THE INFLUENCE OF GEOMETRY ON THE PERFORMANCE OF WASTE STABILIZATION PONDS WITH SPECIAL REFERENCE TO PATHOGEN REMOVAL.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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Abstract

Waste stabilization ponds (WSPs) are shallow, large area, man-made basins, usually rectangular, designed specifically to treat wastes through long retention times, by the processes of natural purification involving the bacteria and algae present in the water. They are simple, partially controlled systems which represent a complex interaction of physical and biochemical processes and are often the sewage treatment method of first choice, especially when good pathogen removal is required. They are particularly suited to developing countries due to the lack of maintenance required.

Current methods of pond design are based on organic loading and temperature but this does not take into account any effects due to depth, pond shape or baffling. The aim of this research project was to determine the effects, in particular on the efficiency of pathogen removal, of pond shape and depth using two experimental waste stabilization pond systems at Caatingueira, near Campina Grande, N.E. Brazil.. Secondary facultative and maturation ponds of different depths (1 to 2 m) and length to breadth ratios (1:1 to 6:1) were studied for a period of 19 months with sampling twice weekly to evaluate the performance of the ponds. The numbers of Faecal coliforms, *Faecal streptococci, Clostridium perfringens, Campylobacter, Salmonella, Vibrio cholerae* and Rotaviruses were determined as well as a variety of physico-chemical parameters. To compliment the routine evaluation of the pond performance a number of 24 hour diurnal experiments were undertaken on a variety of ponds to characterise the changes throughout the diurnal cycle in terms of light, pH, temperature, dissolved oxygen, BOD, chlorophyll-*a* , total suspended solids, faecal coliforms and *Salmonella*.

The results from this research indicate that depth and shape have no significant effect on the effluent of ponds with the same surface loadings. This indicates that the shallower ponds are more efficient in removing pathogens since the same disinfection occurs in ponds with reduced retention times. The findings of this project are important since design engineers may be confident that shaping a pond to fit a particularly oddly shaped site will not compromise the treatment performance attained.

In addition, an investigation into the removal of *Vibrio cholerae* was undertaken in response to publications regarding the efficiency of WSPs in removing this organism (Lesne *et al*, 1991; Anon, 1992; Mezrioui *et al*, 1995). The low numbers of vibrios entering the pond system made it difficult to accurately evaluate the efficiency of their removal but in-pond experiments showed that survival of the treatment process was possible for at least 13 days in facultative and maturation ponds. However, after a maximum of 2 days the number of organisms present was below the critical level which could cause symptoms to occur. The results indicated that the inclusion of an anaerobic pond in the WSP system was necessary for efficient removal of *Vibrio cholerae*.

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To Pops

Glossary of Terms

Abbreviations

BOD5	5 Day 20 °C Biochemical Oxygen Demand, mg l^{-1}
CAMP	*Campylobacter*
Chl-*a*	Chlorophyll-*a*, µg/l
COD	Chemical Oxygen Demand, mg l^{-1}
CP	*Clostridium perfringens*
DO	Dissolved Oxygen
ff	fluorescent foci
FC	Faecal coliforms
FS	Faecal streptococci
HRT	Hydraulic Retention Time, d
PAR	Photosynthetically Active Radiation
PFU	Plaque Forming Units
RS	Raw Sewage
RV	Rotavirus
SAL	*Salmonella*
TEMP	Temperature, °C
TSS	Total Suspended Solids
UV	Ultraviolet
VC	*Vibrio cholerae*
WSP	Waste Stabilization Pond

Principal Notations

A	Area of Pond, m^2
B	BOD contribution, g caput^{-1} d^{-1}
C	Concentration
d	Dispersion number
D	Depth of pond, m
f	Algal toxicity factor
f'	Sulphur O2 demand factor
k1	First order rate constant for BOD5 removal, d^{-1}
kT	First order rate constant for faecal coliform removal, d^{-1}
L	BOD5 concentration, mg l^{-1}; Pond length, m
Li	Influent BOD5, mg l^{-1}
N	Number of faecal coliforms per 100 ml
q	Wastewater flow, l caput^{-1} d^{-1}
Q	Flow, m^3d^{-1}
T	Temperature, °C
v	Kinematic viscosity, m^2s^{-1}
V	Volume of pond, m^3
W	Pond width, m

