69 and 49% respectively, expressed as percent of total solids. These values indicate that organic matter was a significant part of the accumulated sludge. However, complex organic molecules may eventually be degraded when held for several months in facultative sludge lagoons (EPA, 1979). In 1970, Parker presented data revealing 60% reduction of total solids deposited in different anaerobic ponds in Australia; storage time ranged from 5-12 years.

Not all of the volatile solids can be converted by the anaerobic bacteria. Various investigations (quoted in WPCF, 1985) indicate that only 60% to 80% of the measured volatile solids fed to anaerobic digesters are biodegradable, unless industrial wastes or other abnormal characteristics are present.

Some researchers conclude that a significant portion of the settled sludge accumulation results from silt rather than organic solids (Middlebrooks *et al.*, 1965). There may be from 23 to 91 times more silt than organics in some lagoon sludge deposits (Clare *et al.*, 1960). However, the higher concentration of silts than organics in lagoon sludge deposits may be more indicative of lagoons operated in temperate regions than those operated in cold regions (i.e. Alaska).

Many researchers have made theoretical calculations which predict sludge accumulation in municipal sewage lagoons; these calculations are based on an average sewage flow and a yearly sludge contribution from people, and consider only organic materials, neglecting the effect of silt and other inorganic substances that may originate in the sewer system or be washed from the dikes.

The reasons for the discrepancies between the results of this research and values reported from previous studies may be due to differences in climate, organic and hydraulic loading, influent wastewater characteristics and time of sampling and sampling method.

A comparison between sludges accumulated in the anaerobic ponds of waste stabilisation system and primary sludge from conventional wastewater treatment processes is informative. The anaerobic pond sludge settles from raw influent wastewater as does primary sludge in a primary clarifier, but the age of the two types of sludge differs considerably. Comparing the characteristics presented in Tables 9.5 and 9.6 for pond sludges with the range of values for primary sludges presented in Table 9.7 reveals some similarities to digested primary sludge, but not close agreement.

The total solids and the volatile solids percentage in the anaerobic ponds sludges of Al-Samra (12.4%, and 55.5% of TS, respectively) were similar to the upper range of reported values for digested primary sludge, while the total solids percentage range was much higher than the untreated primary sludge. This is reasonable considering the much longer detention time for solids in a pond, expressed in years rather than hours as with primary sludge settling periods. The longer detention time would provide consolidation of solids and digestion in warm climate such as Jordan. The pH values are mid-range of pH values reported for typical digested primary sludge. This is one of the advantages of using anaerobic ponds in treating domestic wastewater.

TABLE 9.7. Typical chemical composition and properties of untreated and digested sludge: (USEPA, 1979)

Item	Untreated primary sludge		Digested primary sludge		Activated sludge
	Range	Typical	Range	Typical	Range
Total dry solids (TS), %	2.0-8.0	5.0	6.0-12.0	10	0.83-1.16
Volatile solids (% of TS)	60-80	65	30-60	40	59-88
pH	5.0-8.0	6.0	6.5-7.5	7.0	6.5-8.0

Table 9.8 presents the results of calculations to estimate the TS that have entered each pond over its operational period, the TS present at the time of sampling the sludge layer, and the percentage of solids unaccounted for. These values were determined using the following equations that have been used by Schneiter *et al.* (1993):

$$TS_i = SS_i \times Q \times t\,(365)\,10^{-3} \qquad \textbf{(eq. 2)}$$

Where
TS_i = total solids into pond over operating time, t, kg;
SS_i = influent suspended solids concentration, mg/L;
Q = average daily inflow, m^3/d; and
t = operating time of lagoon, years.

$$TS_p = A_b\, d \times TS_{mc}\,(10^{-2}) \qquad \textbf{(eq. 3)}$$

TS_p = total solids present in the pond at the time of sampling, kg
A_b = bottom area of pond, m2;
d = depth of sludge layer, cm; and
TS_{mc} = measured concentration of total solids in the sludge layer, g/kg.

$$TS_u = \{1 - (TS_p/TS_i)\}100 \qquad \textbf{(eq. 4)}$$

TS_u = percent total solids unaccounted for, kg.

It is possible only to speculate on the fate of the solids unaccounted for, but some conclusions can be drawn from Table 9.8. Apparently, the solids accumulation in ponds operating in similar climates and conditions even with different operation time (as A1-1 pond, operated 21.5 months after the operation of A2-1, and A3-1) showed no difference. This is not in agreement with Schneiter *et al.* (1993), who found that the solids accumulation in ponds operating in similar climates can differ greatly, in cold regions such as Alaska.

From Table 9.8, the primary anaerobic ponds at Al-Samra had lost around 94-95% of the solids that entered the ponds over the operation period (A1-1 = 5 years; and A2-1 and A3-1 = 7 years). The major "loss" of solids may contribute to successful anaerobic digestion to the organic material which produce simple forms of end products, gases and water. Also, it is suggested that the ponds are undersized and that significant short-circuiting was taking place, allowing solids to pass out of the ponds with effluents to the secondary anaerobic ponds, which is confirmed by the data in Tables 9.3 and 9.4.

TABLE 9.8. Al-Samra primary anaerobic ponds solids summary

Pond	Total solids entering, *$TS_i \times 10^3$ kg	Total solids present, **$TS_p \times 10^3$ kg	Solids unaccounted for, % ***(TS_μ)
A1-1	90403	5524.1	93.9
A2-1	126564	8031.8	93.7
A3-1	126564	5748	95.4

* See equation 2, above,
** See equation 3, above,
*** See equation 4, above.

TABLE 9.9. Physical characteristics of sludge samples from Madaba WSP's

Parameter Average (Range)	Unit	Anaerobic	Facultative	Maturation
ORP	mV	-379 (-200 to -474)	-160 (-73 to -162)	-83 (-60 to -96)
pH	-	6.8 (6.5-7.1)	7.2 (6.9-7.6)	7.8 (7.3-8.9)
TS	g/kg	108 (84-140)	87 (65-119)	57 (32-92)
TS	%	10 (6-14)	7 (3-12)	5 (2-9)
TVS	g/kg	51 (32-76)	42 (28-61)	25 (16-41)
TVS	(% of TS)	47 (39-55)	48 (43-52)	43 (34-54)

From the conventional sewage treatment plant i.e. Jerash, the sludge was grey in colour, and had an extremely offensive odour. The pH values ranged between the 6.4-6.9. Table 9.9 and 9.10 shows the physical characteristics of sludge samples from Madaba WSP and Jerash Treatment Plant, respectively.

TABLE 9.10. Physical characteristics of sludge samples from Jerash oxidation ditch

Parameter Average (Range)	Unit	Settling tank	Thickener	Drying bed
pH	-	6.5 (6.4-6.6)	6.5 (6.4-6.6)	6.8 (6.5-6.9)
TS	%	0.8 (0.6-1.4)	4.5 (3.5-5.5)	11 (11-12)

9.3 Discussion

After 4 years (from March 1989 till March 1993), the average depth and quantity of the sludge in A2-1 anaerobic pond, in this study, was 1.7 times higher than in previous studies (Miqdadi, 1989; Saqqar & Pescod 1994). Based on the measured rate of sludge deposition in the anaerobic ponds, it is projected that the sludge has reached a point where it should be removed (i.e. more than the design limit average of 2 meters). Also there was a difference in the sludge contouring map in pond A2-1 between this study and previous studies (Miqdadi, 1989; Saqqar & Pescod, 1994); this shifting seems to indicate that gas production resuspends bottom sludge and redistributes it again at the bottom of the pond.

The accumulation of sediments in wastewater stabilisation ponds can affect their performances by reducing the pond volume and shortening the hydraulic retention time (Schneiter *et al.*, 1984), by the feedback of soluble organic matter (Bryant & Bauer, 1987, Saqqar & Pescod, 1993) and by the resuspension of particulate matter, increasing turbidity and suspended solids concentration in the overlying water (Parker & Skerry, 1968). As a consequence, periodic desludging of the ponds is necessary. The frequency of desludging depends on the sludge accumulation rate which is the result of the deposition and the decomposition of particulate matter. The rates of both processes are not well known.

The Al-Samra WSP influent deposits much of its suspended solids contents in the anaerobic ponds. There has been no sludge removed from these ponds since the system was commissioned in 1985. From the literature, it is advisable to empty the sludge from the ponds every two years; Ghosh and Kshirsagar (1982) found higher NPK values in the first two layers of the sludge i.e. the deposited solids in last two years of operation of the sewage stabilisation ponds when compared with the deepest layers. They therefore recommended the first two layers of the sludge bed from WSP's should be used as fertiliser for croplands after proper treatment, while the deposits of the lower layers are useful for conditioning sandy as well as lower grade of solids. This is also in agreement with the findings of Carre and Baron (1987).

When sludge is removed, the overall hydraulic retention time in the system will be increased, sludge will not be swept into subsequent ponds during wet-weather flow, and organic loading on the facultative ponds will be reduced. Thus, the average final effluent quality should improve. Comprehensive study has been made of the function of solids in lagoon treatment of wastewater. This work has been reported in the paper by Parker & Skerry (1968). These studies have confirmed earlier observations that the presence of accumulated sludge solids in anaerobic ponds is essential for maximum BOD removal.

The anaerobic ponds in the Al-Samra system probably showed the highest sludge depth values that have been recorded in the literature. Oswald *et al.* (1959) study the drained lagoons of 1.2m depth that had been receiving wastes for about 20 years. About 45cm of sludge was found at the inlet end and this tapered down to about 2.5cm near the outlet end.

Sludge accumulation in anaerobic wastewater stabilisation ponds varies considerably, depending primarily upon the quantity and characteristics of wastewater, the degree of destruction of organic material by digestion, and the concentration of solids carried with effluent, also on detention time, deposition of solids and pattern of deposition throughout the ponds. Consequently, sludge accumulation in ponds depends on the type of community served; the degree of industrialisation and the amount of commercial wastewater reaching the plant; strength of wastewater; the temperature of wastewater and of the atmosphere which affects the destruction of solids and may affect the performance of the pond. These factors will result in different sludge accumulation in different communities.

This investigation showed that sludge is not distributed equally all over the pond. This situation suggested that turbulent flow conditions are occurring at different locations in the anaerobic ponds. Also from this sludge distribution it was concluded that short-circuiting is taking place and flows converges in the vicinity of the inlet and outlet, while it diverges in the middle of the pond. Dead zones are suggested to be around converging and diverging locations. In the facultative ponds nearly uniform settling is taking place, which would indicate that horizontal velocity is nearly zero. Thus no scouring in the sludge layer will occur.

The assumption that all biodegradable VSS will be destroyed on storage for a long time is also supported by EPA (1979), which stated that complex organic molecules may eventually be degraded if held for several months in facultative lagoons. Within the contact time normally associated with anaerobic digesters, volatile solids reduction usually ranges between 35%-60% (EPA, 1979) or 40%-60% (WPCF, 1985). Data presented by Parker (1970) show 60% reduction of total suspended solids deposited in different anaerobic ponds in Australia.

The intensity of anaerobic fermentation in the sludge layer can be estimated by measuring the rate of evolution of gas from the layer (Marais 1970). The gas evolution below 15°C is negligible, but as the temperature increases above 20°C the rate is much higher. At Al-Samra anaerobic ponds sludge temperatures of around 20°C, it is clear that gas evolution occurs in the pond, and that sufficient gases are trapped in the sludge to buoy the sludge to the surface where it forms drifting mats, and the sludge particles settle again to the bottom.

The anaerobic ponds in the Al-Samra system were designed to be desludged when the depth of sludge reached two meters (Binnie & Partners, 1983). The effective detention time and hence the performance of the pond was basically designed on such criteria.

According to the figures shown in Tables 9.3 and 9.4, especially for primary anaerobic ponds, these indicate that the ponds need desludging as soon as possible. According to the design (Binnie & Partners, 1983) the ponds should be desludged after 12.5 years of operation i.e., at the end of 1998. The difference between the design and the results of this research may be for the following reasons:

1. The design assumed that the sludge will be distributed equally in six ponds, indicating that 50% of the removed solids is deposited in the primary anaerobic ponds. The actual performance of the ponds revealed that the quantity of sludge solids that had settled in the primary anaerobic ponds were 1.5 times more (average sludge volume production = 61694 m^3) than in the secondary anaerobic ponds (average sludge volume production = 36758 m^3).

2. The design assumed that the three trains of ponds will be operated simultaneously. The actual operation was different. Train number one was operated 21.5 months after the operation of the second and third trains. It has been in the operation for 6 years while ponds A3-1, A2-1 have been operated for 8 years.

3. The design assumed the population served by Al-Samra WSP, would be 650,327 population equivalents over all the years of operation. The population equivalent served by the plant has changed dramatically from 496,000 in 1985 to 1,352 000 in 1990.

4. The WSP were originally designed to receive 68,000m^3/d, while the flow reached an annual average of nearly 81000m^3/d in 1988, and 120,000 m^3/d in 1992.

CHAPTER TEN

TRACE METALS IN SEWAGE SLUDGES OF JORDAN

10.1 Introduction

Reliable information on sludge composition is needed when designing land application systems in order to minimise the potential for environmental or health problems. Selection of an appropriate technique for sludge disposal depends, to a large extent, on the characteristics of the sludge. The application of liquid or dewatered sludge on land is one of the most effective and attractive methods (Webber *et al.*, 1984) because it has a relatively high content of nutritive elements such as Ca, Mg, P, N, and organic carbon. However, there is a risk that toxic constituents in sludge, such as trace metals and chlorinated hydrocarbons, may accumulate in soil and contaminate ground water or crops and enter the food chains (Dacre, 1980).

Trace elements identified as potentially harmful to plant growth, or as elements whose concentration in crops may reach levels considered to be hazardous to humans and animals, include: Al, As, B, Cd, Cr, Cu, Fe, Pb, Hg, Mn, Mo, Ni, Se, Sb, and Zn. In general, only Cd, Cu, Mo, Ni, and Zn are considered to pose a potentially serious hazard to either crops or the food chain (EPA, 1983).

A variety of factors influences the heavy metal composition of sludges, including the proportion of industrial and residential input, the amount of urban runoff, and the combination of treatment processes used. Thus, sludge composition is variable from one city to another, and even over time at a specific treatment plant.

The term "heavy metals" is used to denote several of the trace elements present in sludge. Concentrations of heavy metals may vary widely, as indicated in Table 10.1. For land application of sludge, concentrations of heavy metals may limit the sludge application rate

and the useful life of the application site. Table 10.1 lists ranges and medians for metals in sludges.

TABLE 10.1. Typical metal content in wastewater sludge (USEPA, 1984)

Metal	Dry sludge (mg/kg)	
	Range	Median
Arsenic	1.1-230	10
Cadmium	1-3,410	10
Chromium	10-99,000	500
Cobalt	11.3-2,490	30
Copper	84-17,000	800
Iron	1,000-154,000	17,000
Lead	13-26,000	500
Manganese	32-9,870	260
Mercury	0.6-56	6
Molybdenum	0.1-214	4
Nickel	2-5,300	80
Selenium	1.7-17.2	5
Tin	2.6-329	14
Zinc	101-49,000	1700

Geochemically, trace elements occur in the natural environment in small concentrations. Physiologically, many (e.g. Cu, Zn, Mo, B) are essential to most living organisms, again in small quantities. Metals can be classified according to the principle of "hard" and "soft" acids and bases (Pearson, 1973) or as class A, class B, and borderline ions (Nieboer & Richardson, 1980). Class A cations (hard acids) preferentially bind to oxygen-containing ligands rather than to nitrogen- and sulfur-containing ligands, whereas the sequence for class B cations (soft acids) is sulfur-containing, nitrogen-containing, and then oxygen-containing ligands. Borderline ions have both class A and class B properties and a high affinity for both oxygen- and nitrogen-containing ligands, and they can also bind to sulfur-containing ligands, especially to sulfhydryl (-SH) groups. Hard acids usually bind to hard bases, and soft acids usually bind to soft bases (Collins & Stotzky, 1992).

Some metals that are classified as class A (e.g., Ca, K, Mg, and Na) are essential for organisms. Some metals (e.g., Cu, Fe, Mn, and Zn) that are required in trace concentrations (micronutrients) are classified as borderline ions. Some metals (e.g., Hg and Pb) that are considered to be pollutants, as they are not necessary for biological functions and are toxic, are classified as class B.

Concentrations of metals are primarily a function of the type and amount of industrial waste that is discharged into the municipal wastewater treatment system. Contamination of raw sewage with heavy metals may potentially cause problems of three types (Lester, 1983):

- Toxic effects on the secondary biological treatment process.
- Through the discharge of final effluent containing excessive concentrations of heavy metals.
- During sludge treatment and disposal.

The chemical characterisation of the sludge affects the following design decisions (EPA, 1983):

- Whether the sludge can be cost-effectively applied to land.
- Which land application options are technically feasible.
- The quantity of sludge which can be applied per unit area of application site, both annually and cumulatively.
- The degree of regulatory control and system monitoring required.

The acidity of a sludge (measured by pH) affects the availability of heavy metals, the pathogen content of the sludge, and the corrosivity of the sludge. High pH (greater than 11) sludges destroy many bacteria and, in conjunction with soils of neutral or high pH, can inhibit movement of heavy metals by plants. Conversely, low pH (less than 6.5) sludges promote leaching of heavy metals and promote greater crop uptake of metals. Leaching of heavy metals can occur at landfills because acid conditions often prevail. Thus, pH affects the suitability of sludge for land application, distribution and marketing, and landfilling (EPA, 1989).

In general, the phytotoxic tolerance of plant species to heavy metals concentrations added to soil and the amounts accumulated by various plant species are highly variable. The amounts present in plant-available form are seemingly more important than the total

quantity in soils. Also the margin between levels considered essential to plant growth and those considered phytotoxic is usually very narrow (EPA, 1983).

There has been no effort to compile information on the composition of sewage sludges in Jordan in the past, consequently there was no established data base concerning the variability of sewage sludges produced in different locations and by different types of sewage treatment processes.

This study was undertaken to determine levels of toxic trace metals in sludges produced at the main wastewater treatment plants in Jordan (Al-Samra WSP, Madaba WSP, and Jerash oxidation ditch treatment plant) and to make a preliminary evaluation of the amenability of these sludges for disposal on land.

The objective of the study described in this chapter is to evaluate the suitability of Jordanian sewage sludges from anaerobic ponds WSP's systems and oxidation-ditch sewage treatment plant for agricultural use, as far as heavy metals are concerned.

10.2 Results

Sludge samples collected from Al-Samra anaerobic WSP's (primary and secondary anaerobic ponds) in November 1992 - March 1993 were analysed for Al, Ag, As, B, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Ni, Pb, Se, Si, Sn, Ti, V, and Zn. In the same period sludge samples collected from Madaba WSP's and Jerash treatment Plant (oxidation ditch) were analysed for Cd, Cr, Cu, Fe, Mn, and Zn only.

For most elements it can be seen that the majority of the samples fall into one or two adjacent concentration ranges, i.e. within a 10- or 100- fold spread. Due to the variable nature of solids content in sludge and the tendency for numerous sludge constituents to be associated with the solid fraction, all sludge composition data should be expressed on an oven-dry solids basis rather than on a wet weight basis (Sommers *et al.*, 1976).

10.2.1 Trace metals in Al-Samra anaerobic ponds sludges

The grand mean, median and range concentrations of twenty trace metals, expressed in terms of dry sludge solids, are shown in Table 10.2 for Al-Samra anaerobic ponds. In order to give a better indication of the distribution of the trace elements in the various anaerobic ponds sludge samples, in addition to ranges and mean contents (arithmetic and geometric), median figures are also reported.

When mean and median values do not agree for many sludge constituents, this indicates that abnormally high or low values may be skewing the mean. The true central tendency for the concentration of a particular parameter in sewage sludges may be more adequately represented by the median rather than the mean (Sommers, 1977). Small differences between median and mean values for most trace metals that are measured in this study, indicate that the mean value is not markedly influenced by unusually high values in a small number of samples.

In an attempt to quantify the extent to which exceptional contamination of sludges occurred, the arithmetic mean:median ratios were calculated; these also are shown in Table 10.2. Higher mean:median ratios were considered to be a higher incidence of excessive contamination compared to what may be considered a normal or background level.

The means were similar to medians for the most of the trace elements in this study (except for Co); this possibly may indicate a normal distribution, without incidence of excessive industrial contamination compared to what may be considered a normal or background level. This may indicate the importance of pretreatment of industrial effluents before connected to the works, or may be one more of the benefits of anaerobic ponds in adsorbing shock loading of different industrial effluents discharge to WSP's. In this study, given the limitations imposed by the number of samples, the mean:median ratios indicated that the range of background concentrations of heavy metals in sludge could be altered by contamination with Co, and less so by Ag and Cr.

Comparing the Al-Samra anaerobic ponds total trace metal median concentrations in Table 10.2 to the median concentrations in primary sludges (Table 10.1) reveals high concentrations of Zn and Ti in the pond sludges. The remaining median total metal concentrations reported for the anaerobic ponds sludges lie below or within the range of median metal concentrations in primary sludges reported by USEPA (1984).

The high Zn concentrations which also exceed the inhibition concentrations (this will be discussed later), may be attributed to the accumulation of the metals in the organic sludge layers over a period of several years, and/or they may be the result of specific influent wastewater sources.

In addition to toxic metals from industrial sources, Li was considered as one of the elements not widely used in industry (Berrow & Webber, 1972); this is confirmed in this study, which the mean of Li were similar to the medians (Table 10.2), possibly indicating a normal distribution unaffected by industrial sources of pollution. Analysis of sludge from Al-Samra anaerobic ponds indicates that common mineral elements such as Al, Fe, and Si, are present in significant quantities.

Inspection of the ranges obtained indicates that extreme variability exists in sludge composition; this creates problems when attempting to generalise about the chemical composition of a sewage sludge. This is neither new nor unexpected, based on the available literature (e.g., Page, 1974; Sommers, 1977). This agree with Sommers (1977), in his statement that, unfortunately, the parameters that are most useful in controlling application rates on agricultural land, i.e., total N, NH_4, and metals, are also those parameters that exhibit the greatest variability.

The variability of sludge composition emphasises the need for a sound sampling and analysis program for sludge in Jordan. The reliability of sludge composition data is improved by obtaining samples of sludge over a 1 to 2 year period, if possible (EPA, 1983).

TABLE 10.2. Ranges, mean, and median of trace metals concentrations in Al-Samra primary and secondary anaerobic ponds sludge samples (mg/kg dry weight basis)

Element	Median	Geometric mean	Arithmetic mean	Range	♠mean : median ratio
Ag	5	5	7	* - 24	1.56
Al	8445	7798	8317	1991 - 13207	0.98
As	2	2	2	* - 3	1.08
B	34	34	36	11 - 89	1.06
Cd	5	5	5	* - 8	0.96
Co	3	5	8	2 - 51	**2.22**
Cr	190	222	263	83 - 669	1.38
Cu	248	231	245	65 - 362	0.99
Fe	16700	16899	17333	8590 - 23676	1.04
Hg	3	2.5	3	0.35 - 5	0.87
Li	4	4	4	2 - 6	1.05
Mn	139	127	133	40 - 175	0.95
Ni	51	47	49	14 - 68	0.97
Pb	160	152	157	58 - 211	0.98
Se	1	1.5	2	1 - 6	1.40
Si	388	402	474	* - 1028	1.22
Sn	0.45	0.39	0.41	* - 0.6	0.92
Ti	224	192	203	* - 316	0.91
V	85	70	78	* - 141	0.91
Zn	2530	2163	2353	433 - 3850	0.93

number of sludge samples = 17 for each metal.

* Concentration is below the detection limit of the element in the final digested solution

♠ Mean = arithmetic mean

10.2.2 Distribution of heavy metal concentrations in sludges of six anaerobic ponds in Al-Samra system

Two way analysis of variance (ANOVA) without replication of the trace metal data showed no significant difference in the concentration of trace metals between the primary and secondary anaerobic ponds in sludge samples ($F < 4.38$, $P = 0.42$) in the Al-Samra system. Due to overloaded conditions in primary anaerobic ponds, the secondary anaerobic ponds are important in helping removal of the trace metal from the wastewater (Table 10.3). As shown in Table 10.3, the removal of Ag, Cr, Hg, and Zn metals from the secondary anaerobic ponds was 2.8, 2.3, 1.8, and 1.6 times more efficient than in primary anaerobic ponds, respectively. However, Co and Si had higher removal efficiency in the primary ponds (by 2.4, and 2.0 times, respectively) than the secondary anaerobic ponds.

The concentrations of trace metals in sludge from each anaerobic pond in the Al-Samra system has been shown in Table 10.4. The distribution of trace metals were not associated with any significant difference between each primary (A1-1, A2-1, and A3-1), or between each secondary (A1-2, A2-2, and A3-2) anaerobic ponds ($F < 3.24$, $P = 0.07$; $F < 3.26$, $P = 0.08$), respectively.

The concentrations of trace metals in relation to depth and surface sludge samples from different primary and secondary anaerobic ponds in the Al-Samra system are given in Table 10.5 and 10.6. Zinc concentrations in the deep sludge samples were greater in all primary anaerobic ponds sites compared to the surface sludge samples. Cadmium concentrations followed distribution patterns similar to those of Zn.

Si concentrations in all cases at surface sludge samples were less than the limits of analytical methods, however, in the deep sludge samples the Si values were very high comparing with the surface sludge samples level, this may indicate movement of Si to a lower depths rather than staying at the surface. Highest removal rate for this metal occurred in the primary anaerobic ponds, while the other metals continued to be removed later by the secondary anaerobic ponds in the Al-Samra system.

It is evident that the concentrations of Ag, Co, Cr, Se, and Ti metals from sludge samples on the surface were double the concentrations in depth samples. In contrast, the concentrations of As, and Hg were 1.9 and 1.8 times, respectively, more in deep sludge compared with surface sludge samples.

TABLE 10.3. Total geometric mean concentrations of trace metals in Al-Samra primary and secondary anaerobic ponds sludge samples (mg/kg dry weight basis)

Element	Total*	Primary	Secondary
Ag	4.61	3.02	8.33
Al	7798	7611	8267
As	1.55	1.45	1.77
B	34	33	34
Cd	4.57	4.05	5.97
Co	4.63	6.00	2.48
Cr	222	174	400
Cu	231	215	277
Fe	16899	17074	16488
Hg	2.49	2.10	3.74
Li	3.58	3.45	3.88
Mn	127	124	137
Ni	47	49	42
Pb	152	147	165
Se	1.46	1.47	1.43
Si	402	523	271
Sn	0.39	0.37	0.40
Ti	192	213	156
V	70	59	96
Zn	2163	1894	2974

*Geometric mean of all sludge samples results of each metal from all primary and secondary anaerobic ponds.

