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WATER AND ENVIRONMENTAL SANITATION SECTION

PATHOGEN SURVIVAL RATE IN FILLED-UP LATRINE PITS

FINAL REPORT
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Foreword

Waste materials, particularly human excreta, are no more considered 'wastes' – they can be converted into resource for human use and benefit. The potential of waste materials particularly of organic ones are being used for the production of fuel, feed, energy and biofertilizer. Judicious and hygienic application of digested excreta can simultaneously reduce pollution owing to the presence of pathogenic microorganisms and worm-eggs/ova.

Human excreta, rich in hosts of pathogenic and commensal microorganisms, during fermentation undergo digestion in filled-up unused latrine pit where a number of physico-chemical and microbiological reactions take place. The resultant reactions culminate into pathogen death. The present study has been designed to experimentally prove as to the length of time required for elimination of pathogens or attaining acceptable level/limit values for safe handling of excreta in agriculture.

The digested excreta devoid of harmful pathogens are excellent source of fertilizer elements like nitrogen, phosphorus, and potassium etc. essential for plant growth. Bangladesh soil is alarmingly deficient in organic matter – vital for the maintenance of soil fertility and productivity. The judicious application of digested/composted human excreta in our agriculture will supplement the use of chemical fertilizers as well as simultaneously help reduce incidence of disease and pollution in our soils and water bodies.

Assistance and cooperation of Dr. T.V. Loung is acknowledged. I am grateful to Engr. Rabiul Islam, former Consultant, UNICEF, Dhaka for his meticulous approach and expert advice in the planning and execution of this research project. I am also indebted to Mr. Nurul Islam, Ex-En, DPHE, Narayangonj and Mr. Nazrul Islam, SAE of Rupganj for their sincere cooperation. I also thank Engr. Sohrab Uddin, Ex-En, Gazipur and staffs for their assistance.

I would also like to thank Mr. Alex Redekopp, Sanitary Engineer & Team Leader, WHO and Engr. Mohammad Mofazzal Huque, National Field Programme Officer, WHO, Dhaka for their advice and useful suggestions.

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List of Abbreviations

cfu	-	Colony forming units
ml	-	millilitre
T.C	-	Total coliform
F.C	-	Faecal coliform
F.S	-	Faecal streptococci
E.coli	-	*Escherichia coli*
MPN	-	Most Probable Number
LWT	-	Low Water Table
HWT	-	High Water Table
gm	-	gram
°C	-	degree calsius
WHO	-	World Health Organization
N	-	Nitrogen
P	-	Phosphorus
K	-	Potassium
TOR	-	Terms of Reference

0.0 EXECUTIVE SUMMARY

The research workplan encompasses two major thrusts:

(a) time required for complete digestion of human excreta for the removal of pathogenic bacteria and worm-eggs in filled-up unused latrine pits, and

(b) possibility of using digested excreta in agriculture as organic fertilizer that involves estimation of fertilizer values for nitrogen, phosphorus and potassium - — three major plant nutrients.

Since digestion of excreta in pits, apart from microbial activity, depends on moisture content as influenced by position of water table and physico-chemical properties of pit soil, two different physiographic units/sites were chosen for pit selection. Towards meeting these criteria, a low water table area at Gazipur (30 feet water table) and the other with high water table area at Rupganj (approx. 10 feet water table) were included in this study. Seasonal temperature variation is negligible between this two areas. An initial time-frame for twelve months was planned for the duration of the study which was later extended to eighteen months; sample testing was scheduled at 2-month interval until 16th month. Three pits from each site were selected for taking average value. From each pit two samples from two different depths (2 feet and 4 feet) were examined.

All samples were dispensed in disinfected plastic containers and put into insulated cool box with ice pack in it. Samples were immediately transported and processed for microbiological examinations and kept at 4°C in a laboratory refrigerator until further use.

Standard microbiological and parasitological techniques were followed for detection and identification of pathogen (faecal) indicator bacteria and worm-eggs. Laboratory examinations for three faecal indicators e.g. (a) **total coliform**, (b) **faecal coliform** and (c) **faecal streptococci** were conducted. For parasite indicators, worm-eggs of (a) **roundworm** and (b) **hookworm** were included for identification and quantification.

The initial count of total coliform (T.C.) at 1st sampling was highest when compared with the other two indicator bacteria e.g. faecal coliform (F.C.) and faecal streptococci (F S). The T.C. count varied between 53,000,000 and 1,200,000 cfu per gram of sludge. The decrease in viable count of T.C. at 2nd sampling was between 0.5 and 1.0 unit followed by similar decrease at 3rd sampling (6 months) where the highest count varied between 700,000 and 60,000 bacteria per gram of sludge. At 4th sampling (8 months), the T.C. count decreased sharply by about 1.0 to 2.0 log units and the average number

ranged between 5,500 to 1,000 per gram. At 5th sampling (10 months) the T.C. count rather decreased insignificantly while the similar trend was maintained at 6th sampling i.e. after 12 months of pit closure where the count ranged between 50,000 and 1,000 per gram of digested excreta. The counts of totoal coliform bacteria (T.C.) fell moderately and ranged between 20,000 and 18000 cells/g of sludge at 7th sampling (14 months). At 8th sampling, the T.C. count (average) decresed further and varied between 3,900 - 4190 viable cells/g. These values are much higher than the acceptable limits.

The count for **faecal coliform** (F.C.) at 1st sampling was relatively lower than T.C. and varied between 750,000 and 110,000 cfu/gm of sludge. The viable indicator bacterial numbers dropped steadily at the 2nd and 3rd sampling where an average decline of more than 1.0 log unit was recorded. The average count was estimated to be 24,000 cfu/gm. The pathogen indicator (F.C.) further decreased markedly at 4th sampling followed by 5th sampling where an average F.C. count of 500 bacterial cfu/gm was estimated. At 6th sampling (12 months), the pathogen indicator count remained almost similar to the level of last sampling. Little or no decrease in **faecal coliform** (F.C.) density was apparent at 7th sampling. The average count ranged between 170 and 510 per gram of digested sludge. Almost similar trend in the death of F.C was recorded at 8th sampling (16 months) where the cell number ranged between 100 - 580/g. These counts are still beyond the acceptable limits for safe handling. The **faecal streptococci** (F.S.) number was almost similar to that of faecal coliform (F.C.) at first sampling where the average F.S. number was 480,000 per gm. The viable count of F.S. decreased steadily until fourth sampling (8th months) where the average number was 100 cfu/gm. With increasing retention time at fifth and sixth sampling the indicator (F.S.) did not drop significantly. Faecal streptococci (F.S.) indicators did decrease slightly or remained static at 7th sampling (14 months). The count was slightly fewer than F.C and varied between 80-90 cells/g of sludge and lies out side the limit values. At 8th sampling the cell number decresed slightly but with no sign of complete extinction.

Concentrations of eggs/ova of two major worms e.g. **roundworm** and **hookworm** as indicator of faecal pollution having disease potential have been determined quantitatively. Particularly the survival/incidence of roundworm eggs (*Ascaris lumbricoides*) is a positive and determinative indicator of wider disease potential.

At 1st sampling the average number of roundworm eggs (*Ascaris lumbricoides*) was 4,300/gm of sludge. The count did drop slightly at the 2nd sampling (2000/gm) . However, at 3rd sampling the eggs decreased sharply to 1300 per gram . The fall in egg number was significant at Gazipur, a dry and low water table zone (LWT) as compared

to Rupganj -- a high water table (HWT) area. The count further fell at 4th sampling to an average of 400 eggs/gm -- the trend continued at the LWT area (44 eggs/gm) more than at HWT zone where a small decrease in the number of eggs (524 eggs/gm) was apparent. The average number of viable eggs remaining at both the water table zones stood at 284 eggs/gm of sludge. At 7th sampling, the count of **roundworm** decreased moderately. With most samples, the fall was significant. The egg count was between 16 and 214 depending on sampling site. Roundworm concentration decreased further at 8th sampling (16 months) and dropped to a low of 5 - 30 eggs/gm of sludge. The numbers were relatively higher with the high water table zone (HWT) as compared to the low water table zone (LWT).

The initial average concentration of **hookworm** eggs (*Ancylostoma doudenale*) was 3500 eggs/gm of sludge. With increasing retention time of excreta in pits, the count tended to decrease rather slowly at 2nd sampling (4 months) with a sharp drop at 3rd sampling (average number of eggs was 150/gm). At 4th sampling (8 months), the count decreased to about 50-60/gm. By the time of 5th sampling the hookworm eggs could not be detected at LWT area (Gazipur) whereas it was 58 eggs/gm at HWT area (Rupganj). At 6th sampling, i.e. by the 12th month, similar trend was observed both at LWT and HWT area–with no sign of complete elimination. The count of **hookworm** at LWT area was nil with all the six samples at 7th sampling, whereas the number ranged between 10-25/g with the HWT. This shows a definite decreasing tendency as compared with earlier samplings. With extended digestion at 8th sampling (16 months), the **hookworm** eggs could nto be detected in any samples regardless of water table position.

It may be mention that the pathogen survival was relatively better with high water table area as compared to low water table zone. This trend in count was most prominent particularly with the survival of round worm eggs.

The background counts of **faecal indicator** bacteria and worm-eggs in field and kitchen garden soils of Gazipur and Rupganj were estimated. It appears from examination that total **coliform** bacteria were present in both field and kitchen garden soils with an avetage of 2,800 cfu or lower in per gram of soils (range 200-3600) of soils. **Faecal coliform** were mostly detected with kitchen garden soils at both the study areas (range: 32-35 cfu/gm) whereas incidence of **faecal streptococci** was uniformly present, also mostly with kitchen garden soils ranging between 20-28 cfu/gm of soils. Wide variation was observed in the incidence of these indicators in kitchen garden soils.

Background counts of worm-eggs was mostly nil to 2-5 cfu/gm particularly with kitchen garden soils. Field soils did not contain either roundworm or hookworm eggs.

The decomposed excreta was found to be potentially rich in fertilizer elements. The average fertilizer values for nitrogen (3.85%), phosphorus (1.82%) and potassium (0.86%) were remarkably high and the values did not significantly fluctuate between sampling intervals although at 14th and particularly at 16th months sampling the decrease was somewhat noteworthy.

1.0 INTRODUCTION AND BACKGROUND

Disease and pollution potential of human excreta are enormous owing to the presence of wide range and high concentrations of pathogens. The more recent fractions of excreta contain viable bacterial pathogens, worms and worm-eggs. Therefore, the temptation to use the material as fertilizer shortly after dewatering must be resisted. The collection, transport, treatment and disposal of human excreta are of utmost importance in the protection of public health.

Human excreta should be regarded as a natural resource to be conserved and reused as organic fertilizer rather than discarded. Apart from carbon, nitrogen, phosphorus and potassium, human excreta contain certain other essential plant nutrients and thus regarded as a useful resource to be used in agriculture. Continuous use of chemical fertilizers (eg Urea, TSP etc.) in soils deficient in organic matter may lead to the destruction of soil physico-chamical properties and soil structure, vital for crop yield and maintenance of soil fertility. Addition of plant nutrients in the form of organic supplements particularly from well digested or composted human excreta, devoid of harmful pathogens or within safety limits, will lead to new dimensions in the conservation and fertility of Bangladesh soils which is losing its fertility potential and is highly deficient in organic fractions – a must for balanced soil properties and crop yield.

UNICEF-Bangladesh has come up with pragmatic and foresighted vision in the economic use and hygienic disposal of human excreta in Bangladesh simultaneously combating environmental pollution and looking in to the possibility of using digested excreta in agriculture. Since human raw excreta contain large number of pathogenic bacteria and parasites, its safe handling and use must be ensured prior to application in agriculture.

Elimination or removal of pathogens in excreta is depended on several environmental factors e.g. moisture, aeration, time, temperature etc. To this end, studies have been planned to examine the duration of time for total removal or attaining of an acceptable limit for safe handling. Another important investigation will determine the fertilizer

value of the digested excreta. This will involve laboratory experimentation of the digested excreta on the elemental composition of certain plant nutrients having fertilizer value. The digestion of the excreta in filled-up unused latrine pits may depend on pit depths and position of water table apart from locations influenced by soil physico-chemical properties.

Bangladesh is a densely populated poor country where although some progress has been made with pure drinking water supply, lack of proper sanitation remains one of the major health problems. Sanitation coverage both in the urban and rural areas is still very low and so is the general awareness on the linkage of water, sanitation and hygiene habits to good health. Most people in Bangladesh are not aware of disease and pollution potential of faecal material. In 1995, the access to proper excreta disposal is around 35% of the population.

Just about a decade back, only 43% of rural families had a latrine. Most latrines were built for privacy and were unhygienic, creating environmental pollution specially of surface water. However, in recent years, the use of sanitary latrines has been steadily increasing. Open air defecation is still a widely practiced habit amongst the poor uneducated rural mass and slum dwellers. However, it is worthy of mention that 26% of the total population now uses sanitary latrines compared to just 10% in 1989. This means that 74% of the population still resort to unhygienic latrine or defecation out in the open. It is estimated that about 28,000 metric tons of faecal matters are deposited/disposed in the public places every day.

The incidence of water and sanitation related diseases e.g. diarrhoea, dysentery and worm infestation has remained the major killer of children under 5 years. The situation will not improve unless there is a vast improvement in sanitation. The Government/ UNICEF target for the use of sanitary latrine is to cover 80% of the population by the year 2000. Homemade pit latrines are relatively cheaper and cover about 60% of sanitary latrines in the country.

In a developing country like Bangladesh, excreta related diseases are very common owing to the presence of high concentrations of viable pathogenic bacteria and worm-eggs. Diarrhoeal diseases are also caused due to such contamination which kills more than 200,000 children annually in our country. Well digested/decomposed human excreta will reduce the survival rate of the pathogens in excreta which can be used as organic fertilizer as is used in China, India, Vietnam, Philippians and certain other countries.

2.0 DETAILS OF THE ASSIGNMENT

2.1 General Objectives

Human excreta contain very high concentrations of viable pathogens of various types having disease potential. Well digested or decomposed excreta should be free from or contain acceptable number of pathogens (e.g. bacteria, parasites and worm-egg) for safe handling and use in agriculture. This study encompasses laboratory examination of human excreta in filled-up and unused latrine pits.

The digestion efficiency in relation to duration of time to eliminate viable pathogens will be studied. The digested sludge in the filled-up pits may be reused as organic fertilizer which will enrich soil fertility by supplying essential plant nutrients simultaneously improving soil physical properties.

2.2 Specific Objective

The specific objective of the research project includes:

- to quantitatively determine the count of indicator bacteria e.g., a) **total coliform**, (b) **faecal coliform**, (c) **faecal streptcocci** and worm-eggs of **(d) roundworm** and **hookworm** and their survival rate over 16 months period.
- to investigate the time period required for the elimination of pathogens (e.g., bacterial indicators, roundworm and hookworm-eggs) in filled-up latrine pits. Count of the test indicator bacteria and worm-eggs upto certain level/limits will be recommended for acceptance for reuse as organic fertilizer in agriculture.
- to assess the fertilizer value of the digested excreta that will involve determination of major nutrient elements like nitrogen, phosphorus and potassium.
- to ascertain whether pit depth, site difference and position of water table will effluence rate of pathogen elimination.

2.3 Scope of the assignment

2.3.1 Time-frame of Study

The research work primarily delineates to investigate the death rate of pathogenic bacteria and worm-eggs at 2-month interval extending upto 16 months. This will ascertain as to whether within this time period the concentration of pathogens in sludge

is eliminated or attains an acceptable limit for safe handling as organic fertilizer. It is expected that with increasing residence time in pits, the viable count of pathogens would decrease or be eliminated to a safe level. However, the desired goal will certainly depend upon environmental factor e.g. temperature, moisture soil reaction (pH) etc.

The scope of this study also encompasses the use of digested excreta as organic fertilizer in agriculture. Laboratory examinations will be limited to estimate the fertilizer value particularly for nitrogen (N), phosphorus (P) and potassium (K).

2.3.2 Limitations

The nature and extent of the research project is bound by specific goals. There is specified guidelines on which this investigation is intended to. Since public pits were selected, there were variations in the closing dates of pits. The number of pits are small, only six. Wider physiographic representation with higher number of pits would be much more representative.

The type of pathogen indicators to be examined was pre-determined in the TOR. Others e.g. microbes like *Clostridium perfringens* or *Bacteroides* could be an added parameter.

Only three fertilizer elements e.g. N, P & K have been mandated for analysis. Others e.g. organic carbon, calcium, magnesium, zinc, copper etc. could be included for comprehensive analysis.

2.4 Team Members

1.	Dr. Sirajul Islam Khan	Professor & Team Leader
2.	Dr. Joseph D. Silva	Research Associate
3.	Mr. Hasem Ali	Technician
5.	Abdur Razzak	Lab Assistant.

3.0 LITERATURE REVIEW

3.1 Pathogens in excreta

A large number of pathogens are abundantly present in human excreta. Of these bacteria, protozoa, worms and worm-eggs including protozoa and viruses cause wider spectra of diseases. The faeces of healthy person contain large number of commensal bacteria of

many genera and species. The species of bacteria found in normal excreta and their numbers will vary amongst communities. Because the bacteria are ubiquitous and numerous in the faeces of healthy people, a number of them have been used as indicator of faecal pollution. Indicator bacteria usually do not cause disease, can be easily identified and counted that also indicate the presence of pathogenic bacteria in faeces. Since isolation, identification and counting of pathogenic bacteria are troublesome, often imprecise and time consuming, the use of indicators have been recommended as an indirect approach that reflects the pathogen potential of the excreta. The most widely used indicator has been the faecal coliform *Escherichia coli (E.coli)*– the main component of the coliform groups. There are two principal groups of coliform bacteria:

(a) the faecal coliform (comprising mainly the *E. Coli* and the thermotolerant *Klebsiella pneumoniae*, and

(b) total coliform group that includes and comprises four bacterial species.

The former are exclusively faecal in origin, whereas the latter, although commonly found in faeces also occur naturally in unpolluted soils and waters.

The faecal streptococci (or group D streptococci) are mostly found in the intestines of man and other warm-blooded animals. These bacteria generally survive somewhat longer than faecal coliform and is better indicator of excreted bacterial pathogens. The following is a list of important indicators and pathogenic bacteria normally present in human excreta:

A. Indicators:

a). Coliform group

Totoal Coliform : *E coli* (37°C), *Klebsiella, Enterobacter* and *Citrobacter.*

b) Faecal Coliform : *E. coli* (45°C)

Klebsiella pneumoniae (thermotolerant) (45°C)

B. Other indicator bacteria : *Streptococcus faecalis, Clostridium perfrigens. Bifidobacterium, Bacteroides.*

C. Pathogens of enteric origin : *Salmonella Shigella, Vibrio cholerae Yersinia, Campylobacter*, etc.

The persistence and prevalence of the above mentioned indicators and pathogenic bacteria will depend upon various factors e.g organic and inorganic nutrients, temperature, duration of digestion, soil reaction, moisture content etc.

Protozoa

The protozoan agents appear as cyst in faecal material. Many species of protozoa can infect man and cause diseases like diarrhoea and dysentery. Infective forms of these protozoa are often passed as cysts in the faeces and man is infected when he ingests them. Under optimum conditions of temperature and moisture they survive 60 to 80 days in the soil (Pahren *et al* 1979). Only three species of human intestinal protozoa are considered to be pathogenic e.g. *Entamoeba histolytica, Giardia lamiblia* and *Balantidium coli*

Helminths (worms)

The intestinal parasite e.g. roundworm and hookworm are probably of greater public health significance than the protozoan agents in terms of sludge reuse. Many species of parasitic worms or helminths, have human hosts. Some can cause serious illness, but a number of them generate few symptoms.