θ	Coefficient of temperature activity; Mean Hydraulic Retention time
λv	Volumetric organic loading, g $m^{-3}d^{-1}$
λs	Surface organic loading kg ha^{-1} d^{-1}

Prefixes

F	Facultative
M	Maturation
A	Anaerobic

Subscripts

a	Anaerobic
e	Effluent
f	Facultative
i	Influent
m	Maturation

Chapter 1 Introduction and Literature Review

1.1 Wastewater Treatment

Auden was attributed with the saying "few have died from lack of love, many have died from lack of water" (Rogers, 1985). In the developing world some 38% of people in urban areas and 85% of people in rural areas do not have adequate sanitation and 14 and 56% respectively do not have an adequate water supply (WHO, 1987). The increasing population of the world, particularly in urban areas, is placing increasing demands upon water resources since water is a commodity which people customarily, but mistakenly, regard as a free resource to be used or wasted at will. As demand increases the economical and efficient conversion of wastewater into water suitable for human consumption is becoming increasingly important. It is believed that the provision of sanitation and potable water to poor communities in developing countries will help reduce the rate at which people, especially children, suffer from, and frequently die of, diarrhoea. Indeed Esrey *et al.* (1985) reviewed 67 studies from 28 countries and found that investment in water supply and sanitation can reduce diarrhoea morbidity and mortality rates by a median of 22% and 21% respectively. The acquisition of sanitation alone has been shown to bring a 25% reduction in the rates of diarrhoea in young children in rural Lesotho (Daniels *et al.*, 1990).

Sanitation may be achieved in a variety of ways from latrines to flush toilets. In high density urban areas where water is available in the home some form of flush toilet will be preferred by most people. Sewerage in turn creates problems of removal and disposal of large volumes of wastewater.

1.2 Composition of Sewage

Wastewater from a community consists of domestic and industrial waste. Domestic sewage is made up of faeces, urine and sullage and is approximately 99.9% water and 0.1% solids (Tebbutt 1970). Fresh domestic waste has a high suspended solids content and contains enormous quantities of micro-organisms. Most of the bacteria present in faeces are responsible for the degradation of food in the human gut. Some will be pathogenic, capable of causing faeco-oral diseases such as diarrhoea, cholera and typhoid. Other micro-organisms present may include pathogenic protozoa, viruses and parasite eggs, depending on the diseases that are present in the contributing population (see Table 1.1). These will cause serious infections where washing and drinking water is inadequately treated or the wastewater used for irrigation.

The large number of chemicals present in sewage makes it impossible to list them all, which is why sewage is generally characterised using other parameters such as organic strength, suspended solids and faecal bacterial numbers. A typical chemical composition of sewage is found in Table 1.2.
The composition of excreta is variable. The weight of individual wet faeces varies depending on age and diet of the person but may range from 20 g to 1.5 kg per day (Gotaas, 1956). The quantity of faeces produced per capita in developing countries tends to be greater than that produced in developed countries in wet weight terms, 400 g as opposed to 150 g. The concentration of pathogens passed on by an infected person may vary widely. A person infected with a small number of nematode worms may only pass on a few grams of eggs a day but a person infected with Vibrio cholera may pass 10^{13} vibrios a day (Feachem *et al*, 1983).

Table 1.1 Pathogens Excreted in Faeces.

Pathogen	Disease
Viruses:	
Adenovirus	Numerous conditions
Enterovirus:	
Poliovirus	Poliomyelitis, paralysis and others
Echovirus	Numerous conditions
Coxsackie viruses	Numerous conditions
Hepatitis A virus	Infectious hepatitis
Rotaviruses, Norwalk agent and others	Diarrhoea
Reoviruses	Numerous conditions
Bacteria:	
Campylobacter fetus spp *jejuni*	Diarrhoea
Pathogenic *E. coli*	Diarrhoea
Salmonella:	
S. typhi	Typhoid fever
S. paratyphi	Paratyphoid fever
Other Salmonella	Food poisoning
Shigella spp.	Bacillary dysentry
Vibrio:	
V. cholerae	Cholera
other Vibrios	Diarrhoea
Yersinia enterocolitca	Diarrhoea and septicemia
Protozoa:	
Balantidium coli	Diarrhoea, dysentry & colonic ulceration
Entamoeba hystolytica	Colonic ulceration, amoebic dysentry & liver abscess
Helminths:	
Schistosoma spp.	Schistosomiasis
Taenia spp.	Tapeworms
Ascaris spp.	Nematode worms