TABLE 10.4. Characterisation of each anaerobic ponds sludge trace metals in the Al-Samra system (mg/Kg dry weight basis) *

Element	Primary anaerobic ponds			Secondary anaerobic ponds		
	A1-1	A2-1	A3-1	A1-2	A2-2	A3-2
Ag	4	3	2	3	11	11
Al	10511	5119	8904	10860	8732	6829
As	2	1.20	2	2	2	2
B	36	32	33	38	35	32
Cd	6	3	4	8	6	5
Co	3	7	7	3	2	2
Cr	211	137	190	579	348	383
Cu	300	153	241	362	292	229
Fe	18682	14157	18772	21400	17065	13982
Hg	4	1.33	2	4	4	4
Li	4	3	4	6	4	3
Mn	146	92	143	175	141	117
Ni	54	40	54	51	47	34
Pb	182	118	157	209	174	139
Se	0.88	1.40	1.81	2.20	1.24	1.34
Si	298	467	1028	**	189	388
Sn	0.50	0.20	0.50	0.30	0.35	0.55
Ti	216	182	223	227	138	146
V	99	66	46	141	100	77
Zn	2830	1317	2112	3850	3110	2500

*Results expressed as geometric mean concentration, $n \leq 4$

** Concentration is below the detection limit of the element in the final digested solution

TABLE 10.5. Geometric mean concentrations of trace metals in all surface sludge samples of Al-Samra primary and secondary anaerobic ponds
(mg/kg dry weight basis)

Element	Total (surface) *	Primary (surface)	Secondary (surface)
Ag	6.94	4.83	11
Al	7602	7335	8459
As	1.26	1.18	1.50
B	34	33	37
Cd	4.31	3.65	6.71
Co	5.65	7.76	2.18
Cr	248	188	572
Cu	221	199	304
Fe	17607	17514	17890
Hg	2.09	1.68	4.03
Li	3.91	3.61	4.83
Mn	127	120	151
Ni	45	45	45
Pb	151	142	181
Se	1.98	1.99	1.96
Si	**	**	**
Sn	0.36	**	0.36
Ti	233	250	199
V	64	48	115
Zn	1956	1680	3089

* Geometric mean of all surface sludge sample results of each metal from all primary and secondary anaerobic ponds.

TABLE 10.6. Total geometric mean concentrations of trace metals in deep sludge samples of Al-Samra primary and secondary anaerobic ponds (mg/g dry weight basis)*.

Element	Total (deep)	Primary (deep)	Secondary (deep)
Ag	2.60	1.61	5.32
Al	8292	8501	7987
As	2.43	2.53	2.28
B	32	34	31
Cd	5.22	5.36	5.02
Co	2.86	2.78	3.00
Cr	170	137	235
Cu	258	270	240
Fe	15314	15818	14588
Hg	3.79	4.13	3.35
Li	2.95	3.07	2.79
Mn	129	137	118
Ni	51	61	38
Pb	153	160	144
Se	0.71	0.60	0.90
Si	402	523	271
Sn	0.41	0.37	0.49
Ti	130	146	108
V	83	90	74
Zn	2753	2716	2810

* Except Pond A1-2, no deep sludge sample could be obtained from this site because sample insufficiently viscous to stay in the sludge sampler pipe as the pipe withdrawn from the pond.

10.2.3 Efficiency of metal removal from wastewater in Al-Samra WSP's system

The concentration of total heavy metals in the influent, subsequent ponds and final effluent, and the removal efficiency (% removal) through the second train at Al-Samra WSP's System are shown in Table 10.7.

The As, Cd, and Co levels in the influent wastewater of the Al-Samra system were less than the instrumental detection limit; reflecting the lack of industrial effluent discharging these elements to Al-Samra influent.

For Al, Cr, Cu, Hg, Pb, and Zn the removal efficiency from both primary and secondary anaerobic ponds together, were typically greater or equal to 75%; the highest removal value was recorded for Al (83%), with the exception being chromium (33%). It seems from these results that the primary and secondary anaerobic ponds play a more important role in the removal of most trace metals that have been examined (Table 10.7), especially for Pb, Hg, and Cu, than the subsequent facultative and maturation ponds.

This is in agreement with Lester (1983), who found that the Pb, Cu, and Zn were the metals most readily removed in primary sedimentation tanks, whilst Ni and Cr were the least readily removed. Brown *et al.* (1973) found that Cr, Cu, and Pb were removed more efficiently from wastewater in secondary treatment plants than in the primary treatment process. The enlarged particles of activated sludge secondary treatment microbial floc served to absorb all chromium forms and reduced soluble Cr^{+6}, as well as absorbing finely-divided suspended particles containing chromium.

The trace metal concentrations in Al-Samra anaerobic ponds sludge samples range from 40-600 times more concentrated than the influent wastewater trace metal concentrations. This indicates that the highest concentrations of most trace metals will be found in the sludge layer. From the literature (Moshe *et al.*, 1972; Jenne & Luoma, 1977) it appears that the removal of heavy metals in a pond system would include several mechanisms such as complexation with organic and inorganic compounds, adsorption, precipitation, and sedimentation.

Metal removal efficiencies from wastewater have been found to be 80% for Cd, 60% for Cu, 79% for Pb, 1% for Ni, and 50% for Zn (Oliver & Cosgrove, 1975). These are merely estimates because other treatment plants may be more or less efficient in metal removal (Brown *et al.*, 1973). Also they found that metals such as Zn, Cu, Cd, Ni, and Pb are present largely in the solid phase rather than in solution, which is in agreement with the results of this study. Even though total concentrations may be > 1000 mg/kg, soluble metals are commonly < 5 μg/ml (Bradford *et al.*, 1975; Sommers *et al.*, 1972). Exchangeable, sorbed, organic-matter bound, carbonate, and sulfide forms of Cu, Zn, Cd, Zn, Pb are all present, in varying proportions, in anaerobic sewage sludges (Stover *et al.*, 1976). These chemical reactions remove the ions from the solution and concentrate metals in the solid phase (EPA, 1983). The black colour associated with anaerobically digested sludges is attributed to the formation of insoluble FeS (EPA, 1983).

Comparing the literature studies on the concentration of heavy metals in wastewater, the results of this study showed that the heavy metals concentrations in UK (Lester, 1983), and USA (Brown *et al.*, 1973) raw wastewater and sludge samples were much higher than Jordan values, results that reflect the lower level of industrialisation in Jordan.

Copper, zinc and nickel are known to retard the anaerobic process due to metal toxicity. The concentrations that are toxic to a particular system is dependent upon the chemical composition of the waste stream. To be toxic these metals must be in solution. These metals easily form insoluble salts by reacting with hydroxides, carbonates, sulfides or phosphates. These anions are common in anaerobic systems and in most cases metal toxicity is not a problem. However, waste streams containing high concentrations of the heavy metals or complexing agents can develop sufficiently high concentrations of soluble metals to cause toxicity (Pfeffer, 1970).

TABLE 10.7. Metal concentrations in raw wastewater and their % removal through the second train in Al-Samra WSP System

Pond	Heavy metals concentration (mg/l)								
	Al	As	Cd	Co	Cr	Cu	Hg	Pb	Zn
Influent*	1.7	<0.002	<0.005	<0.02	0.03	0.08	0.0023	0.08	0.78
A2-1	0.8	<0.002	<0.005	<0.02	0.04	0.06	0.0008	0.02	0.42
A2-2	0.3	<0.002	<0.005	<0.02	0.02	0.02	<0.0005	0.18	0.18
F2-1	0.2	<0.002	<0.005	<0.02	0.01	0.01	<0.0005	0.03	0.12
F2-2	0.2	<0.002	<0.005	<0.02	0.02	0.01	<0.0005	<0.02	0.10
F2-3	0.1	<0.002	<0.005	<0.02	0.02	0.01	<0.0005	<0.02	0.09
F2-4	<0.1	<0.002	<0.005	<0.02	0.02	0.01	<0.0005	<0.02	0.10
M2-1	<0.1	<0.002	<0.005	<0.02	0.01	0.02	<0.0005	<0.02	0.10
M2-2	<0.1	<0.002	<0.005	<0.02	0.02	0.01	<0.0005	0.07	0.11
M2-3	<0.1	<0.002	<0.005	<0.02	0.01	0.02	<0.0005	0.02	0.10
M2-4	<0.1	<0.002	<0.005	<0.02	0.02	0.02	<0.0005	0.03	0.10
Effluent	<0.1	<0.002	<0.005	<0.02	0.01	0.02	<0.0005	<0.02	0.09
Removal (%)♦	82	-	-	-	33	75	78	75	77

Note: The sign (<) followed by a number indicates that the element concentration is below the instrumental detection limit.

* Average of heavy metals concentrations from Ain-Ghazal, Zarqa, and Hashmia domestic sewage inflow city site, before entering Al-Samra WSP.

♦ Removal percentage from both primary and secondary anaerobic ponds; except for Pb metal only from primary anaerobic ponds.

The most important anion in the control of metal toxicity is sulfide. Metal sulfides salts have very low solubilities. Additional sulfide can easily be added to the process for control of metal toxicity.

Collins and Stotzky (1992) results suggested that the toxicity of some heavy metals to microorganisms varies with pH, because the hydrolysed speciation forms of these metals, which occur at higher pH values, bind on the cell surface and alter the net charge of the cell. This change in charge could affect various physiological functions of the cell, as well as its interactions with other cells and inanimate particulates in the environment.

Toxic metals tend to be associated with the suspended solids of sewage (El-Nennah & El-Kobbia, 1983) and thus would not limit the use of wastewater for irrigation or recharge. This implies that the heavy metals are more concern in the reuse of sludge in agriculture than in the reuse of effluents.

Wasay *et al.* (1993) studied the interaction of heavy metal ions (Mn, Cr, Ni, Cu, Zn, Cd and Pb) with the extracted organic matter from the sludge in a sewage oxidation pond. The results showed that the distribution of heavy metals was between 60 to 97% associated with the solid waste (sludge) of the oxidation pond. The adsorption/removal efficiency of metal ions onto the sludge ash was more than 90% and 97%, respectively, in the pure system. They found good agreement suggesting that sediment and humic/fulvic acids have an important role in the mobility, dispersion and sedimentation of metal ions in an aquatic environment.

10.2.4 Comparing metal levels from three different wastewater treatment plant sludges

Table 10.8 shows the results of the metal concentrations of the sludges from the three sewage treatment plants in Jordan. The data are summarised presenting the geometric mean values, expressed on a dry weight basis, of the different trace metals. In general the sludges produced in the three treatment plants present a very variable composition. Similar variation in the composition of sludges from other geographical areas were found by several authors (Page, 1974; Sommers, 1977).

The sludge metal concentrations are not uniform because sewage sludge is a product having a relatively variable composition. The metal concentrations in Al-Samra anaerobic ponds sludge samples, as well as Madaba WSP's, were found to be within the range of metal contents in wastewater sludges reported by USEPA (1984) (Table 10.1). The exception was Jerash treatment plant, were all the metal concentrations were lower than the USEPA range detected level from different wastewater sludges.

Considering the geometric average levels of metals in Jordanian sludges from the three different treatment plants (Table 10.8) the following comments can be made. Sludges were primarily domestic from Jerash and Madaba treatment plants. The Jerash oxidation-ditch system had the lowest concentrations of all metals examined. The Al-Samra anaerobic ponds sludges had the highest concentrations of metals, for example Fe, 16899mg/kg dry weight; Zn, 2163mg/kg dry weight; and Cu, 231 mg/kg dry weight (Table 10.8).

As the wastewater proceeds through a treatment system, trace elements gradually accumulate in the wastewater sludges with time; this may explain the higher concentration of trace metals in Madaba WSP's compared with the treatment plant at Jerash, and/or may also be due to lack of industrial contribution to the raw sewage.

Considering the data in Table 10.8, the sludges produced from industrial cities (Amman and Zarqa) contained much higher concentrations of Zn, Cu, Cr. Thus, the sludges produced in cities without industrial activity (Madaba and Jerash) were suitable for agricultural utilisation, while sludges obtained from Al-Samra may have some limitations to agricultural purposes.

TABLE 10.8. Comparison of geometric means of metal concentrations in sludge samples from the three wastewater treatment plants (mg/kg dry weight basis)

Element	Treatment plant		
	Jerash (n = 3)	Madaba (n = 3)	Al-Samra (n = 17)
Population served	16000	19000	1352000
Cd	*	3	5
Cr	*	1.2	222
Cu	21	21	231
Fe	111	1725	16899
Mn	9	97	127
Zn	74	293	2163

* Concentration is below the detection limit of the element in the final digested solution

It is clearly observed that there were relationships between the quantity of wastewater treated and the trace metal concentrations, which is increased with increasing volume of wastewater entering the plant; the lowest metal concentrations were found in Jerash treatment plant which had the lowest volume of wastewater flow (1500 m^3/d, Table 6.1, chapter six). Al-Samra anaerobic ponds had the highest metal concentrations in sludge, due to the large volume of wastewater inflow to the system (120000 m^3/day), which serves more than one million population as well as many industrial effluents.

It is not always true that with increasing size of the plant a high level of trace metals should be expected in sludge. This was the conclusion in the EPA (1983) report, when they compared the relation between quantity of wastewater treated and the chemical composition of sludges in more than 60 treatment plants in Indiana City. They found that the proportion and specific nature of industrial and other non domestic inputs into the sewage treatment plants were of greatest significance.

It is recognised that the heavy metal concentrations in sludges are not constant, even within a single treatment facility. This variation has been discussed by numerous authors including Page (1974), Bradford *et al.* (1975), and Sommers *et al.* (1976). The variability in metal content of sludges from city to city is a reflection of the variability of sources of metals entering the various treatment plants.

Since it appears that metal levels in sludges may be one of the principal factors in determining acceptable rates of sludge application to land, good environmental management will require the identification and regulation of major sources of metals to sewage treatment plants in Jordan. In this regard, it should be noted that the present data show a significant correlation between degree of industrialisation and elevated metal levels in sludges. The principal non-industrial sources of metals to sewage systems are probably plumbing fixtures and street runoff. Also it seems that there was relatively satisfactory treatment of industrial wastewater, prior to its mixture with domestic wastewater in the system of Al-Samra.

The data also indicate that the typical sewage sludge from a city relatively free of industrial inputs will still have adequate metal levels to warrant carefully controlled application rates to soils. Metal constituents present in plumbing systems, urban runoff, etc., can continue to enter the waste treatment system, resulting in a sludge containing significant amounts of metals.

10.2.5 Sludge trace metal concentration in Jordan and guidelines

The use of sewage sludge on agricultural land must be regulated to minimise heavy metal contamination of soils. Current sludge use guidelines for control of heavy metal contamination in soils are based on different assumptions (Webber *et al.*, 1983). In this chapter, the assumption is that heavy metal concentrations in sludge applied to agricultural land should not exceed defined limits. The guidelines for maximum permissible heavy metal concentrations in sludge considered to be acceptable for use on agricultural land are used to assess the analysed sludge suitability for utilisation on agricultural land.

Except for Zn in Al-Samra anaerobic pond sludge samples, levels of trace metals in Jordanian local sludges from Al-Samra anaerobic ponds, Madaba WSP's, and Jerash wastewater treatment plant sludges (Tables 10.2 and 10.8) are significantly lower than the recommended limit values of USEPA and EEC for application on agricultural land (Tables 10.9 and 10.10).

The mean and/or maximum Zn level in Al-Samra anaerobic ponds sludge samples, were higher than the suggested limit from several countries such as USEPA, Canada, France and Germany. On a 5% confidence level the Zn concentration is below EEC and USEPA mandatory guidelines, but the upper 5% confidence interval (2732 mg/kg dry weight basis) exceeds Canada recommended maximum limit (1850 mg Zn/kg dry weight).

On the other hand, the upper 5% confidence interval of the Co level is within the recommended limit values of all these countries. While the maximum range of Co levels (51 mg/kg dry weight basis) in Al-Samra anaerobic ponds sludge samples, were higher than the suggested limit from Sweden (50 mg/kg dry weight basis).

Table 10.9 and 10.10 show the various limits of metal concentrations in sludge samples which is recommended for use on agricultural land from different countries, included three European Countries, Canada, and USEPA (regulations 40 CFR Part 257,1992) and EEC (directive 86/278/EEC) guidelines.

Data on the ranges and typical trace element levels found in normal agricultural soils, unaffected by waste from mining or other industrial are presented in Table 10.8. Comparing the findings from this present study indicates that sludges contain (on a dry matter basis) values similar or less to those found in soils (Table 10.2) for Co, Fe, Li, Mn, Ni, Sn, Ti, V from Al-Samra anaerobic ponds, for Cr, Cu, Fe, Mn from Madaba; all metals tested from Jerash (Table 10.7).

TABLE 10.9. Guidelines on pollutant concentrations in sewage sludge accepted for use on agricultural land, together with comparable trace metal values for soils

Element*	EEC	USEPA (1992)		Soils (Total content)♠		Element
		♦Pollutant concentration Table 3 of §503.13	♦Ceiling concentration for the pollutant Table 1 of §503.13	Normal range	Typical value	
Ag	-	-	-	<1	<1	Ag
Al	-	-	-	-	-	Al
As	-	41	75	-	-	As
B	-	-	-	2-100	10	B
Ba	-	-		100-4000	1000	Ba
Cd	20-40	39	85	0.01-0.7	0.1	Cd
Co	-	-	-	1-40	15	Co
Cr	-	1200	3000	5-1000	100	Cr
Cu	1000-1750	1500	4300	2-100	20	Cu
Fe	-	-	-	10^4 - 2 x 10^5	40 000	Fe
Hg	16-25	17	57	-	-	Hg
Li	-	-		5-200	50	Li
Mn	-	-	-	100-3000	800	Mn
Mo	-	18	-	<1-5	1	Mo
Ni	300-400	420	420	5-500	50	Ni
Pb	750-1200	300	840	2-200	30	Pb
Se	-	36	100			Se
Si	-	-				Si
Sn	-	-		<1-10	3	Sn
Ti	-	-		10^3 - 2 x 10^4	4000	Ti
V	-	-		20-500	100	V
Zn	2300-4000	2800	7500	10-300	80	Zn

* Units mg/kg dry weight

* Annex 1B Limit values for heavy metal concentrations in sludge for use in agriculture, in "Directive Council on the Protection of the Environment, and in Particular the Soil, When Sewage Sludge is Used in Agriculture," 86/278/EEC, 12 June 1986, appearing in the Official Journal of the European Communities, L181, vol. 29 (4/July), pp 6-12.

♦ See Appendix 10.1, for more details.

♠ Berrow and Webber (1972)

TABLE 10.10. Standards or limits from different countries for concentrations of trace metals in sludge recommended for use on agricultural land

Element*	[b]Canada	[C]France	[a]Germany	[a]Sweden
Al	-	-	-	-
Ag	-	-	-	-
As	75	-	-	-
B	-	-	-	-
Cd	20	40	20	15
Co	150	-	-	50
Cr	-	1000	1200	1000
Cu	-	1000	1200	3000
Fe	-	-	-	-
Hg	5	10	25	8
Li	-	-	-	-
Mn	-	-	-	-
Mo	20	-	-	-
Ni	180	200	200	500
Pb	500	800	1200	300
Se	14	100	-	-
Si	-	-	-	-
Sn	-	-	-	-
Ti	-	-	-	-
V	-	-	-	-
Zn	1850	3000	3000	10000

* Units mg/kg dry weight; these guidelines are based on the assumptions that heavy metal concentrations in sludge applied to agricultural land may not exceed defined limits.

[a] Huckers (1980) Activities of Working Party 5 " Environmental Effects of Sludge" introductory remark. In Characterisation, Treatment and Use of Sewage Sludge. Ed. P. L'Hermite and H. Ott. D. Reidel Publishing Company, London. PP.624-636.

[b] In Municipal Sewage Sludge Management: Processing, Utilisation and Disposal. C. Lue-Hing, D. R. Zenz and R. Kuchenrither. Vol. 4, Water Quality Management Library. Technomic Publishing Co., Inc. Lancaster.1992.

[C]Water Research Center (1992) Sewage sludge: current disposal practice and future developments in selected countries. Report no. FR0265.

In Madaba WSP the levels of Cd and Zn are high; while the levels of Ag, B, Cd, Cr, Cu, Pb, and Zn from anaerobic ponds of Al-Samra were very high compared with the normal agricultural soil levels. This confirmed Sterritt and Lester's (1980) statement that sludge heavy metal concentrations may exceed soil concentrations by two orders of magnitude or more.

The range of A2-1 pond trace metal concentrations in sludge samples from this study were compared with the previous study on trace metal values in the sludge of the same pond (A2-1) done by Miqdadi (1989). Al, Cd, Co, Cu, Mn, Ni, and Pb were lower in the present study than in the study carried by Miqdadi (1989) (Table 10.11), while Fe, and Li concentrations had not increased since then. In contrast, Cr, V, and Zn concentrations were higher in the present study.

This may be indicative of some success in limiting industrial effluent discharges of metals of current interest while the same may not have occurred for metals of lesser interest or not included in the guidelines. Of the metals studied, Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, and Zn are included in European countries standards (Table 10.10) and USEPA and EEC (Table 10.9) guidelines and most of these metals have been of concern for several years. On the other hand, the results emphasise the need to restrict the discharge of effluents from leather tanning, ore mining and metal industries which might have caused the increase of Cr and Zn in Al-Samra anaerobic ponds sludges.

TABLE 10.11. Comparison the trace metal ranges of A2-1 primary anaerobic pond sludge samples with the previous study (Miqdadi 1989)

Element*	This study (1993)	Miqdadi (1989)♣
Al	1991-8550	3242-12227
Ag	1.1-9.1	-
As	0.61-2.6	1.67-5.08
B	11.3-88.8	32.9-38.2
Ba	-	177.3-307.6
Cd	0.86-5.3	3.94-7.84
Co	1.7-50.7	1.67-113.26
Cr	83.1-200	42.73-181.97
Cu	65-247	182-307
Fe	8590-21218	10023-21470
Hg	0.35-4	-
Li	1.7-3.8	1.52-3.64
Mn	40-142	81-162
Mo	-	5-54
Ni	13.8-68.4	28.4-110
Pb	58-185	98-366
Se	0.6-6.3	<5.10-10.70
Si	467-467	879-2924
Sn	0.2-0.2	-
Ti	140-236	-
V	49-89	30.4-37.7
Zn	433-2670	974.2-2222

♣ Primary anaerobic pond (A2-1) sludge in the Al-Samra system

* units mg/kg dry weight basis.

Discussion

Metals removed by the WSP treatment processes are concentrated in the sludge. In an anaerobic pond this sludge will be partially anaerobically digested during the storage time. Digestion reduces the volume of the sludge and further concentrates the metals. This is can explain the higher values of most metals (i.e. As, Cd, Cu, Hg, Ni, Si, V, and Zn) in the anaerobic pond's deep sludge samples than the surface sludge samples.

The presence and high concentration of one of those metals in sewage sludge samples can refer to the type of industries that are concentrated in the cities of Amman, Russaifa, Zarqa, and Sukhneh, and the pretreatment of industrial effluent before discharging to ASWSP system. More than thirty different industries including batteries, chlorine, soft drinks, yeast, detergent, beer, rubber, paper, leather tanning, plastic, paints, steel and casting industries, phosphate mining, and oil refining are based in the Amman-Zarqa catchment area.

In general, the high level of Zn found in Al-Samra anaerobic ponds calls for some comments. Contents up to 3000 mg/kg in the dry matter are normal even for sludges from sewage works receiving little or no industrial effluent (Berrow & Webber, 1972). The source of these high levels is not clear; zinc compounds present in pharmaceuticals, cosmetics, metal finishing, ore mining, inorganic chemicals, porcelain enamelling, steel and rubber may contribute; dissolution of Zn from galvanised metal by rainwater must play a considerable part. The source of Cd might be from the metal finishing industries, while Cr is mainly from the metal finishing and leather tanning industries. Copper, lead and nickel mainly derive from metal finishing, ore mining and inorganic chemicals industries.

Other metal, such as Co found in high value in some sludge samples at Al-Samra anaerobic ponds, could also be related to the presence of particular industries. Cobalt, Cu, Cr, and Ni probably come from electroplating, foundry processes and alloy production. Such unusual elements as Ag and Cd levels, normally present in soils in very small traces (Tables 10.2, 10.8 and 10.9), appeared in Al-Samra and Madaba but not Jerash sludge

samples. Halides of Ag are used extensively in the photographic industry and considerable quantities of Ag are also used for electroplating.

The increasing acidity of soils has proved to liberate the bound pool of metals, which may lead to increased availability and uptake of metal ions in plants (Pahlsson, 1989). When soil become acidic, Cd, Zn, and Ni from sludge application could seriously reduce crop yields (Chang *et al.*, 1981). However, the potential hazard and impact of trace metal elements may be minimised by maintaining favourable soil conditions, selecting the proper crop and carefully controlling the sludge inputs to supply adequate but not excessive nitrogen for the crop. Under suitable management, even repeated sludge applications do not appear to damage crops.

Chemical and physical properties of Jordanian soils (mainly from the Amman, Zarqa, King Talal area, and Jordan valley) were analysed by El-Khattari (1986); the results showed that the soils are calcareous with total carbonate ranges from 16 to 49%. The soil pH ranged from 7.3 to 7.9. Soil texture varied from a clay loam to sandy loam with a clay content ranged from 7 to 32%.

The results obtained indicate that most of the sewage sludges produced in Jordan are adequate for agricultural soils, however, the maximum value of Zn concentration from Al-Samra anaerobic ponds sludges exceeded the European countries and USEPA guidelines. Most heavy metals become less soluble, thus less available to plants in alkaline conditions due to their precipitation.

Arsenic, cobalt, mercury, molybdenum, and selenium concentrations in Jordanian sludges generally are very low and may have no significant effect on plant composition. It is known that As, Cr, Pb and Hg may accumulate in the roots of plants but are not translocated to the above-ground tissues. Copper exhibits limited translocation and seldom occurs at concentrations greater than 20 mg/kg dry weight in above-ground plant tissues (Black *et al.*, 1984). Cadmium, Zn and Ni have been found to be taken up to an appreciable extent by plants, and generally increase with increasing amounts of metal applied to soil in sludge. However, large differences in the concentrations of these metals may also be related to different plant species, different plant parts and year to year

variations. Soil pH is the most important soil factor affecting plant uptake of Cd, Zn, and Ni. Soil texture, cation exchange capacity, and organic matter content may also affect uptake of these metals (EPA, 1983).

The determination of the sources of heavy metals which are polluting sewage and prevention of these entering into the urban sewage systems without pretreatment will make it possible to obtain sludges suitable for agricultural use. This could contribute to making up the shortage of organic amendments in semi-arid to arid regions like Jordan.

Before adopting such an application locally, however, the characteristics of the local soil and prevailing environmental conditions should be taken into consideration. When transferring heavy metal guidelines for sludge use on agricultural land from one region to an other it could be interesting to compare the conditions under which the guidelines were made with the actual conditions in the target region.

A direct transformation of western guidelines to Jordan may not be appropriate. In some respects, the guidelines are based on conditions which differ from those found in Jordan. With respect to heavy metals, two factors, soil pH and amount of atmospheric deposition, seem more favourable in Jordan for the use sewage sludge on agricultural land than in many European countries. An important source of many heavy metals to arable land in Europe is atmospheric deposition. The predominant input of Pb to soil comes from atmospheric deposition, and automobiles are normally considered the main source of Pb emission, therefore atmospheric deposition of Pb is expected to be a lower on Jordanian arable land than on arable land in Europe. However, in Europe emission of Pb has decreased tremendously in the last decade because increased use of unleaded petrol.

To prevent toxicity to humans or animals from crops grown on treated agricultural lands, guidelines relating to metal concentrations in sludge and maximum additions to land should be developed in Jordan. These guidelines and those of other jurisdictions should be followed scrupulously, and accurate records kept of treated wastewater and sludge applications to ensure maximum health protection and minimum adverse environmental impact.

In general, As, Cr, Co, Pb, Hg and Se in Jordanian sludges utilised on agricultural land are not likely to present a significant environmental health hazard. Although Cu, Zn and Ni buildup in the soil may result in phytotoxicity and reduced crop yields, toxicity to animals is very unlikely. Animals have high tolerances for these metals and apparently healthy crops do not present a hazard. Notable exceptions are Cu toxcities to sheep and possibly cattle, which are associated with very low Mo intake. Cadmium in the sludge is the greatest concern, because its buildup in soil may result in toxicity to both crops and animals. It is taken up by crops and accumulates in the liver and kidneys of animals. There is concern that healthy crops with enhanced Cd concentrations might contribute to toxicity, particularly in humans. Humans have a long life span during which accumulation can occur (EPA, 1983).