Helminths (except for *Strongyloides*) do not multiply within the human host and this is of great importance in understanding their transmission and disease causing potential. The worms or helminths are classified into two main groups: the roundworms (nematodes)and that are flat in cross section. The flatworms are further divided into two groups e.g. i) the tapeworm (cestode) and the flukes (trematodes). The round worm may cause mechanical obstruction (Ascariasis), rectal prolapse (Trichuriasis) etching around the anus (Enterobiasis) or anemia (hookworm). The following are a few parasites having disease potential in human:

Round worm : *Ascaris lumbricoides*

Whip worm : *Trichuris trichiura*

Pin worm : *Enterobius vermicularis*

Hook worm : *Ancylostoma duodenale, Necator americanus*

Tape worm : *Taenia saginata, Taenia solium*

Viruses

Viruses may infect the intestinal tract and is discharged with faeces and may infect new human hosts by ingestion or inhalation. The concentration of viruses per gram of human faeces may exceed 10^9 virus particles. Usually they cannot multiply outside suitable host (i.e. they are obligate parasites). The excreted viruses may survive many weeks in the environment.

Five groups of pathogenic viruses are particularly important:

(a) adeno viruses, (b) enteroviruses (including polioviruses), (c) hepatitis A virus (d) reovirus and (e) diarrhoea causing viruses e.g. rotavirus. Human excreta may contain certain other types of viruses. Echo virus and coxsackie virus infections (within entero virus) can cause wide range of diseases e.g. fever, meningitis, respiratory illness, paralysis etc.

3.2 Survival of Pathogens in Environment

Survival of pathogenic bacteria, protozoa, worm-eggs etc. will depend on various pysico-chemical and biological factors e.g. pH, temperature, moisture, aeration, presence of toxic chemical substances or nutritional stresses etc. Negative interactive effect of environmental factors may often adversely affect survival rate. Soil reaction (pH) and temperature followed by moisture content of the excreta positively contribute to the organisms' survival potential. Besides this, the organisms intrinsic properties may also play important note.

The concentration of pathogens from the time of excretion usually declines with increasing incubation time. Protozoa and viruses will always decrease in numbers following excretion but bacteria may multiply in a nutrient rich environment like human excreta. Viruses usually die out within 3 months while protozoal concentration decrease relatively faster at about 20-30°C. The death rate of bacterial indicators varies and at similar temperature ranges usually between 2-3 months (Feachem *et al* 1983). Reports on survival rate for pathogenic bacteria and indicators in tropical environment is rather scarce. However, with the increase in temperature between 55-65°C the fall in the viable count is quite short. Lowering of moisture content or exposure to sunlight will also quicken the death process. Most pathogens including indicator bacteria survive well at 5-10°C and rapidly die at high temperature (> 40°C).

Most worm/ova die out within months whereas the eggs of roundworm *Ascaris lumbicoides* may survive more than one year (Feachem, 1983).

The death of pathogens will also depend on type of digestion -- whether it is aerobic or anaerobic. Under anaerobic condition fermentation will proceed steadily and most

aerobic pathogen will die out. This will also depend on temperature and duration of the digestion.

3.3 Risks of excreta to public health

Excreta are the storehouse of numerous pathogens. Enteric or pathogenic bacteria, protozoa, viruses worm and worm-eggs cause more than fifty types of diseases and frequently transmit from the excreta of an infected person to another person by various means. After defecation the disease causing agents (the pathogens) in excreta, if not properly disposed/digested, serve as potential threat to diarrhoea, dysentery, gastroenteritis, anemia, poliomyelitis, typhoid, food poisioning, cholera, septecemia, ascariasis and hosts of other diseases. Use of undigested excreta in agriculture or aquaculture may pose serious threat to public health. Dissemination of live pathogenic bacteria or infective worm-eggs involves high risks particularly by the handlers of such excreta for agricultural use. The only sure way to protect the health of the farmers is to use fully digested pathogen-free excreta or numbers of pathogens will be within safe limits for handling.

3.4 Digested Excreta as Organic Fertilizer

Human excreta are biodegradable natural resource that can be used as biofertilizer. Huge amount of human excreta are being deposited everyday (about 28,000 m.t/d) and remain unutilized or wasted. Moreover, it is acting as a vehicle for disease and environmental polluting agent. Decomposed excreta/sludge can provide a rich source of nitrogen and other essential nutrients (e.g P,K, Ca, micro nutrients etc.) for agriculture and aquaculture. Unused well decomposed excreta from pit latrines can be used as orgnaic or biofertilizer.

The safe use of excreta will depend on retention time, temperature of digestion and moisture content. Application of heat or thermophilic digestion will enhance pathogen death rate. Compositing of decomposed excreta with household or municipal organic wastes will also reduce pathogen survival potential. In this case temperature (around 55°C aerobic digestion) affect will be most effective. The compost thus produced is useful as soil conditioner. This will help retain and improve soil physical properties besides supplying essential nutrients to plants. The use of chemical fertilizers may be fully or partially supplemented by the cheaply produced excreta based compost. Addition of organic matter in the form of decomposed excreta could usher a milestone in restoring soil fertility.

3.5 Pathogen Indicators and International Limits

Faecal indicator bacteria are excreted alongwith pathogenic bacteria (please see Section 4.1) and are good indicators of environmental pollution by faecal material of recent origin. Faecal indicators are relatively easy to culture and identify, survive longer and have dependable correlation with the existence of pathogens. Pathogen identifications are difficult, laborious, expensive and often difficult to reproduce results accurately. Hence indicators are preferred for detection over pathogens. However some of the faecal indicators (e.g. *E.coli, S. faecalis* could themselves be opportunistic pathogens.

The faecal indicator bacteria include the coliform, the faecal coliforms E. Coli faecal streptococci, *Clostridium perfringens, Pseudomonas aeruginosa Bifidobacterium, Bacteroides* and others that are excreted in large numbers by healthy persons or worm-blooded animals.

Considering various factors, convenience and convincing criteria only a few of the above faecal indicator bacteria and parasitic eggs are quantitatively determined for safe handling of digested sludge. Determination of total coliform suggests only presumptive estimation of pollution. Of late, the incidence of following bacterial pathogen indicators and worm-eggs have been strongly recommended by the World Bank studies and WHO as pollution indicators with certain limit values. These are :

a) Faecal coliform (mostly *E.coli*)

b) Faecal streptococci and

c) Roundworm eggs (mainly *Ascaris*)

The following guideline/limit values of pathogens in digested excreta for use in agriculture have been recommended by World Bank (1983) and WHO (1989).

Pathogens	World Bank (1983)	WHO (1989)
Faecal coliform	100/100gm	1000/100 gm
Faecal streptococci	100/100gm	-
Ascaris eggs	10/100 gm	1/100 gm

4.0 METHODOLOGY OF STUDY

4.1 Field Sampling

Two areas representing low (Gazipur) and high (Rupganj) water tables were selected for study. Three pits were selected from each area and two samples were dug from each pit. In total 2x3x2 = 12 samples were collected for microbiological and parasitological analyses. Sampling was done after every 2-month interval upto eighth sampling (16 months) After 8 months, fertilizer values for N, P & K were determined in six samples -- representing one sample from each pit. Sampling procedure for collecting excreta samples from filled-up unused pits was carefully designed and executed. Specially designed metal core sampler was designed and used to sample about 250 gm of samples from 2 and 4 feet depth of each pit.

4.2 Laboratory Tests

Laboratory examinations for pathogen indicators were conducted for following bacteria and worm-eggs:

a) Total coliform

b) Faecal coliform

c) Faecal streptococci

d) Roundworm eggs/ova and

e) Hookworm eggs/ova

The tests were performed in the laboratories of the Department of Microbiology, University of Dhaka, which is well-equipped to perform these tests. Internationally accepted and executed techniques were followed (Standard method for the examination of water and wastewater, 1992, USA) for the isolation, identification and quantification of pathgen indicator bacteria. For identification and quantification of worm-eggs, the following books/manuals have been used:

1) "An Illustrated Manual of Parasitology". R.M. Cable, (1960) Burges, Minneapolis, U.S.A.

2) "Diagnostic Parasitology". L.S. Garcia and L.R.Ash (1975). The C.V.Mosby & Co., St.Louis, U.S.A.

4.3 Interpretations of Results

Results for each sampling for three bacterial and two worm-eggs tests were compared with the subsequent samples done at every 2-month interval until the 16th month. The viable counts for bacterial pathogen indicators and worm-eggs were very high at the beginning of pit closure. With increasing residence time of excreta in pits, the counts gradually decreased but not to a level acceptable for safe handling. Counts particularly for worm-eggs varied on the basis of depth of water table. Results were also interpreted on the duration of incubation time and moisture concentration as influenced by water table. Acceptability of limits as mentioned in Section 3.5 were the guideline values. However, for Bangladesh considering its environmental conditions and high temperature, as characteristic of a tropical country, the limit values for acceptability for handling digested excreta may be relaxed and a slight upward value has been recommended for Bangladesh. The values are as follows:

Pathogen indicator bacteria/worm-eggs		Counts/100 gm
Total coliform	--	3500
Faecal coliform	--	2000
Faecal streptococci	--	2000
Roundworm eggs (*Ascaris*)	--	500

5.0 FIELD SAMPLING

5.1 Field Visits

As per Eng. Rabiul Islam's discussion with Mr. J.K. Baral, Deputy Chief of UNICEF Dhaka Division on 22/06/95, it was decided that areas in Narayanganj and Gazipur districts would be selected for pathogen survival study in filled-up pits. This decision was based on low and high water tables at Gazipur and Narayanganj respectively.

On 25.06.95 myself and Engr. Rabiul Islam visited Narayanganj DPHE Division Office and met Mr. Nurul Islam, Ex-EN, DPHE. According to his suggestions Rupganj area in Narayanganj was selected for studies.

On 26.06.95 and on 03.07.95 myself and Engr. Rabiul Islam visited Gazipur sadar Ex-En office. We received all co-operation from Mr. Sohrab Uddin, Ex-En. Mr. Shahjahan Mia, SAE Gazipur Sadar assisted us in selecting sites and pits.

5.2 Site Selection

The decision for site selction in two different areas was made earlier, representing high and low water tables that might influence decomposition of sludge and death rate of pathogens. The water table at Gazipur rests about 32 feet whereas at Rupganj water table lies at about 10 feet below surface soil.

Following our earlier visits and discussions on 26/06/95 and 03/07/95, the village Bhurulia under Gazipur Pourasabha, in Gazipur district was chosen for pit-selection, representing the low water table zone. Two villages e.g. Mahmudabad and Brahmingaon in Rupganj Sadar in Narayanganj district were selected for pits representing high water table zone.

5.3 Selection of pits

Three pits from low water table (LWT) area which have been recently filled-up have been selected in the village Bhurulia, Ward no. 1 under Gazipur Pourasabha. As per contract, three filled-up pits have been selected for pathogen survival study from this low water table area. Each pit was sampled at two different heights — i.e at 2 and 4 feet depth. The details of pit selection are as follows:

Pit#1.

For convenience, pits have been numbered. Pit #1 has been selected in the residence of Mr. Kafiluddin of village Bhurulia. The pit was completely filled and was closed on 05/07/95).

Pit#2.

Pit #2 has been selected from the premises of Mr. Hasan Ali of the same village. The filled-in pit has been closed on the day of our visit, (09/07/95). (Johra Kahtoon's pit was later selected, the pit was closed on 25/5/95).

Pit#3.

Pit #3 was selected in the homestead of Mr. Moslem Uddin. This pit was closed about a month back (on 07/06/95, as reported).

All the pits were selected with the assistance of Mr Shahjahan Miah, SAE. The pits were immediately covered with a layer of surface soil and secured by placing concrete rings on which pit number and initiation of experimental dates were marked.

For selection of pits at high water table (HWT) area, myself along with Engr. Rabiul Islam of UNICEF and Mr. Abdur Razzaque, SAE, VS of DPHE visited Rupganj area on 12/07/95. From the DPHE, Executive Engr. Nurul Islam and Mr. Khalilur Rahman, Senior SAE of Narayanganj district also accompanied us and extended all cooperation and assistance in selecting site and pits for pathogen study.

Three filled-up pits from the village Mahmudabad (2 pits) and Brahmingaon (1 pit) in Murapara Union, P.S. Rupganj under Narayanganj district were selected. The following are the details of pit selection from HWT area (Rupganj):

Pit#4

Pit #4 was selected form the residence of Mr. Abdul Malek Mia of village Mahmudad. The filled-in pit was closed on 12/06/95.

Pit #5

This pit has been selected from the homestead of Mr. Abdul Awal of the same village (as in pit #4). Arrangements have been made to close thepit on 17/07/95, and this would constitute a recently filled-in pit.

Pit #6

Pit # 6 has been selected from the residential area of Mr. Manik Mia of village Brahmingaon. As reported, this pit was closed on 12/05/95.

Arrangement have been made to earmark and protect the pits as described in case of pits from Gazipur area.

5.4 Care and protection of pits

To ensure proper management, safeguard and marking of the pits, I have visited the village Bhurulia in Gazipur area on 25/07/95 and found things were in proper order. As per our earlier visits and suggestions, pits have been properly secured with concrete rings, painted white, that bore pit number and date of initiation of experiments Mr. Shahjahan Mia, SAE Gazipur accompanied me to the site during our visit. It was decidced that first sampling would be done on 31/07/95 from Gazipur area.

Similar arrangements have been made for Rupgonj area. Mr. Nurul Islam, Executive Engr. & Mr. Nazrul Islam, SAE Rupgonj have extended all possible assistance. Sampling from this area will was done on 01/08/95.

5.5 Sampling Equipment and Tools

A newly designed core sampler has been fabricated/devised to take sludge sample from pre-determined depths. The sampler has been made of aluminum alloys with the provision of suction, facilitating intake of desired volume of sludge. A sketch and cross-sectional view of the sampler has been drawn in the next page.

5.6 Field Sampling Technique

Prior to sampling, the sampler was cleaned with detergent/soap and finally rinsed in water containing antiseptic solution (dettol in water) and/or wiped with soft cloth and tissue paper. The suction liners/gasket was lightly greased for smooth sliding in and out. After each sampling, the lower part of the sampler was washed repeatedly and finally rinsed/soaked in disinfectant and was ready for next sampling. This was done to avoid cross contamination between samples.

The sludge samples were carefully dispensed into disinfected plastic containers, having screw-cap system and was marked appropriately.

5.7 Sample Preservation and Transportation

The sludge samples (in plastic containers) were then immediately put into insulator box having ice in it. The provision of ice keeps the temperature inside the insular box around 4-6°C. At this temperature the pathogens do not die out nor do they multiply within a few hours. The samples were then transported to the laboratory and processed for analytical purpose. The samples were kept at 4°C in a laboratory refrigerator until further use.

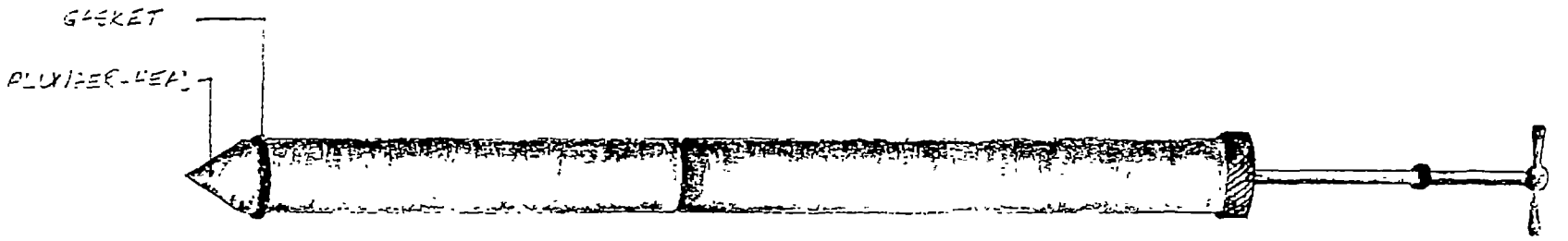
6.0 LABORATORY TESTS

6.1 Test Methodology

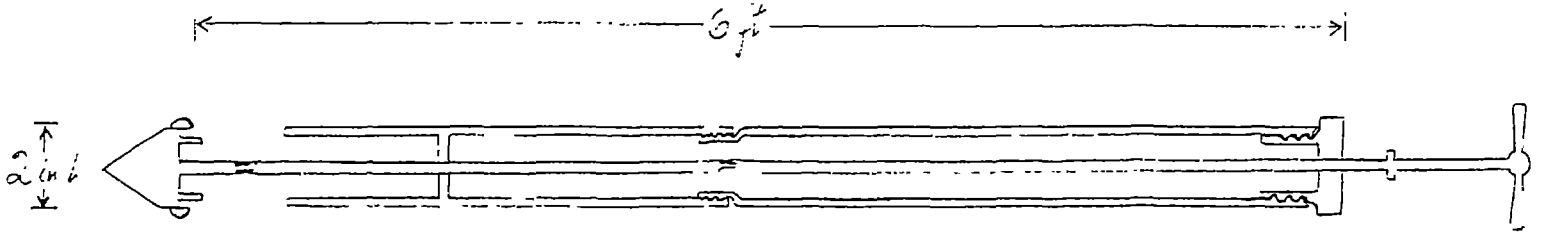
Standard test methodology were followed for microbiological examination of pathogen indicator bacteria and worm-eggs. Since the concentrations of pathogens are usually very high, particularly at the beginning of experimentation, 10-fold serial dilutions of 5.0g of sample (wet weight) were made in sterile saline and shaken in vortex mixture for uniform dispersion of bacterial cells from adhering clay or silt particles. Aliquot (0.1 ml) from each dilution was spread on appropriate culture media (described in Section 7.3). The indicator bacterial count was determined following spread plate and/or MPN technique.

DESIGN OF THE SLUDGE - SAMPLER.

(Approximate sketch.)



a) Normal view



b) Cross-sectional view

The inoculated plates (petri dish) were incubated at 37°C for the appearance of colonies of total coliform (24 h) and faecal streptococci cells (48-72h). For the detection of faecal coliforms, the plates were incubated at 44.5°C for 24 h.

For the identification of parasites, tests were done by the sedimentation technique and calculations were done by following the technique of Hall (1981) and methodologies mentioned earlier (Section 4.2).

6.2 Laboratory Equipment

The following laboratory equipment were used for the identification and quantification of pathogens:

a) Vortex mixture
b) Low and high powered microscopes
c) Constant temperature water bath
d) Laminar air flow cabinet
e) Autoclave
f) Incubators (37°C and 44.5 C)
g) Colony counter
h) Filtration unit
I) Centrifuge

6.3 Media and Reagents used

The following culture media and reagents were used for the isolation, detection and quantification of pathogen indicators:

a) MacConkey agar
b) Lactose broth
c) Brilliant green bile broth
d) Eosine Methylene Blue agar
e) m-FC agar
f) K-F Streptococcus agar
g) IMViC media
h) Oxidase media
I) Sugar fermentation media
j) Physiological saline
k) Bacteriological dyes
l) Acids/Alkalis

6.4 Pathogen Indicator Bacteria and Worm-eggs

Presumptive tests for faecal indicators particularly for total coliform and faecal coliform were done on Lactose Broth followed by growth and gas production in Brilliant Green Lactose Bile Broth (BGLB).For qualitative tests the turbid growth was streaked on

MacConkey Agar where pink/reddish colonies (after incubation at 37°C overnight) were indicative of coliform bacteria. These were later confirmed by Growth on EMB and biochemical tests. For quantitative estimation, 10-fold serial dilution of a measured concentrations were plated and incubated. For faecal coliform detection, m-FC agar medium was used and incubated at 44.5° C for 24 h. Blue colonies were indicative of faecal coliform. Later biochemical tests were performed for confirmation. For faecal streptococci, K-F streptococcus agar was used where brownish red colonies were indicative of streptococci. Finally microscopic and biochemical tests were done for confirmation. Test procedures for the identification and quantification of worm-eggs have been described in Section 7.1.