Source: Feachem *et al.* (1983)

Table 1.2 Typical Composition of Untreated Domestic Sewage

Constituent	Concentration*		
	Strong	Medium	Weak
Solids, Total:	1200	720	350
Dissolved, Total:	850	500	250
Fixed	525	300	145
Volatile	325	200	105
Suspended, Total:	350	220	100
Fixed	75	55	20
Volatile	275	165	80
Settleable solids (ml/l)	20	10	5
BOD5, 20 °C	400	220	110
Total Organic Carbon	290	160	80
COD	1000	500	250
Nitrogen (Total as N)	85	40	20
Organic	35	15	8
Free Ammonia	50	25	12
Nitrates	0	0	0
Nitrites	0	0	0
Phosphorus, (Total as P)	15	8	4
Organic	5	3	1
Inorganic	10	5	3
Chlorides	100	50	30
Alkalinity (as CaCO3)	200	100	50
Grease	150	100	50

* all values mg/l except where stated.

Source: Metcalf and Eddy (1972) in Benefield and Randall (1980).

1.2.1 Organic Strength

The organic strength of sewage is normally expressed in terms of the oxygen demand exerted by the waste matter during oxidation. The most commonly used parameters are Chemical Oxygen Demand (COD, where the wastes are chemically oxidised), and Biochemical Oxygen Demand (BOD, where the wastes are biologically oxidised through bacterial degradation). COD measurements may be obtained quickly but give no information on the biodegradable characteristics of the waste. The oxygen demand after five days of biodegradation is the most commonly used since this can be measured in a fairly short time and generally has a fairly consistent relationship with the ultimate biochemical demand. The BOD contribution per capita can vary from around 25 grams/day in African developing countries to about 60 grams/day in the USA and Western Europe (Arthur, 1982). The total organic load produced by a community can be estimated from the number of people in the community, a knowledge of the probable BOD5 per capita production and any industrial and commercial contributions. A typical design figure for an urban area would be 40-50 grams BOD5 per capita per day (although this figure will vary with differing customs, religions etc.) with a wastewater contribution of about 100 litres/capita/day. Hydraulic load is a function of water availability with about 80% of water consumption ending up as sewage. Assuming the construction of sewers to be fair so that infiltration of groundwater is kept down to about 15% of wastewater flow this gives a raw sewage with a BOD5 of 350 mg l^{-1} assuming daily contributions of 40 g BOD5 and 100 litres wastewater.

1.2.2 Pathogenic Bacteria present in Wastewater.

1.2.2.1 Salmonella. In humans, salmonellosis most commonly occurs as an acute gastroenteritis with diarrhoea and abdominal cramps. Fever, nausea

and vomiting are frequently found as additional symptoms. There are over two thousand serotypes known to man and all are pathogenic the most serious being *Salmonella typhi* which causes typhoid fever. Reported concentrations (per 100 ml) in sewage vary considerably. Phirke (1974) reported 7 - 250 in India, 2 - 41 in South Africa (Grabow and Nupen, 1972), 500 in Baltimore, USA (Olivieri *et al*, 1978), up to 2.3 in Finland (Hirn 1980), up to 7240 in England (Jones, 1977) and 670 in Holland (Kampelmacher and van Noorle Jansen, 1970).

1.2.2.2 Shigella. Shigellosis is an acute diarrhoeal disease caused by bacteria of the genus *Shigella.* The symptoms range from mild diarrhoea to a severe disease accompanied by vomiting, fever, cramps and blood, mucus and pus in the stools. Shigellae are gram-negative, non-motile rods belonging to the family Enterobacteriaceae and closely resembling *E. coli* and *Salmonella* (Feachem *et al,* 1983). Some 40 serotypes are known. Sewage may contain between 10 and 10^4 *Shigella* per litre (Feachem *et al,* 1983).