The predominant input of Pb to soil comes from atmospheric deposition and automobiles are normally considered the main source of Pb emission. Zn is the only heavy metal examined which exceeds USEPA guidelines. Zn is also an essential micronutrient, and a moderate presence seems beneficial for both plants and animals. Soil pH is a primary factor that affects the bioavailability of Zn in soils, and high pH values tend to be associated with low Zn availability to plants (Verma & Singh, 1991). Therefore, a relatively high limit value of Zn may be acceptable in Jordan.

The sewage works from which the samples were collected serve communities ranging in size from small rural towns up to a major industrial city. Madaba WSP serve Madaba city (population served 19000); Jerash extended aeration system serves 16,000 (Table 10.8). Al-Samra WSP was designed to serve Amman and Zarqa city (population served 1,352,000). A correlation with the size of town was found. In contrast, the findings of Berrow and Webber (1972) were that there was no correlation with the size of town for any trace element except Cr, for which the total content tended to rise steadily with population. Forty-two representative samples of sludges collected from different cities and towns through England and Wales.

Berrow and Webber (1972) have reported the average trace metal content of about 42 UK sludge samples. Comparing the results of their study with the trace metal values found in Jordan sludges in this study, the mean trace metal levels in UK were much higher in sludge samples than in Jordan, reflecting the lower level of industrialisation in Jordan.

Concentrations of Cd, Cr, Cu, Ni and Pb are significantly lower than the maximum concentrations recommended in EEC guidelines. Compared with other governmental guidelines for use of sludge in agriculture (Webber *et al.*, 1983; Tjell, 1985) a similar picture can be seen in most countries; acceptable sludge maximum concentrations are significantly higher than concentrations of Cd, Cr, Cu, Ni and Pb found in Al-Samra, although some countries, i.e. Canada, the Netherlands, USEPA and Switzerland, would find the concentration of Zn unacceptably high for agricultural use. However, many countries accept a higher concentration of Zn than shown in Table 10.10, e.g. Denmark, Finland, and Norway.

Three samples of sludge were analysed from Jerash treatment plant and Madaba WSP's. All concentrations of heavy metals are significantly lower than EEC recommended maximum concentrations. The same conclusion can be based on other countries guidelines.

To summarise the discussion, it can be said that, although the concentrations of heavy metals in sludges are lower and/or within those concentrations usually reported in literature, and lower than the maximum recommended concentrations of heavy metals, except for Zn, for sludges which would be considered good quality for application to agricultural land, it cannot be concluded that the sludge from Al-Samra, Madaba, and Jerash is suitable for agricultural application in Jordan unless more research is done on the uptake of heavy metals by plants, which varies with different factors. Further research is needed to assess the uptake of heavy metals by plants under local environmental conditions, soil and irrigation water characteristics.

Jordan is located in arid and semi-arid climate zones. Most soils in Jordan have poor aggregate stability. This is usually manifested by crust formation at the soil surface, extensive surface runoff of rainwater, and substantial soil erosion. Increasing aggregate stability can be achieved by increasing soil organic matter (Abu-sharar, 1993). Additionally, Epstein *et al.* (1976) reported an increase in the water-holding capacity of an Aquic Hapludult soil amended with sewage sludge and sludge compost. The properties of sewage sludge as a soil conditioner may be very useful for Jordanian agriculture. An increase of the water holding capacity of soil amended with sewage sludge and sludge compost has been reported. Increased aggregate stability can be achieved by increased soil organic matter.

As a first step, clearly it is important to develop guidelines for Jordan before land application of sewage sludge can become an acceptable and desirable practice.

CHAPTER ELEVEN

PERSISTENCE OF INDIGENOUS *ASCARIS* EGGS AND INDICATOR BACTERIA IN SLUDGES TREATED ON DRYING BEDS

11.1 Introduction

There are many sludge disinfection processes available to the design engineer. However, there is no universally accepted process which is ideal for most locations as there is for wastewater disinfection. The information available in the literature on this subject indicates that more research and development must be done before the technology of sludge disinfection is equal to that of water or wastewater disinfection.

In Jordan, land disposal of sewage sludge was not practiced until now, due to the small quantity of sludge produced from sewage treatment plants and the small population. Increasing population, and increasing wastewater connections, leads to high production of sludge, a need to characterise the quantity and quality and a need to establish how best to treat it and dispose of it. There is also need to set standards and regulations to protect public health and avoid nuisance problems arising from the use sludge as fertiliser on agriculture lands. It was a matter of urgent national importance that research should be carried out to determine what methods of sludge treatment might economically be employed for the conversion of sewage sludge into a safe fertiliser.

Jordan is one of the countries blessed with an abundance of solar energy, the use of which can play an important role in meeting some of the national energy requirements. The methods which are adopted in semi-arid developing countries for handling and disposal of sludge should conform to the local environment. Beneficial meteorological and

socio-economic conditions should be considered in planning systems for sludge collection, treatment, and disposal.

Drying beds should be considered as one of the important methods for sludge treatment, especially for developing countries. This is for many reasons: for example, it requires less energy and consequently running costs are lower; it does not require skilled operating staff; capital cost is minimal, not needing spare parts to be imported from developed countries (Pescod, 1971).

Questions concerning potential disease transmission and other pollutants have prevented greater application of sewage sludge on land. Previous chapters (Chapters Seven and Eight) have shown that indicator bacteria and parasites are associated with the sludge suspended solids, and are thereby concentrated in the sewage sludge. Also *Ascaris* eggs are the most commonly found parasite present in Jordanian sludges. The eggs of *Ascaris* are not in an infective stage to man when they enter the sewage treatment system, but may develop to infectivity, as has been recognised in Jerash treatment plant.

Several papers were referred to in the literature review describing sand drying beds design; however, specific research on drying waste stabilisation ponds sludges was notably lacking. From a review of the literature, there exists a need for the investigation of wastewater anaerobic pond sludge accumulation, characterisation, and ultimate disposal, especially when considering the Mediterranean semi-arid region.

One of the objectives of this study is to attempt to fill some of the gaps in our knowledge on the occurrence of human parasites and bacterial pathogens in municipal sludges in Jordan, which were discussed in Chapter Seven, and to investigate selected methods for inactivating those pathogens. In order to make cost effective decisions on the type of treatment process to adopt, it is necessary to have clear-cut objectives regarding the ultimate use of the treated sludge and the minimum acceptable bacterial and parasitological quality.

The data and characteristics of the sludge from anaerobic ponds in the Al-Samra waste stabilisation ponds (ASWSP), and Jerash (oxidation ditch) treatment plant (JTP), is summarised in Table 11.1; it was observed that the concentrations of total helminth eggs

range between 0-1015 eggs/g of total solids. *Salmonella* sp. and faecal streptococci range between 10^3-10^6 and 0- 10^7CFU/g of total solids, respectively; while faecal coliforms range between 10^5-10^7 MPN/g of total solids (all counts expressed on a dry weight basis). This value is far in excess of the "Class A" criterion in the USEPA Regulations (1992) for land application and using the sludge as a fertiliser.

TABLE 11.1. Range of helminth eggs and bacterial indicators in the three wastewater treatment plant sludge samples, counts/g of total solids (dry weight basis)

Agent	Al-Samra	Madaba	Jerash
Total helminth	45-1015	0-150	288-619
TC	10^5-10^8	10^6-10^8	10^7-10^9
FC	10^5-10^7	10^5-10^7	10^6-10^7
FS	0-10^2	10^3-10^5	10^5-10^7
Salmonella spp.	10^3-10^5	10^4-10^6	10^3-10^5

The anaerobic ponds at Al-Samra have never been emptied since they were commissioned in 1985, and prolonged storage in anaerobic conditions may reduce egg viability. However, as ponds work as a continuous process, it is assumed that the most recently deposited eggs will have suffered little loss in viability, and that to achieve complete safety from helminthological risks WSP sludge would still require further treatment or storage.

USEPA (1992) regulations, "Class A" requirements must be met for sludge applied in bulk to agricultural land, forest, public contact site, reclamation site, lawn, or home garden; or if the sludge is to be sold or given away in bags or other containers. The less restrictive "Class B" is for bulk application to land where food crops meet various criteria, e.g. of time and distance between sludge application and crop harvest. There are several versions of "class A" requirements, all versions requiring: Faecal coliforms less than 1000 MPN per gram of total solids, or the density of *Salmonella* spp. in the sewage

sludge be less than 3 MPN per 4 grams of total solids at the time the sewage sludge is used or disposed. The sewage sludge is "Class A" with respect to viable helminth eggs when the density of viable helminth eggs in sewage sludge after treatment is less than one per four grams of total solids (all counts expressed on a dry weight basis).

As shown in Chapter Ten heavy metal concentrations in the sludge of anaerobic ponds in Al-Samra waste stabilisation ponds are within the USEPA and EC guideline values for all metals but zinc, which slightly exceeds the guideline value. In this case the geometric average Al-Samra sample value is slightly less than either the USEPA or the EC criteria, while the maximum value is within the stated EC range, but slightly exceeds the USEPA value. Nutrients in Al-Samra anaerobic pond A2-1 sludge samples were measured by Miqdadi (1989). He found the concentrations lower than those usually quoted in literature. The fertiliser value of sludge was found to be worth JD 2.84/tonne of dry matter.

The choice of natural open drying beds for pathogen treatment of sludge would be based on economics, low maintenance and energy requirements, and the lack of needs for imported spare parts or skilled and qualified operators or technicians to run drying beds. If it can also be demonstrated to be an effective method of treatment, easily incorporated into current sludge stabilisation techniques, this would be an additional advantage. It is known that high temperatures and desiccation are effective lethal factors for *Ascaris* eggs; Jordan is a semi-arid country with low humidity, rainfall and high evaporation rate especially during the summer season as well an air temperature range during the year between 7-26°C (Table 11.2). These facts suggest that sludge drying beds could well be an effective method for eliminating parasite eggs, particularly in warmer geographic locations.

The overall objective of this chapter was to evaluate the effectiveness of sludge storage in natural drying beds as a method of inactivating pathogens, by studying the survival of parasitic eggs, indicator bacteria, pathogens such as *Salmonella*, in two different types of sand or gravel drying beds as a basis for evaluating the suitability of the sludge for land application as fertiliser. An additional objective was to detect changes in sludge characteristics which could be correlated with pathogen inactivation.

The objectives will be categorised as follows:

1- The applicability of using combined treatment processes, i.e. using partially digested stored sludges from anaerobic waste stabilisation ponds followed by natural open drying bed storage for five months (during summer season), will be investigated as a feasible and simple approach for the inactivation of *Ascaris* eggs and bacterial pathogens in sludges.

2- Drying bed studies will be initiated using raw domestic sludge (from Jerash oxidation ditch plant) at full scale and stored sludges from Al-Samra anaerobic ponds as a pilot plant study. In this way the best stabilisation conditions can be determined for producing sludges to meet PFRP (Process to Further Reduce Pathogens) criteria in USEPA Regulations (1992) for land application.

3- A comparison will be made of the effectiveness of this treatment between conventional wastewater treatment plant (oxidation ditches) sludges and Al-Samra anaerobic ponds sludges. Also to investigate the difference and the effectiveness of this treatment in using sand or gravel media in drying beds.

4- The effect of seasonal variations on the effectiveness of drying beds will be investigated for inactivation of *Ascaris* eggs and other bacterial pathogens, using oxidation ditch sludges from Jerash.

5- A comparison of the Crystal violet staining method with the incubation method to evaluate which is the more accurate method to detect *Ascaris* eggs viability in sludges that had been dried in natural open drying beds

The experimental investigations were carried out during the summer period (April-August 1994) for both Al-Samra and Jerash treatment plant sludges, comparing sand and gravel drying beds. During the winter season a study was carried out for 3 months on field scale gravel drying beds, only for Jerash treatment plant sludges.

The present study will be carried on the real infected (indigenous pathogens) wastewater treatment plant sludges without artificial seeding of parasite eggs or indicator bacteria.

11.2 Study Area, Experimental Work and Methodology

11.2.1 Locations of drying beds

The in-field natural open drying beds are located in Jordan; the bases for selecting the wastewater treatment plants are: (1) method of treatment; (2) size of plant;

11.2.2 Air drying beds design and characteristics

All studies for this chapter were carried out in two similar dimensions of open natural sand or gravel pilot-scale drying beds using for treatment sludges from anaerobic ponds at Al-Samra site (Fig. 11.1); while for treatment raw sludge from JTP, two full-scale open natural sand or gravel drying beds (Fig. 11.2) were used. Table 11.2 summarise the details of the drying beds that have been used in this study.

Sludge will dewater by drainage; underdrains are perforated 15 cm plastic pipes laid with open joints. The drainage pipes are adequately supported and covered with three layer of different diameter size coarse gravel (A = 19-37mm; B = 6-19mm, C = 0.5-10mm, Fig. 11.3 and 11.4), with the relatively coarser materials at the bottom. A sand layer is laid on top of the gravel layer. The sand media characteristics are: clean, hard particles, no clay or silt, or organic matter, effective size of 0.3-0.75 mm with uniformity coefficient less than 4.0.

Sludge from the anaerobic ponds at Al-Samra and Jerash treatment plant was well distributed on to the beds to give a depth of 35 and 30cm, respectively. Al-Samra drying beds design was similar to the full scale Jerash dewatering drying beds, but on a pilot plant scale.

FIG. 11.1. Pilot-scale of the natural open drying bed at ASWSP site

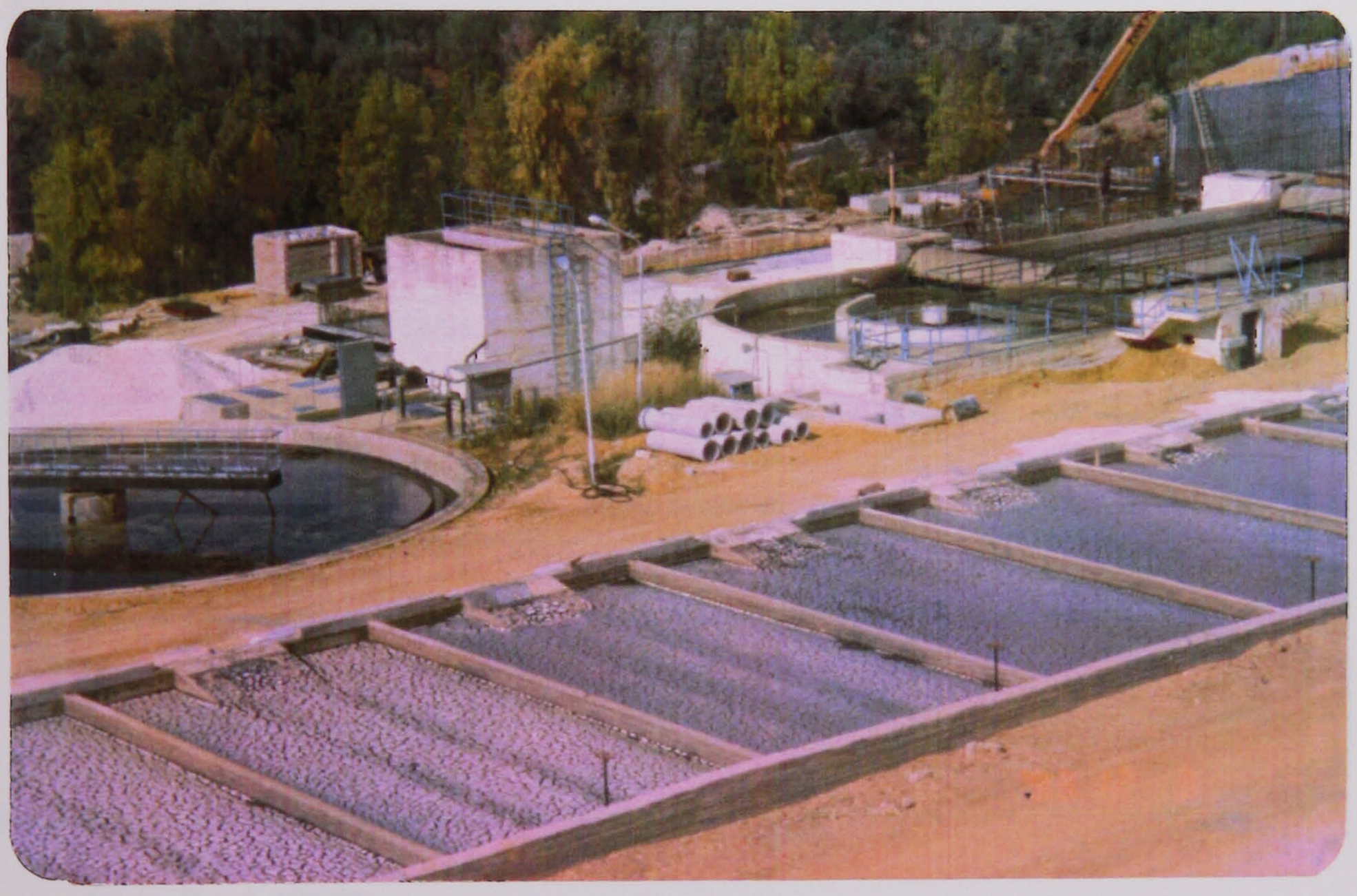


FIG. 11.2. Full-scale natural open drying bed at JTP site

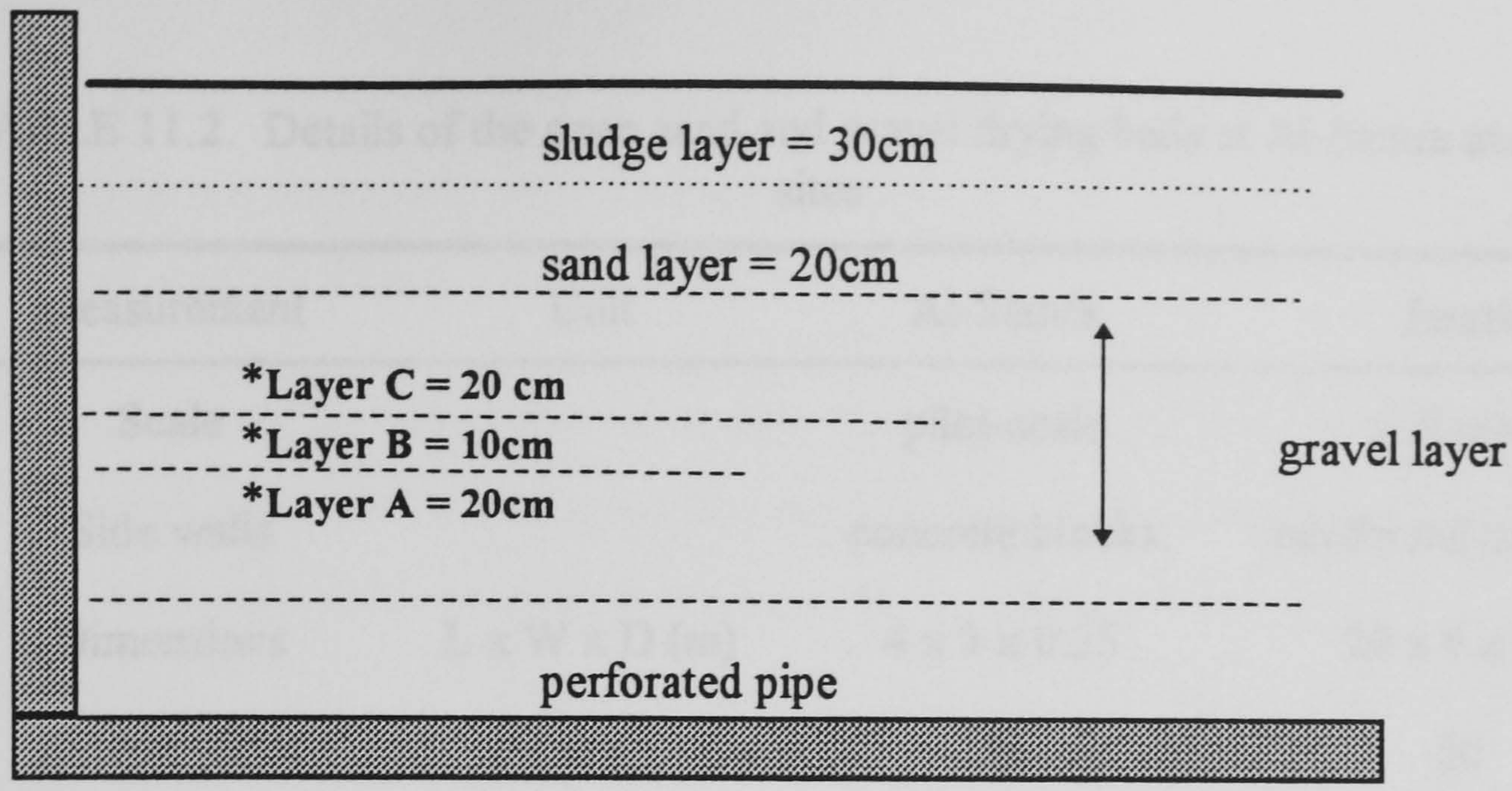


FIG. 11.3. General arrangement of the sand sludge drying beds

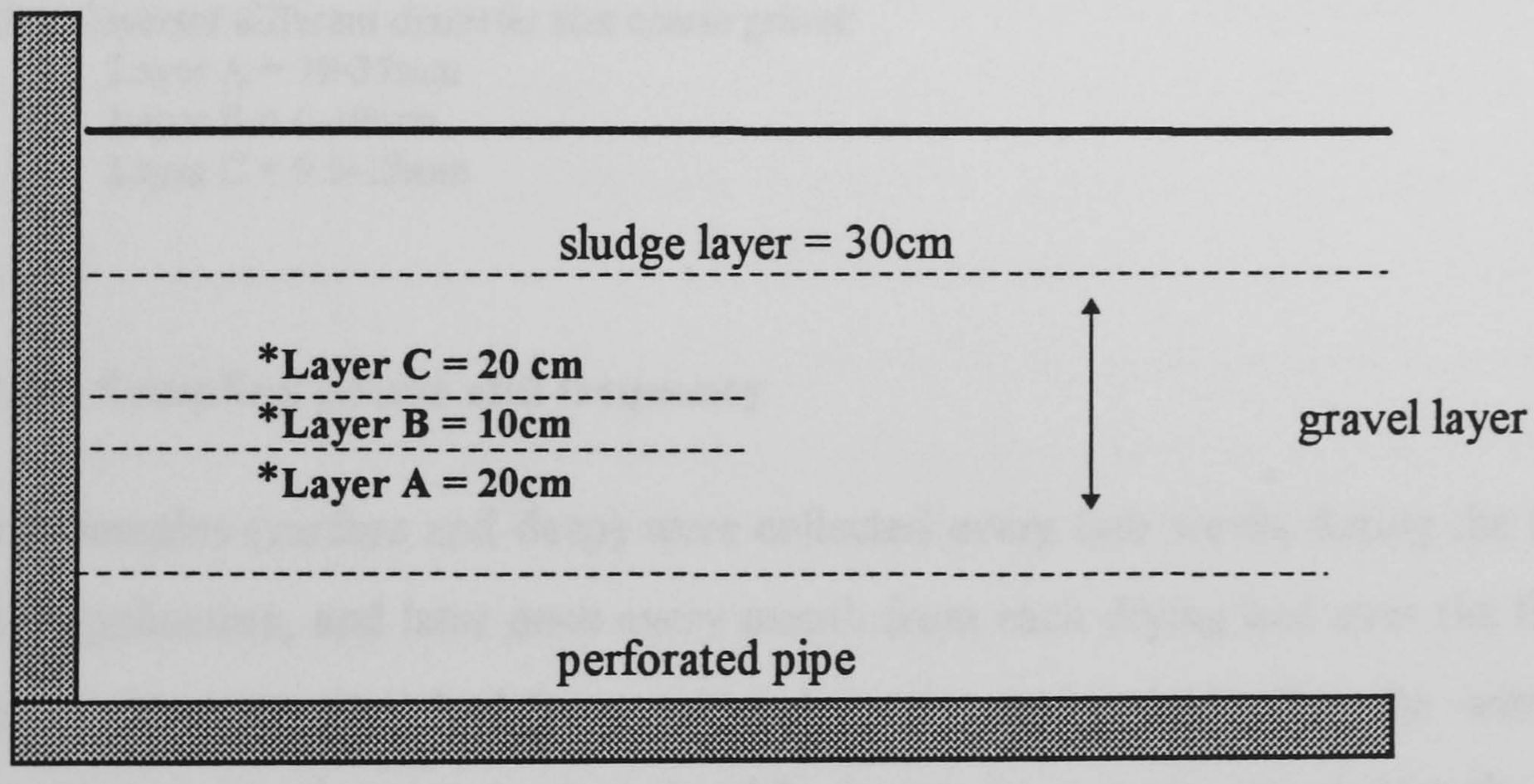


FIG. 11.4. General arrangement of the gravel sludge drying beds

* three layersof different diameter size coarse gravel:
  Layer A = 19-37mm
  Layer B = 6-19mm
  Layer C = 0.5-10mm

TABLE 11.2. Details of the open sand and gravel drying beds at Al-Samra and Jerash sites

Measurement	Unit	Al-Samra	Jerash
Scale		pilot-scale	full-scale
Side walls		concrete blocks	reinforced concrete
Dimensions	L x W x D (m)	4 x 3 x 0.35	20 x 6 x 0.6
Sludge depth	cm	35	30
Sand depth	cm	20	20
Coarse gravel layer depth	*A + B + C (cm)	20 + 10 + 20	(16 to 25) + 10 + 20
Drying time	months	5	5

* three layersof different diameter size coarse gravel:
Layer A = 19-37mm
Layer B = 6-19mm
Layer C = 0.5-10mm

11.2.3 Sampling points and frequency

Grab samples (surface and deep) were collected every two weeks during the first month after application, and later once every month from each drying bed over the five months period. Various sites had been selected in the drying beds (for the winter season experiment nine sites were selected, while during the summer experiment the sites were reduced to four, as the basis of statistical analysis). An important point was that a sample taken from any portion of the drying bed should be representative of the parasite and bacterial population in the entire drying bed.

11.2.4 Measuring abiotic and biotic sludge parameters

Certain abiotic parameters were measured to determine their influence on the survival of the pathogenic organisms. The parameters measured and the reference to the procedures used are listed in Chapter Six, Table 6.7.

The abiotic measurements were air temperature (°C), sludge temperature (°C), moisture content (%), weather (rain, evaporation rate), colour of the sludge, pH, total solids and total volatile solids (%), and depth of sludge (cm). While biotic measurements were type and counts of intestinal nematodes, viability and the stage of development of *Ascaris* eggs, total coliform counts (MPN/g), total faecal coliform counts (MPN/g), presumptive *Salmonella* sp. counts (CFU/g), faecal streptococci counts (CFU/g).

11.2.5 Statistical analysis

EXCEL package was used for the statistical analysis of the data. Analysis of variance (Two-Way ANOVA Without Replication) and regression analysis were performed in order to determine what effects, if any, abiotic parameters could have on the viability of the microorganism being monitored. Arithmetic means were calculated for replicate readings for abiotic data. The four biological parameters (total coliforms, faecal coliform, faecal streptococccci, *Salmonella* spp.) were expressed as geometric means. The percent inactivation over time was calculated for *Ascaris* eggs. Also, the regression analysis was made for the natural log of each biological parameter (except for *Ascaris* where actual values were used) versus percentage of total solids.