6.5 Fertilizer Element Analysis (N,P & K)

Fertilizer values for nitrogen, phosphorus and potassium were chemically analysed from the 4th sampling (after 8th month of pit closure). Six samples were analysed from six pits at each sampling time. The samples were air dried and ground to pass 200 mesh sieve and stored in screw capped plastic containers. Nitrogen was analysed by Microkjeldahl method, phosphorus by ammonium phosphomolybdate method and potassium was analysed following flame photometry (Black, 1965).

7.0 LABORATORY RESULTS OF BI-MONTHLY SAMPLING

7.1 Report on 1st sampling (after 2 months)

Twelve samples from two different areas were examined for concentration of pathogens in decomposed human excreta. Three filled-up pits from Gazipur and another 3 from Rupganj area were sampled respectively on 31-07-95 and 01-08-95. From each pit, two samples were collected from two pre-designated depths (e.g, 2 and 4 feet).

The newly fabricated sampler was used for sampling. Between sampling, the apparatus was cleaned, washed and finally rinsed with disinfectant so as to preclude chances of cross-contamination. The test samples were put into presterile containers, appropriately marked and dispensed in insulator box, with ice pack in it. The samples were immediately transferred to the laboratory and kept at 4° C until examination. Sample nos. 1-6 were from Gazipur area and the samples bearing nos. 7-12 were from Rupjanj area.

The concentration of pathogen indicator bacteria particularly total coliform was very high that ranged between 7.5×10^7 and 1.4×10^6 cfu/gm of sludge. The counts of faecal coliform was relatively lower and varied between 7.5×10^5 and 1.1×10^5 cfu/gm. Almost similar values were obtained for faecal streptococci (Table-1).

The counts of roundworm and hookworm were much less than indicator bacteria. Their counts ranged between 100 to more than 5,000 per gm of sludge (Table-1:Appendix I&II).

Table - 1

REPORT ON FIRST SAMPLING

Examination of Bacterial Indicators and worm-eggs

(1st sampling - approx. one month after pit closure)

31/07/95 and 01/-8/95

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	5.3×10^{7}	7.5×10^{5}	7.1×10^{5}	Low (100-1000)	Low (100-1000)
	2 (4′ depth)	2.8×10^{6}	7.0×10^{5}	2.6×10^{5}	Moderate (2000-5000)	Moderate (2000-5000)
2 (Gazipur) 25/05/95	3 (2′ depth)	2.0×10^{7}	7.4×10^{5}	2.2×10^{5}	Low (100-1000)	Moderate (2000-5000)
	4 (4′ depth)	2.10×10^{6}	1.1×10^{5}	1.49×10^{5}	Low (100-1000)	Nil
3 (Gazipur) 07/06/95	5 (2′ depth)	2.5×10^{6}	6.5×10^{5}	5.0×10^{5}	High >5000	Low (100-1000)
	6 (4′ depth)	1.4×10^{6}	2.2×10^{5}	1.3×10^{4}	Moderate (2000-5000)	Low (100-1000)
4 (Rupganj) 12/06/95	7 (2′ depth)	7.1×10^{6}	6.3×10^{5}	2.0×10^{4}	High >5000	Low (100-1000)
	8 (4′ depth)	1.2×10^{6}	5.2×10^{5}	1.02×10^{5}	High >5000	Low (100-1000)
5 (Rupganj) 17/07/95	9 (2′ depth)	1.03×10^{7}	5.8×10^{5}	1.7×10^{5}	Moderate (2000-5000)	Moderate (2000-5000)
	10 (4′ depth)	2.35×10^{6}	2.5×10^{5}	1.0×10^{6}	Low (100-1000)	Low (100-1000)
6 (Rupganj) 15/05/95	11 (2′ depth)	5.9×10^{6}	2.5×10^{5}	2.0×10^{5}	High >5000	Low (100-1000)
	12 (4′ depth)	3.5×10^{6}	4.4×10^{5}	4.0×10^{4}	Moderate (2000-5000)	Moderate (2000-5000)

7.2 Report on 2nd sampling (after 4 months)

Twelve samples representing six pits from two sampling sites e.g., Gazipur and Rupganj were done, after two months of 1st sampling, respectively on 01/10/95 and 02/10/95. The samples were processed and analysed for bacterial indicators and worm-eggs.

Incidence of Bacterial Pathogen Indicators

On the examination of the 2nd sampling done after two months (of the 1st sampling), it was observed that viable numbers of almost all the indicators declined between 0.1 to 2.0 log depending on types of indicators. The count of total coliforms (T.C) fell between 0 2 to 1.0 log unit within two months from 1 to 10 million of viable cells/gm of sludge.

The viable number of faecal coliform fell between 0.1 to 0.5 log with most samples. There was a decrease of about 1.0 log unit of viable cells with only one sample. The numbers still appear to be very high and fall outside the acceptable limit.

The concentration of faecal streptococci (F.S) fell sharply when compared with the other two bacterial indicators. The decrease in numbers decreased between 1.0-2.0 log units. In some cases the fall was minimal and ranged between 0.1 to 0.5 log unit (Table-2; Appendix-I & II)

Relative Incidence of Roundworm and Hookworm-eggs.

Of all the worm-eggs detected, the incidence of *Ascaris lumbricoides* eggs were predominant. The intensity of incidence of *Ascaris* eggs ranged between low to high (100-5000/gm) while one-third of the sample was representative of moderate intensity infestation. From the date of closing the pits to the 2nd sampling time (about 3-4 months), the *Ascaris* eggs did not decrease or die-off or did it very insignificnatly.

Amongst the other worm-eggs detected, the prevalence of hookworm (*Ancylostoma duodonale*) was predominant. However, the concentration was of moderate range. The eggs of tape worm (*Taenia saginata*), whipworm (*Trichuris trichiura*) and pinworm (*Enterobius vermicularis*) also appeared to have persisted until about 4 months after closure of pits. Although their percent incidence was relatively low.

Table - 2

REPORT ON SECOND SAMPLING

Examination of Bacterial Indicators and worm-eggs

(2nd sampling - approx. four months after pit closure)

01/10/95 and 02/10/95

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	1.2×10^{6}	1.1×10^{5}	3.0×10^{4}	Low (100-1000)	Low (100-1000)
	2 (4′ depth)	4.8×10^{5}	3.5×10^{4}	1.0×10^{4}	Moderate (2000-5000)	Moderate (2000-5000)
2 (Gazipur) 25/05/95	3 (2′ depth)	3.0×10^{6}	4.0×10^{5}	1.2×10^{4}	Low (100-1000)	Moderate (2000-5000)
	4 (4′ depth)	1.1×10^{6}	9.0×10^{4}	1.5×10^{4}	Low (100-1000)	Nil
3 (Gazipur) 07/06/95	5 (2′ depth)	1.8×10^{6}	2.1×10^{5}	5.0×10^{3}	High >5000	Low (100-1000)
	6 (4′ depth)	8.4×10^{5}	1.0×10^{5}	3.0×10^{3}	Moderate (2000-5000)	Low (100-1000)
4 (Rupganj) 12/06/95	7 (2′ depth)	1.0×10^{6}	2.5×10^{5}	1.0×10^{3}	High >5000	Low (100-1000)
	8 (4′ depth)	5.8×10^{5}	1.1×10^{5}	2.0×10^{4}	Moderate (2000-5000)	Low (100-1000)
5 (Rupganj) 17/07/95	9 (2′ depth)	2.2×10^{6}	4.1×10^{5}	1.5×10^{3}	Moderate (2000-5000)	Moderate (2000-5000)
	10 (4′ depth)	1.1×10^{6}	2.1×10^{5}	1.3×10^{4}	Low (100-1000)	Low (100-1000)
6 (Rupganj) 15/05/95	11 (2′ depth)	2.0×10^{6}	4.8×10^{5}	0.75×10^{4}	Moderate (2000-5000)	Low (100-1000)
	12 (4′ depth)	2.0×10^{5}	1.1×10^{5}	3.0×10^{4}	Moderate (2000-5000)	Low (100-1000)

7.3 Report on 3rd sampling (after 6 months)

Twelve samples representing six filled up latrine pits, from two sampling sites e.g. Gazipur and Rupganj were examined in the laboratory for the survival of pathogen indicator bacteria and worm-eggs/ova in the unused pits. The sampling was done on 02/12/95 and 03/12/95 at Gazipur and Rupganj respectively.

Survival of bacterial pathogen indicators

With the increasing period of incubation time, the viable numbers of pathogen indicator bacteria declined remarkably. The number of total coliform counts decreased by approximately 0.5 to 1.0 log unit. The viable cell number ranged between 70,000 and 6,000 per gram. The number decreased more with increasing depth in most cases.

The decrease in the viable cell concentration of faecal coliform was also significant. In most cases the cell death rate was around 1.0 log unit after the last sampling, two months ago. This depicts a favourable picture over the fall in viable numbers of faecal coliform. The viable number ranged between 1800 and 8,000 per gram of the digested excreta.

The number of faecal streptococci also fell remarkably. In most cases the decrease in viable number was more than one log unit. The viable numbers fell from 3,000 per gram between 40 and 300 per gram of digested sludge. This represents a significant decrease with most samples and indicates a positive response to longer residence time in unused pits. (Table-3; Appendix I & II).

Concentrations of Roundworm and Hookworm - eggs

The presence of *Ascaris* eggs/ova are indicative of their persistent character in the digested excreta. The incidence of *Ascaris* and other worm-eggs have now been attempted to express quantitatively. Except for two samples (sample Nos 4 & 5), most samples contained considerable number of *Ascaris* eggs. The number was low with sample number 1, 2 and 3 and ranged between 40-140/gm of sludge. With other samples, the concentration of *Ascaris* ova/eggs ranged between 420 to 7,413 per gram. The highest concentration was detected with sample numbers 7, 8, 10 and 11. More than 50% cases, the number of *Ascaris* ova was still very high even after 6 months of residence in unused pits.

With other worm-eggs the incidence of the hook-worm, *Ancylostoma duodanale* was most prevalent followed by *Trichuris trichiura*. Only three samples e.g. sample nos. 2, 4 and 5 were found to be free of any worm-eggs/ova. The eggs/ova of *Ancylostoma* varied between 41 and 325 per gram of decomposed sludge while the number of *Trichuris* ova/eggs ranged between 42 and 320 per gram. Only four samples (e.g. 7, 8, 10 and 12) contained both *Ancylostoma* and *Trichuris* eggs/ova while the other samples contained either of the *Ancylostoma* or *Trichuris* ova/eggs (Table-3; Appendix I & II).

Table - 3

REPORT ON THIRD SAMPLING

Examination of Bacterial Indicators and worm-eggs

3rd sampling - approx. six months after pit closure

(02/12/95 and 03/12/95)

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	2.0×10^{5}	8.0×10^{3}	0.5×10^{3}	42	41
	2 (4′ depth)	6.0×10^{4}	1.5×10^{3}	6.0×10^{3}	40	Nil
2 (Gazipur) 25/05/95	3 (2′ depth)	1.2×10^{5}	5.0×10^{3}	3.0×10^{3}	140	42
	4 (4′ depth)	3.5×10^{5}	3.0×10^{3}	1.0×10^{3}	Nill	20
3 (Gazipur) 07/06/95	5 (2′ depth)	8.0×10^{5}	1.5×10^{4}	1.4×10^{3}	Nill	4
	6 (4′ depth)	4.0×10^{5}	8.0×10^{4}	1.2×10^{3}	745	92
4 (Rupganj) 12/06/95	7 (2′ depth)	8.0×10^{5}	5.0×10^{4}	0.8×10^{3}	7,413	325
	8 (4′ depth)	1.0×10^{5}	2.0×10^{4}	1.8×10^{3}	2,065	245
5 (Rupganj) 17/07/95	9 (2′ depth)	7.0×10^{5}	5.0×10^{4}	1.2×10^{3}	420	42
	10 (4′ depth)	3.0×10^{5}	1.0×10^{4}	1.7×10^{3}	1,025	223
6 (Rupganj) 15/05/95	11 (2′ depth)	1.5×10^{5}	8.0×10^{3}	0.4×10^{3}	2,053	280
	12 (4′ depth)	1.0×10^{5}	1.0×10^{4}	0.8×10^{3}	1,680	140

7.4 Report on 4th sampling (after 8 months)

Sixteen samples of which 12 from 6 filled up pits and 4 samples, each two representing kitchen garden and field soils, from Gazipur and Rupganj were examined for the survival of pathogen indicator bacteria and worm-eggs/ova. The sampling was done on 01/02/96 and 02/02/96 at Gazipur and Rupganj respectively.

Survival of Pathogen Indicator Bacteria

The decrease in number of the viable indicator bacterial was relatively slow with the increasing residence time. The concentration of total coliform cells decreased by approximately 1.0 to 0.5 log units with most samples. The viable cell number ranged between 55,000 and 1,000 per gram of sludge samples of which sample number 1,2 & 3 showed remarkable decline. With increasing depth the cells decreased slowly.

The viable cells of faecal coliform bacteria decreased rather slowly. In most cases the cell death rate varied between 0.7 and 0.1 log units. The concentration of cells ranged between 4,000 and 120 per gram of decomposed sludge (Table - 4).

The decrease in the number of faecal streptococci was minimal when compared with the other indicator organisms. The cell number fluctuated between 0.5 and 0.1 log units. The cell counts ranged between 30 and 220 per gram of sludge. This still lies beyond the acceptable limits for safety. (Table - 4; Appendix I & II).

Kitchen Garden and Field Soils

Laboratory examination with kitchen garden soils of Gazipur and Rupganj reflects that both the soils contain high number of total coliform bacteria with low concentrations of faecal coliform and faecal streptococci cells. The cell numbers were relatively higher at Gazipur than at Rupganj. Field soils at both the sites contained considerable number of total coliform (30,000 to 42,000 per gram). Whereas no faecal streptococci was detected in any of the field samples. Gazipur field soils only contained 200 cells of faecal coliform per gram of soil sample (Table - 4).

Concentration of Roundworm and Hookworm eggs

The concentration of roundworm eggs (*Ascaris*) decreased significantly with most samples except for sample numbers 1,2 & 3. Only four samples e.g. sample nos. 7,8,11 & 12 were found to contain high counts of *Ascaris* eggs followed by a decreasing trend with the other samples. Even after eight months of pit closure, most samples still harbour high number of *Ascaris* eggs .

The incidence of hookworm (*Ancylostoma duodenale*) and other worms-eggs was most prevalent followed by whipworm (*Trichuris trichiura*). The counts of eggs with both the types of worms ranged between 21 and 200 per gram of sludge. Three samples e.g., sample nos. 4,5 & 12 did not contain any eggs regardless of types. It is unusual that even after eight months of pit closure, eggs of hookworm and whipworm are still present with most sludge samples (Table - 4a).

Kitchen Garden and Field Soils

Both at Gazipur and Rupganj, kitchen garden and field soils did not contain any eggs of roundworm (*Ascaris*). Only soils from Gazipur found to harbour worm-eggs; their number ranged between 30 and 100 per gram of soil whereas they were absent in Rupganj soils (Table - 4a).

Table - 4

REPORT ON FOURTH SAMPLING

Examination of Bacterial Indicators and worm-eggs

4th sampling - approx. eight months after pit closure

(01/02/96 and 02/02/96)

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	1.1×10^{3}	2.2×10^{2}	0.3×10^{2}	45	30
	2 (4′ depth)	1.0×10^{3}	1.2×10^{2}	2.2×10^{2}	35	Nil
2 (Gazipur) 25/05/95	3 (2′ depth)	1.7×10^{3}	3.5×10^{2}	1.7×10^{2}	110	200 (*Trichuris*)
	4 (4′ depth)	2.0×10^{4}	2.2×10^{2}	0.7×10^{2}	30	21 (*Trichuris*)
3 (Gazipur) 07/06/95	5 (2′ depth)	4.2×10^{4}	3.8×10^{2}	1.3×10^{2}	Nil	Nil
	6 (4′ depth)	5.5×10^{4}	4.0×10^{3}	0.8×10^{2}	330	Nil
4 (Rupganj) 12/06/95	7 (2′ depth)	4.5×10^{4}	2.0×10^{3}	0.4×10^{2}	2,250	60
	8 (4′ depth)	2.1×10^{4}	1.0×10^{3}	1.4×10^{2}	1,150	200
5 (Rupganj) 17/07/95	9 (2′ depth)	3.3×10^{4}	2.2×10^{3}	0.9×10^{2}	220	130
	10 (4′ depth)	2.5×10^{4}	$5\ 0\times10^{2}$	1.2×10^{2}	650	22
6 (Rupganj) 15/05/95	11 (2′ depth)	2.0×10^{4}	4.0×10^{2}	0.4×10^{2}	1,130	105
	12 (4′ depth)	1.5×10^{4}	3.0×10^{2}	0.7×10^{2}	1,260	150

Table - 4a

Examination of kitchen garden and field soils for pathogen indicator bacteria and worm-eggs

Sampling dates : 1/2/96 and 2/2/96

Area	Sample Type	Sample No	Pathogen Indicator Bacteria (per gram)			Roundworm & Whookworm (per gram)	
			Total Coliform	Faceal Coliform	Faecal Streep.	Roundworm *(Ascaris)*	Whookworm *(Ancylostoma)*
Gazipur	Field Soils (GSF)	1	3.0×10^{4}	2.0×10^{2}	Nil	Nil	100
	Kitche Garden Soils (GKS)	2	4.5×10^{5}	3.5×10^{2}	1.2×10^{4}	Nil	30
Rupganj	Field Soils (RES)	3	$4\,2\times10^{4}$	Nil	Nil	Nil	Nil
	Kitchen Garden Soil (RKS)	4	4.0×10^{5}	1.5×10^{2}	1.1×10^{2}	Nil	Nil

7.5 Report on 5th sampling (after 10 months)

Twelve samples of digested human excreta from six pits representing two different areas and each pit constituting two samples of two different depths, were examined for concentration/survival of pathogens after about ten months of pit closure. Additionally, four samples, representing two different sites (e.g. Gazipur & Rupganj), each two representing kitchen garden and field soils from one area were also microbiologically examined for background concentration of pathogen indicator bacteria and worm-egg (roundworm of hookworm), if any.

The digested sludge samples from pits were also analysed for fertilizer elements e.g nitrogen, phosphorus and potassium.

Survival of Pathogen indicator bacteria

With the increase in digestion period to about ten months the number of viable indicator bacteria decreased rather slowly. The concentration of **total coliform cells** fell rather sluggishly. However, the decrease in number was apparent with almost all the samples, irrespective of sites and pit depths. In most cases this decrease was in fraction of log units. The viable cell number ranged between 50,000 and 1000 per gram with the most sludge samples.

The concentration of viable cell number of **faecal coliform** also did not decrease significantly. Rather the cell number almost remained close to that recorded in the 4th sampling time. Although in most cases cell number fell but it was in the fractional range of one log unit. The count of coliform cells ranged between 100 to about 400 hundred per gram of sludge sample (Table-5).