11.3 Results

Chapters seven and eight indicated that at temperatures and conditions prevailing in anaerobic pond and oxidation-ditch treatment plant sludges pathogens are still present in high numbers. In this chapter the results are presented on abiotic and climate data, parasite survival, bacterial survival and statistical analysis. Analysis of variance (two-way without replication) was used to find the difference in performance between using sand or

gravel drying beds and the difference between anaerobic pond or oxidation-ditch sludges, and the effect of drying time on abiotic and biotic parameters.

11.3.1 Abiotic parameters and meteorological conditions

Table (11.3) below and Figures 11.5 to 11.9 summarise the prevailing climatic data obtained by an automatic weather station on Al-Samra site during the period of the study. As is very well-known, the performance of drying beds is particularly influenced by the meteorological conditions: wind, temperature, rainfall, sunshine and evaporation.

TABLE 11.3. Meteorological Parameters during the study period from Al-Samra meteorological station.

Year		Sunshine	Daily temperature	Rainfall	Evaporation
Unit		hours	°C	mm/month	mm/month
1992	Average	8.7	16.8	10.5	12.3
	Minimum	4	6	0	4.4
	Maximum	12.3	26.3	56	31.5
1993	Average	8.4	18.2	2.8	13
	Minimum	5.2	6.8	0	2.7
	Maximum	12.2	25.7	8	40.5
1994	Average	8	18	17	4.9
	Minimum	5.1	7.7	0	-0.78
	Maximum	10.4	25	68	9.7

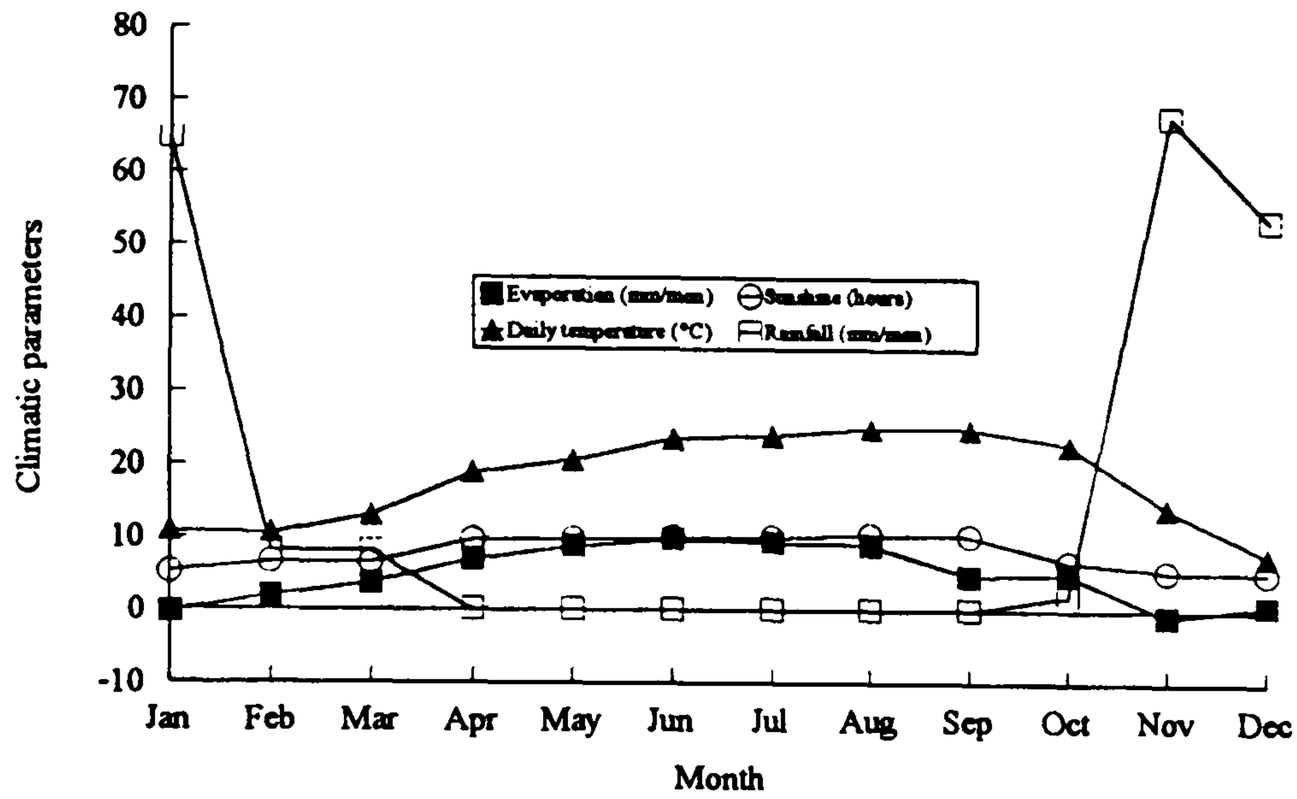


FIG. 11.5. Monthly average meteorological parameters in ASWSP site, 1994

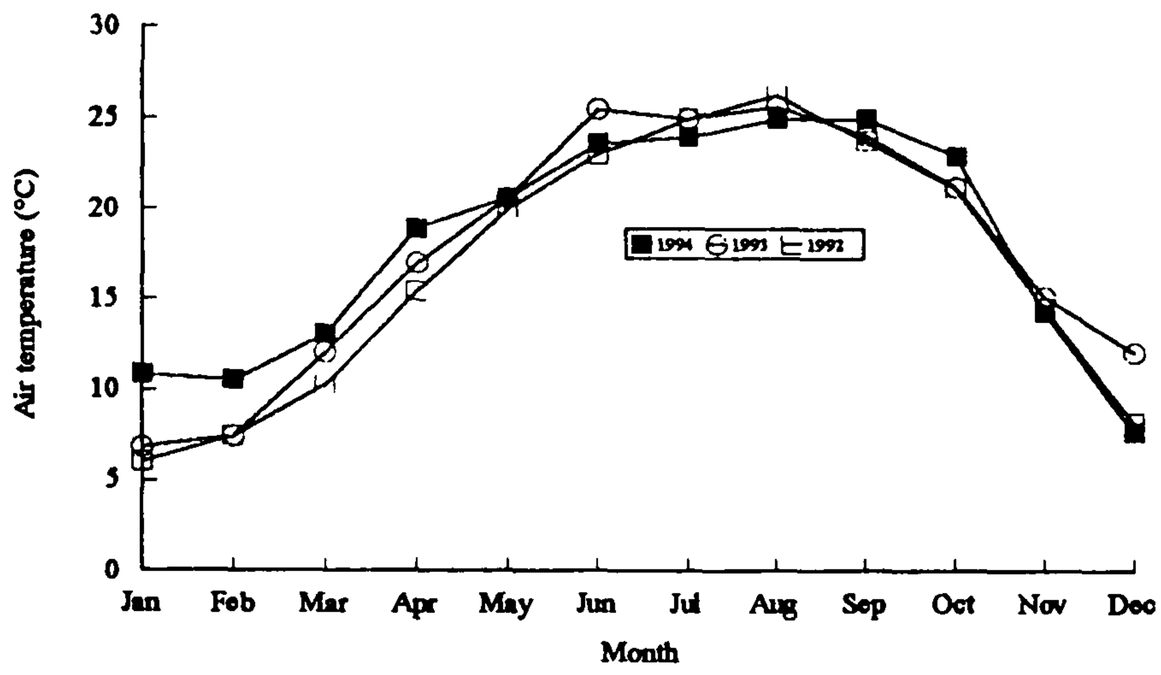


FIG. 11.6. Monthly means of air temperature at ASWSP site during the study period (1992-1994)

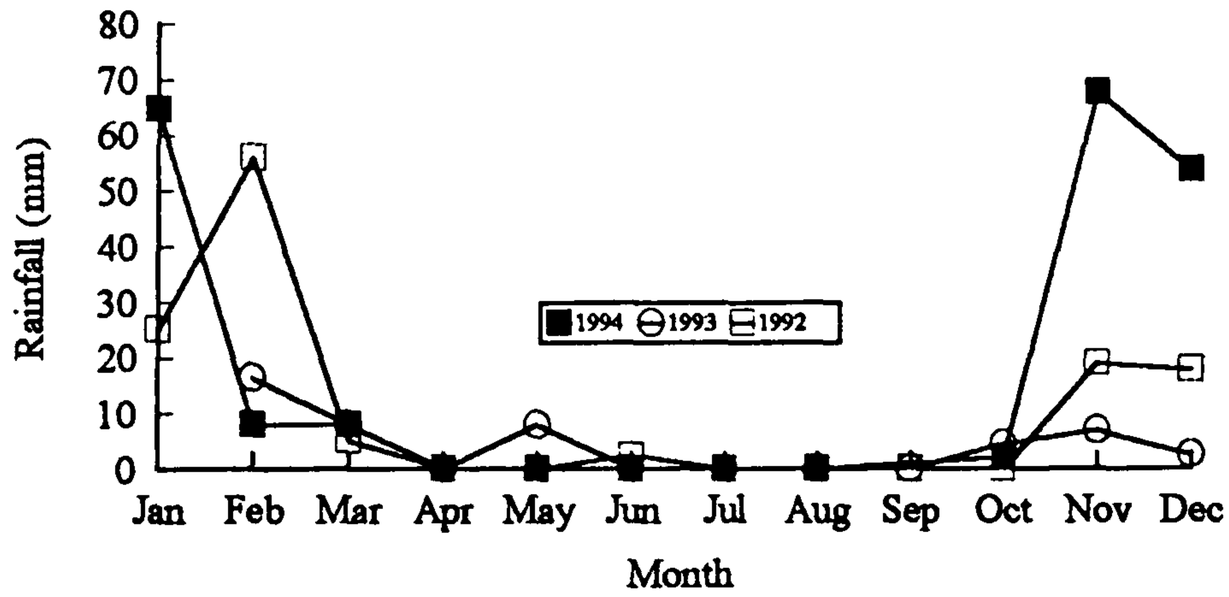


FIG. 11.7. Monthly means of the rainfall (mm/month) at ASWSP site during the study period (1992-1994)

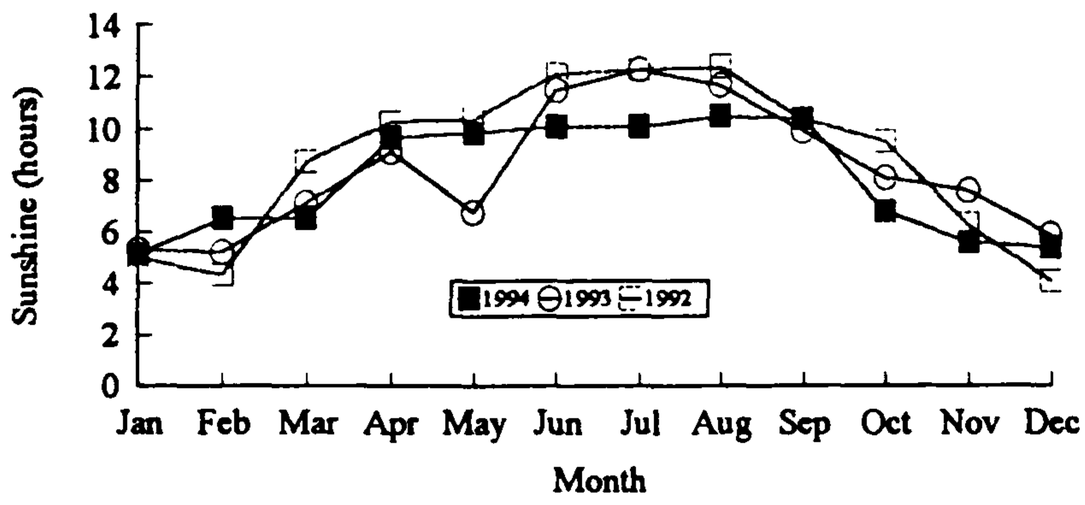


FIG. 11.8. Monthly means of the number of sunshine hours per day at ASWSP site during the study period (1992-1994)

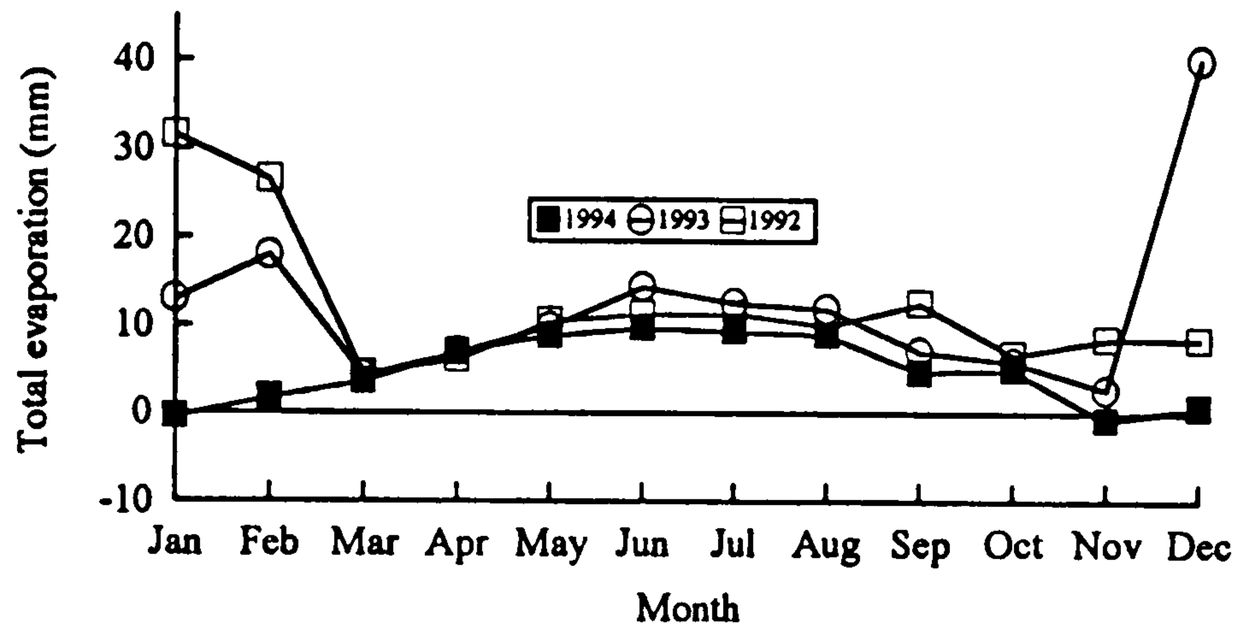


FIG. 11.9. Monthly means of the total evaporation (mm/month) at ASWSP during the period study (1992-1994)

11.3.2 Temperature

Comparison of media and sludge temperatures during the summer season (May-September, 1994) for both Al-Samra and Jerash drying beds are illustrated in Figure 11.10 and 11.11.

Although the temperature of the sand and gravel increased considerably at times during summer season, the temperature of the sludge showed slightly higher values than the gravel or sand temperature. The maximum temperatures noted were 43°C and 45°C for gravel and sand respectively, in Al-Samra drying beds; while the temperatures of the sludge were 46°C and 47°C on gravel or sand, respectively. The maximum temperature at Jerash drying beds (sand or gravel) was noted to be 44°C, while for sludges it was 45°C. Referring to the analysis of variance test results (Table 11.4 and 11.5), this showed there was no significance difference between sand or gravel temperatures ($F < 6.0$) for both sludge types. This implies that choosing either of those media in the drying bed design did not make any difference to the temperature effect achieved.

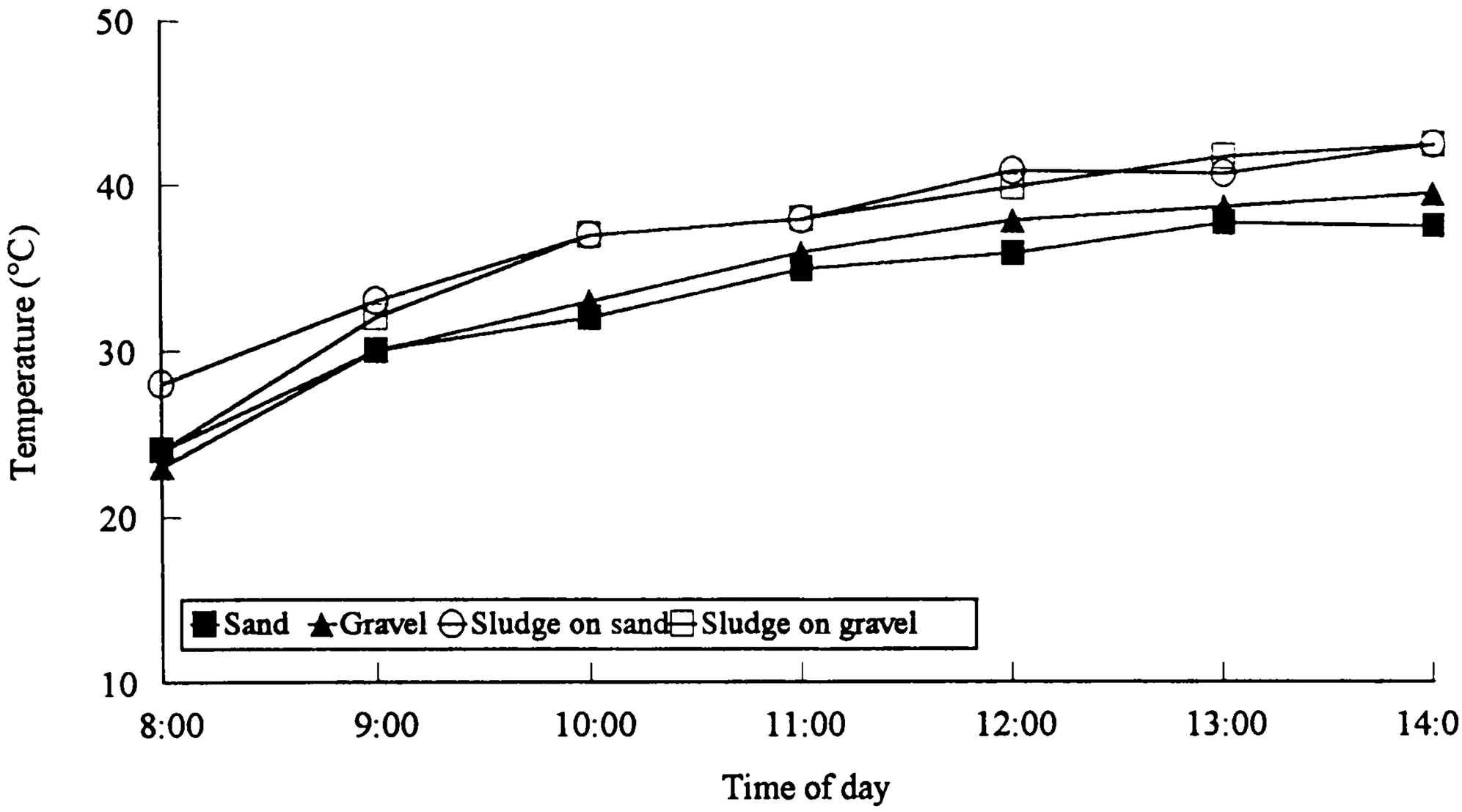


FIG. 11.10. Changes in average daily temperature with time for sludge, gravel and sand at ASWSP dryings beds

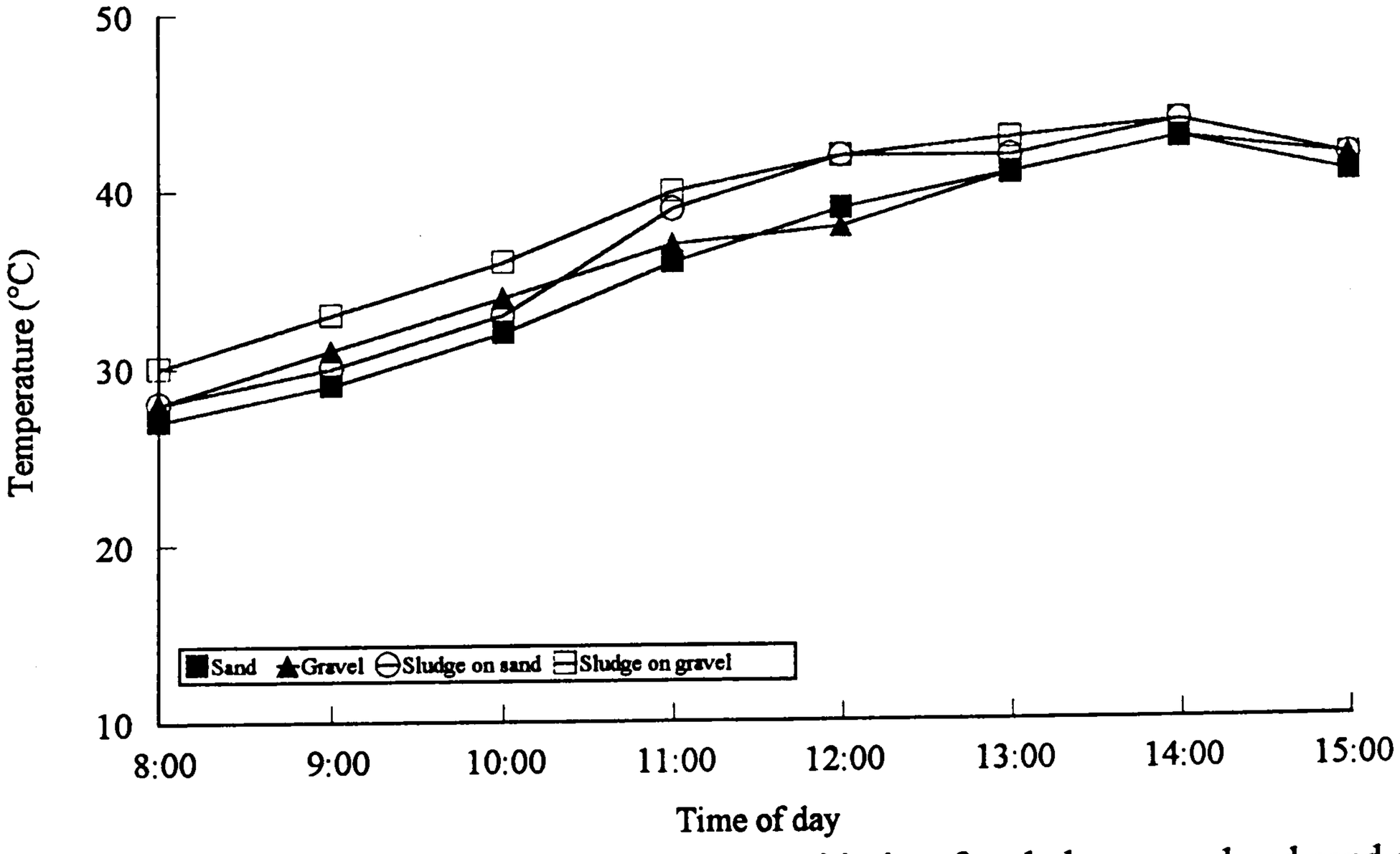


FIG. 11.11. Changes in average daily temperature with time for sludge, gravel and sand at JTP drying beds

TABLE 11.4. Summary of analysis of variance results comparing Jerash and Al-Samra drying beds with drying bed type (sand or gravel), time, abiotic and biotic variables

Parameter	Variance	ANOVA					
		*Jerash			**Al-Samra		
		P-value	♠F-value	Critical F-value	P-value	♠F-value	Critical F-value
a) Abiotic							
Sludge depth (cm)	Drying bed type	0.5	0.5	7.7	0.03	10	7.7
	Time	8×10^{-6}	630	6.4	5×10^{-5}	243	6.4
Sludge temperature (°C)	Drying bed type	0.01	14	5.6	0.15	3	6
	Time	3×10^{-4}	173	3.8	1×10^{-5}	98	4.3
Gravel and sand temperature (°C)	Drying bed type	0.06	5	5.6	0.1	4	6
	Time	1.9×10^{-6}	97	3.8	4×10^{-6}	137	4.3
pH	Drying bed type	0.6	0.3	7.7	0.06	6	6.6
	Time	0.05	6	6.4	0.05	5	5.1
Total solids (%)	Drying bed type	0.03	10	7.7	0.6	0.4	6.6
	Time	0.05	2.7×10^{4}	6.4	0.01	10	5.1
b) Biotic							
Total coliforms	Drying bed type	0.13	4	7.7	0.98	5×10^{-4}	6.6
	Time	5.9×10^{-5}	224	6.4	0.01	11	5.1
Faecal coliforms	Drying bed type	0.75	0.1	7.7	0.9	0.01	6.6
	Time	3×10^{-4}	96	6.4	0.02	8	5.1
Salmonella spp.	Drying bed type	0.42	0.8	7.7	0.9	0.02	6.6
	Time	3.8×10^{-4}	88	6.4	8×10^{-3}	12	5.1
Faecal Streptococci	Drying bed type	0.7	0.1	7.7	0.3	1	6.6
	Time	7×10^{-5}	212	6.4	0.8	0.4	5.1

ANOVA: Two-Way Without Replication, significant at the $\alpha = 0.05$ level

*Comparing sand and gravel dry beds at Jerash treatment plant site.

**Comparing sand and gravel dry beds at Al-Samra WSP site.

♠Experimental F-value, if it is > than F-critical this mean there is significant difference in treatment with different types of drying beds; if P > 0.1 this mean there is no significant difference.

TABLE 11.5. Summary of analysis of variance results comparing sand or gravel drying beds with two different sludge type (ASWSP or JTP), time, abiotic and biotic variables

Parameter	Variance	ANOVA					
		♣Gravel			*Sand		
		P-value	♠F-value	Critical F-value	P-value	♠F-value	Critical F-value
a) Abiotic							
Sludge depth (cm)	Sludge type	0.02	11	6.6	0.14	3	7.7
	Time	2×10^{-4}	55	5.1	4×10^{-3}	25	6.4
Sludge temperature (°C)	Sludge type	0.5	0.7	7.7	0.9	0.03	7.7
	Time	0.1	3.8	6.4	0.2	2.4	6.4
Gravel or sand temperature (°C)	Sludge type	0.7	0.2	18.5	0.7	0.2	10.1
	Time	0.3	2.3	18.5	0.6	0.8	9.3
pH	Sludge type	0.9	0.03	5.99	0.04	7.8	7.7
	Time	0.02	6.3	4.3	0.2	2	6.4
Total solids (%)	Sludge type	0.5	0.6	5.99	1	2	7.7
	Time	6×10^{-7}	249	4.3	2×10^{-4}	111	6.4
b) Biotic							
Total coliforms	Sludge type	0.4	0.7	5.99	0.53	0.5	7.7
	Time	4×10^{-3}	12	4.3	5×10^{-2}	23	6.4
Faecal coliforms	Sludge type	0.1	4.1	5.99	0.02	13	7.7
	Time	9×10^{-3}	9	4.3	5×10^{-4}	80	6.4
Salmonella spp.	Sludge type	0.7	0.2	6.6	0.5	0.6	7.7
	Time	3 x 10-3	18	5.1	0.06	5	6.4
Faecal Streptococci	Sludge type	2×10^{-5}	142	5.99	4.2×10^{-5}	373	7.7
	Time	0.02	6	4.3	1×10^{-2}	14	6.4

ANOVA: Two-Way Without Replication, significant at the $\alpha = 0.05$ level

♣Comparing the difference between treatment primary and partially digested sludge on gravel sludge drying beds samples from Jerash and Al-Samra sewage treatment plant, respectively.

*Comparing the difference between treatment primary and partially digested sludge treated on sand sludge drying beds samples from Jerash and Al-Samra sewage treatment plant, respectively.

♠Experimental F-value, if it is > than F-critical this mean there is significant difference in treatment with different types of sludges; if P > 0.1 this means there is no difference.

11.3.3 Total solids and sludge thickness

The changes in percentage of total solids and reduction in sludge thickness with time for sludges applied to the sand and gravel beds are shown in Figure 11.12 and 11.13.

The rate of moisture removal from the sludge changed during dewatering. The rapid dewatering rate which occurred in the first three days was almost entirely because of drainage, while the rest represents the effects of evaporation.

Sludge from ASWSP consisting of 94% water content, was dewatered consistently to less than 4% moisture in 67 days using an applied depth of 35cm. The pan evaporation rate was between 5 and 10mm/day. After 35 days in Jerash drying beds (starting with an average of 98% moisture content) the sludge had dried to thin crusts. During the winter season the Jerash gravel drying beds moisture content declined only from 98% to 88% within a 3 months period, and the sludge thickness decreased from 30 cm to 6 cm within one month. Mosquito larva invaded the sludge in the gravel drying bed at Jerash treatment plant during the winter season, and a high odour nuisance occurred throughout the winter season study. Sludge removed from the anaerobic ponds dried without the creation of a nuisance.

The values of moisture content at both drying beds at Jerash and Al-Samra sites dropped after 35 days and 67 days, respectively, and then remained constant for the remainder of the storage period. The constant value at Al-Samra drying beds was 4% moisture content and that at Jerash was 8% and 7% for the gravel and sand drying beds, respectively. The final sludge cake thicknesses at Jerash sludges were comparable at roughly 3cm to 5cm for the sand and gravel beds, respectively, while with partially digested anaerobic ponds sludges at Al-Samra were 4 and 8cm for the sand and gravel beds, respectively. The changes in moisture content and decline in sludge cake thickness provide an indication of the dewaterability and evaporation of the anaerobic pond and oxidation ditch sludges.

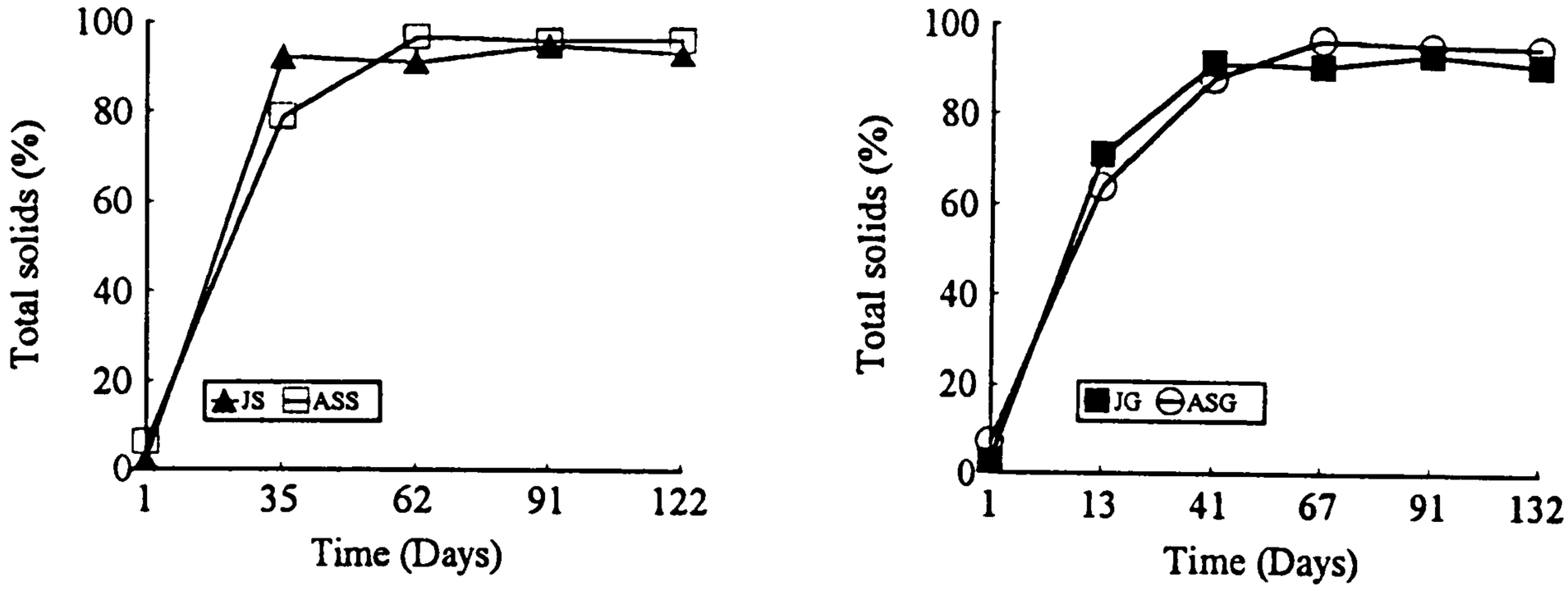


FIG. 11.12. Changes in total solids content with time for sludges applied to the sand and gravel drying beds

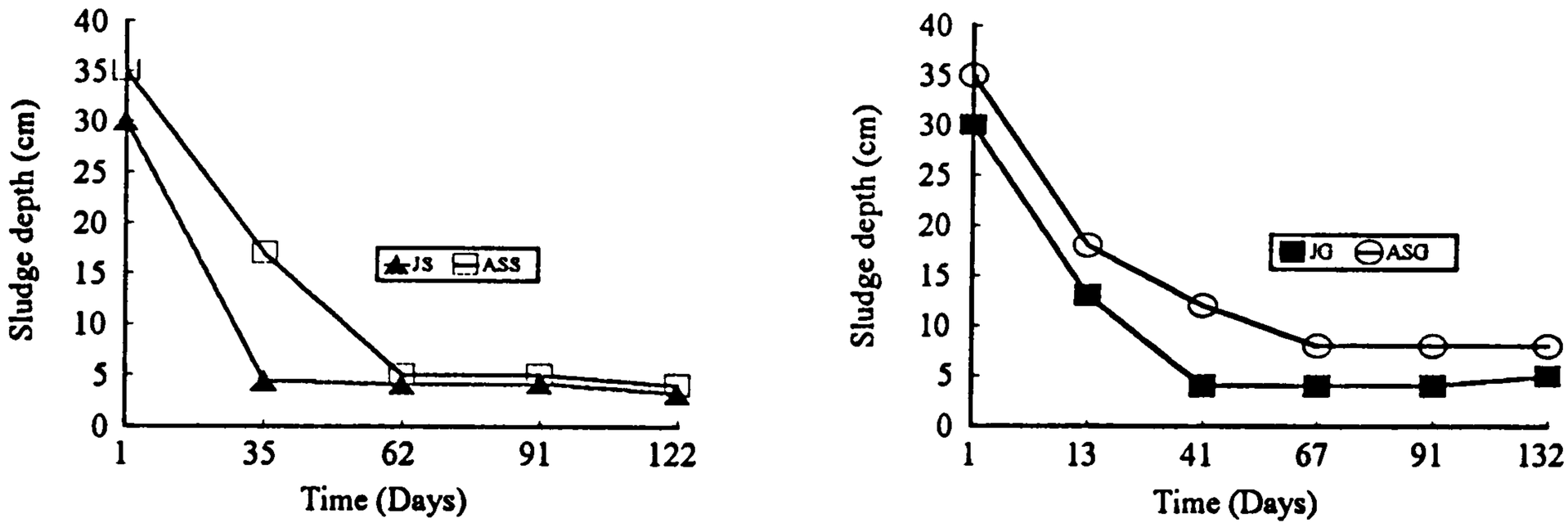


FIG. 11.13. Changes in sludge depth with time for sludges applied to sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

Statistical analysis proved that there was no effect of using different media (sand or gravel) on the sludge depth when oxidation ditch sludge was applied ($P > 0.5$, $F < 7.7$, Table 11.4). On the other hand, when anaerobic pond sludges were applied to gravel or sand beds, a significant difference in sludge depth occurred. This is might be explained by the fact that sludges from anaerobic ponds were not as homogenous as oxidation-ditch sludges, and had a lot of debris, seeds, hair, etc. The results also showed that with different types of sludges on gravel drying beds, the sludge depth will also be affected ($P = 0.02$, $F > 6.6$, Table 11.5). In contrast, when sand drying beds were used, the final

sludge depth was similar whichever type of sludge was applied ($P > 0.1$, $F < 7.7$, Table 11.5). It might be that the small pore size between sand particles helped to retain most of sludge particles on the surface of sand, and little was lost through the pores, in contrast to the situation with gravel drying beds.

The changes in percentage of total volatile solids with time in the sludges applied to the four drying beds are shown in Figure 11.14. The results indicate that there were only small variations in the percentage of volatile solids through the treatment period (132 days) with anaerobic pond sludge, which was already partially digested during storage in anaerobic conditions for about 7 years. In contrast, oxidation-ditch sludge showed no stability during the drying period.

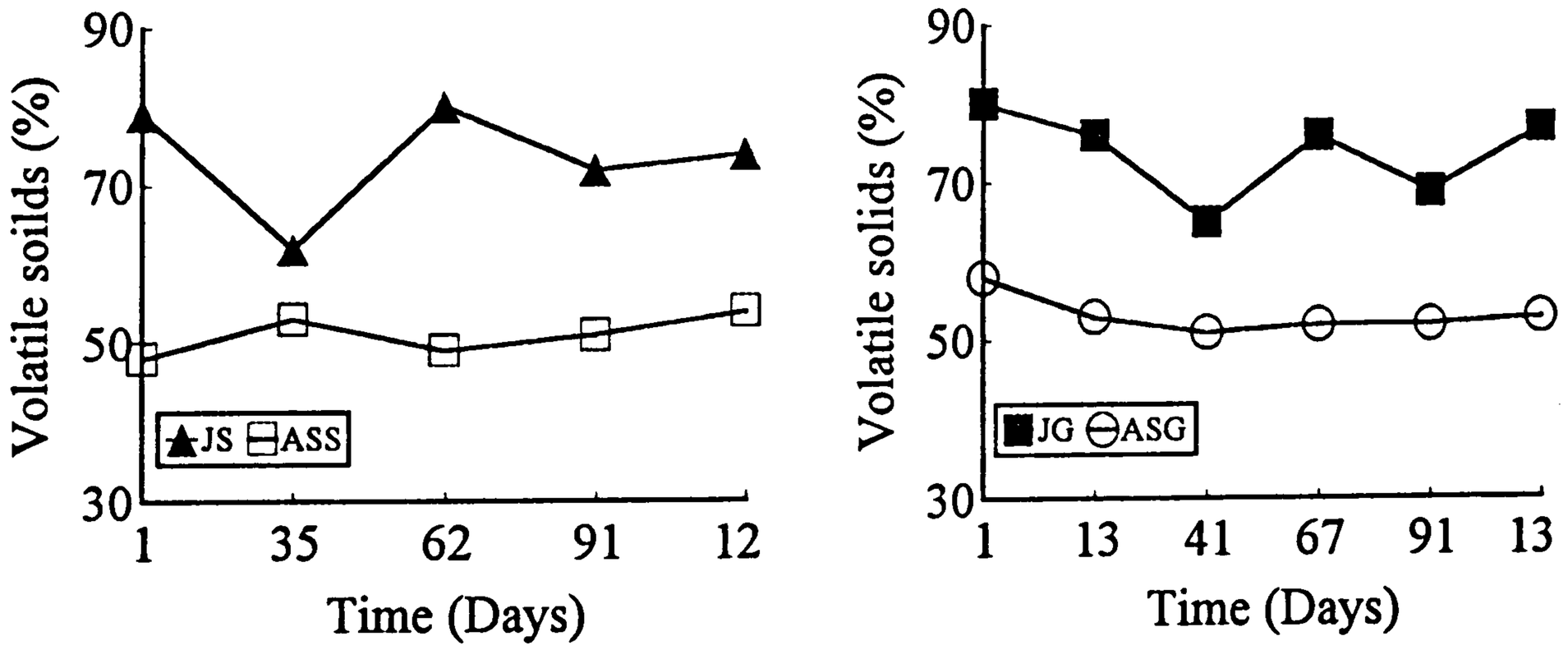


FIG. 11.14. Changes in volatile solids content with time for sludges applied to the sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

11.3.4 pH

Changes of pH values with time of ASWSP and JTP sludges applied to the sand and gravel beds are shown in Figure 11.15.

Referring to the statistical analysis, this showed that the pH value was not affected by the treatment period on sand drying beds with the two different sludge types ($P = 0.05$; Table 11.4); significant variations between the pH values of sludge types only occurred when gravel drying beds were used ($P = 0.9$, $F < 6.0$; Table 11.5).

Although there were slight decreases in values in all drying beds sludges with time, the range (6.0-8.0), were all within the range of tolerance of nematode eggs and bacterial cells. Thus, it appears unlikely that the disappearance of the organisms can be explained on this basis.

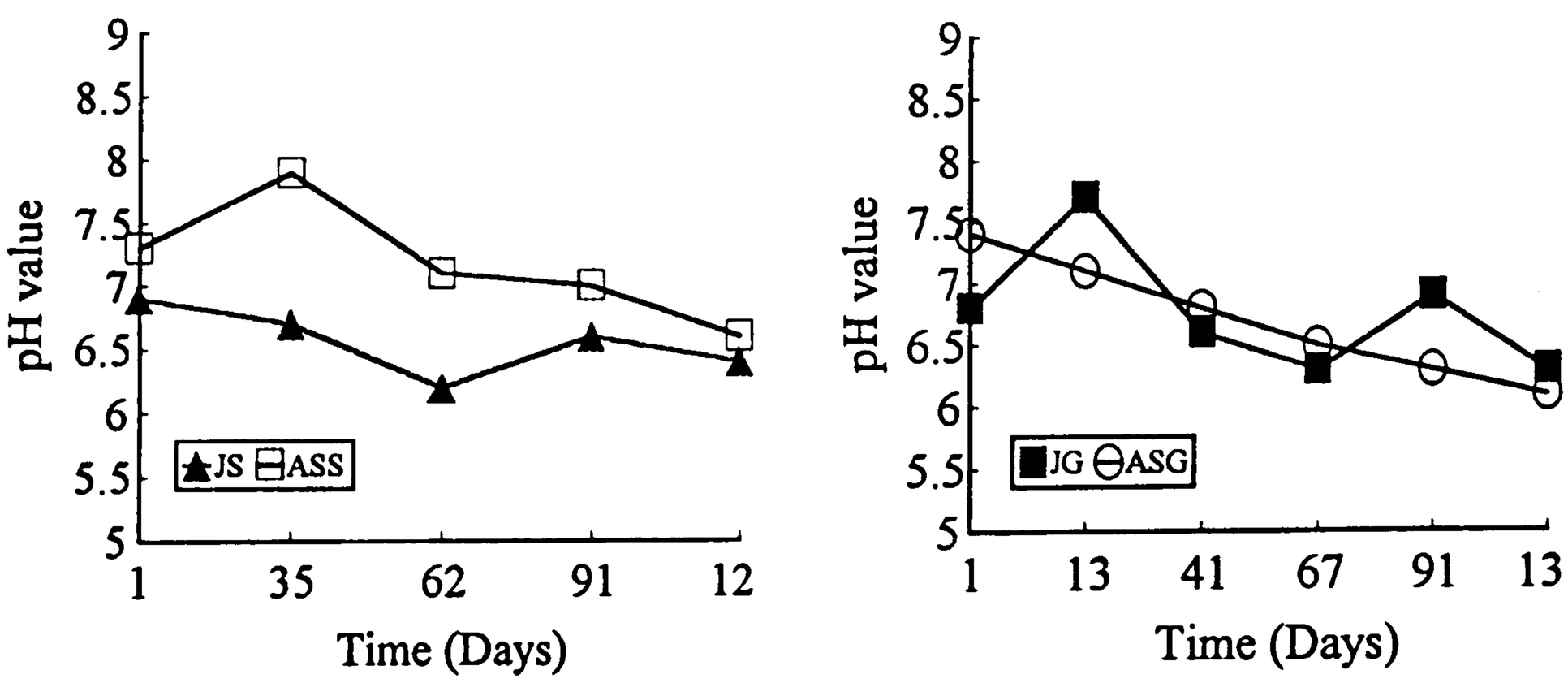


FIG. 11.15. Changes in pH value with time for sludges applied to the sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

11.3.5 Summary of statistical analysis

The difference in the sludge type loaded on sand drying beds was not associated with any difference observed in the abiotic parameters ($P > 0.05$, except pH values) that were measured (Table 11.5). The difference in the sludge type loaded on gravel drying beds did not show any significant impact on the abiotic parameters ($P > 0.5$, $F < 6.0$; Table 11.5); there were only significant differences in the sludge depth ($P < 0.03$, $F > 6.6$; Table 11.5) between the two gravel drying beds that were loaded with different sludge types.

11.3.6 Bacterial survival

The log numbers of total coliforms and faecal coliforms in raw sludge and the survival time in drying beds are presented in Figures 11.16 and 11.17. The *Salmonella* spp. and faecal streptococci isolations are indicated in Figures 11.18 and 11.19. All counts are expressed on a dry weight basis.

Coliform counts from sludges applied to the sand and gravel drying beds from both JTP and ASWSP sludges are shown in Figure 11.16. In all drying bed sludges the coliform counts were higher than 10^2 MPN/g total solids. While faecal coliform counts were less than 100 MPN/g total solids after 132 days following the application of the sludge to all drying beds (Fig. 11.17), except at Al-Samra gravel drying bed where faecal coliforms reduced to 10^3 MPN/g total solids. As for faecal coliforms, the total coliform counts recorded the lowest reduction in the Al-Samra gravel drying bed (Table 11.6). *Salmonella* spp. showed more sensitivity to desiccation than other tested indicators bacteria. No *Salmonella* spp. were recorded after 122 and 132 days of application of the sludge on sand and gravel respectively, in both Al-Samra and Jerash sludges (Table 11.6 and Fig. 11.18).

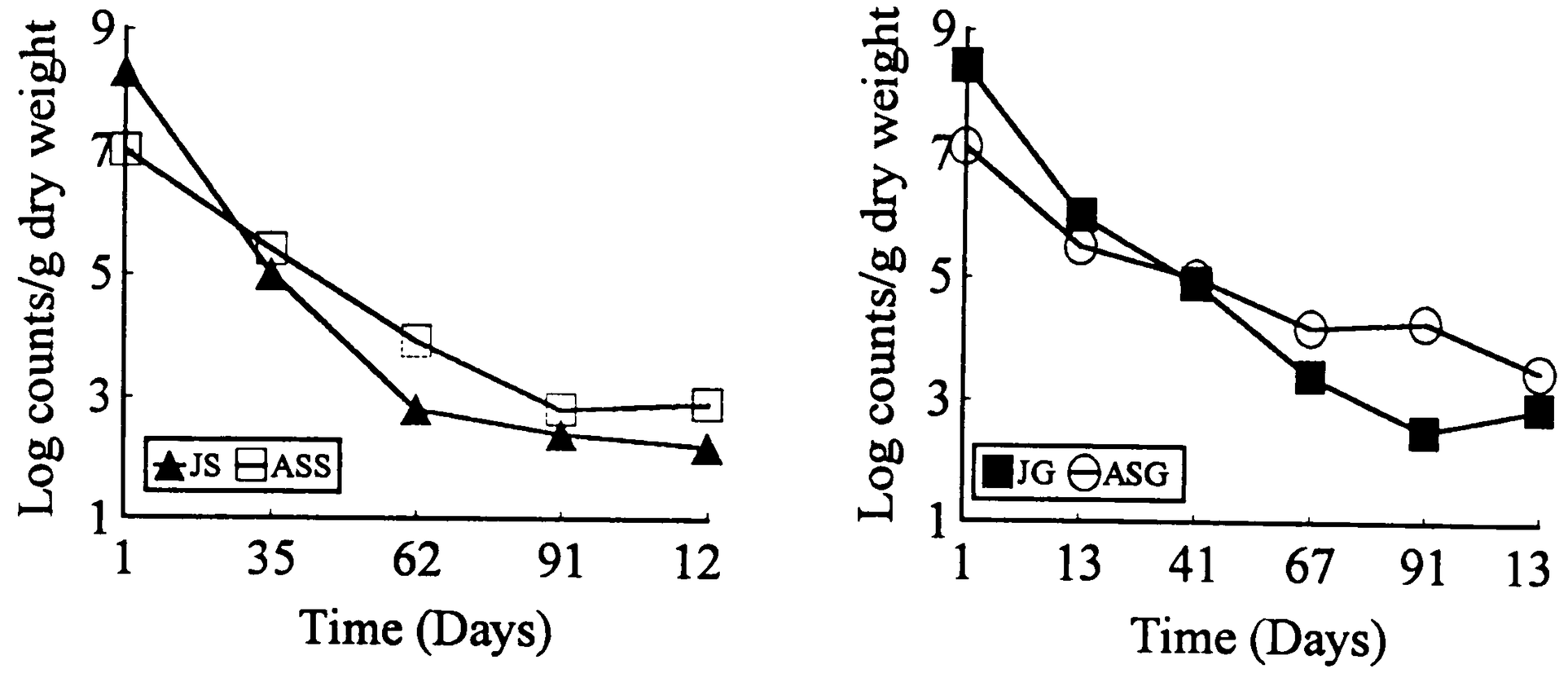


FIG. 11.16. Reduction in total coliform counts with time for sludges applied to the sand and gravel drying beds

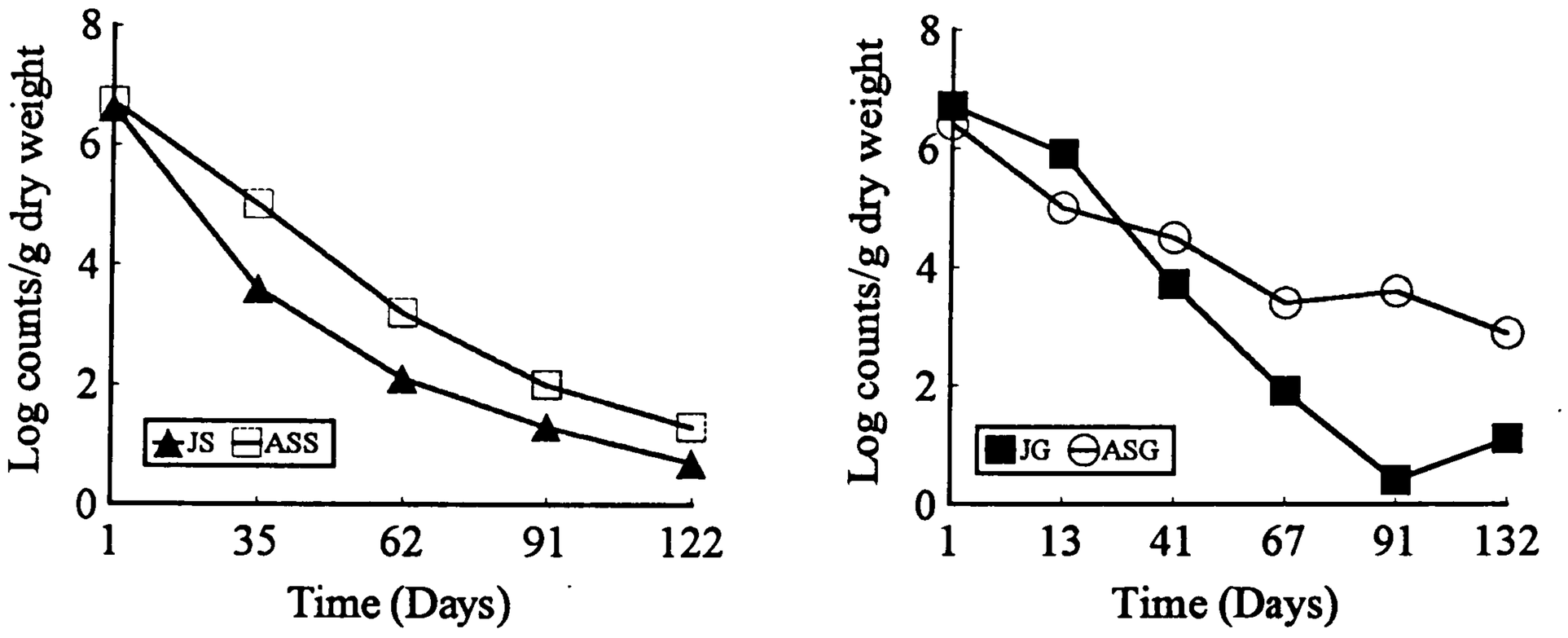


FIG. 11.17. Reduction in total faecal coliform counts with time for sludges applied to the sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

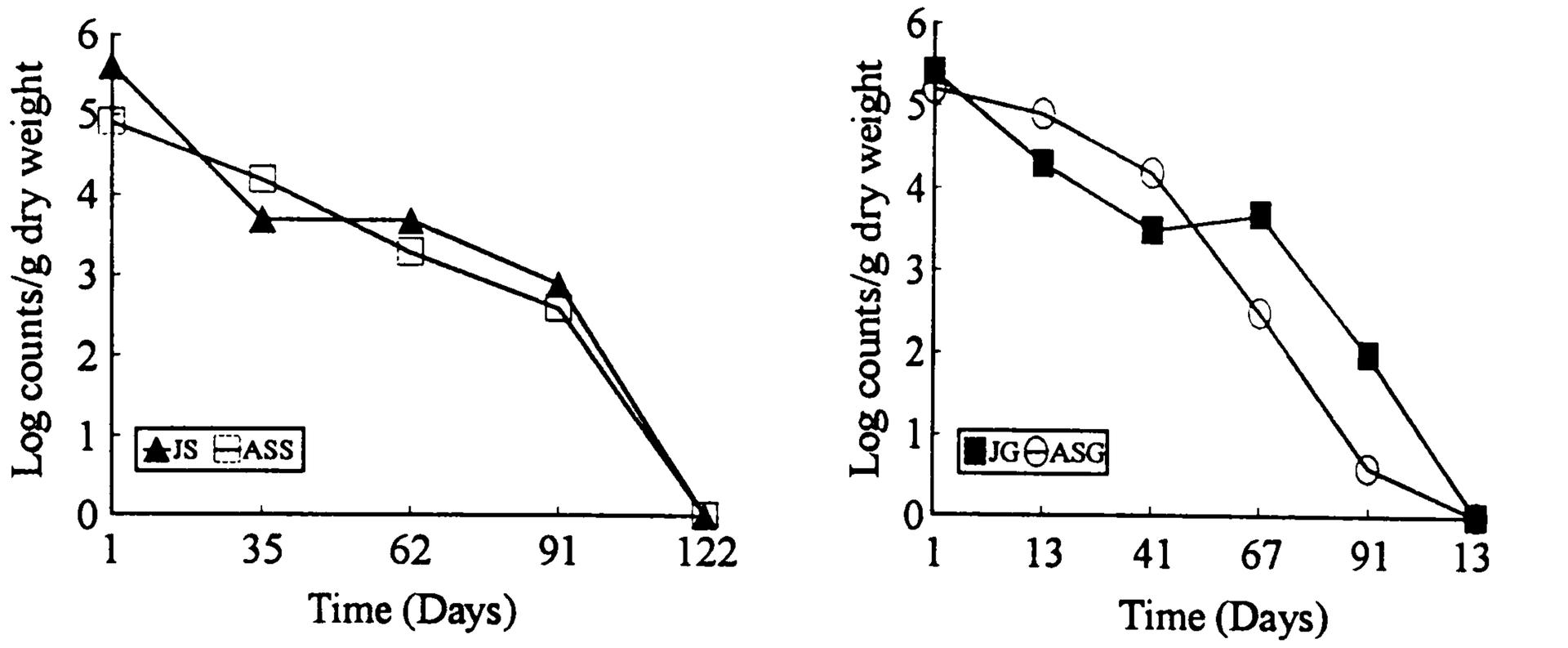


FIG. 11.18. Reduction in *Salmonella* spp. counts with time for sludges applied to the sand and gravel drying beds