The decrease in number of viable cells of **faecal streptococci** was rather notable as compared to total coliform and faecal coliform cells. However, the decreasing trend was more apparent to about 0.5 log unit to almost negligible with most samples. The viable cell number varied between 20 to 240 cells per gram of decomposed excreta and appears to be low (Table - 5; Appendix I & II).

Concentration of Roundworms and Hookworms in digested sludge

The concentration of roundworms (e.g. *Ascaries*) and hookworm (e.g. *Ancylostoma*) and other worm-eggs declined gradually. The decrease was rather remarkable with most samples except for sample numbers 7, 8 & 11. The number of roundworm was moderate with the sample numbers 10, 12 & 6. The remaining samples showed gradual decrease with increasing time of residence.

The incidence of hookworm decreased sharply. Sample nos. 1 to 6 and 9 did not contain any egg/ovum of any type of hookworm including tape and whipworm. Only one

sample e.g. sample no. 11 was found to contain 30 eggs of hookworm (*Ancylostoma*) per gm of sludge whereas the sample numbers 7,8,10 and 11 contained ova/egg of *Trichuris* which ranged between 60 and 100 per gram. This finding reflects that with increasing time the ova/eggs of roundworm and hookworm have been decreasing slowly (Table - 5; Appendix - I & II).

Prevalence of Pathogens in kitchen garden and field soils

Kitchen garden and field soil samples from both Gazipur and Rupganj were microbiologically examined for the prevalence of pathogen indicator bacteria and worm-eggs.

The concentration of total coliform with both field and kitchen garden soils at Gazipur and Rupganj appears to be high. The numbers were relatively higher with the kitchen garden soils as compared with the field (agricultural) soils. The number ranged between 65,000 to 21000 depending on sampling site. The incidence of faecal coliform was recorded only with kitchen garden soils whereas field soils were not found to contain any viable cells of faecal coliform. Faecal streptococci cells were detected with three samples out of four. Only Gazipur field soil was not found to contain any faecal streptococci cells. The number of such cells ranged between 110 and 27,000 per gram of soil sample.

The incidence of roundworm and hookworm was found to be nil or minimal. Only kitchen garden soils of both Gazipur and Rupganj contained 4 and 3 cells/gram respectively. The incidence of hookworm particularly *Ancylostoma* was only with Gazipur and Rupganj kitchen garden soils respectively containing only 2 and 5 ova/eggs per gram of soil. This observation suggests that kitchen garden soils are most frequently contaminated with human faeces and hence the prevalence of pathogens are apparent although their numbers are not very high. (Table-5a).

Concentration of fertilizer elements in decomposed human excreta

Six samples from six pits from two different areas were chemically analysed for the concentration of three major fertilizer elements e.g. nitrogen, phosphorus and potassium. The results of 8 and 10 months sampling show that the decomposed sludge contain a very high concentration of N.P. & K. This suggests that the decomposed/ digested sludge has the potential for using as organic fertilizer (Table-6). However, the sludge mast be free from attendant pathogens or their number should be within acceptable limit for safe handing.

Table - 5

REPORT ON FIFTH SAMPLING

Examination of Bacterial Indicators and worm-eggs

5th sampling - approx. ten months after pit closure

(04/04/96 and 05/04/96)

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	1.0×10^{3}	1.7×10^{2}	0.2×10^{2}	29	Nil
	2 (4′ depth)	0.9×10^{3}	1.0×10^{2}	1.3×10^{2}	27	Nil
2 (Gazipur) 25/05/95	3 (2′ depth)	1.5×10^{3}	2.5×10^{2}	1.2×10^{2}	57	Nil
	4 (4′ depth)	1.7×10^{4}	2.0×10^{2}	0.8×10^{2}	30	Nil
3 (Gazipur) 07/06/95	5 (2′ depth)	4.0×10^{4}	3.0×10^{2}	1.1×10^{2}	Nil	Nil
	6 (4′ depth)	5.0×10^{4}	2.5×10^{3}	0.8×10^{2}	210	Nil
4 (Rupganj) 12/06/95	7 (2′ depth)	4.0×10^{4}	1.1×10^{3}	0.3×10^{2}	1900	100 (*Trichuris*)
	8 (4′ depth)	3.0×10^{4}	2.7×10^{2}	2.4×10^{2}	780	100 (*Trichuris*)
5 (Rupganj) 17/07/95	9 (2′ depth)	3.1×10^{4}	1.7×10^{2}	1.1×10^{2}	187	Nil
	10 (4′ depth)	2.1×10^{4}	4.2×10^{2}	1.2×10^{2}	510	30 (*Trichuris*)
6 (Rupganj) 15/05/95	11 (2′ depth)	8.0×10^{3}	3.0×10^{2}	0.3×10^{2}	830	30 *Ancylestoma* 60 (*Trichuris*)
	12 (4′ depth)	1.2×10^{4}	2.0×10^{2}	0.4×10^{2}	530	Nil

Table - 5a

Examination of kitchen garden and field soils for pathogen indicator bacteria and worm-eggs

Sampling dates : 04/04/96 and 05/04/96

Area	Sample Type	Sample No.	Pathogen Indicator Bacteria (per gram)			Roundworm & Whookworm (per gram)	
			Total Coliform	Faceal Coliform	Faecal Streep.	Roundworm(*Ascaris)*	Whookworm/ others *(Ancylostoma)*
	Field Soils (GSF)	1	2.1×10^{3}	Nil	Nil	Nil	Nil
Gazipur	Kitche Garden Soils (GKS)	2	$4\,2\times10^{4}$	4.3×10^{2}	2.7×10^{3}	4	2 *(Ancylostoma)*
	Field Soils (RES)	3	4.0×10^{3}	Nil	1.1×10^{2}	Nil	Nil
Rupganj	Kitchen Garden Soil (RKS)	4	6.5×10^{4}	4.1×10^{2}	2.0×10^{3}	3	5 *(Ancylostoma)*

Table - 6

Concentration of fertilizer elements (N, P & K) in decomposed human excreta (Component part of 4th & 5th report)

Sampling area	Pit No	**1st sampling after 8 months** (3rd and 4th Feb., 1996)			**2nd sampling after 10 months** (4th and 5th April, 1996)		
		Chemical composition, %			Chemical composition, %		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
Gazipur	1	4.92	2.21	1.10	4.78	2.11	0.98
	2	4.61	2.01	0.93	4.39	2.0	0.92
	3	5.33	1.97	1.02	5.39	1.87	1.01
Rupganj	4	3.90	1.88	0.87	4.01	1.79	0 82
	5	3.89	2.1	0.82	3.9	1 83	0.87
	6	4.01	1.97	1.1	3 82	1.89	1.12

7.6 Report on 6th sampling (after 12 months)

Decomposed sludge samples on 6th sampling (12 months) were analyzed for pathogen indicator bacteria e.g. **Total Coliform** (TC), **Faecal Coliform** (FC) and **Faecal Streptococci** (FS) including worm eggs/ova for **Roundworm** and **Hookworm**. Field and kitchen garden soils from both Gazipur and Rupganj were also examined for background count. Digested excreta samples from both sites were chemically estimated for nitrogen, phosphorus and potassium concentrations.

Pathogen indicator bacteria

At sixth sampling i.e. approximately after 12 months of pit closure the numbers of **total coliform bacteria,** with most samples, remained static or almost similar. Few samples showed decreasing tendency. The highest count was around 44,000 per gram while the lower levels ranged between 1,000 and 2,000 cfu/gm with most samples. The higher levels/numbers were with the high water table area (Rupganj) while with the low water table area e.g. Gazipur, the TC cell counts were slightly lower (Table - 7; Appendix I & II).

The fall in concentration of **faecal coliforms** also showed similar trend or remained static with little tendency to gradual decrease. The per gram viable numbers ranged between 470 (Rupganj) and 150 at Gazipur respectively high and low water table zones. The numbers of **faecal streptococci** also did not decrease appreciably. With some samples the viable numbers increased slightly while the count decreased with certain other samples (Table 7). However, the overall fluctuation was too small to reckon with. The viable coccoids ranged between 140 to 30 cells per gm and appears to be above the acceptable limits suggested for Bangladesh (Table - 7; Appendix I & II).

Counts of Roundworm and Hookworm-eggs

The incidence of **Roundworm** is still apparent with most samples. However the numbers of eggs/ova decreased rather slowly. The per gram number of eggs varied between 1760 and 25. The numbers were relatively lower with the low water table area (Gazipur) as compared with the high water table area, (Rupganj); where with most samples the egg/ova concentration varied between 200 to 600 per gram. The **hookworm** eggs/ova were nil at Gazipur with all the six samples while at Rupganj, a high water table area, still a few eggs could be counted. The egg numbers ranged between 30 and 210 per gram of sludge. (Table - 7: Appendix I & II).

Incidence of pathogens at field and kitchen garden soils

Field and kitchen garden soils were sampled from both Gazipur and Rupganj area. At both the sites, the number of **total coliforms** ranged between 1,2000 and 10,000 per gram of soils. The higher counts were apparent with kitchen garden soils (Table- 7a). Except for one sample at Rupganj (RFS), the samples contained 50 to 500 **faecal coliform** cfu/gm of soils indicating the probable contamination of kitchen garden and field soils with faecal materials. (Table - 7a).

The similar trend was also observed with the incidence of **faecal streptococci** cells. Surprisingly all the samples representing both field and kitchen garden soils, the incidence of FS was detected although their numbers were low (50-200/gm) with most samples except for Rupganj kitchen garden soils that contained 2,400 cfu/gm (Table - 7a).

Analysis for fertilizer elements N,P & K

The concentrations of fertilizer elements e.g., nitrogen, phosphorus and potassium were still very high. When compared with the 1st sampling, the fertilizer values for NP & K remained almost constant with negligible variation between samples (Table - 8).

Conclusions

a) The laboratory examinations for pathogen content in decomposed sludge after about 12 months digestion reveal that the cells/eggs concentration did not decrease appreciably. Rather the counts with a number of samples for both bacterial pathogen indicators and worm-eggs remained static or in a few cases showed slight but insignificant increase. This is probably because of increased moisture content due to rains.

b) The count of **roundworm** decreased slowly while the **hookworm** concentration decreased further at Rupganj; their incidence at Gazipur was nil with all the samples.

c) The fertilizer values with all the six sludge samples remained almost static when compared with earlier samplings. However with a few samples, slight decrease in fertilizer values was observed.

Table - 7

REPORT ON SIXTH SAMPLING

Examination of Bacterial Indicators and worm-eggs

6th sampling - approx. twelve months after pit closure

(01/06/96 and 03/06/96)

Pit no. (with closing dates)	Sample no. (with depth)	Pathogen Indicator Bacteria (per gram)			Roundworm worm and Hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm	Hookworm
1 (Gazipur) 05/07/95	1 (2′ depth)	2.0×10^{3}	2.0×10^{2}	0.3×10^{2}	Nil	Nil
	2 (4′ depth)	1.1×10^{3}	2.3×10^{2}	1.4×10^{2}	35	Nil
2 (Gazipur) 25/05/95	3 (2′ depth)	1.6×10^{3}	2.6×10^{2}	1.2×10^{2}	42	Nil
	4 (4′ depth)	1.6×10^{4}	2.3×10^{2}	1.1×10^{2}	25	Nil
3 (Gazipur) 07/06/95	5 (2′ depth)	4.3×10^{4}	2.5×10^{2}	1.0×10^{2}	Nil	Nil
	6 (4′ depth)	4.4×10^{4}	2.4×10^{3}	0.7×10^{2}	165	10
4 (Rupganj) 12/06/95	7 (2′ depth)	4.5×10^{4}	2.0×10^{3}	0.8×10^{2}	1760	89
	8 (4′ depth)	3.0×10^{4}	2.6×10^{3}	2.2×10^{2}	610	210
5 (Rupganj) 17/07/95	9 (2′ depth)	2.9×10^{4}	1.5×10^{2}	1.0×10^{2}	200	Nil
	10 (4′ depth)	2.0×10^{4}	4.7×10^{2}	1.1×10^{2}	382	30
6 (Rupganj) 15/05/95	11 (2′ depth)	1.0×10^{4}	3.7×10^{2}	0.5×10^{2}	610	93
	12 (4′ depth)	1.1×10^{4}	2.1×10^{2}	0.3×10^{2}	460	Nil

Table - 7a

Examination of kitchen garden and field soils for pathogen indicator bacteria and worm-eggs

Sampling dates : 01/06/96 (Rupganj) and 03/06/96 (Gazipur)

Area	Sample Type	Sample No.	Pathogen Indicator Bacteria (per gram)			Roundworm & Whookworm (per gram)	
			Total coliform	Faceal coliform	Faecal streep.	Roundworm	Whookworm
	Field Soils (GSF)	1	1.0×10^{4}	0.5×10^{2}	0.6×10^{2}	Nil	Nil
Gazipur	Kitche Garden Soils (GKS)	2	1.2×10^{5}	5.0×10^{2}	2.0×10^{2}	Nil	Nil
	Field Soils (RES)	3	2.2×10^{4}	Nil	1.5×10^{2}	Nil	Nil
Rupganj	Kitchen Garden Soil (RKS)	4	7.0×10^{4}	6.4×10^{2}	2.4×10^{3}	Nil	Nil

Table - 8

Concentration of fertilizer elements (N,P & K) in decomposed human excreta

Comparison of fertilizer values at 14 months with that of 8 months of digestion

Sampling area	Pit No.	1st sampling after 8 months of pit closure (3rd and 4th Feb., 1996)			4th sampling after 14 months of pit closure (16th and 28th Aug., 1996)		
		Chemical composition, %			Chemical composition, %		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
Gazipur	1	4.92	2.21	1.10	4.80	2.27	1.94
	2	4.61	2.01	0.93	4.40	2.10	0.95
	3	5.33	1.97	1.02	5.40	1.90	1.12
Rupganj	4	3.90	1.88	0.87	3.97	1.86	0.84
	5	3.89	2.1	0.82	3.72	2.10	0.98
	6	4.01	1.97	1.1	3.90	2.00	1.00

7.7 Report on 7th sampling (after 14 months)

Digested human excrta from six filled-up and unused pits at two different sites (Gazilpur amd Rupganj) were sampled for pathogen survival study. Twelve samples from 6 pits were analyzed for (a) **Total Colform (T,C), (b) Faecal Colforms (F.C) and (c) Faecal Streptococci (F.S)** as pathogen indicator bacteria. Included in the examinations were also **(d)Roundworm and (e) Hookworm** eggs/ova. Field and Kitchen garden soils from both Gaxipur, a low water table area (LWT), and Rupganj-a high water table area (HWT) were also nanalyzed for pathogens as background count.

Pathogen Indicator Bacteria

At secenth sampling i.e. approximately after 14 months of pit closure, the viable numbers of total coliform (T.C) remained more or less static particularly at HWT zone (Rupganj) whereas the numbers fell slightly at LWT area (Gazipur). The average concentrations of viable bacterial counts (T.C) ranged between20,000 and 18,000 cells/s of sludge in both the sites. The highest number occurred at HWT zone and the lowest counts were apparent with LWT area (Table- 9; Figures 1 and 6)

Little or no decrease in the concentration of faecal coliform (F.C) was observed at both the sampling sites regardless of the position of water tables. The viable cells of F.C. ranged between 170 and 510 per gram of digested soudge (Table 9a; Figures 2 and 7).

Almost similar trend in the viability or decrease in the concentrations of faecal streptococci (F.S) was recorded at both the sampling sites. However, the numbers were slightly fewer than F.C. and the average counts ranged between 80-90/g of sludge respectively at HWT & LWT zones (Table-9; Figure 3 & 8).

Concentrations of Worm-eggs/ova

The concentrations of **roundworms** decreased moderately but still not within the safety limit of use of handling. Numbers with most samples decreased by more than half and shows a steady declining trend. The viable egg count is relatively higher (214/g) with the HWT area at Rupganj as compared to LWT at Joydevpur, where the count is much lower— only 16 eggs/g of sludege (Table-9; Figures 4 and 9).

The eggs/ova of **hookworm** were nil at Gazipur (LWT zone) with all the six samples while at Rupganj (HWT) still a few eggs/s (10-25) remains with only three samples that definitely shows steady decrease (Table 9; Figures 5 & 10).

Incidence of Pathogens at Field and Kitchen Garden Soils

Field and kitchen garden soils from Gazipur & Rupganj were examined for the background count of pathogen indicator bacteria and worm-eggs. The occurrence of T.C., F.C. and F.S in field soils only lends credence to their probable adaptation with natural soils. Slightly higher numbers that were apparent with kitchen garden soils is obvious and suggests faecal contamination. The indicator bacterial counts ranged between 10,000 and 3000/g of soil for T.C. while for F.C. it did fluctuate between 20 and 110 cells/g of sludge.

The eggs/ova of **hookworm** were nil at Gazipur & Rupganj were examined for the background count of pathogen indicator bacteria and worm-eggs. The incidence of these indicators appears to be relatively lower as compared to the sixth sampling. The occurrence of F.C., F.C., and F.S. in field soils only lends credence to their probable adaptation with natural soils. Slightly higher numbers that were apparent with kitchen

garden soils is obvious and suggests faecal contamination. The indicator bacterial counts ranged between 10,000 and 300/g of soil for T.C. while for F.C. it did fluctuate between 20 and 110 cells/g of sludge. The number of F.S. was unusually high with one sample and was nil with another sample (Table 9a).

Chemical Analysis for Fertilizer elements—N.P. & K.

The concentrations of three major fertilizer elements e.g. nitrogen, phsophorus and potassium did not change appreciably. With increasing digestion/decomposition time, the nutrient levels fell slightly or insignificantly at the 7th sampling. The decrease or variation in the concentrations between the six sludge samples, representing six pits, were negligible (Table 10).

Concluding Remarks

I. The laboratory examinationis at seventh sampling (after approximately 14 months of pit closure) reveal that the pathogen indicator bacteria have markedly decreased over time (Table 9; Figures 1-10). However, the counts with most pathogens are not still within the range of safe handling for use as biofertilizer in agriculture.

II. The fertilizer values for N.P &K. are very high and a small decrease appers to be negligible. The digested sludge has potential for use in our agriculture. However the sludge still harbours a few harmful pathogens and might need special treatment prior to safe handling (Table 10).