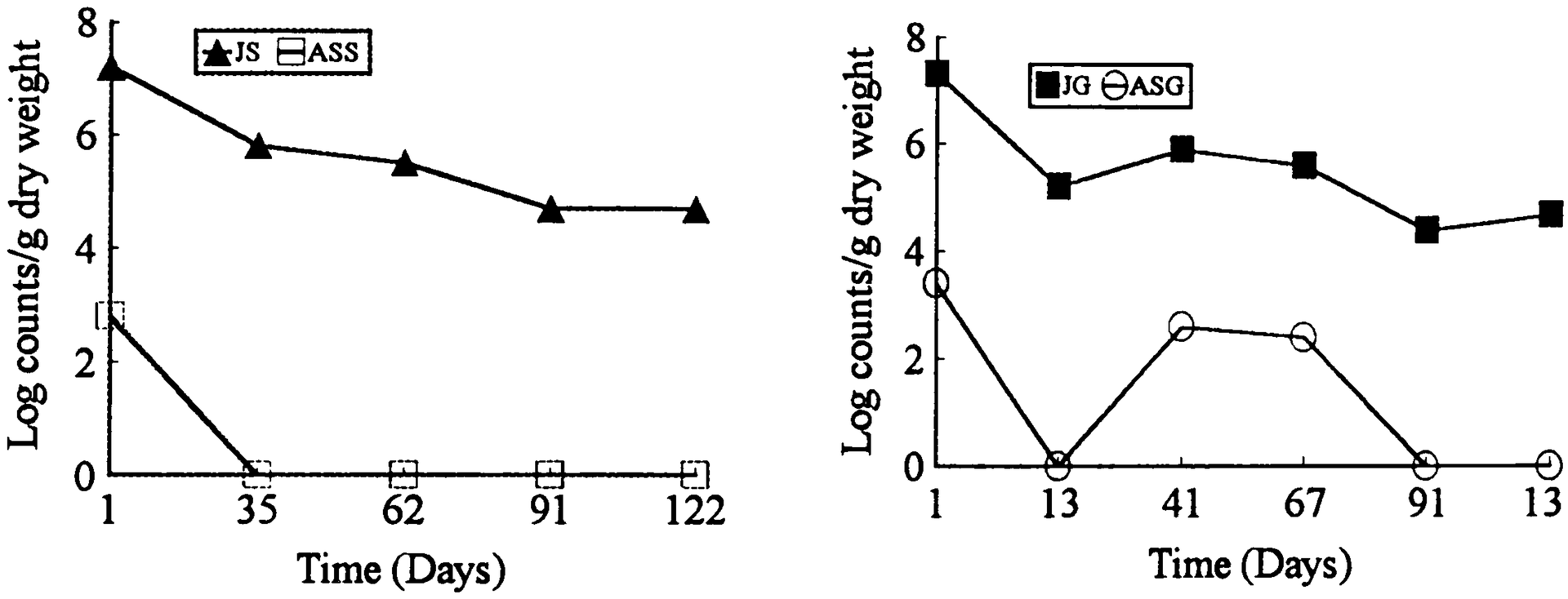


FIG. 11.19. Reduction in faecal streptococci counts with time for sludges applied to the sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

TABLE 11.6. Log reduction of indigenous bacterial counts in sludge after five months detention time, applied on both drying bed types from both systems, in summer and winter season

Species (Log_{10})	Al-Samra		Jerash		
	Summer		Summer		Winter
	Gravel	Sand	Gravel	Sand	Gravel
TC	3.6	4.1	5.5	6.1	1
FC	3.5	5.4	5.6	5.9	0.3
Salmonella sp.	5	5.6	5	5.2	2.2
FS	3	2.8	2.6	2.5	0.9
Total solids increased to	96%	96%	92%	95%	14%

The results from Table 11.6 and Figures 11.16 and 11.17 showed that coliform and faecal coliform counts decreased more rapidly in JTP sludges than at Al-Samra drying beds, while *Salmonella* counts reached undetectable levels in all drying beds sludges after 132 days. Referring to Tables 11.4 and 11.5, the inactivation of bacterial indicators and *Salmonella* spp. were significantly increased with time in the four drying beds ($P \leq 0.06$, $F > 4.3$). Faecal streptococci in ASWSP sludges was not associated with any significant reduction with time in sand or gravel drying bed types ($P = 0.8$, $F < 5.1$; Table 11.4, Fig. 11.19). This is due to the original fluctuation in faecal streptococci numbers in anaerobic ponds, so the statistical analysis cannot predict the changes.

Also the results indicate that there were no significant differences when using different types of sludge on gravel drying bed in the inactivation of indicator bacteria and *Salmonella* spp. (Table 11.5). Only faecal streptococci showed significantly higher counts within the Jerash sludges compared with ASWSP sludges ($P < 10^{-4}$, $F > 6.0$; Table 11.5; Fig. 11.19). In contrast, faecal coliforms showed higher in numbers in ASWSP sludges than JTP sludges and showing more resistance to drying when sand drying beds were used ($P = 0.02$, $F > 7.7$; Table 11.5; Fig. 11.17). Using different media (sand or gravel) in

drying bed did not show any significant difference in inactivating bacterial indicator and pathogens from both Jerash and Al-Samra sludges ($F < 7.7$, $F < 6.6$ respectively; Table 11.4).

During winter, bacterial inactivation using gravel drying beds for Jerash sludges was very ineffective, with a range between 1 to 2.2 $\log_{10}$ colony counts reduction (Table 11.6, Fig. 11.20). Over the same period sludge water content reduced from 98.6 to 88% during storage of two months on drying beds, with air temperatures ranging between 10-15°C and rainfall ranged between 3-65 mm/month during the winter study period (Nov. 1993-Jan. 1994).

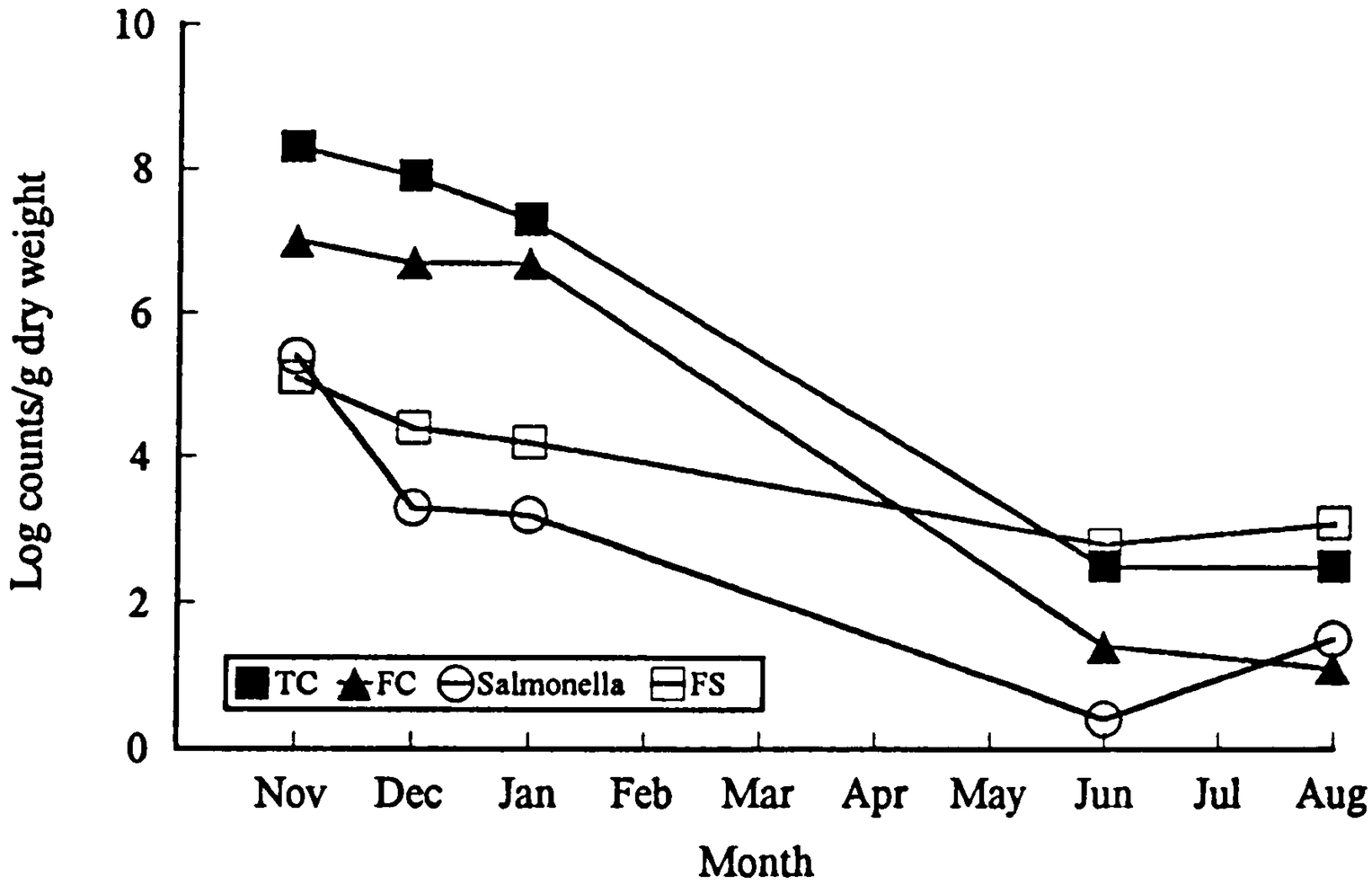


FIG. 11.20. Reduction in bacterial counts with time in oxidation ditch sludges during winter and summer season on gravel drying beds

Exponential regression analysis provided a mathematical relationship between indicator bacterial counts and *Salmonella* spp. with the percentage of total solids, which is shown in Table 11.7. In general, these functions were statistically significant with correlation coefficients 0.91 to 0.87 for total coliform counts and total faecal coliform counts,

respectively. The inactivation of total coliforms, faecal coliforms, and *Salmonella* counts was observed to increase exponentially at higher total solids content in sludge. Reducing faecal streptococci counts was observed to have weak correlation coefficient (r = 0.42), and showed weakly inactivation with the increasing in total solids percentages. The results in Table 11.7 lead to the conclusion that other factors i.e. sunshine, sludge temperature, season, nutrients, competition, etc. may contribute with total solids for inactivation of *Salmonella* spp. and faecal streptococci.

TABLE 11.7. Regression analysis of the effects of total solid contents on viable, total helminth eggs, bacterial indicators and pathogens counts from the sludge drying beds

Pathogens	Function $*y = \propto e^{\beta x}$	Correlation coefficient (r)	r-squared
Total coliforms	Bacterial counts = $19.1\ e^{-0.12TS}$	0.91	0.83
Faecal coliforms	Bacterial counts = $17.2\ e^{-0.12TS}$	0.87	0.75
Salmonella spp.	Bacterial counts = $13\ e^{-0.085TS}$	0.69	0.47
Faecal streptococci	Bacterial counts = $8.0x10^6 - 2x10^6$ ln(TS)	0.42	0.33
Total helminth eggs	Egg counts = $4.6\ e^{-0.04TS}$	0.84	0.71
Viable *Ascaris* eggs	Egg counts = $3.7\ e^{-0.04TS}$	0.88	0.79

*y = natural logarithmic eggs or bacterial counts (except faecal streptococci which had logarithmic plot [$\propto + \beta$ ln(x)]) in drying beds sludges (counts/g dry weight);
x = Percentage of total solids.

11.3.7 *Ascaris* eggs survival

Results shown in Figures 11.21 and 11.22 illustrate that drying bed treatment provided effective *Ascaris* egg reduction in both JTP and ASWSP sites. During the summer period, treatment anaerobic pond sludges on sand or gravel (May to September, 1994) revealed that all the eggs had degenerated when the percentage of total solids was recorded as more than 88%.

During the summer season experiments on drying beds, when the range of air temperature recorded was between 25-37°C and no rain fell, a die off of the *Ascaris* eggs occurred much more rapidly than the bacterial cells. After 35 days exposure of the eggs in the Jerash sludge to the natural drying conditions in open drying beds, 100% of the viable eggs had been inactivated, with reduction of the moisture content from 98% to 7.5%. In the winter season, the viability of *Ascaris* eggs showed 78% reduction (from 127 to 28 viable eggs/g) within 30 days, and after 60 days the viability decreased to 8 eggs/g (94% reduction); only slight decrease in moisture content was noticed (99% reduced to 88% after 60 days). The reduction in *Ascaris* eggs viability in winter confirmed that there are many synergistic factors playing a role in reducing egg viability, in addition to desiccation. A good correlation coefficient of 0.84 was found between total helminth eggs and the percentage of total solids in sludge.

In the surface layer of anaerobic pond sludges in sand drying beds, the moisture content reduced to 21% after 41 days, when all *Ascaris* eggs showed degeneration. However, in the deep layers of the same drying bed, the viability of *Ascaris* eggs showed 94% reduction for the same period exposure, were the moisture content reduced only from 94% to 62%.

For anaerobic pond sludges on a gravel drying bed after 13 days exposure period, in deep layers the viability of *Ascaris* eggs showed only 45% reduction (from initial viable eggs counts 40 reduced to 22 eggs/g after treatment in drying beds), when moisture content reach 76%. Surface layers showed higher reduction in moisture content (36%) than the deep layers at the same exposure period of the same site, with 91% reduction of *Ascaris* eggs viability (reduced to 4 eggs/g) (Fig. 11.21). Increasing the drying exposure time to 41 days, reduced the moisture content to 68% and 12% in the deep and surface layers, respectively, and the viability of *Ascaris* eggs was reduced by 70 and 100%, respectively.

Indigenous *Ascaris* eggs in oxidation-ditch sludges were inactivated within 35 days and 67 days by using either sand or gravel open drying beds, respectively. On the other hand,

anaerobic pond sludges showed high reduction in viability of *Ascaris* eggs after 62 days using sand and 91 days using gravel drying beds.

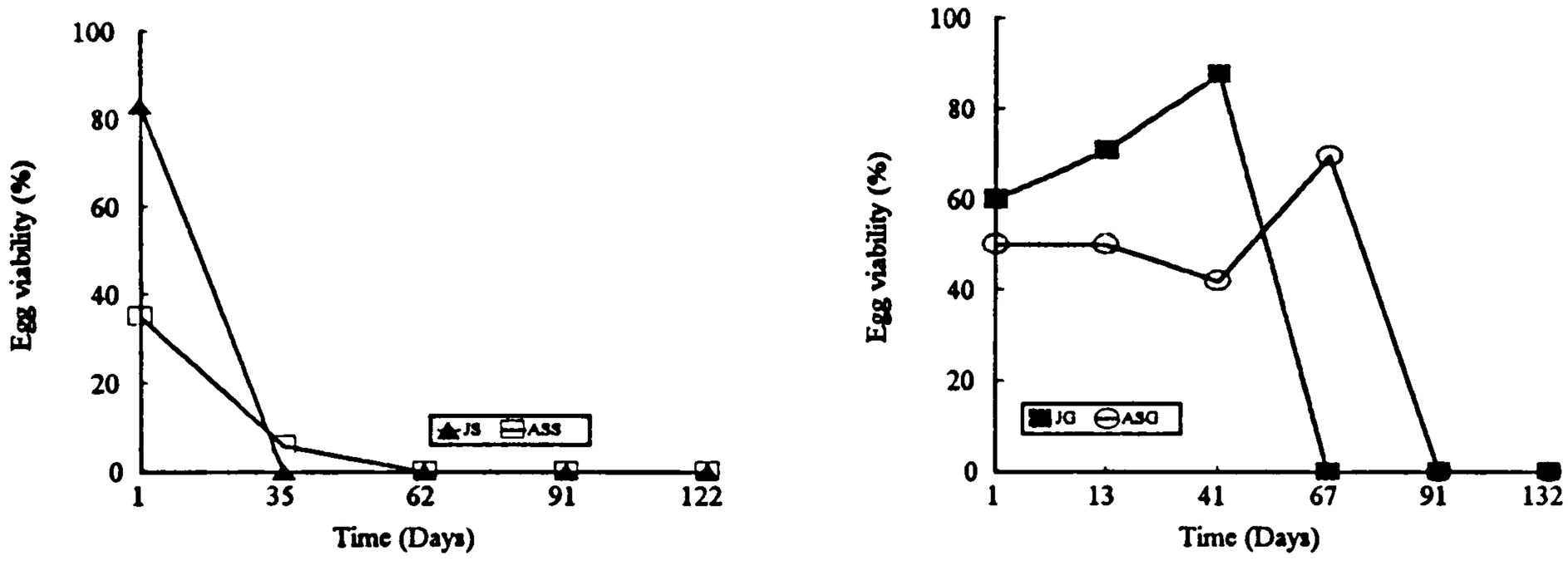


FIG. 11.21. Reduction in the viability of *Ascaris* eggs with time, for sludges applied to the sand and gravel drying beds

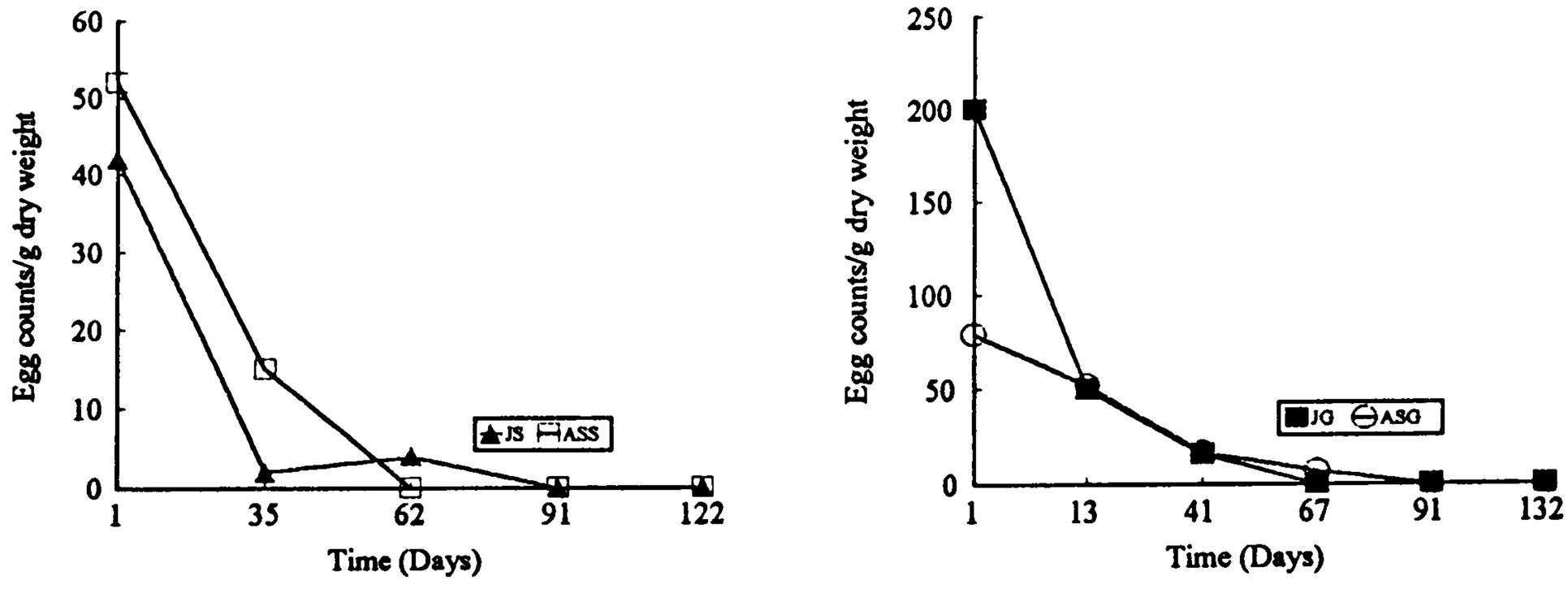


FIG. 11.22. Reduction in *Ascaris* egg counts with time for sludges applied to the sand and gravel drying beds

(Note: JS = JTP sludges treated on sand drying beds, ASS = ASWSP sludges treated on sand drying beds; JG = JTP sludges treated on gravel drying beds; ASG = ASWSP sludges treated on gravel drying beds).

The influence of total solid content (inverse of moisture content) of sludge in drying beds was statistically analysed using regression analysis, comparing the densities of helminth eggs and the viability of *Ascaris* eggs in all drying beds sludges to the different total solids content. The natural logarithmic transformed egg counts had a regression equation fit which indicated a statistically significant or good relationship occurred between total solid content and egg counts or viable egg counts. The results of this analysis are shown in

Table 11.7. In general, these functions were statistically significant with correlation coefficients ranging from 0.8 to 0.9 for viable *Ascaris* eggs and total helminth eggs counts (viable and non-viable), respectively. The inactivation of *Ascaris* eggs and reducing total helminth counts was observed to increase exponentially at higher total solids content in sludge. The 100 percent inactivation of total helminth eggs was found to be at approximately 22% sludge moisture content.

Using the Crystal violet staining method to assess *Ascaris* viability in sludges during the drying periods in drying beds showed weak correlation coefficient (0.44) with the incubation method. The percentage of eggs stained with crystal violet (presumed non-viable) was lower than the percentage of dead eggs determined by using the incubation method. This is due to the shrinkage and breaks in the shell of the eggs as a result of desiccation, although the cells inside the eggs were not sufficiently damaged physiologically to take up the stain.

Figures from 11.23 - 11.28 show the system for pumping the anaerobic pond sludge to the ASWSP drying bed; view for JTP sludges on gravel drying bed during the late winter season; also the difference between the oxidation ditch and anaerobic pond sludges after drying period on gravel or sand drying beds.

FIG. 11.23. View showing the system for pumping the anaerobic pond sludge to the ASWSP drying bed

FIG. 11.24. Showing the tomato and camomile plants growing on oxidation-ditch sludges on the gravel drying bed during the late winter season

FIG. 11.25. Showing the difference between the oxidation ditch (left) and the anaerobic pond (right) sludges from surface layer after 132 days drying period on drying beds

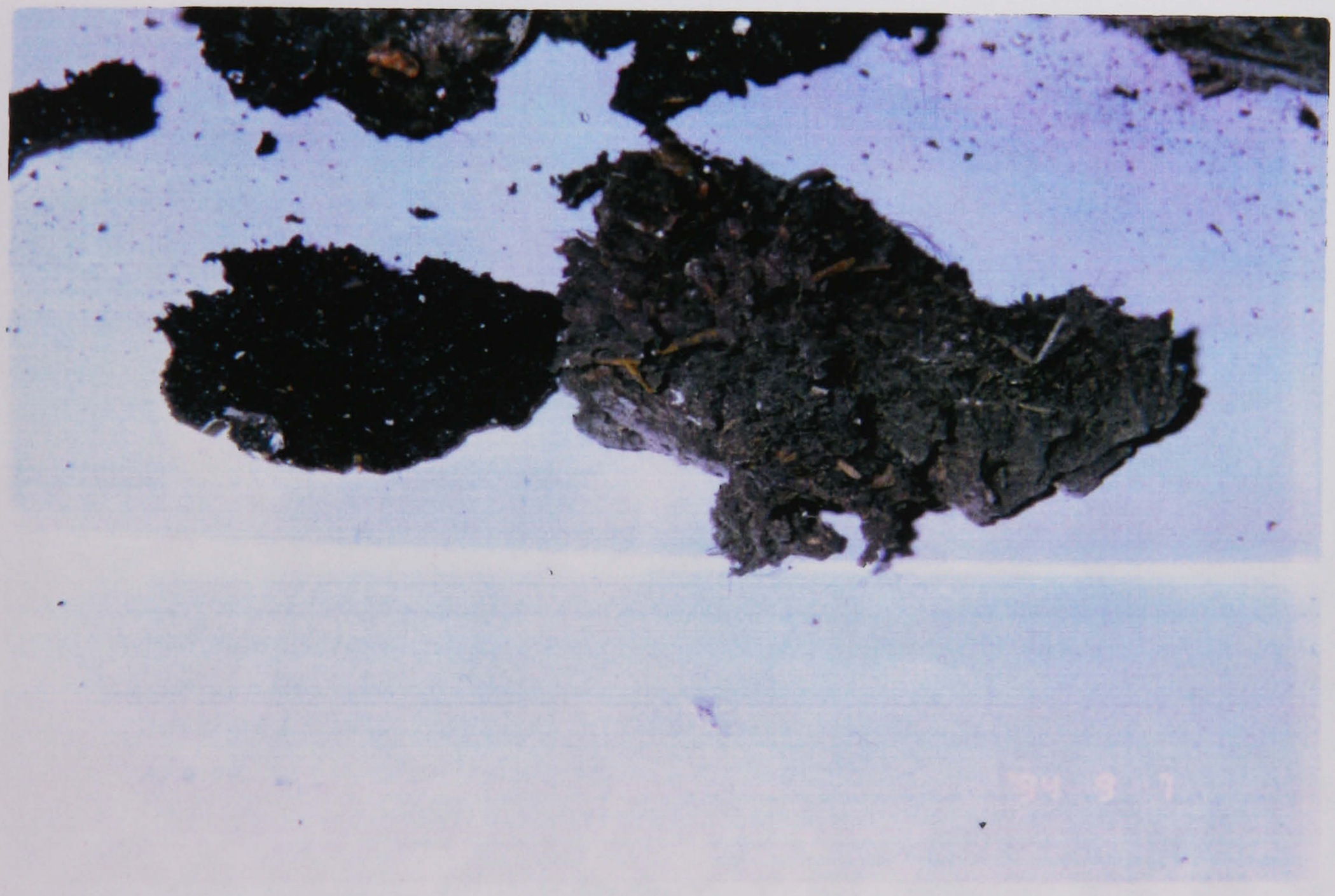


FIG. 11.26. Showing the difference between the oxidation ditch (left) and the anaerobic pond (right) sludges from deep layer after 132 days drying period on drying beds

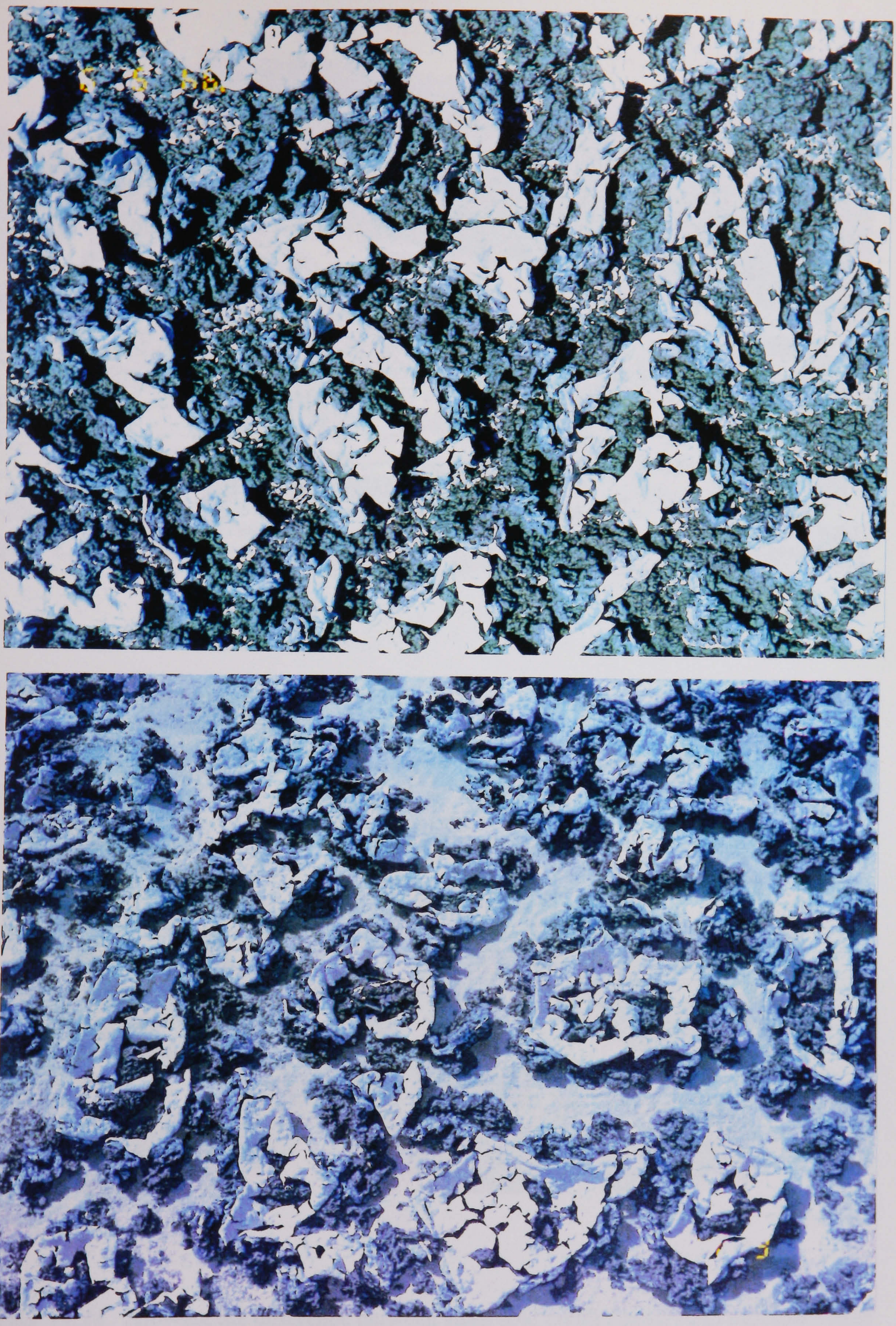


FIG. 11.27. Showing the difference between oxidation-ditch sludges after drying period of 132 days on gravel (upper) or sand (lower) drying beds

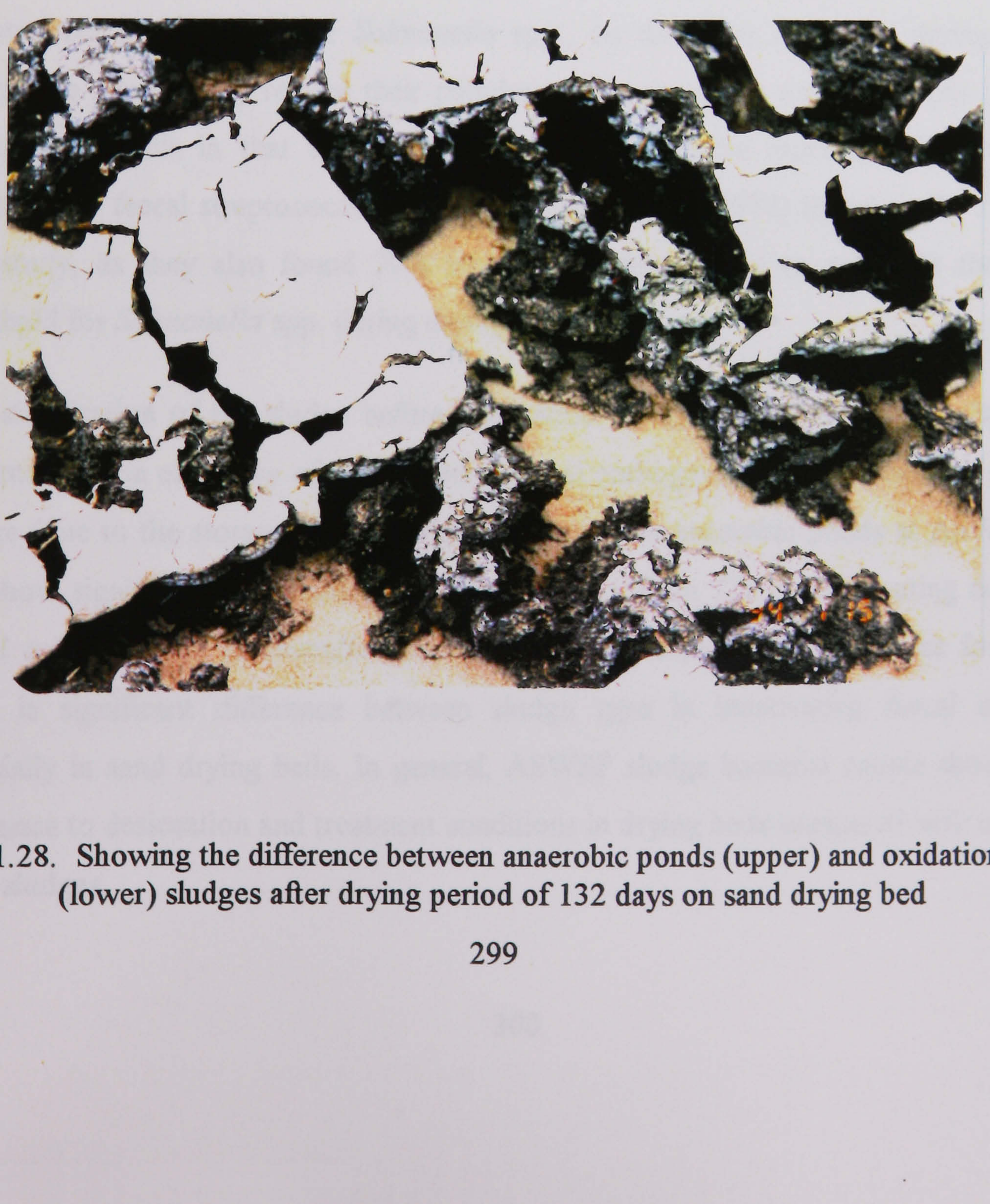


FIG. 11.28. Showing the difference between anaerobic ponds (upper) and oxidation-ditch (lower) sludges after drying period of 132 days on sand drying bed

11.4 Discussion

Applying digested sludge from anaerobic ponds to sand or gravel drying beds produces a sludge cake with a moisture content near 4% after 62 and 67 days in sand and gravel drying beds, respectively, at a temperature range between 25-37°C, with no rainfall and maximum evaporation rate of 9.7 mm/month. These drying periods for Al-Samra are significantly longer than with the Jerash sludge to achieve a stable depth on both sand and gravel. The different dewatering periods may be related to the dissimilar sludge total solids concentrations of Jerash and Al-Samra. Pescod (1971) has mentioned that 60% dry solids would be desirable for lifting sludge. The resulting sludge cake was easily handled and suitable for ultimate disposal. Drying beds need little operator attention, but are usually restricted to digested sludge because raw sludge is smelly, attracts insects, and does not dry well when applied at reasonable depths (Outwater, 1994), a finding which is confirmed in this present study.

Natural drying on drying beds was not very effective in reducing coliforms and faecal streptococci populations. For *Salmonella* spp., on the other hand, the drying process appears to significantly reduce their populations. It should be noted that this is not an unexpected result in that these bacteria are known to be more fastidious than are coliforms or faecal streptococci. Brandon and Neuhauser (1978) found similar results to this study, as they also found 20% moisture content seems to represent the critical threshold for *Salmonella* spp. during drying conditions.

The stabilisation of the sludge before application to drying beds appears to have little influence on the efficiency of drying beds to treat pathogens. Partially anaerobic digested sludge (due to the storage of the sludge for 7 years in anaerobic ponds at ASWSP) did not show significant difference with the raw sludge from JTP in inactivating coliforms, faecal coliforms and *Salmonella* when gravel drying beds used. But it was found that there is significant difference between sludge type in inactivating faecal coliforms especially in sand drying beds. In general, ASWSP sludge bacterial counts show higher resistance to desiccation and treatment conditions in drying beds compared with oxidation ditch sludges.

It appeared that the most likely explanation for the resistance of total and faecal coliforms to drying conditions in ASWSP sludges, was that anaerobic digestion of the sludge in anaerobic ponds caused degradation of most of the organic material to simple substances that can be readily taken up by microorganisms, and this situation may help bacteria to survive in higher numbers without high competition from other organisms for food. In contrast to oxidation ditch sludge, where the nutrients are still in complex forms and not readily available to be taken up by microorganisms; this causes high competition between microorganisms for food. This means that the organic material can be utilised best by microorganisms that are adapted to the environment. *Salmonella* spp. is an intestinal type accustomed to favourable uniform temperature and a readily available supply of simple nutrient materials. Other coliform bacteria and faecal streptococci fared better than *Salmonella spp.* in the sludge, but were less able than many of the other bacteria present to survive in the environment. Fuller and Litsky (1950) proved that *E. coli* can live longer in sludge if nutrients are available, and they eliminated the possibility that anti-biotic agents or bacteriophage had been responsible for *E. coli* disappearance from dried sludge.

The survival of *Salmonella* spp. in JTP sludges treated on gravel drying in winter season showed a 2 logs decrease in their counts with air temperatures ranging between 10-15°C and rainfall ranged between 3-65 mm/month; this might be explained that the organism disappeared because of competition with other microorganisms better adapted to survive in the environment, also this proved that many factors other than desiccation play a role in inactivating bacteria.

During the summer season, the results of this study showed that *Salmonella* spp. were inactivated within 5 months of exposing to natural drying conditions. The indicator bacterial counts decreased between 4 to 6 logs for total and faecal coliforms but the faecal streptococci decreased only 3 logs. Similar findings were reported by Reimers *et al.* (1989) but they used lagoon sludge storage treatment; they found indicator bacterial counts decreased two to six logs for total and faecal coliforms but faecal streptococci decreased very little even when sludge was stored in lagoons for fifteen months.

The bacterial survival in drying beds reported in this study supports one main conclusion regarding the effects of water loss on the survival of bacteria in sludge. This is that loss of water by evaporation in itself does not appear to be an adequate method for reducing the bacterial population in sludge.

In general, the results from the literature showed that conventional sludge stabilisation treatment processes (i.e. mesophilic anaerobic or aerobic digestion) are not completely effective in destroying parasite eggs. Drying beds, however, appear to be very effective in destroying parasites, which is also confirmed with Reimers *et al.* (1981) findings in USA sludge drying beds.

In contrast, Bahaskaran *et al.* (1956) studies showed that the parasites survived up to 51 days when the moisture content in the samples was 3.1 per cent. The viability of the parasites was, however, reduced to 10% when the moisture content dropped down to this level. They concluded that if drying has to be used as a method of destruction of parasites in sludge it is necessary to dry the sludge to a very low level of moisture which is not, however, practicable.

Keller (1951) found a further decrease in *Ascaris* egg viability occurs during drying, but he refers to this decrease as due to mould activity, rather than to slow desiccation. He found that during drying on drying beds the eggs are attacked by moulds, and thus the eggs are destroyed by mould activity rather than by slow desiccation. The writer has come to the conclusion that mould activity is far more influential in the destruction of the eggs than slow desiccation which may only retard or temporarily suppress the active development of the eggs, but in most cases will not inactivate them completely.

In 1943, Cram reported that *Ascaris* eggs were not completely inactivated in sludge drying beds unless the moisture content was less than 5%. Reimers *et al.* (1981) found that certain drying bed conditions such as previous sludge stabilisation, high temperature, and low moisture content appear to inactivate parasite eggs synergistically between 60% to 5% sludge moisture content. In the present study, inactivation of viable *Ascaris* eggs in sludge drying beds was observed at moisture contents less than 21%. The inactivation of *Ascaris* eggs in drying beds is probably due to more factors than

desiccation alone. Temperature, oxygen content, solar radiation, exposure time, mould activity etc., may also affect survival of the eggs.

In the present study the results showed, that indigenous *Ascaris* eggs in oxidation-ditch sludges were inactivated within 35 days and 67 days by using either sand or gravel open drying beds, respectively. On the other hand, anaerobic pond sludges showed high reduction in viability of *Ascaris* eggs after 62 days using sand and 91 days using gravel drying beds.

The influence of sludge type on drying bed performance in inactivating parasite eggs had also been shown by Reimers *et al.* (1981). In contrast to the results of this study, they found that anaerobically digested sludge showed a high percentage reduction (97%) of viable *Ascaris* eggs when using drying beds; compared with aerobically digested sludge where only 75% reduction in *Ascaris* egg viability occurred. Clearly one of the factors affecting the performance of drying beds is the type of sludge; whether it is raw sludge or has been through one of the stabilisation processes. The variation between the results of this study and Reimers *et al.* (1981) finding may be attributed to the quality and the source of the sludge itself, i.e. from ponds or conventional treatment plant. Sludge from anaerobic ponds, for example had a lot of debris, sand and seeds, not very homogenous with high total solids content; it will end up after drying as a thick sludge, more difficult to dry than primary sludge from conventional treatment plants (homogenous, with no debris, less total suspended solids, which forms a thin layer of sludge after drying).

In the present study it was found that climatic conditions were generally favourable for dewatering sludge on drying beds in Jordan, except when heavy rainfall during the winter season prolonged the drying time.

The results of this study give a clear picture of the effects that the weather contributed to the elimination of the coliforms, faecal streptococci and parasite eggs from the surface sludge on drying beds. The more rapid elimination of pathogens from the surface of the sludge on the drying beds suggests that even spreading sewage sludge on the surface of

the soil under dry conditions would more rapidly eliminate pathogenic organisms than if sludge storage lagoons were used to treat pathogens. Reimers *et al.* (1989) found that sludge storage in lagoons took 15 months to inactivate *Ascaris* eggs; for *Salmonella livingstone* and polioviruses inactivation occurred within six months, which is a very long period compared with the results found in this study when drying beds were used.

The absence of *Ascaris* eggs from sludges that have been treated on drying beds cannot guarantee safety from other pathogenic organisms, such as *Salmonella* spp.. The results of the present study indicate that the absence of total coliforms from **dried sludge** should be adequate insurance against danger from pathogenic intestinal types of bacteria, such as *Salmonella* and nematode eggs. Additional evidence would be needed to assure saftey from certain other diseases, such as poliomylitis. Little is known about the survival of protozoa and viruses in dried sludge. Fuller and Litsky (1950) through their discussion of laboratory scale, artificially seeded *E. coli*. in dried sludge, proved that the absence of *E. coli* from dried sludge should indicate that there is nothing to fear from *Salmonella* spp., but their results could not be extrapolated to assure safety of sludge from parasites.

Because the disposal of sludge onto land provides an effective solution to sludge management problems with a somewhat unrealised potential, offers added benefits for dried sludge treated on drying beds, and is especially suited to semi-arid countries such as Jordan where the land is plentiful, land application of dried treated sludge is considered to be a most suitable means for anaerobic pond sludge disposal, based on the results of this study.

Developing Jordanian regulations is a very important and urgent priority before sludge application to land starts to be practiced. This will require detailed sampling and analysis of sludge to identify and characterise the sludge constituents, so as to determine whether sludges are suitable for land application. Maximum annual loading rates may be prescribed, as well as permissible cumulative loading rates, depending on whether the land is used for agricultural or non agricultural purposes.

11.5 Summary

The results have shown that *Ascaris* eggs were found to be inactivated before coliforms in drying beds, so that *Ascaris* eggs cannot be considered to act as a good indicator for other pathogens removal in drying beds. Coliforms were unexpectedly resistant to low moisture content in air dried sludge. This is borne out by high residual coliform counts in both ASWSP and JTP sludges (as well faecal streptococci in JTP sludges). This underlines the importance of coliform counts in assessing sludge disinfection by using air drying procedures. *Salmonella* and *Ascaris* eggs cannot be used as hygiene indicators because their susceptibility to desiccation in sludge treatment differs considerably from that of coliforms and faecal streptococci.

Applying the sludges from anaerobic ponds or oxidation ditches to sand or gravel drying beds produces a handleable sludge cake with a moisture content near 4% after 2 months. It provides an easily applied and relatively economic solution to sludge disposal problems associated with pathogen control especially of helminth eggs and *Salmonella* spp.

The sewage sludge after drying bed treatment during the summer season met "Class A" criterion in the USEPA Regulations (1992) with respect to viable helminth eggs, because the density of viable helminth eggs in sewage sludge after 67 days treatment is less than one per four grams of total solids. Also after at least two months treatment of sludge on drying beds, faecal coliforms (MPN) reduced to less than 1000 per gram of total solids, and the density of *Salmonella* sp. in the sewage sludge was less than 3 MPN per 4 grams of total solids after around 132 days natural drying treatment. "Class A" criterion in the USEPA Regulations (1992) is for land application and using the sludge as a fertiliser. 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. It also should be confirmed that the bacteriological counts are not increased due to regrowth problems at the time when the sewage sludge is used or disposed, and

that enteric virus counts are low, but these aspects were not addressed in the present study.

Finally, the die-off data in both anaerobic pond and oxidation-ditch sludges on gravel or sand drying beds, indicated pathogen reductions within 5 months, and was in accordance with USEPA Regulations with processes to further reduce pathogens (PFRP) in sludge, treatment processes, or process schemes.

This research suggest that sludge drying beds can be an effective method for eliminating parasite eggs, particularly in warmer geographic locations. The treated sludge can be considered of suitable quality for application to agricultural land particularly from the parasitological characteristics. It would be best to store the sludge in a holding pond, and then to apply a quantity of sludge to a small number of sludge drying beds, in order to minimize the cost of drying bed construction.

Extensive future research is needed to define the applicability of using the sludge for agriculture under the conditions prevailing in Jordan with respect to pathogens (viruses, protozoa), vector attraction, nutrition value, toxic organic elements, choice of crops, soil, etc.

CHAPTER TWELVE

CONCLUSIONS AND RECOMMENDATIONS

12.1 Summary and Conclusions

This research objective included survey, laboratory and field studies. First, an evaluation of the present status of intestinal parasitic infections was carried out, especially the incidence of *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia* spp. in the Jordanian population.

Second, laboratory investigations were conducted on the development of a new technique to detect the viability of *Ascaris* eggs, by comparing 4 different vital stains, and evaluating the versatility of the staining method, and the advantages and disadvantages inherent in the method.

The field studies consisted of a year-long investigation of indigenous parasites and indicator pathogens in domestic waste sludges in Jordan. This investigation has resulted in new information concerning: (1) the types and concentrations of resistant stages of parasitic helminth eggs and bacterial indicators in Jordanian sludges, especially from waste stabilisation ponds sediments; (2) characterisation and quantity of sludge from anaerobic ponds at the Al-Samra system; (3) the distribution of sludge and pathogens over the bottom of the pond; (4) the level of heavy metals in sludge. This information is helpful in making a preliminary evaluation of the suitability of these sludges for disposal on land. (5) Field investigations also were conducted on the effect of sludge treatment processes on the inactivation of parasite eggs and bacterial pathogens by using open natural drying beds.

A survey of available literature, together with the results of this study, indicates **a need for a universally accepted definition of a viable** *Ascaris* egg. Conclusions are presented individually for the three major phases of the study.

12.1.1. Phase one: Survey of Health Statistics

- A total of 22,214 patients were examined in the period from 1990-1994 at the Central Health Ministry Laboratory. Of these 3,352 (15%) were found positive for intestinal parasites. Eight helminth species were identified, five nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Enterobius vermicularis*, and *Strongyloides stercoralis*; one trematode (*Schistosoma mansoni*); and two cestodes (*Hymenolepis nana*, and *Taenia saginata*).

- Protozoan infections occurred in 81% of all positive cases, helminthic and intestinal nematode infections in 19% and 7% respectively. Furthermore, the commonest helminth infections were *Hymenolepis nana.* The least common parasite found was *Trichuris trichiura* 0.045%.

- There was a 55% and 37% reduction in the prevalence of intestinal nematodes and intestinal helminth infections respectively from 1990 until 1994 [specifically, the reduction for *Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia saginata* and *Hymenolepis nana* was 60%, 65%, 64%, and 30% respectively]. In contrast, for the protozoan infections there was an increase of 14% in the prevalence of this group in the same period (1990 to 1994).

12.1.2. Phase two: Evaluation of Vital Staining Method

- A staining technique has been developed in conjunction with research studies of *Ascaris* eggs in sludge. A series of 4 vital stains (Crystal violet, Meldola's blue, Methylene blue eosin-borax, and Nile blue stain) have been assessed in the laboratory to determine whether exclusion or inclusion of them correlated with viability as assessed by the incubation method. In conclusion, it is suggested that Crystal violet as a vital staining method has potential for use in the rapid assessment of viability of *Ascaris* eggs. The data indicate, for the experimental conditions investigated, a close agreement between Crystal violet viability values and embryonation of eggs using the

Incubation method, with a correlation coefficient of 0.979, after 5-10 min applying the stain at 21°C ± 2. A short staining period is employed to facilitate routine examination and to minimise changes in the samples.

- Furthermore, the combined results at pH 4.5, 7.2, and 10; and with temperatures 4°C, 21°C and 37°C gave a good correlation between the viabilities determined by the Incubation and Crystal violet staining method, with a correlation coefficient of 0.927. Also, after *Ascaris* eggs were treated with high temperature (64°C) for different contact times, Crystal violet shows the best spontaneous detection of changes in egg viability, although the differences between stains are slight. This means that Crystal violet stain had the highest correlation with incubation method, and was more precise than for the other stains tested.

- The four vital stains showed striking differences between non-viable and viable decorticated eggs, and showed good correlation (Crystal violet =0.979; Meldola's blue = 0.945, Methylene blue eosin-borax = 0.978, and Nile blue = 0.963) with embryonation (the Incubation method) as a method for determining the viability of *Ascaris* eggs, 5-10 min after applying the stain at 21°C ± 2. This leads to the conclusion that, all vital stains used in this experiment with these conditions had similar good correlation with incubation method. In general, there was no impact of contact time on the uptake of the all tested stains by viable eggs over the range of conditions investigated.

- Temperature and pH treatment of eggs had a significant impact on the uptake of stain and affected the prediction of viability using Meldola's blue, MBEB, and Nile blue staining method with correlation coefficient of 0.31, -0.117, and 0.35 respectively. However for Crystal violet, there was no impact of temperature and pH treatment on the uptake of the stain, and there was a high correlation coefficient (0.927) with the Incubation method.

- The method for application of the stain on eggs to detect their viability was as follows: one drop from the decorticated egg suspension was placed on a clean glass slide; the end of a wire loop touched a drop of vital stain and was mixed thoroughly

with the eggs; after 5-10 minutes stained and unstained eggs were counted using a light microscope.

- Washing *Ascaris* eggs with distilled water had a significant impact on the uptake of the vital stain by the dead eggs and affected the prediction of viability. Statistical analysis of the washing experiment showed that it had a negative impact on the prediction of the viability of *Ascaris* eggs at different pH values and temperatures for Crystal violet, Meldola's blue and MBEB stain. Only Nile blue sulphate stain proved to have a good correlation between washing and non-washing experiments.

- The four vital stains tested in this study show no evidence of toxicity, and proved excellent as culture media after 30 days incubation, whereas non-viable eggs which stained blue never proliferate. Viable *Ascaris* eggs suspended in these stains embryonate and give very healthy and active larvae that also hatch and swim in the stain suspension, which suggests a possible use for these stains in determining the effects of various agents (e.g., chemicals) upon the viability of eggs in culture, without sacrificing the cultures.

- Treatment performed on decorticated undeveloped eggs had a significant impact on the uptake of the stain by the eggs and had an effect on the prediction of viability using the Crystal violet staining method. For temperatures 37°C, 50°C, and 60°C a weak correlation was observed between incubation method (% of motile embryos) and predicted (using Crystal violet vital stain) viabilities with a correlation coefficient, 0.26, 0.33, and 0.47 respectively. But as the temperatures increased, 70°C, 80°C, 90°C, and 105°C, good to strong correlation was observed between observed and predicted viabilities with correlation coefficients of 0.67, 0.68, 0.59, and 0.99, respectively. UV radiation also showed effect on the prediction of viability using Crystal violet, giving a correlation coefficient of 0.26 with Incubation method. The results showed there was a moderate correlation coefficient of 0.57 between the staining method using Crystal violet and the incubation method in detecting *Ascaris* egg viability in anaerobic pond sludge samples. The results show that higher eggs

counts were predicted to be viable by using the staining method than was found after incubation in all study samples.

- In conclusion, the Crystal violet staining method is not an absolute measure of full development of *Ascaris* eggs, it is however within certain parameters a strong indicator of the state of the eggs. A better application of the stain method would be to use it as a presumptive test by using Crystal violet stain (as a quick method to be used for routine purposes). It is suggested that if greater than 60% of eggs 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 less than 60% of the eggs take up the stain the confirmation of the eggs mortality must be obtained by using incubation method.

- The direct morphological microscopic method to detect the viability of *Ascaris* eggs proved to be not as accurate as the culturing method, as many eggs appeared in this study as healthy eggs, with no change in the morphology of the eggs but never-the-less losing their ability to develop. Only when degeneration is apparent can death be confirmed, and this may take many weeks or months. The study leads to the conclusion that living and dead eggs cannot be distinguished with certainty by direct observation under a microscope.

12.1.3. Phase three: Field Studies on Helminth Enumeration and Survival in Jordanian Sludges

- Anaerobic ponds are noted for their ability to store sludge for a long period; in the case of Al-Samra anaerobic ponds they had the capability to store a huge volume of sludge (36,600 m^3 dry weight basis) from 1985-1993 in six anaerobic ponds. The maximum depth recorded was 4.7m near the outlet in the primary anaerobic ponds A2-1. The mean sludge depths in the first sets of the ponds are approximately 1.5 times more than the mean sludge depths in second anaerobic ponds.

- The results showed that approximately equal average sludge depths occurred for ponds A1-1, and A3-1 of the same type and size, operating in a similar climate and conditions even though A3-1 pond had operated for 21.5 months longer than the A1-1 ponds. However, this was not found for ponds A2-1 and A3-1, even though they started operation on the same day.

- The sludge in primary and secondary ponds from WSP's was black in colour, granular in texture, compact, and had no objectionable odour. The sludge in all anaerobic ponds were generally in the range between pH 6.5 and 8.4; the pH increased with depth. The moisture content of the sludge was between 78%-99% in the primary ponds, while in the secondary ponds the range was between 82%-91%. From the deeper sludge sample data, moisture contents were mostly lower than the surface (78%-86%). The deep layers of anaerobic ponds in the Al-Samra system was at an advanced stage of stabilisation.

- Silting is limited mainly to the primary and secondary anaerobic ponds. The annual sludge accumulation rates estimated from mean sludge depth and operational period for the primary anaerobic ponds A1-1, A2-1 and A3-1 ponds were 29, 35, and 22cm/year, respectively. While for the secondary anaerobic ponds A1-2, A2-2, and A3-2, the annual sludge accumulation rate were 12, 25, and 20 cm, respectively.