Table - 9

REPORT ON SEVENTH SAMPLING
Examination of bacterial indicators and worm-eggs
7th Sampling - approx. fourteen months after pit clouser
(16/08/96 and 20/08/96)

Pit No. (with closing dates)	Sample No. (with depth)	Pathogen indicator bacteria (per gram)			Roundworm and hookworm (per gram)	
		Total coliform	Faecal coliform	Faecal strep.	Roundworm (*Ascaris*)	Hookworm & other eggs (*Ancylostoma*)
1 (Gazipur) 05/07/95	1 (2′ depth)	3.3×10^3	2.1×10^2	0.4×10^2	Nil	Nil
	2 (4′ depth)	2.7×10^3	1.9×10^2	1.1×10^2	15	Nil
2 (Gazipur) 25/05/95	3 (2′ depth)	3.8×10^3	2.0×10^2	$0\ 8\times10^2$	18	Nil
	4 (4′ depth)	3.2×10^3	1.1×10^2	1.0×10^2	12	Nil
3 (Gazipur) 07/06/95	5 (2′ depth)	1.8×10^4	1.8×10^2	0.9×10^2	Nil	Nil
	6 (4′ depth)	7.0×10^3	1.5×10^2	1.0×10^2	55	Nil
4 (Rupganj) 12/06/95	7 (2′ depth)	2.0×10^4	1.5×10^3	0.7×10^2	410	15
	8 (4′ depth)	1.5×10^4	8.0×10^2	1.0×10^2	235	25
5 (Rupganj) 17/07/95	9 (2′ depth)	$2\ 6\times10^4$	1.8×10^2	0.9×10^2	105	Nil
	10 (4′ depth)	1.5×10^4	1.7×10^2	$0\ 8\times10^2$	110	Nil
6 (Rupganj) 15/05/95	11 (2′ depth)	2.5×10^4	2.4×10^2	0.9×10^2	210	10
	12 (4′ depth)	2.0×10^4	1.3×10^2	$0\ 4\times10^2$	218	Nil

Table - 9a

Examination of kitchen garden and field soils for pathogen indicator bacteria and worm-eggs

Sampling dates : 16/08/96 (Rupganj) and 20/08/96 (Gazipur)

Area	Sample Type	Sample No.	Pathogen Indicator Bacteria (per gram)			Roundworm & Hookworm (per gram)	
			Total coliform	Faceal coliform	Faecal streep.	Roundworm	Hookworm
Gazipur	Field Soils (GSF)	1	3.0×10^{3}	0.4×10^{2}	Nill	Nil	Nil
	Kitche Garden Soils (GKS)	2	1.1×10^{4}	0.8×10^{2}	0.3×10^{2}	Nill	Nill
Rupganj	Field Soils (RES)	3	3.5×10^{3}	0.2×10^{2}	1.5×10^{2}	Nil	Nil
	Kitchen Garden Soil (RKS)	4	3.8×10^{3}	1.1×10^{2}	1.0×10^{3}	Nill	Nill

Table - 10

Concentration of fertilizer elements (N, P & K) in decomposed excreta

(comparison between 1st and 4th sampling)

Sample dates : 16/08/96 (Rupganj) and 20/08/96 (Gazipur)*

Sampling area	Pit No.	1st sampling after 8 months of pit closure (3rd and 4th Feb., 1996)			4th sampling after 14 months of pit closure (16th and 28th Aug., 1996)		
		Chemical composition, %			Chemical composition, %		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
Gazipur	1	4.92	2.21	1.10	4.40	2.25	1.00
	2	4.61	2.01	0.93	4.15	2.00	0.90
	3	5.33	1.97	1.02	5.35	1.87	1.12
Rupganj	4	3.90	1.88	0.87	4.42	1.73	0.85
	5	3.89	2.1	0.82	3.81	1.99	0.88
	6	4.01	1.97	1.1	3.85	1.80	0.95

* A comparison with the concentration of 1st sampling for NP&K reveals very little difference with the 4th sampling in the concentrations of fertilizer values.

7.8 Report on 8th sampling (after 16 months of pit closure)

The final sampling for digested excreta from sit pits representing two sites, at two different depths, with low water table area (LWT) at Gazipur and a high water table zone (HWT) at Rupganj, was examined for pathogen survival study. Twelve sludge samples were ($3 \times 2 \times 2$) = 12 were examined for (a) total coliform (T.C), (b) faecal coliform (F.C), and fecal streptococci (FS) bacteria. The samples were also examined for worm-eggs that included (d) **roundworm** and (e) **hookworm**. For a background count of pathogens, adapted with the field or kitchen garden soils, sampling was also done from both Gazipur and Rupganj area.

Pathogen Indicator Bacteria

Microbiological analysis of the digested sludge sample after 16 months of pit closure reveals that the counts of **total coliform** (T.C) fell only insignificantly at both the high and low water table zones. The average number of T.C. at LWT (Gazipur) was 3900/gm; at HWT i.e. at Rupganj it was 4190/g of sludge (Table 11; Figures 1 and 6) small decrease was apparent only with **faecal coliform** (F.C) counts at the low water table zone. The numbers are still high that ranges between 100 (LWT) and 600 per gram (at HWT) of sludge. The counts do not suggest that the F.C. may not altogether die out from the digested sludge within a short span of the (Table 11; Figures 2 and 7).

With the **faecal streptococci** (F.S.) the counts decreased slighty with both the test sites regardless of water table positions. The average number of F.S. at Gazipur (HWT) was 60 per gram whereas it was 70 per gram with the HWT (Rupganj). The viable cell numbers of F.S. are still not within the safety limits of handling (Table 11; Figures 3 and 7).

Concentrations of worm-eggs/ova

The concentration of roundworm eggs decreased further as compared with the 7th sampling. The eggs were low (5/gm) at LWT whereas it was relatively higher (30/g) at HWT (Gazipur). Only two samples were devoid of **roundworm** eggs with the LWT zone. The fall of egg counts with the HWT was considerable and might disappear soon. (Table 11; Figures 4 and 9).

The significant observation was made in relation to the disappearance of **hookworm**-eggs with both the water table zones. Not a single worm was detected with any of the 12 sampels examined (Table 11; Figure 5 and 10).

Incidence of Pathogens at Field and Kitchen Garden Soils

To quantitatively assess background count of pathogen indicator bacteria and worm-eggs, 2 field, and 2 kitchen garden soils, were examined in Gazipur and Rupgang soils. Field and kitchen garden soils from both the sites were found to contain considerable total **coliform bacteria**. The counts ranged bacteria 2000 - 3,600 per gram, and appears to be slightly lower than the counts obtained in pits with digested sludge. This indicates a significant incidence of total coliforms with the natural samples, obviously higher with the kitchen garden soils, probably contaiminated with faeces.

The counts with F.C. and F.S. were lower and was only apparent with kitchen garden soils (30-35 cells/g). The number of F.S. was slightly lower than F.C. that ranged between 20 and 28 cells per gram of kitchen garden soils (Table 11a).

Chemical Analysis for Fertilizer elements — N, P and K.

Chemical analysis for three major fertilizer elements e.g. N, P and K after 16 months of digestion in unused pits appears to have slightly reduced concentration with all the test elements. The decrease at 8th sampling appears to be moderate as compared with earlier samplings. It indicates that with increasign digestion, probably owing to leaching or complexation, the concentrations might have been reduced, with all the 3 fertilizer elements as compared with the values at 1st sampling (Table 12).

Concluding Remarks

I. With extended incubation/digestion of the sludge samples at the 8th sampling (after 16 months of pit closure), the counts of the pathogen indicator bacteria, e.g. (a) **total coliform**, (b) **faecal coliform** and the (c) **faecal streptococci** decreased remarkably when xompared with the 1st sampling. But the decline in viable indicator bacteria was nil or minimal when compared with the 6th and 7th samplings. The counts with all the 3 indicators are still high and are not within the range of safe handling in agriculture as biofertilizer. (Figures 1 - 10).

II. Examination on worm-eggs of **roundworm** and **hookworm** reveals that the former is still apparent, although at a very low number (5-12/g) at LWT zone (Gazipur), their relatively higher incidence was dected in HWT samples of

Rupganj that ranged between 20-39 egg/g. These counts are not within the range of safe handling of digested sludge in agriculture.

III. The fertilizer values of digested human excreta for N.P and K are still high, even though there has been small decrease in concentrations with all the 3 nutrient elements. Special treatments like compositing or heat treatment might eliminate remaining pathogens in sludge to a satisfactory level at this or similar level of digestion period.

Table - 11

REPORT ON EIGHTH SAMPLING

Examination of Bacterial Indicators and worm-eggs

8th sampling - approx. fourteen months after pit closure

(15/10/96 and 16/10/96)

Pit No. (with closing dates)	Sample No. (with depth)	Pathogen Indicator Bacteria			Roundworm and Hookworm	
		Total coliform	Faecal coliform	Faecal strep	Roundworm (*Ascaris*)	Hookworm & other eggs (*Ancylostoma*)
1 (Gazipur)	1 (2′ depth)	3.0×10^3	0.9×10^2	0.3×10^2	Nil	Nil
05/07/95	2 (4′ depth)	2.8×10^3	0.8×10^2	0.7×10^2	5	Nil
2 (Gazipur)	3 (2′ depth)	3.6×10^3	0.7×10^2	0.5×10^2	10	Nil
25/05/95	4 (4′ depth)	$3\ 0\times10^3$	1.0×10^2	0.60×10^2	7	Nil
3 (Gazipur)	5 (2′ depth)	3.4×10^3	1.7×10^2	0.75×10^2	Nil	Nil
07/06/95	6 (4′ depth)	8.0×10^3	1.0×10^2	0.8×10^2	12	Nil
4 (Rupganj)	7 (2′ depth)	1.0×10^4	1.5×10^2	0.3×10^2	20	Nil
12/06/95	8 (4′ depth)	1.2×10^4	5.5×10^2	$0\ 8\times10^2$	29	Nil
5 (Rupganj)	9 (2′ depth)	2.1×10^4	1.7×10^2	0.9×10^2	40	Nil
17/07/95	10 (4′ depth)	1.4×10^4	1.0×10^2	0.8×10^2	32	Nil
6 (Rupganj)	11 (2′ depth)	2.1×10^4	2.1×10^2	0.75×10^2	15	Nil
15/05/95	12 (4′ depth)	1.7×10^4	1.0×10^2	0.7×10^2	39	Nil

Table - 11a

Examination of kitchen garden and field soils for Pathogen indicator bacteria and worm-eggs

Sampling dates : 15/10/96 and 16/10/96

Area	Sample Type	Sample No.	Pathogen Indicator Bacteria (per gram)			Roundworm & Hookworm (per gram)	
			Total coliform	Faceal coliform	Faecal streep.	Roundwor m	Hookworm
Gazipur	Field Soils (GSF)	1	2.0×10^3	Nil	Nil	Nil	Nil
	Kitche Garden Soils (GKS)	2	$3\ 3\times10^3$	0.3×10^2	0.2×10^2	Nil	Nil
Rupganj	Field Soils (RES)	3	2.1×10^3	Nil	Nil	Nil	Nil
	Kitchen Garden Soil (RKS)	4	3.6×10^3	0.35×10^2	0.4×10^2	Nil	Nil

Table - 12

Concentration of fertilizer elements (N,P and K) in decomposed excreta

Comparison of fertilizer values between 8 and 16 months of digestion

Dates: 15/10/196 and 16/10/96

Sampling area	Pit No.	1st sampling after 8 months of pit closure (3rd and 4th Feb., 1996)			5th sampling after 16 months of pit closure (15th and 16th Oct., 1996)		
		Chemical composition, %			Chemical composition, %		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
Gazipur	1	4.92	2.21	1.10	4.10	2.15	0.88
	2	4.61	2.01	0.93	3.90	1.94	0.83
	3	5.33	1.97	1.02	4.72	1.73	1.00
Rupganj	4	3.90	1.88	0.87	3.35	1.70	0.82
	5	3.89	2.1	0.82	3.74	1.69	0.80
	6	4.01	1.97	1.1	3.29	1.73	0.84

8. INTERPRETATIONS

Laboratory examinations of digested excreta for three indicator bacteria e.g. (a) **total coliform** (T.C.) (b) **faecal coliform** (F.C.) (c) **faecal streptococci (F.S)** including worm-eggs of (d) **roundworm** and (e) **hookworm** were carried out following standard techniques. Examinations of excreta samples at first sampling started at about weeks after pit closure. The initial concentrations of all the three pathogen indicators were very high particularly of T.C. **Total coliform** count is considered to be presumptive indication of faecal pollution. The average count was very high that stood at 53,000,000 cfu/per gram of sludge.

With increasing retention time in pits the counts of T.C decreased gradually until the 3rd sampling where the average count was 318,000 per gram. The count further decreased at 4th and 5th sampling to a few thousand (21,000 cfu/gm). The numbers were slightly higher at Rupganj (HWT area) than at Gazipur (LWT area). At 6th sampling similar trend was observed in the survival pattern of pathogens (Table - 7; Appendix-II. Figure 1 and 6). With increasing digestion in closed pits at 7th and 8th sampling respectively at 14 and 16 month after pit closure, the number of **total coliform bacteria** did not decrease or decreased insignificantly. At appears that the total coliform bacterial cells might have adopted with the sludge or soil samples and would persist even under adverse growth conditions (Table 9 and 11; Figures 1 & 6).

The initial count for **faecal coliform** (F.C.) was relatively lower than T.C. Concentration of F.C. at first sampling ranged between 750,000 and 110,000 cells per gram. The count gradually started declining sharply until 4th sampling (8 months) where the numbers still averaged 1000 F.C./gm. This trend continued until the 6th sampling with no sign of complete removal/elimination as yet (Table-7; Appendix-I, & II). The **faecal coliform** (F.C) counts decreased slightly or remained almsot static even during 7th and 8th sampling. It appears that even at 16 months of digestion in pits F.C cells did not become extinct. The cells somewhat managed to attach with or strongly adhere to clay/soil particles and survive at refractory nutrient condition (Table 9 and 11; Figure 2 & 7).

The initial count for **faecal streptococci** (F.S.) was slightly lower than F.C. and varied between 710,000 and 40,000 per gm of sludge. With duration of digestion continued and sampling at 2nd and 3rd revealed further elimination of F.S. to 1650 cfu/gm of sludge. The count sharply decreased at 4th and 5th sampling where the number ranged between 90-100 /gm. At the 6th sampling the count remained almost static, still with no sign of complete removal (Tables-7; Appendix-I & II). Similar trend in survival pattern was observed with **faecal streptococci** (F.S). On close observation if appears that the counts

remained almost similar to 7th sampling whereas at 8th sampling a slight but insignificantt decrease is apparent. Naturalization with physico-chemical environments of pit prevented their complete elimination. (Table 9 and 11, Figure 3 and 8).

Concentrations of worm-eggs were remarkably lower than that of bacterial indicators. Counts of **roundworm** and **hookworm** eggs are indicative of faecal pollution having disease potential. The average count of **roundworm** (mostly *Ascaris lumbricoides*) at 1st sampling was 2,200/gm (range 1000-5000). The numbers remained almost similar (2,000/gm) at 2nd sampling with considerable decrease at 3rd (1,300/gm) and 4th samplings (600/gm). The count further dropped at 5th sampling to about 400 eggs/gm with an average decrease to about 250-300 eggs/gm at the 6th sampling. The numbers were relatively lower at Gazipur area when compared with Rupganj. (Tables 1-7; Appendix-I & II). Concentration of **roundworms** did decreased to about half at 7th sampling as compared with 6th sampling. Most sludge samples still contain roundworm eggs particularly with high waer table zone at Rupganj. At the 8th sampling, the numbers reduced further to a very low level (5-39/gm). These eggs/ova appear to be very resistant to distruction or death while most worm-eggs would die out at such a long period of digestion. (Tables 9 and 11, Figure 4 and 9).

The count of **hookworm** eggs (*Ancylostoma duodenate*) was relatively fewer than **roundworm** at initial concentrations with an average of 4,600 eggs/gm (range: 100-5000). The density of **hookworm** eggs did drop at second sampling with an average of 2,300 eggs/gm. The count decreased significantly at third and fourth sampling to an average of about 210 eggs/gm . It further dropped to about 60 eggs/gm at 4th sampling. At 5th and 6th sampling, the count at Gazipur (LWT area) was nil, whereas at Rupganj (HWT area) the average count was 60 eggs/gm of sludge (Table-5 ; Appendix-I & II). The density of hookworm at 7th sampling also decreased remarkably and was nil with most samples except for two. At 8th sampling no eggs/ova of **hookworm** could be detected in any samples of decomposed excreta (Table 9 & 11, Figures 5 & 10). Eggs/ova of hookworm are much more susceptible to physico chemical condition of pit and die out relatively easiliy as compared to roundworm.

Observation on background concentrations of faecal indicator bacteria (as mentioned earlier) in field and kitchen garden soils of Gazipur and Rupganj reveals that the count of **total coliform** bacteria ranged between 20,000 and 40,000 cells per gm of field soil while increasing numbers occurring with the kitchen garden soils was recorded. Counts of **faecal coliform** were mostly detected in kitchen garden soils the concentration of which ranged between 50 and 400 cell/gm. The incidence of **faecal streptococci** was

rather ubiquitous although their counts were slightly lower (50-120 cfu/gm) as compared to **faecal coliform.**

The incidence of pathogenic indicator was apparent with field and kitchen garden soils of both Gazipur and Rupganj. However the incidence of **total coliform** was apparent, although at a lower level than concentration in excreta suggests that they might have been adapted to the agricultural or kitchen garden soils. **Feacal coliform** and **feacal streptococci** incidence was occasionally recorded although at a very low or insignificant number with one or two exceptions. These low numbers at 7th and 8th sampling could suggest a background count to be associated with most kitchen garden soils. Field soils in most cases do not contain F-C or F.S cells (Tables 9a and 11a; Figures 1 to 10).

The concentrations of wormeggs in field and kitchen garden soils was nil to negligible in quantity. The proportion of incidence of **roundworm** and **hookworm** in one or two cases was almost similar where their counts and were in the range of 2-5 eggs/gm of soil mostly with kitchen garden soils (Tables 4a, 5a & 6a). The concentration of **roundworm band hookworm** was nil with both at Gazipur and Rupganj field and kitchen garden soils. (Tables 9a & 11).

The fertilizer values of nitrogen, phosphorus and potassium in decomposed/digested sludge were much higher as compared to normal organic fraction of soils. Particularly the concentration of nitrogen was reasonably high with an average concentration of 4.40% followed by phosphorus (2.0%) and potassium (about 1.0%). The fertilizer value at 14 and 16 months of sampling slightly decreased, particularly at the 8th sampling time. But still the fertilizer values have potential to be used in agriculture for physico-chemical enrichment of agriculture soils (Tables 9a & 11a; Figures 1 to 10).

9. FINDING

Faecal materials in pits initially have high concentrations of **total coliform** bacteria followed by **faecal coliform** and **faecal streptococci**. The count at first sampling i.e. weeks after pit closure was a few millions to 53,000,000 per gram of excreta.

With increasing duration of digestion in unused filled-up pits, the number of viable **total coliform** dropped to about 21,000/gm after 12 months of digestion. This fall in bacterial numbers was due to biochemical reactions produced during fermentation of nutrient rich organic excreta by coliform bacteria. During fermentation increase of temperature could

also be a factor for bacterial death rate. Another cause of decline could be due to production of toxic end production of bacterial metabolism.

Similar trend in decrease of viable count of faecal coliform and faecal streptococci was detected. Initial count of faecal coliform was about 480,000 per gram which fell to about 400-500 cfu/gm. The third indicator bacterium **faecal streptococci** had also an average of 470,000 cfu/gm which also dropped to 5-100 cfu/gm of sludge. The fall of these two indicator bacteria to about 50-500 cfu/gm signifies that after certain period of time due to production of toxic substances and acid reaction produced during fermentation. However, it is evident that pathogen indicator bacteria have decreased drastically from very high initial numbers to about hundred or so per gram of sludge. This level of count in per gram of sludge is still not safe for handling as organic fertilizer.

Examination of worm-eggs particularly of **hookworms** and **roundworms** demonstrates that from the initial concentration of about 4500 eggs/gm the count decreased to nil or a few eggs of **hookworm,** and to about hundred per gram for **roundworms** by 12 months of digestion in pits. The **roundworm** eggs are reported to be very persistent and resist destruction under variety of adverse environmental condition. The count is still not within safe handling for **roundworm** - the most important indicator eggs for faecal pollution detection having disease potential.