- The measured total volatile solid content (TVS) of the sludge in the anaerobic ponds ranges between 47 to 73% of the total solids (TS). The top layers of the sludge are less digested than the bottom layers. The anaerobic pond sludge reveals some similarities to digested primary sludge. The longer detention time would provide consolidation of solids and digestion in a warm climate such as Jordan. From the less conventional sewage treatment plant i.e. Jerash, the sludge was grey in colour, and had an extremely offensive odour. The pH values ranged between the 6.4-6.9.

- The primary anaerobic ponds at Al-Samra had lost around 94-95% of the solids that entered the ponds over the operation period. It is suggested that the ponds are undersized and that significant short-circuiting was taking place, allowing solids to pass out of the ponds with effluents to the secondary anaerobic ponds.

- The primary removal mechanism of nematode eggs in WSP is thought to be sedimentation. However, it is clear from the data that other hydraulic mechanisms prevent complete sedimentation in ponds where the overflow rate is smaller than the settling velocity of the eggs.

- The results show that sludge from different systems may influence the survival of parasite eggs in different ways. The sludge from Jerash treatment plant provides a good conditions for parasite eggs to survive and develop. It is clear that only robust eggs can be detected and withstand the harsh conditions which prevail in the anaerobic pond sludges of waste stabilisation systems.

- Generally, the average total helminth eggs counts were found to be highest in Jerash sludges (313 eggs/g dry weight basis), followed by Al-Samra sludges (303 eggs/g dry weight basis), with the lowest counts observed at Madaba WSP (64 eggs/g dry weight basis). *Ascaris lumbricoides* were the most helminth eggs found.

- Primary anaerobic ponds cannot remove human intestinal nematode eggs efficiently and consistently, especially in overloaded conditions, where interrupted sedimentation can occur due to the gas production prevailing from anaerobic conditions, so it is advisable to be followed with secondary anaerobic ponds.

- The results showed that the highest egg counts (except for *Trichuris* eggs), were in the secondary rather than the primary anaerobic ponds at Al-Samra where twice the number of helminth eggs were found in the secondary compared with the primary anaerobic ponds. Furthermore, the results demonstrated that *Ascaris* eggs are not a good indicator for other helminth egg removal in WSP, because they are the heaviest among the helminth eggs, and most easily removed during the settling process. The results of this study showed high numbers of *Ascaris* eggs in the primary and secondary anaerobic ponds while *Taenia saginata* was predominant in secondary anaerobic ponds.

- Of the two different treatments investigated here, WSP's (specifically anaerobic ponds) were the most efficient in destroying and inhibiting the development of the cell

inside the eggs in the sludge layer, compared with oxidation ditch treatment. Apparently the Jerash sewage treatment plant had favourable conditions for the eggs to develop; in Jerash sludge samples, 63% of the *Ascaris* eggs were in the multi-cell and larval stage, and only 12 % of the eggs in one-cell stage, and they appeared viable by microscopic observation.

- Some of the *Ascaris* eggs that were isolated from Al-Samra anaerobic ponds showed a blackened appearance, which made it impossible to evaluate the internal cell stage. It must be assumed that this pigmentation was expressive of a degenerative process leading to the relatively imminent destruction of the egg. The observations from Jerash treatment plant, confirmed that there was no noticeable effect of the process upon the *Ascaris* eggs. None of the examinations revealed any evidence of blackening or any other alteration of the eggs from the normal appearance.

- The levels of indicator bacteria were found to be in the same range for the Madaba and Al-Samra samples, while a difference can be observed between WSP's and Jerash sludge samples; the results show at least 2 $\log_{10}$ counts higher of indicator bacteria in Jerash sludge samples. This confirmed that the microbiological quality of WSP sludges is better than that from less conventional sewage treatment plants, i.e. oxidation ditch.

- *Salmonella* spp. showed higher mean counts than reported in the literature in all treatment plant sludges that have been tested in this study, but within the range levels reported.

- The concentrations of total coliform, faecal coliform, and *Salmonella* spp. in sludge samples at Al-Samra anaerobic ponds decreased slightly with depth, with only 1.2 times higher counts in the surface than in the deep sludge layer. With respect to the faecal streptococci, another pattern is observed, since the concentrations appear 4.2 times higher in the surface than in the deep sludge layer.

- In this study the indicator and pathogen analysis (i.e. faecal coliforms and streptococci, *Salmonella* spp., and helminth eggs) of sludges from different treatment plants show levels in excess of those considered acceptable for sludge applied in bulk

to agricultural land, forest, public contact sites, reclamation sites, lawns, or home gardens (EPA, Class A Regulations). The sludge would therefore have to undergo a process to significantly reduce pathogens (PSRP) before they can safely be applied to agricultural land or used as a soil amendment.

- The removal of *Ascaris* eggs in anaerobic ponds may be related to the dimensions of the particles which settle towards the middle of the ponds, where high counts of eggs are also found to occur. In contrast, the bacteriological counts appear as a random distribution, with no concentration gradient established along the pond (except for *Salmonella* spp.).

- The statistical analysis of distribution of viable *Ascaris* eggs in A1-1 pond sediments shows that neither the length nor width of the pond seem to have much effect on the percentage of egg viability in A1-1 pond. Closer examination of the percentage viability of *Ascaris* eggs in sludge along the anaerobic pond length at Al-Samra shows that there was a slight decline in the viability of eggs in the middle of the pond. The majority of eggs settled out in a mean distance of ≈ 155m from the inlet and also near the corners, and the higher number of eggs detected in sludge samples, the higher is the probability to find viable eggs.

- The results showed no significant difference in the concentration of trace metals between the primary and secondary anaerobic ponds in sludge samples in the Al-Samra system. Due to overloaded conditions in the primary anaerobic ponds, the secondary anaerobic ponds are important in helping removal of the trace metal from the wastewater. The removal of Ag, Cr, Hg, and Zn metals from the secondary anaerobic ponds was 2.8, 2.3, 1.8, and 1.6 times more efficient than in primary anaerobic ponds, respectively. However, Co and Si had higher removal efficiency in the primary ponds (by 2.4, and 2.0 times, respectively) than the secondary anaerobic ponds.

- The distribution of trace metals were not associated with any significant difference between each primary or between each secondary anaerobic pond. The total concentration of As, Cd, Hg, Si and Zn in the deep layers were greater than surface

sludge samples. However the concentrations of Ag, Co, Cr, Se, and Ti metals from sludge samples on the surface were double the concentrations in depth samples.

- For Al, Cr, Cu, Hg, Pb, and Zn the removal efficiency from both primary and secondary anaerobic pond wastewater together, was typically 75% or more; the highest removal value was recorded for Al (83%), with the exception being chromium (33%). It seems from these results that the primary and secondary anaerobic ponds play a more important role in the removal of most trace metals that have been examined, especially for Pb, Hg, and Cu, than the subsequent facultative and maturation ponds. The results shows that the anaerobic pond could achieve a high removal of metals and shows that the removed metals accumulate in the sludge of the primary and secondary anaerobic ponds.

- Sludges produced in cities without industrial activity (Madaba and Jerash) were suitable for agricultural utilisation, while sludges obtained from Al-Samra may have some limitations to agricultural purposes, based on their metal content.

- Except for Zn in Al-Samra anaerobic pond sludge samples, levels of trace metals in Jordanian local sludges from Al-Samra anaerobic ponds, Madaba WSP's, and Jerash wastewater treatment plant sludges are significantly lower than the recommended limit values of USEPA and EEC for application on agricultural land. The mean and/or maximum Zn level in Al-Samra anaerobic ponds sludge samples, were higher than the suggested limit from several countries such as USEPA, Canada, France and Germany.

- A direct transformation of western guidelines to Jordan may not be appropriate. In some respects, the guidelines are based on conditions which differ from those found in Jordan. With respect to heavy metals, two factors, soil pH and amount of atmospheric deposition, seem more favourable in Jordan for the use sewage sludge on agricultural land than in many European countries.

- In most cases, even the high values of Zn probably should not be considered prohibitive to the application of these sludges on soils in Jordan. It would be necessary in these cases, however, to apply smaller quantities of sludge than those recommended when all the heavy metal contents are within the prescribed limits. In addition, the

major part of the soils under cultivation in Jordan are calcareous with total carbonate ranges from 16% to 49%. The soil pH ranges from 7.3 to 7.9.

- Sludge drying beds are the simplest and cheapest form of treatment to reduce water content to a level that would be lethal to *A. lumbricoides* eggs. In addition, in countries where cost and infrastructure are limiting factors, drying beds are a better solution than prolonged storage. An additional advantage can be concluded from this study, that drying beds were demonstrated to be an effective method of treatment, easily incorporated into current sludge stabilisation techniques particularly in warmer geographic locations.

- In general, the results from the literature showed that conventional sludge stabilisation treatment processes (i.e. mesophilic anaerobic or aerobic digestion) are not completely effective in destroying parasite eggs. From this present study drying beds, however, appear to be very effective in destroying parasites.

- Indigenous *Ascaris* eggs in oxidation-ditch sludges were inactivated within 35 days and 67 days by using either sand or gravel open drying beds, respectively. On the other hand, anaerobic pond sludges showed high reduction in viability of *Ascaris* eggs after 62 days using sand and 91 days using gravel drying beds. Parasitic eggs had degenerated when the percentage of total solids was recorded as more than 88%.

- The reduction in *Ascaris* eggs viability in winter confirmed that there are many synergistic factors playing a role in reducing egg viability, in addition to desiccation. A good correlation coefficient of 0.84 was found between total inactivated helminth eggs and the percentage of total solids in sludge. The inactivation of *Ascaris* eggs in drying beds is probably due to more factors than desiccation alone. Temperature, oxygen content, solar radiation, exposure time, mould activity, type of sludge, type of media used in drying beds etc., may also affect survival of the eggs.

- The difference in the sludge type loaded on **sand** drying beds was not associated with any difference observed in the sludge depth, sludge temperature, media temperature, and total solid contents (except pH values) that were measured. The difference in the sludge type loaded on **gravel** drying beds did not show any significant impact on the

abiotic parameters; there were only significant differences in the sludge depth between the two gravel drying beds that were loaded with different sludge types.

- There was no effect of using different media (sand or gravel) on the sludge depth when oxidation ditch sludge was applied. On the other hand, when anaerobic pond sludges were applied to gravel or sand beds, a significant difference in sludge depth occurred.

- In all drying bed sludges, the coliform counts were higher than 10^2 MPN/g total solids, while faecal coliform counts were less than 100 MPN/g total solids after 132 days following the application of the sludge to all drying beds, except at Al-Samra gravel drying bed where faecal coliforms reduced to 10^3 MPN/g total solids. *Salmonella* spp. showed more sensitivity to desiccation than other tested indicator bacteria. No *Salmonella* spp. were recorded after 122 and 132 days of application of the sludge on sand and gravel respectively, in both Al-Samra and Jerash sludges

- Exponential regression analysis provided a mathematical relationship between reducing indicator bacterial counts and *Salmonella* spp. with the percentage of total solids. In general, these functions were statistically significant with correlation coefficients 0.91 to 0.87 for total coliform counts and total faecal coliform counts, respectively. Reducing faecal streptococci counts were observed to have a weak correlation coefficient ($r = 0.42$) with increasing total solids percentages.

- The stabilisation of the sludge before application to drying beds appears to have little influence on the efficiency of drying beds to treat pathogens. Partially anaerobic digested sludge (due to the storage of the sludge for 7 years in anaerobic ponds at ASWSP) did not show significant difference with the raw sludge from JTP in inactivating coliforms, faecal coliforms and *Salmonella* when gravel drying beds used. But it was found that there is significant difference between sludge type in inactivating faecal coliforms especially in sand drying beds. In general, ASWSP sludge bacterial counts show higher resistance to desiccation and treatment conditions in drying beds compared with oxidation ditch sludges.

- During the summer season, the results of this study showed that *Salmonella* spp. were inactivated within 5 months of being exposed to natural drying conditions. The indicator bacterial counts decreased between 4 to 6 logs for total and faecal coliforms but the faecal streptococci decreased only 3 logs.

- The results of the present study indicate that the absence of total coliforms in **dried sludge** should be adequate insurance against danger from pathogens such as *Salmonella* and nematode eggs.

- The sewage sludge after drying bed treatment during the summer season met the "Class A" criterion in the USEPA Regulations (1992) with respect to viable helminth eggs, because the density of viable helminth eggs in sewage sludge after 67 days treatment is less than one per four grams of total solids. Also after at least two months treatment of sludge on drying beds, faecal coliforms (MPN) reduced to less than 1000 per gram of total solids, and the density of *Salmonella* sp. in the sewage sludge was less than 3 MPN per 4 grams of total solids after around 132 days natural drying treatment. "Class A" criterion in the USEPA Regulations (1992) is for land application and using the sludge as a fertiliser. 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. It also should be confirmed that the bacteriological counts are not increased due to regrowth problems at the time when the sewage sludge is used or disposed, and that enteric virus counts are low, but these aspects were not addressed in the present study.

- Finally, the die-off data in both anaerobic pond and oxidation-ditch sludges on gravel or sand drying beds, indicated pathogen reductions within 5 months, and was in accordance with USEPA Regulations with processes to further reduce pathogens (PFRP) in sludge, treatment processes, or process schemes. This research concludes that sludge drying beds can be an effective method for eliminating parasite eggs, particularly in warmer geographic locations. The treated sludge can be considered of suitable quality for application to agricultural land particularly from the parasitological characteristics.

- Developing Jordanian regulations is a very important and urgent priority before sludge application to land starts to be practiced.

The following can be summarised pertaining to functions and advantages of anaerobic waste stabilisation ponds:

- Control odour and make the sludge less putrescible for appropriate diposal;
- Reduce the population of pathogens, however inactivation of helminths eggs or pathogenic bacteria at mesophilic temperatures is never complete.
- Reduction of the sludge volume, by a substantial decrease in the amount of suspended solids, and consolidation, and sometimes by the improvement of dewatering properties, can thereby ease handling and transport;
- The results of the present study show that anaerobic ponds could achieve a high removal of metals and shows that the removed metals accumulate in the sludge of the primary and secondary anaerobic ponds;
- The results of this study highlight the importance of secondary anaerobic ponds especially in countries that have high strength of wastewater, overloaded primary anaerobic pond, and in developing countries where there is low maintenance and attention towards the performance of the ponds. The addition of secondary anaerobic ponds may be necessary to ensure that the effluent quality is within the WHO guidelines for reuse, depending on the initial concentration of eggs per liter.

It must be stated that still further studies are needed to complete and elucidate the findings of this study. It is hoped that the results reported herein will stimulate similar work investigating viability methods for intestinal parasitic eggs, and the effects of moisture on bacterial and parasitic eggs survival and inactivation in sludge systems.

12.2 Recommendations

The results of this 3 years study on the incidence and persistence of parasites in sewage sludge indicate that additional information can be obtained by further research. The specific areas recommended for additional research on the fate of parasites in wastewater sludges are as follows:

- A survey of available literature and this study results indicates a need for a universally accepted definition of "**VIABILITY**" specifically "**viable parasitic eggs**".

- The development of a standard analytical method for parasitologic examination of sewage sludges. 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).

- Information is needed on the effect of gases such as H_2S, NH_3, CO_2, etc. on the viability of *Ascaris* eggs and bacteria.

- Further studies should investigate the effects of environmental conditions on the reliability of viability detection using the staining method. Also further work should investigate the selection of vital stains that can be used on corticated *Ascaris* eggs. Furthermore, selection of vital stains which can differentiate between viable and dead *Trichuris* eggs, would be extremely useful.

- An intensive investigation is required to clarify the effects of both regular and irregular changing temperature conditions on the rate of development and viability of *Ascaris* eggs.

- More information is required on the fate of *Enterobius* eggs during sewage treatment, in wastewater, and sludge treatment;

- Extensive future research is needed to define the applicability of using the sludge for agriculture under the conditions prevailing in Jordan with respect to pathogens (viruses, protozoa), vector attraction, nutrition value, toxic organic elements, choice of crops, soil, etc.

- Future research is required on the destruction of *Vibrio cholera* or protozoa cysts during nightsoil or sludge treatments.

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Appendix 2.1
USEPA REGULATIONS FOR SLUDGE USE

Under the requirements of the Resource Conservation and Recovery act (RCRA), the USEPA in the Federal Register (Volume 44, No. 179, Thursday, September 13, 1979) Interim Final, and Proposed Regulation (Criteria), 40 CFR Part 257 "Criteria for Classification of Solid Waste Disposal Facilities and Practices." Under these criteria, sludge is defined as a solid waste. Also under the Criteria, untreated or raw sewage sludge cannot be applied to, incorporated into, or injected shallowly into the land surface. Appendix II of the criteria defines the processes and the process conditions for acceptable treatment of sewage sludge for pathogen reduction before use on land. Appendix II A defines "Processes to significantly Reduce Pathogens". Appendix IIB of Criteria defines the acceptable "Processes to Further Reduce Pathogens".

TABLE A. Pathogen Treatment Processes: Processes to Further Reduce Pathogens (PFRP)

1. **Composting**: Using either in-vessel or static aerated pile composting, the temperature of the sewage sludge is maintained at 55°C or higher for 3 days. Using windrow composting, the temperature of the sewage sludge is maintained at 55°C or higher for 15 days or longer. During this period, a minimum of five windrow turnings are required.

2. **Heat drying**: Sewage sludge is dried by indirect or direct contact with hot gases to reduce the moisture content of the sludge to 10% or lower. Either the temperature of the gas in contact with sludge exceeds 80°C or the wet bulb temperature of the gas in contact with the sludge as the sludge leaves the dryer exceeds 80°C.

3. **Heat treatment**: Liquid sludge is heated to a temperature of 180°C or higher for 30 min.

4. **Thermophilic aerobic digestion**: Liquid dewatered sludge is agitated with air or oxygen to maintain aerobic conditions, and the mean cell residence time for the sewage sludge is 10 days at 55°C to 60°C.

5. **Beta ray irradiation**: Sewage sludge is irradiated with beta rays from an accelerator at dosage of at least 1.0 Mrad at room temperature (ca. 20°C).

6. **Gamma ray irradiation** : Sewage sludge is irradiated with gamma rays from certain isotopes such as ^{60}Co and ^{137}Ce, at dosages of at least 1.0 Mrad at room temperature (ca. 20°C).

7. **Pasteurisation**: The temperature of the sludge is maintained at 70°C or higher for at least 30 min.

TABLE B . Pathogen Treatment Processes: Processes to Significantly Reduce Pathogens (PSRP)

1. **Aerobic digestion**: Sewage sludge is agitated with air and oxygen to maintain aerobic conditions for a mean cell residence time and temperature between 40 days at 20°C and 60 days at 15°C.

2. **Air drying**: Sludge is dried on sand beds or on paved or unpaved basins for a minimum of 3 months; during 2 of the 3 months, the ambient average daily temperature is above 0°C.

3. **Anaerobic digestion**: Sludge is treated in the absence of air for a mean cell residence time and temperature of between 15 days at 35 to 55°C and 60 days at 20°C.

4. **Composting**: Using either in-vessel, static aerated pile, or windrow composting methods, the temperature of the sludge is raised to 40°C or higher for 5 days. For 4 h during the 5 days, the pile temperature must exceed 55°C.

5. **Lime stabilisation**: Sufficient lime is added to the sludge to raise the pH of the sludge to 12 after 2 h of contact.

Appendix 4.1

USE OF n- BUTANOL FOR VIABILITY DETERMINATION OF *ASCARIS* EGGS

The Stien-Scwartzbrod 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 increase 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 x 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* and wastewater only.

Appendix 4.2

BUFFER SOLUTIONS AS A BIOCHEMICAL TRIGGERS

Sodium Bicarbonate Buffer (pH 10)

A: 0.2 M solution of anhydrous sodium carbonate (21.1g in 1000ml).

B: 0.2 M solution of sodium bicarbonate (16.8g in 1000ml).

Take 27.5 ml of A solution with 22.5 ml of solution B, then diluted to a total of 200 ml, the pH equal 10 and it will not change appreciably on dilution.

Acetoacetic Acid Buffer (pH 4.5)

Weight crystalline sodium acetate 5g, add 3.6 ml glacial acetic acid, make up to 1000ml with distilled water.

0.1 N Sulfuric Acid pH (1.5)

Add 2.8 ml of sulfuric acid (H_2SO_4) solution to make up 1000ml of distilled water.

0.1 N Sodium Hydroxide (pH 12)

Add 4g of sodium hydroxide (NaOH) make it up to one liter by distilled water.

Phosphate Buffer Solution (pH 7.2):

Dissolve 34.0g potassium dihydrogen phosphate (KH_2PO_4), in 500ml reagent-grade water, adjust to pH 7.2±0.5 with 1 N sodium hydroxide (NaOH), and dilute to 1 L with reagent-grade water. Add 1.25 ml stock phosphate buffer solution and 5.0 ml magnesium

chloride solution (81.1g $MgCl_2$ 6 H_2O/L reagent grade water) to 1L reagent-grade water (Standard Method, APHA, 1992).

Appendix 6.1

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 6.2

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 discard the supernatant, retain the residue.

4. The light solids and fat which interfere with floatation are removed by formol-ethanol extraction of Allen and Ridley (1970).

5. Suspended the residue in 100ml of formol saline solution (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 minutes.

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 x100 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).

Appendix 6.3

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), was used as detailed below. The technique is convenient as eggs are extracted and removed immediately into a solution of 0.1N H_2SO_4 in which they are left for incubation.

Method:

1. 100 ml of 2.62% sodium hypochlorite (50% dilution of Chloros) 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 liquor 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 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 μm 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 0.1N 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 N 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 1μm 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 N/litre sludge = (X x 50) Y 13.333

where: 1000ml/75ml = 13.333

Appendix 7.1

STOKES' LAW FOR THE CALCULATION OF SETTLING VELOCITIES

The theoretical settling velocities (Us) of human intestinal helminth eggs most commonly found in wastewater can be calculated using Stokes' Law as follows (Ayres, 1992):

$Us = g\,(p_s-p)d^2 / 18\mu$

where: Us = settling velocity, m/s

g = acceleration due to gravity ($9.18 m/s^2$)

p = density of the suspending fluid (1000)

p_s = density of particles (i.e specific gravity x 1000, kg/m^3)

d = characteristic linear dimension for a conservative estimate

μ = molecular viscosity of the suspending fluid at any temperature. (for a water at 20°C, $\mu = 1.01 \times 10^{-3}$).

The settling velocities *A. lumbricoides*, *T. trichiura*, *A. duodenale*, and *Taenia* spp. eggs in water at 20°C were calculated from the Table below.

TABLE D. Theoretical settling velocities of intestinal helminth eggs at 20°C in water

	A. lumbricoides	*T. trichiura*	*A. duodenale*	*Taenia* spp.
d max	75×10^{-6}	58×10^{-6}	60×10^{-6}	40 x 10-6
Specific gravity	1.11	1.15	1.055	1.225
p_s	1111	1150	1055	1225
Us ($m\ s^{-1}$)	3.4×10^{-4}	2.7×10^{-4}	1.1×10^{-4}	1.9×10^{-4}
Us ($cm\ min^{-1}$)	2.04	1.62	0.66	1.14

Appendix 8.1

CALCULATIONS OF SETTLING VELOCITY AND SEDIMENTATION DISTANCE

The theoretical settling velocity (Us) of helminth eggs in wastewater at 20°C can be approximated using Stokes law as 3.4×10^{-4} m/s (Appendix 7.1). The use of an idealised continuous flow sedimentation tank as a model to predict the removal of helminth in ponds was tested. WSP do not behave as perfect plug flow reactors and their effective volume is always reduced by dead zones, particularly at the corners, so it was anticipated that the model would need to provide a generous margin of error to account for this.

The settling velocity and the sedimentation distance of intestinal helminths was found by using a method similar to that used by Ayres (1992). Assuming that nematode eggs are removed by gravity, the likelihood of their removal can be calculated from the minimum settling velocity (Us*) for any given pond as:

$$Us^* = D/t^* \qquad \text{(eq. 1)}$$

where: D = pond depth and t* = mean hydraulic retention time, seconds.

The mean hydraulic retention time in a continuous flow tank is given by:

$$t^* = AD/Q \qquad \text{(eq. 2)}$$

where: AD = tank volume, m^3 (A = area) and Q = flow rate through tank, $m^3\ s^{-1}$.

Us* can also be defined as:

$$Us^* = Q/A \qquad \text{(eq. 3)}$$

which is also known as the overflow rate. All particles with a settling velocity, Us, greater than the minimum settling velocity or overflow rate (Us*) will be removed within one retention time (Ayres, 1992).

At Al-Samra the estimated flow rate (Q) during the period of operation of pond A1-1 was $103500 m^3/d$, ($1.979\ m^3\ s^{-1}$) and the area of A1-1 pond was $3.17 \times 10^4\ m^2$.

Therefore $Us^* = Q/A = 6.2 \times 10^{-5}$ m s^{-1}.

As Us for some intestinal helminth eggs in Appendix (7.1) was greater than Us^* (6.2×10^{-5} m s^{-1}), the model implies that *A. lumbricoides* (Us = 3.4×10^{-4} m s^{-1}), *T. trichiura* (Us = 2.7×10^{-4} m s^{-1}), *A. duodenale* (Us = 1.1×10^{-4} m s^{-1}), and *Taenia* spp. (Us = 1.9×10^{-4} m s^{-1}),.would be removed in one retention time in pond A1-1, even allowing a large margin of error.

To determine the distance (L) at which A. *lumbricoides* eggs may be expected to sediment out: assuming that the pond was 260m long, by using equation

$$Us = Q/A = Q/BL \qquad \text{(eq. 4)}$$
$$Us = Us^*$$

where: B = breadth, L = length
therefore:

$$L = Q/B\ Us \qquad \text{(eq. 5)}$$
$$L = 22.4m$$

Appendix 10.1

USEPA CODE CRITERIA FOR CLASSIFICATION OF SOLID WASTE DISPOSAL PRACTICES

United States Environmental Protection Agency, Code of Federal Regulations 40 CFR Part 257, Criteria for Classification of Solid Waste Disposal Practices, Final rule, November 25, 1992.

Part 503-Standards for the use or disposal of sewage sludge. Subpart B-Land application. §503.13: Pollutant limits:

(a) Sewage sludge

(2) If bulk sewage sludge is applied to agriculture land, forest, public contact site, or a reclamation site, either:

(i) the cumulative loading rate for each pollutant shall not exceed the cumulative pollutant loading rate for the pollutant in Table 2 of §503.13, or

(ii) the concentration of each pollutant in the sewage sludge shall not exceed the concentration for the pollutant in Table 3 of §503.13.

(3) If bulk sewage sludge is applied to a lawn or a home garden, the concentration of each pollutant in the sewage sludge shall not exceed the concentration for the pollutant in Table 3 of §503.13.

(4) If sewage sludge is sold or given away in a bag or other container for application to the land, either:

(i) the concentration of each pollutant in the sewage sludge shall not exceed the concentration for the pollutant in Table 3 of §503.13.

(b) Pollutant concentrations and loading rates-sewage sludge

(3) Pollutant concentrations

TABLE C. Of §503.13--- Pollutant concentrations

Pollutant	mg/kg dry weight basis
Arsenic	41
Cadmium	39
Chromium	1200
Copper	1500
Lead	300
Mercury	17
Molybdenum	18
Nickel	420
Selenium	36
Zinc	2800