The incidence of background concentrations of faecal indicators (T.C., F.C. & F.S.) in the field and kitchen garden soils reflect that kitchen garden soils are prone to contamination by faecal materials. Their numbers was as high as 500 cfu/gm of sludge. Normal field soils were almost devoid of **faecal coliform.** The relatively high concentration of **total coliform** in field and kitchen garden soils reflects that the T.C.s could have been adapted or naturalized with field conditions and could only serve as presumptive indicator.

The incidence of worm-eggs of both **roundworm** and **hookworm** in field soils at 8th sampling was nil or was present in insignificant (2-4 cfu/gm) numbers as compared to digested excreta (Figures 1 - 10).

The fertilizer value of digested sludge appears to be high particularly for nitrogen (3.85%) followed by phosphorus (1.82%) and potassium (0.86%). The fertilizer values slightly decreased at 14 and 16 months of digestion. These have potential for use in our agricultural soils poor in organic matter. These concentrations are very high as compared to even very rich organic fractions/organic matter normally encountered in soil.

10. CONCLUDING REMARKS

Laboratory examinations on pathogen survival rate in filled-up latrine pits at two different water-table zones was studied. The sampling schedule was at 2-month interval until 16 months.

Tests for three pathogen indicator bacteria e.g. (a) **total coliform** (T.C.), (b) **faecal coliform** (F.C.) and (c) **faecal streptococci** (F.S.) including eggs of (d) roundworm and (e) **hookworm** also as faecal pollution indicator were carried out. With increasing digestion time (up to 16 months in filled-up unused pits), counts of most pathogens decreased remarkebly to a very low level but did not completely die-off Only eggs of **hookworm** were not detected at 14th and 16th months in both the water table zones.

On quantitative examination it was revealed that the initial count of T.C. of around 50 million cfu/gm of sludge dropped to about an average of 3,800 **total coliform** cells/gm. This indicates considerable number of T.C. cells after 16 months of digestion in closed pits. The slowing down of survival rate around 6th, 7th and 8th sampling is indicative of adaption/naturalization of total coliform cells with the sludge. Although T.C.serves as a presumptive indicator of pollution, further decrease in their number is desirable.

The persistence of **faecal coliform** (F.C) in sludge samples although at a very low rate suggests its pollution potential. Initial number of FC of about 500,000 cfu/gm gradually dropped to about 100-168 cfu/gm with duration of digestion time upto 16 months. It is evident from laboratory testing that this fall in numbers is associated with extended time, temperature and depletion of nutrients or generation of toxic metabolic end products. However, the count still poses potential threat to disease. Total destruction of **faecal coliform** may not be attained considering the nature and environmental conditions to which the F.C. are exposed under tropical weather conditions.

The initial average concentration of the **faecal streptococci** (F.S.) was relatively fewer than faecal coliform count and stood at 470,000 cfu/gm. With extended duration of digestion, the cell number decreased gradually and at 8th sampling (16 months) the count ranged between 60-70 cfu/gm of digested sludge.

The status of **faecal streptococci** is comparable with **faecal coliform** – both of which decreased to very low level. But this count (60-70 cfu/gm) in sludge is still higher and as such not within safety range of handling. Further drop is expected with extended digestion period but complete elimination may not be attained owing to several nutritional and environmental factors intrinsic within fermenting/digesting excreta in pits

The survival rate of worm-eggs e.g. **roundworm** met almost similar fate as has occurred with faecal indicator bacteria described above. Although their initial count was much lower (about 3000eggs/gm) – their persistence in sludge was remarkable particularly for **roundworm** at LWT area at Rupganj. The number of roundworm eggs decreased rather steadily at both the test sites. Their count per gram stood between 5-30/gm at 8th sampling. With extended time at 16 months the count of **hookworm** was completely extinct from both the LWT and HWT zones. **Roundworm** cells are relatively resistant to decay/destruction, the eggs of hookworm decreased faster and was completely eliminated at 16 months owing to their susceptibility to adverse environmental conditions.

Background counts of pathogen indicator bacteria and **roundworm** and **hookworm** eggs in field and kitchen garden soils of Gazipur and Rupganj area indicate that even the field and kitchen garden soils contain considerable number of **total coliform** bacteria (2000-3000 cells/gm of soils) which is slightly lower than the counts recorded at 16-month digestion with digested sludge samples. The occurrence of **faecal coliform** and **faecal streptococci** was more in kitchen garden than in field soils indicates faecal pollution of these soils. Their occurrence in field soils particularly of **faecal coliform** was very low or nil (30-35/gm). The counts of worm-eggs were also nil or very low reflecting the necessity of further reduction in numbers of indicator bacteria or worm-eggs in pit samples. Comparable higher numbers of pathogen indicator bacteria in sludge samples suggests the role of organic and inorganic nutrients that supported better adaptation of the pathogens in pits than in kitchen garden or field soils.

The chemical analysis of digested excreta for **nitrogen, phosphorus** and **potassium** as fertilizer elements suggests that the digested faecal materials contain high concentrations of the three major fertilizer elements respectively at 3.85, 1.82 and 0.86 percent. The fertilizer values almost remained steady until 14 months of digestion; their values decreased somewhat at 16 months of digestion but still have excellent potential for use as orgnaic fertilizer.

11. RECOMMENDATION

It appears from the laboratory examinations that the initial concentrations of pathogens, both **bacterial indicators** and **parasitic eggs**, were very high that ranged between 500,000 to about 50,000,000 per gram of sludge. A comparison of the initial and final counts, after 16 months of digestion in pits reveals that more than 90% of the

pathogens/indicators have been eliminated. Although the count of survivors are much lower for FC and FS, 60-260/gm of sludge, **total coliform** number was much higher (about 4000/g) still poses potential threat to health and hygiene. This level of survival rate is beyond the limit of acceptance for safe handling when compared with international standard (World Bank, 1983: WHO, 1989; Please see Section 3.5). However, the decreasing trend of pathogens with increasing residence time in pits are encouraging. The long persistence of pathogens is indicative of their adaptation or colonization with the sludge in pits.

Since most pathogen indicator bacteria including the worm-eggs of **roundworm** were not completely eliminated even at 16 months of digestion in pits, it may be suggested that under tropical weather condition of Bangladesh, the pathogens survives better and hence probability of adaptation increases as compared to temperate environmental conditions.

The limit values for safe handling of digested excreta set by World Bank studies (1983) and WHO (1989) appear to be stringent for Bangladesh conditions. Hence a relaxed limit values for pathogens have been suggested for safe handling of excreta. A comparison of the proposed limit values for Bangladesh with that of World Bank and WHO is given below:

Guideline values of pathogens in digested excreta for use in agriculture

Pathogens	World Bank (1983)	WHO (1989)	Recommneded limit values for Bangladesh
Faecal coliform	100/100 gm	1000/100 gm	2000/100 gm
Faecal strep.	100/100 gm	-	2000/1000 gm
Ascaris **eggs**	10/100 gm	1/100 gm	500/100 gm

Since the pathogens did not completly die-off even at 16 months of digestion in pits, special arrangements may have to be taken to **sundry** or **composting** of the sludge with kitchen/organic wastes. Exposing the sludge to a temperature of around 50-60°C might accelerate complete elimination or attaining safe limit values for pathogen handling. It will hope fully be safe for using in agriculture as organic or biofertilizer.

APPENDICES

APPENDIX - I

(List of Tables)

Table 1

Survival Rate of Total Coliform in filled-up pits

Pit No. (with closing dates)	Sample No. with depth	Concentration of Total Coliform (per gram)							
		1st Sampling 31/7/95 & 01/8/95	2nd Sampling 1/10/91 & 2/10/95	3rd Sampling 2/12/95 & 3/12/95	4th Sampling 1/2/96 & 2/2/96	5th Sampling 4/4/96 & 5/4/96	6th Sampling 1/6/96 & 3/6/96	7th Sampling 16/8/96 & 20/8/96	8th Sampling 15/10/96 & 16/10/96
1 (Gazipur) 05/07/95)	1 (2′ depth)	5.3×10^7	1.2×10^6	2.0×10^5	1.1×10^3	1.0×10^3	2.0×10^3	3.3×10^3	3.0×10^3
	2 (4′ depth)	2.8×10^6	4.8×10^5	6.0×10^4	1.0×10^3	0.9×10^3	1.1×10^3	2.7×10^3	2.8×10^3
2 (Gazipur) 25/05/95)	3 (2′ depth)	2.0×10^7	3.0×10^6	1.2×10^5	1.7×10^3	1.5×10^3	1.6×10^4	3.8×10^3	3.6×10^3
	4 (4′ depth)	2.1×10^6	1.1×10^6	3.5×10^5	2.0×10^4	1.7×10^4	1.7×10^4	3.2×10^3	3.0×10^4
3 (Gazipur) 07/06/95)	5 (2′ depth)	2.5×10^6	1.8×10^6	8.0×10^5	4.2×10^4	4.0×10^4	4.0×10^4	1.8×10^4	3.4×10^3
	6 (4′ depth)	1.4×10^6	8.4×10^5	4.0×10^5	5.5×10^4	5.0×10^4	5.0×10^4	7.0×10^3	8.0×10^3
4 (Rupganj) 12/06/95)	7 (2′ depth)	7.1×10^6	1.0×10^6	8.0×10^5	4.5×10^4	4.0×10^4	4.0×10^4	2.0×10^4	1.0×10^4
	8 (4′ depth)	1.2×10^6	5.8×10^5	1.0×10^5	2.1×10^4	3.0×10^4	3.0×10^4	1.5×10^4	1.4×10^4
5 (Rupganj) 17/07/95)	9 (2′ depth)	1.0×10^7	2.2×10^6	7.0×10^5	3.3×10^4	3.1×10^4	3.1×10^4	2.6×10^4	2.1×10^4
	10 (4′ depth)	2.3×10^6	1.1×10^6	3.0×10^5	2.5×10^4	2.1×10^4	2.1×10^4	1.5×10^4	1.4×10^4
6 (Rupganj) 15/05/95)	11 (2′ depth)	5.9×10^6	2.0×10^6	1.5×10^5	2.0×10^4	8.0×10^3	8.0×10^3	2.5×10^4	2.1×10^3
	12 (4′ depth)	3.5×10^6	2.0×10^5	1.0×10^5	1.5×10^4	1.2×10^4	1.2×10^4	2.0×10^4	1.7×10^4

Table : 2

Survival Rate of Faecal Coliform in Filled-up Pits

Pit No. (with closing dates)	Sample No. with depth	Concentration Faecal Coliforms (per gram)							
		1st Sampling 31/7/95 & 01/8/95	2nd Sampling 1/10/91 & 2/10/95	3rd Sampling 2/12/95 & 3/12/95	4th Sampling 1/2/96 & 2/2/96	5th Sampling 4/4/96 & 5/4/96	6th Sampling 1/6/96 & 3/6/96	7th Sampling 16/8/96 & 20/8/96	8th Sampling 15/10/96 & 16/10/96
1 (Gazipur) 05/07/95)	1 (2′ depth)	7.5×10^5	1.1×10^5	8.0×10^3	2.2×10^2	1.7×10^2	2.0×10^2	2.1×10^2	0.9×10^2
	2 (4′ depth)	7.0×10^5	3.5×10^4	1.5×10^3	1.2×10^2	1.0×10^2	2.3×10^2	1.9×10^2	0.8×10^2
2 (Gazipur) 25/05/95)	3 (2′ depth)	7.4×10^5	4.0×10^5	5.0×10^3	3.5×10^2	2.5×10^2	2.6×10^2	2.0×10^2	0.7×10^2
	4 (4′ depth)	1.1×10^5	9.0×10^4	3.0×10^3	2.2×10^2	2.0×10^2	2.3×10^2	1.1×10^2	1.0×10^2
3 (Gazipur) 07/06/95)	5 (2′ depth)	6.5×10^5	2.1×10^5	1.5×10^4	3.8×10^2	3.0×10^2	2.5×10^2	1.8×10^2	1.7×10^2
	6 (4′ depth)	2.2×10^5	1.0×10^5	8.0×10^4	4.0×10^3	2.5×10^3	2.4×10^3	1.5×10^2	1.0×10^2
4 (Rupganj) 12/06/95)	7 (2′ depth)	6.3×10^5	2.5×10^5	5.0×10^4	2.0×10^3	1.1×10^3	2.0×10^3	1.5×10^2	1.5×10^2
	8 (4′ depth)	5.2×10^5	1.1×10^5	2.0×10^4	1.0×10^3	2.7×10^2	2.6×10^3	8.0×10^2	5.5×10^2
5 (Rupganj) 17/07/95)	9 (2′ depth)	5.8×10^5	4.1×10^5	5.0×10^4	2.2×10^3	1.7×10^2	1.5×10^2	1.8×10^2	1.7×10^2
	10 (4′ depth)	2.5×10^5	2.1×10^5	1.0×10^4	5.0×10^2	4.2×10^2	4.7×10^2	1.7×10^2	1.0×10^2
6 (Rupganj) 15/05/95)	11 (2′ depth)	2.5×10^5	4.8×10^5	8.0×10^3	4.0×10^2	3.0×10^2	3.7×10^2	2.4×10^2	2.1×10^2
	12 (4′ depth)	4.4×10^5	1.1×10^5	1.0×10^4	3.0×10^2	2.0×10^2	2.1×10^2	1.3×10^2	1.0×10^2

Table : 3

Survival Rate of Faecal Streptococci in Filled-up Pits

Pit No. (with closing dates)	Sample No. with depth	Concentration of Faecal Streptococci (per gram) 1st Sampling 31/7/95 & 01/8/95	2nd Sampling 1/10/91 & 2/10/95	3rd Sampling 2/12/95 & 3/12/95	4th Sampling 1/2/96 & 2/2/96	5th Sampling 4/4/96 & 5/4/96	6th Sampling 1/6/96 & 3/6/96	7th Sampling 16/8/96 & 20/8/96	8th Sampling 15/10/96 & 16/10/96
1 (Gazipur) 05/07/95)	1 (2′ depth)	7.1×10^5	3.0×10^4	0.5×10^3	0.3×10^2	0.2×10^2	0.3×10^2	0.4×10^2	0.3×10^2
	2 (4′ depth)	2.6×10^5	1.0×10^4	6.0×10^3	2.2×10^2	1.3×10^2	1.4×10^2	1.1×10^2	0.7×10^2
2 (Gazipur) 25/05/95)	3 (2′ depth)	2.2×10^5	1.2×10^4	3.0×10^3	1.7×10^2	1.2×10^2	1.2×10^2	0.8×10^2	0.5×10^2
	4 (4′ depth)	1.5×10^5	1.5×10^4	1.0×10^3	0.7×10^2	0.8×10^2	1.1×10^2	1.0×10^2	0.6×10^2
3 (Gazipur) 07/06/95)	5 (2′ depth)	5.0×10^5	5.0×10^3	1.4×10^3	1.3×10^2	1.1×10^2	1.0×10^2	0.9×10^2	0.7×10^2
	6 (4′ depth)	1.3×10^4	3.0×10^3	1.2×10^3	0.8×10^2	0.8×10^2	0.7×10^2	1.0×10^2	0.8×10^2
4 (Rupganj) 12/06/95)	7 (2′ depth)	2.0×10^4	1.0×10^3	0.8×10^3	0.4×10^2	0.3×10^2	0.8×10^2	0.7×10^2	0.3×10^2
	8 (4′ depth)	1.0×10^5	2.0×10^4	1.8×10^3	1.4×10^2	2.4×10^2	2.2×10^2	1.0×10^2	0.8×10^2
5 (Rupganj) 17/07/95)	9 (2′ depth)	1.7×10^5	1.5×10^3	1.2×10^3	0.9×10^2	1.1×10^2	1.0×10^2	0.9×10^2	0.9×10^2
	10 (4′ depth)	1.0×10^6	1.3×10^4	1.7×10^3	1.2×10^2	1.2×10^2	1.1×10^2	0.8×10^2	0.8×10^2
6 (Rupganj) 15/05/95)	11 (2′ depth)	2.0×10^5	0.75×10^4	0.4×10^3	0.4×10^2	0.3×10^2	0.5×10^2	0.9×10^2	0.7×10^2
	12 (4′ depth)	4.0×10^4	3.0×10^4	0.8×10^3	0.7×10^2	0.4×10^2	0.3×10^2	0.4×10^2	0.7×10^2

Table : 4

Survival Rate of Roundworm-eggs in Filled-up Pits

Pit No. (with closing dates)	Sample No. with depth	Concentration of Roundworm-eggs (*Ascaris lumbricoides*) (per gram)							
		1st Sampling 31/7/95 & 01/8/95	2nd Sampling 1/10/91 & 2/10/95	3rd Sampling 2/12/95 & 3/12/95	4th Sampling 1/2/96 & 2/2/96	5th Sampling 4/4/96 & 5/4/96	6th Sampling 1/6/96 & 3/6/96	7th Sampling 16/8/96 & 20/8/96	8th Sampling 15/10/96 & 16/10/96
1 (Gazipur) 05/07/95)	1 (2′ depth)	Low (100-1000)	Low (100-1000)	42	45	29	Nil	Nil	Nil
	2 (4′ depth)	Moderate (2000-5000)	Moderate (2000-5000)	40	35	27	35	15	5
2 (Gazipur) 25/05/95)	3 (2′ depth)	Low (100-1000)	Low (100-1000)	140	110	57	42	18	10
	4 (4′ depth)	Low (100-1000)	Low (100-1000)	Nil	30	30	25	12	7
3 (Gazipur) 07/06/95)	5 (2′ depth)	High > 5000	High > 5000	Nil	Nil	Nil	Nil	Nil	Nil
	6 (4′ depth)	Moderate (2000-5000)	Moderate (2000-5000)	745	330	210	165	55	12
4 (Rupganj) 12/06/95)	7 (2′ depth)	High > 5000	High > 5000	7,413	2,250	1,900	1760	410	20
	8 (4′ depth)	Hig > 5000	Moderate (2000-5000)	2,065	1,150	780	610	235	29
5 (Rupganj) 17/07/95)	9 (2′ depth)	Moderate (2000-5000)	Moderate (2000-5000)	420	220	187	200	105	40
	10 (4′ depth)	Low (100-1000)	Low (100-1000)	1,025	650	510	382	110	32
6 (Rupganj) 15/05/95)	11 (2′ depth)	High > 5000	Moderate (2000-5000)	2,053	1,130	830	610	210	15
	12 (4′ depth)	Moderate (2000-5000)	Moderate (2000-5000)	1,680	1,260	530		218	39

Table : 5

Survival Rate of Hookworm-eggs in Filled-up Pits

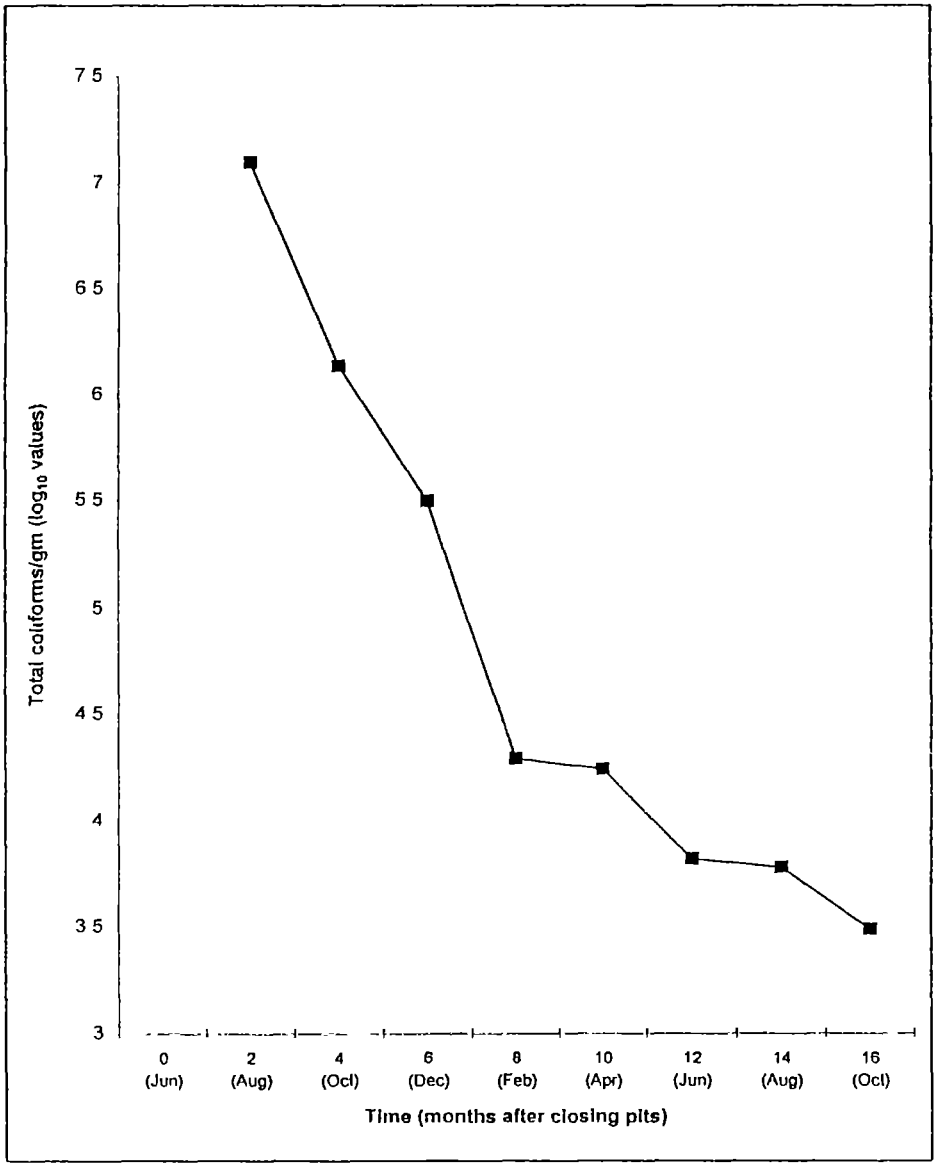
Pit No. (with closing dates)	Sample No. with depth	Concentration of Hookworm-eggs (*Ancylostoma*) (per gram)							
		1st Sampling 31/7/95 & 01/8/95	2nd Sampling 1/10/91 & 2/10/95	3rd Sampling 2/12/95 & 3/12/95	4th Sampling 1/2/96 & 2/2/96	5th Sampling 4/4/96 & 5/4/96	6th Sampling 1/6/96 & 3/6/96	7th Sampling 16/8/96 & 20/8/96	8th Sampling 15/10/96 & 16/10/96
1 (Gazipur) 05/07/95)	1 (2' depth)	*Ancylostoma* Low (100-1000)	*Ancylostoma* Low (100-1000)	41 *(Ancylostoma)*	30 *(Ancylostoma)*	Nil	Nil	Nil	Nil
	2 (4' depth)	*Enterobius* Moderate (2000-5000)	*Ancylostoma* Moderate (2000-5000) *Trichuris* moderate	Nil	200 *(Trichuris)*	Nil	Nil	Nil	Nil
2 (Gazipur) 25/05/95)	3 (2' depth)	*Ancylostoma* Moderate (2000-5000)	*Ancylostoma* Moderate (2000-5000) *Trichuris* mod. (2000-5000) *Taenia saginata* (2000-5000)	42 *(Trichuris)*	21 *(Trichuris)*	Nil	Nil	Nil	Nil
	4 (4' depth)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3 (Gazipur) 07/06/95)	5 (2' depth)	*Ancylostoma* Low (100-1000)	*Ancylostoma* Low (100-1000) Trichuris (Moderate)	Nil	Nil	Nil	Nil	Nil	Nil
	6 (4' depth)	*Ancylostoma* Low (100-1000)	*Ancylostoma* law (100-1000) *Trichuris* (100-1000)	92 *(Ancylostoma)*	60 *(Ancylostoma)*	Nil	10	Nil	Nil
4 (Rupganj) 12/06/95)	7 (2' depth)	Nil	*Ancylostoma* Low (100-1000)	325 *(Ancylostoma)* 320 *(Trichuris)*	200 *(Ancylostoma)* 110 *(Trichuris)*	100 *(Trichuris)*	89	15	Nil
	8 (4' depth)	Nil	*Ancylostoma* Low (100-1000) *Enterobius* (2000-5000)	245 *(Ancylostoma)* 175 *(Trichuris)*	130 *(Ancylostoma)* 60 *(Trichuris)*	100 *(Trichuris)*	210	25	Nil
5 (Rupganj) 17/07/95)	9 (2' depth)	Nil	*Ancylostoma* Moderate (2000-5000)	42 *(Ancylostoma)*	22 *(Ancylostoma)*	Nil	Nil	Nil	Nil
	10 (4' depth)	Nil	*Taenia saginata* Low (100-1000)	223 *(Ancylostoma)* 140 *(Trichuris)*	105 *(Ancylostoma)* 125 *(Trichuris)*	30 *(Trichuris)*	30	Nil	Nil
6 (Rupganj) 15/05/95)	11 (2' depth)	Nil	*Ancylostoma* Low (100-1000)	280 *(Ancylostoma)*	150 *(Ancylostoma)* 30 *(Trichuris)*	30 *(Ancylostoma)* 60 *(Trichuris)*	93	10	Nil
	12 (4' depth)	Nil	Nil	140 *(Ancylostoma)* 84 *(Trichuris)*	Nil	Nil	Nil	Nil	Nil

APPENDIX - II

(List of Figures)

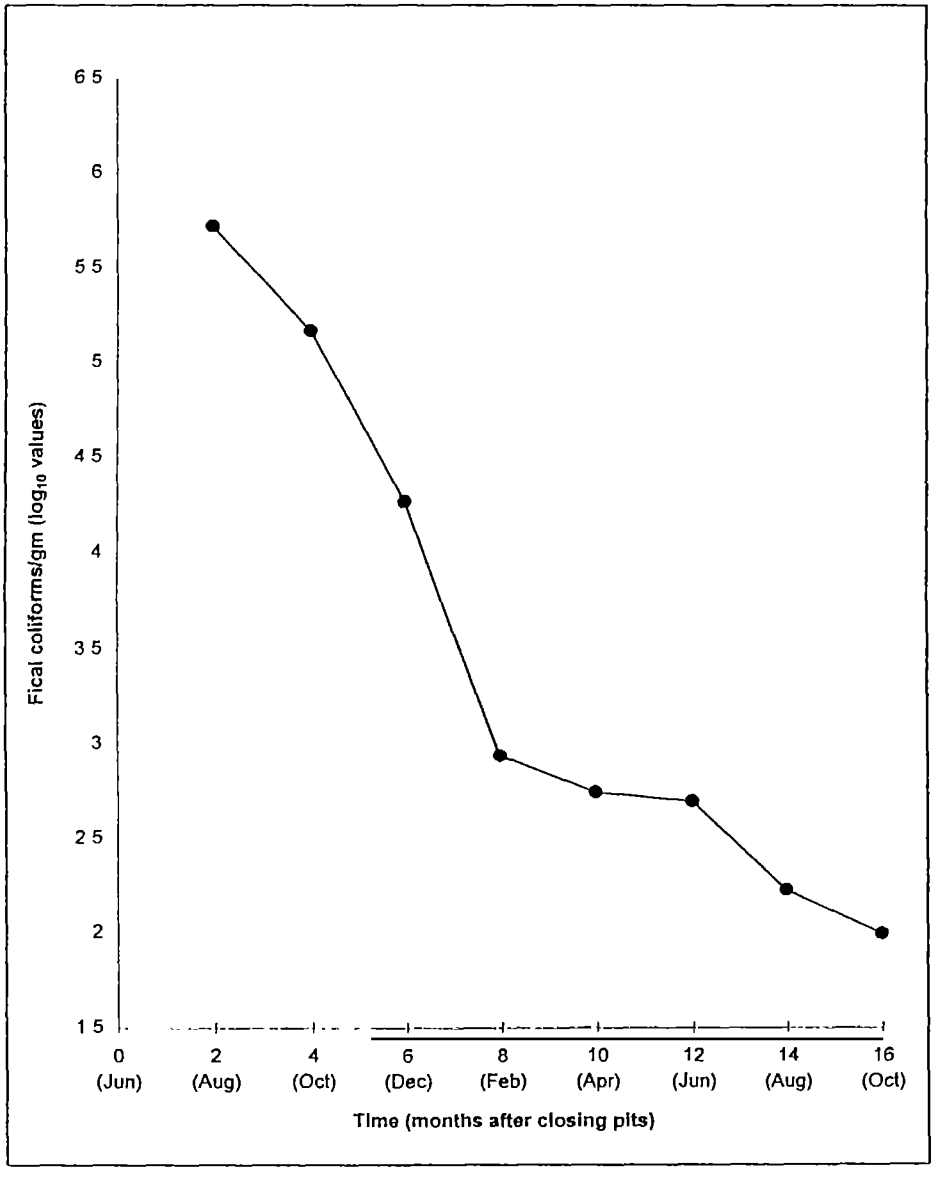
SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

Figure-1 : Count of total coliform bacteria in sludge
Study site : Gazipur, LWT (dry) area



SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

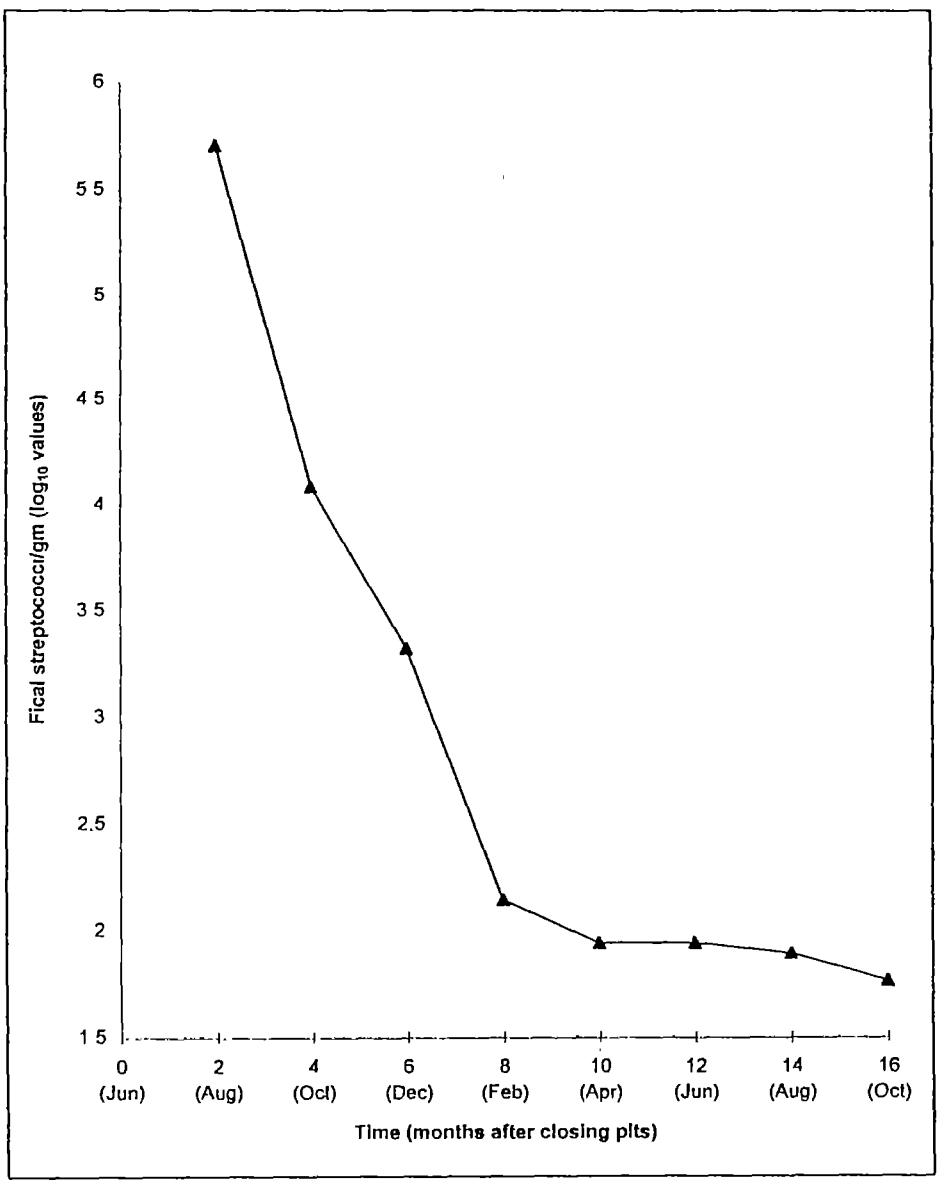
Figure-2 : Count of total coliform (mostly *E.coli*) in sludge
Study site : Gazipur, LWT (dry) area



Slk xls(6th rep graph)

SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

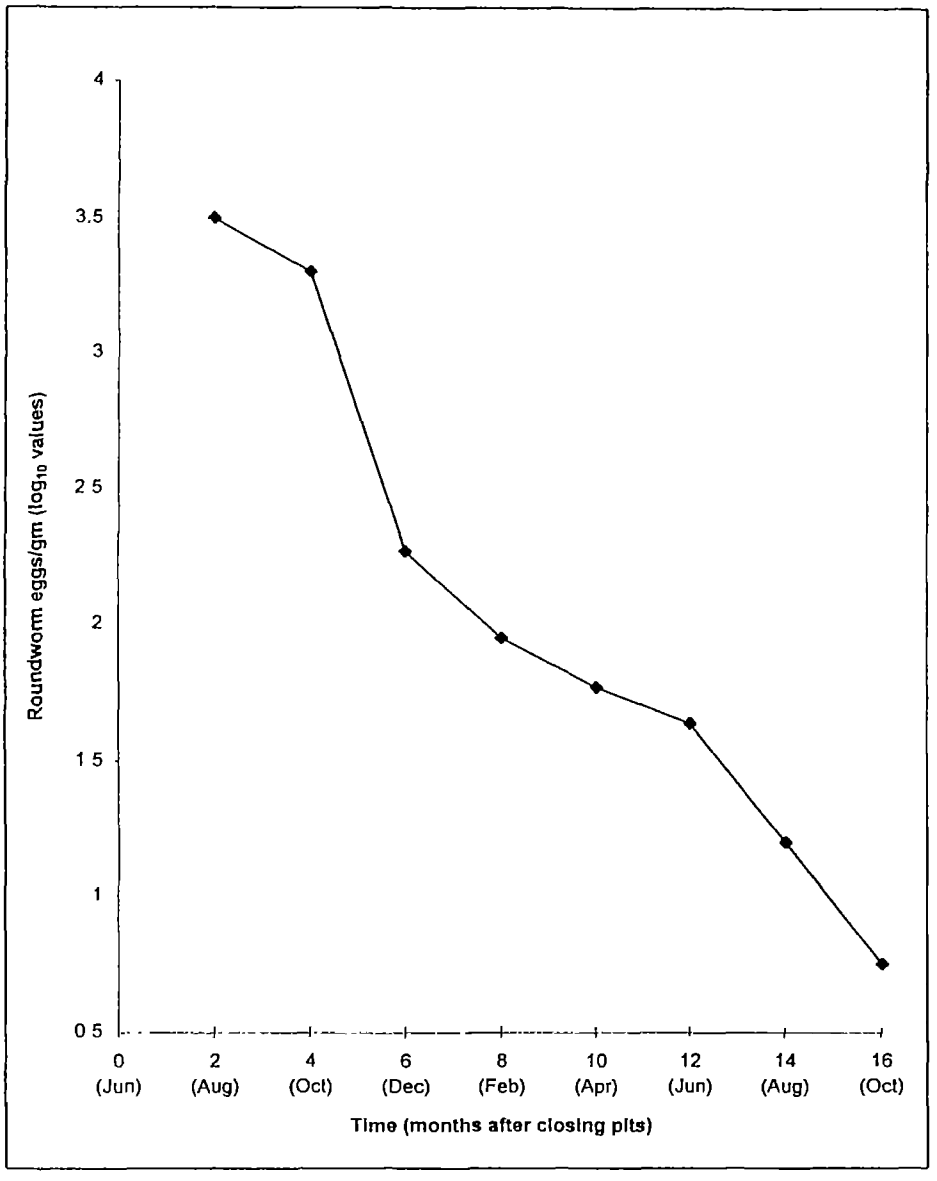
Figure-3 : Count of fecal streptococci in sludge
Study site : Gazipur, LWT (dry) area



Sfk xls(6th rep graph)

SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

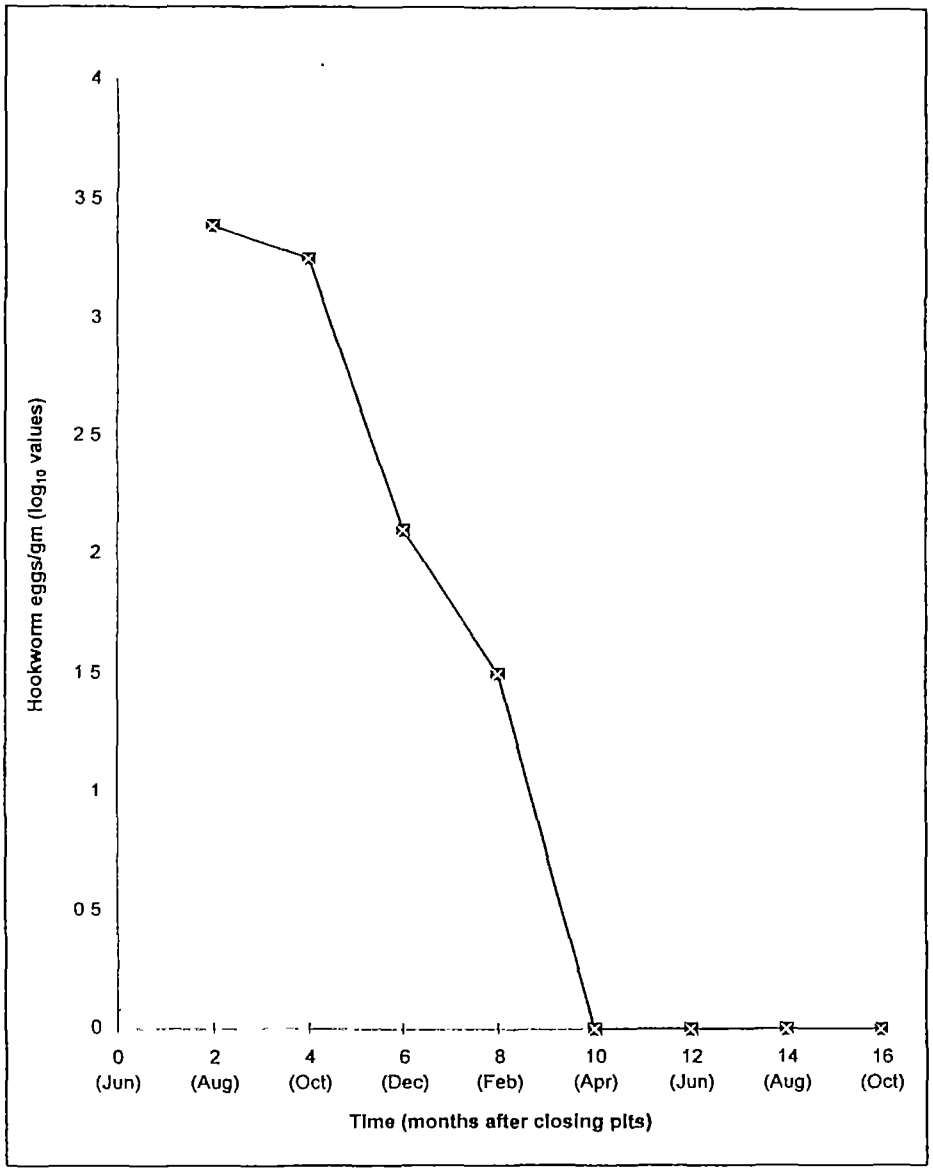
Figure-4 : Count of roundworm eggs/ova in sludge
Study site : Gazipur, LWT (dry) area



Sik xls(6th rep graph)

SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

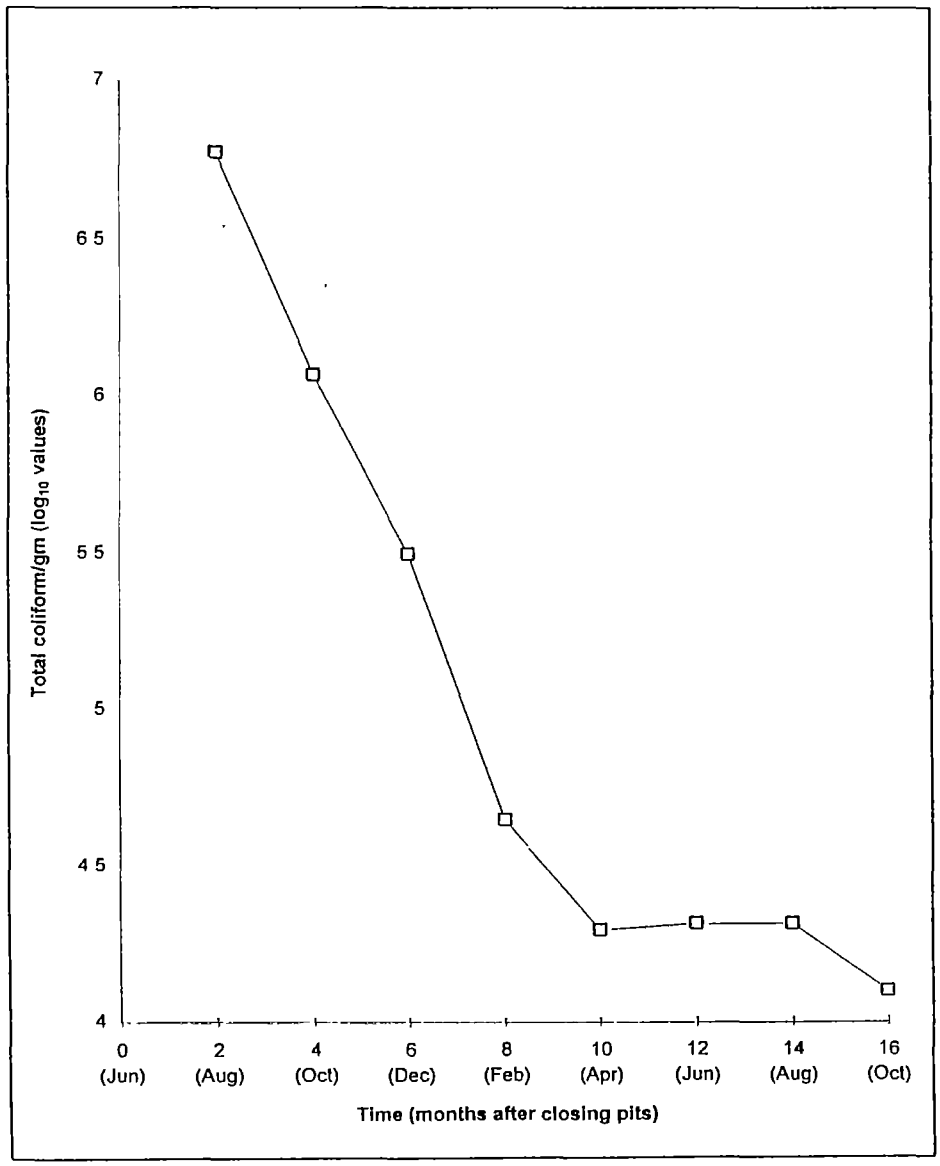
Figure-5 : Count of hookworm eggs/ova in sludge
Study site : Gazipur, LWT (dry) area



Sik xls(6th rep graph)

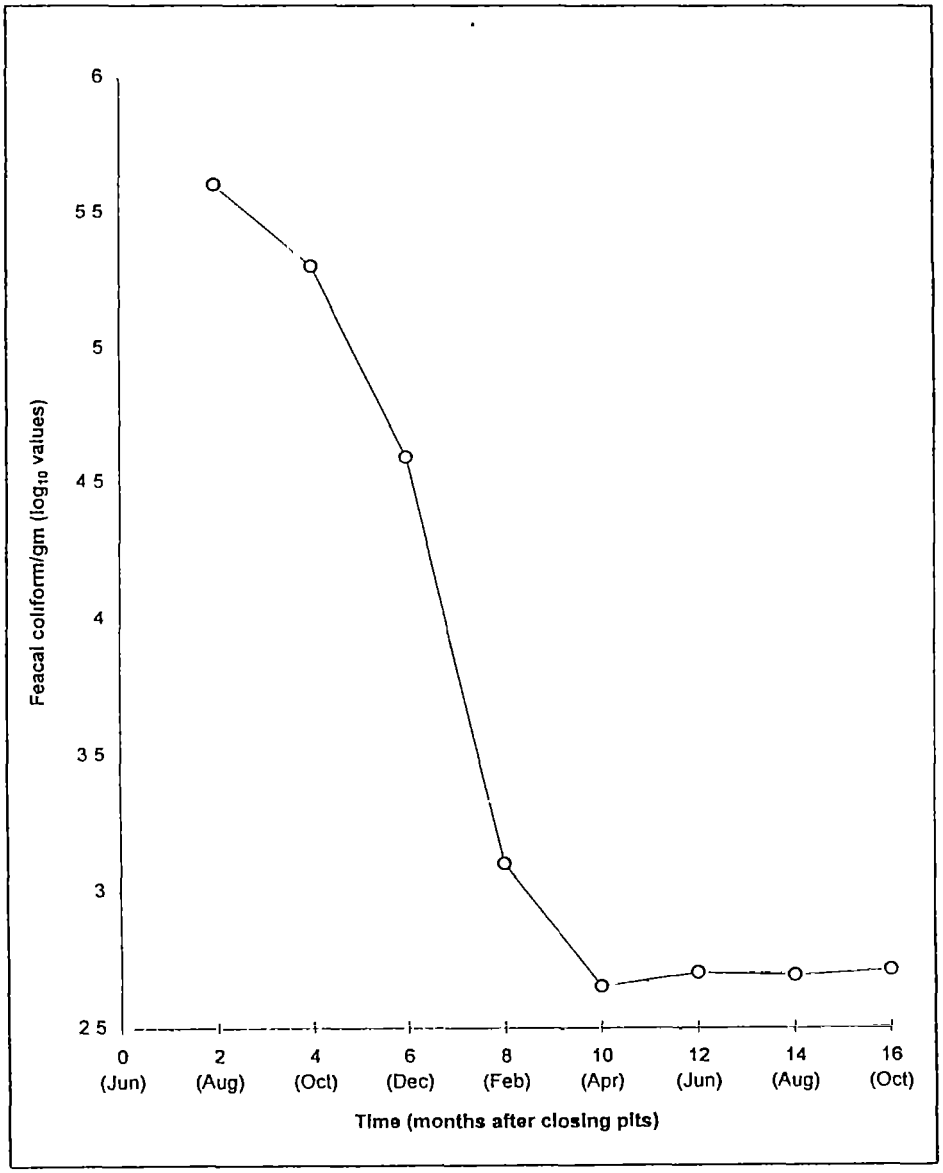
SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

Figure-6 : Count of total coliform bacteria in sludge
Study site : Rupganj, HWT (wet) area



SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

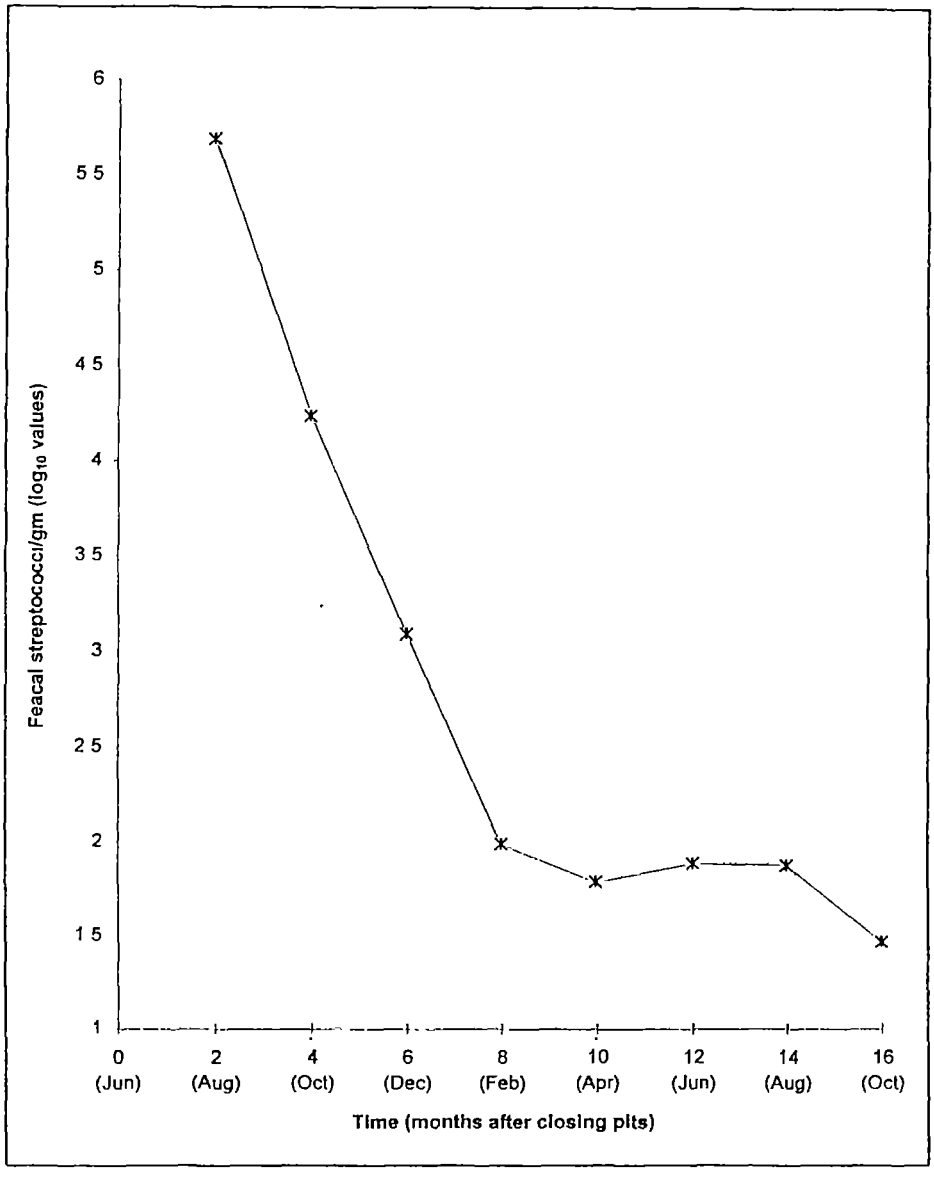
Figure-7 : Count of feacal coliform (mostly *E. coli*) in sludge
Study site : Rupganj, HWT (wet) area



Sik xls(6th rep graph)

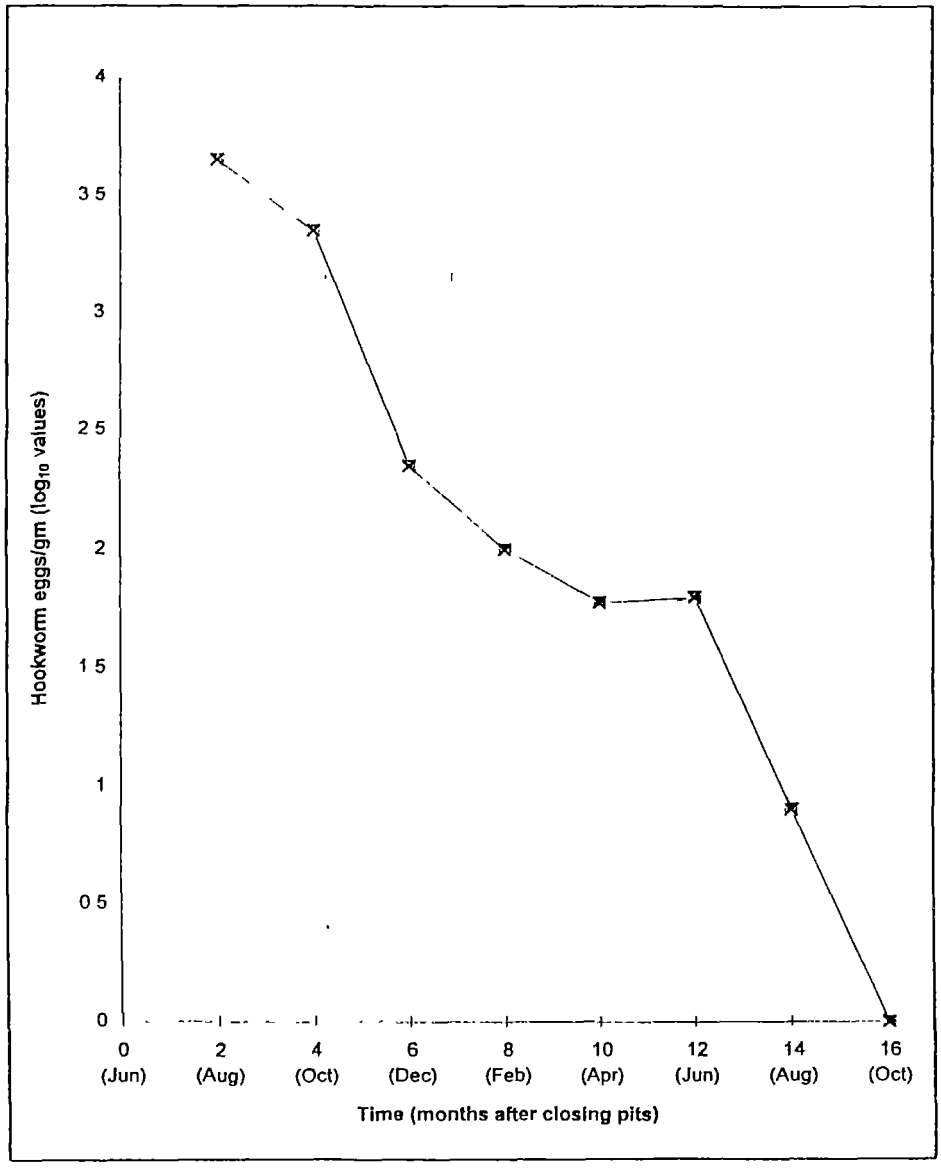
SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

Figure-8 : Count of feacal streptococci in sludge
Study site : Rupganj, HWT (wet) area



SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

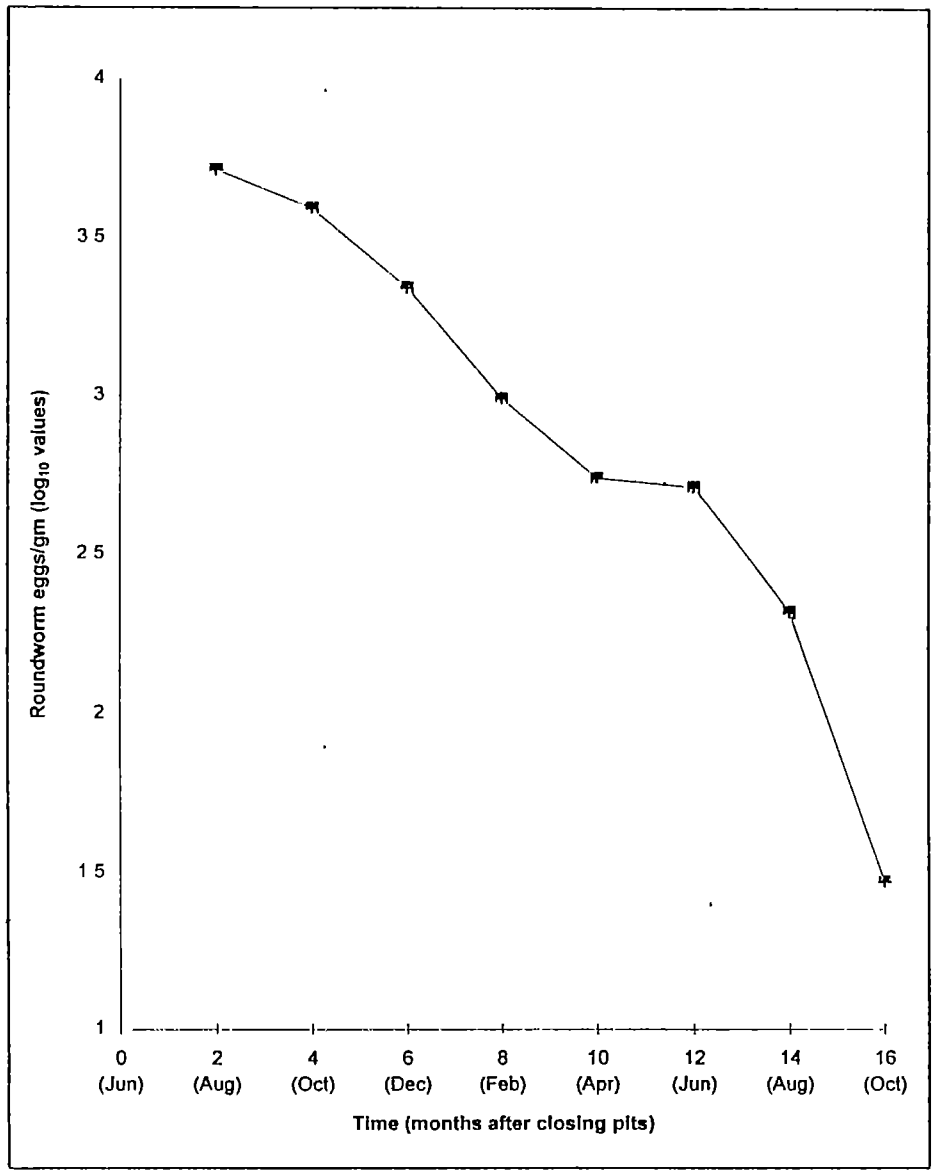
Figure-10 : Count of hookworm eggs/ova in sludge
Study site : Rupganj, HWT (wet) area



Sik.xls(6th rep graph)

SURVIVAL RATE OF PATHOGENS IN FILLED-UP PITS

Figure-9 : Count of roundworm egg/ova in sludge
Study site : Rupganj, HWT (wet) area



Sik xls(6th rep graph)