1.2.2.3 *Vibrio cholerae.* Cholera is probably the best known and most feared of diarrhoeal diseases. It is caused by bacterial infection of the small intestine. The causative organism, *Vibrio cholerae,* exists in two biotypes - classical and El Tor (01). Both can cause acute intestinal disease with symptoms such as vomiting, muscle cramps and diarrhoea. If untreated, the patient becomes rapidly dehydrated and may go into shock and die (Feachem, 1983). Sixty percent or more of untreated classical cholera cases die, whereas El Tor is generally regarded as a milder infection with a lower fatality rate and a higher proportion of asymptomatic infections. However, evidence from Bangladesh (Khan and Shahidullah, 1980) suggests that El Tor virulence may be increasing since the severity and number of cases appears to be increasing. Cholera was introduced to Africa 20 years ago where it quickly spread to 30 of the 46

countries of the region. In January 1991, epidemic cholera emerged in Peru (probably from a Chinese freighter which released contaminated bilge water into Lima harbour), causing 200,000 cases and killing 1500 people, and spread to 7 other countries in Latin America reaching Brazil in early 1992. Cholera is transmitted by the faecal-oral route from person to person. Infective doses are high in healthy adults, Hornick *et al* (1971) required 10^8 classical *V. cholerae* in water to produce diarrhoea in 50% of adult volunteers and 10^{11} organisms to produce cholera-like diarrhoea. In contrast the minimum infective dose reported by Levine, Black and Clements (1984, cited in West, 1989) is much lower being in the range 10^3 to 10^5 live organisms. Reported concentrations of *V. cholerae* in sewage vary. Kott and Betzer (1972) estimated between 10 and 10^4 *V. cholerae* per 100 ml during the cholera epidemic of 1970 in Israel. Daniel and Lloyd (1980) reported mean concentrations of 2600 and 160 in strong sewage in two refugee camps in Bangladesh.

1.2.2.4 Campylobacter. Campylobacters are microaerophilic, gram-negative, motile, slender, curved or spiral bacteria. *Campylobacter* enteritis is an enteric infection caused by *Campylobacter fetus* subspecies *jejuni*. In some patients the symptoms are profuse and watery diarrhoea often accompanied by strong abdominal pain, headache and fever (Feachem, 1983). Stools containing blood and mucus are common, especially in children. *C. fetus* ssp. *jejuni* is excreted by a wide range of animals and birds. Infected persons may excrete 10^6-10^9 *C. jejuni* per gram faeces (Feachem *et al.*, 1983).

1.2.3 Faecal Indicator Bacteria.

Contamination by sewage is the greatest danger associated with water for drinking, bathing, recreational use and irrigation. Normally, the monitoring for the presence of specific pathogenic bacteria, viruses and other agents in

water is impracticable and, indeed, unnecessary for routine control purposes. Any pathogenic micro-organisms present in water are usually greatly outnumbered by, and in general tend to die out more rapidly than, the normal commensal bacterial flora of the human or animal intestine (Feachem *et al.*, 1983). The search for organisms indicative of faecal pollution instead of for the pathogens themselves is universally accepted for monitoring the microbial pollution of water supplies. Ideally, the finding of these bacteria should denote the potential presence of intestinal pathogens. Indicator bacteria should be abundant in faeces and sewage; absent or at least very small in number from all other sources; capable of easy isolation, identification, and numerical estimation; unable to grow in the aquatic environment. They should be more resistant than pathogens to disinfectants such as chlorine, as well as to environmental stress. In practice, there is no organism that consistently meets all these criteria, but most of them are fulfilled by *E. coli*.

1.2.3.1 Faecal coliforms. FC are the bacteria normally chosen as indicators of bacterial water quality, and they are expressed as numbers per 100 ml. The group is mainly composed of *Esclierichia coli*. These organisms are exclusively faecal in origin, excreted in large numbers and their presence in a water body, therefore, indicates contamination with faecal material. They are a group of bacteria which are normally present in the intestine of healthy people and at levels of 10^6 to 10^9 per gram of faeces (Feachem *et al.*, 1983). Faecal coliforms are suitable for use as indicator bacteria since they are a normal member of the intestinal flora of healthy people, are easy and safe to handle, and easy to detect and count.

1.2.3.2 Faecal streptococci or Lancefield group D streptococci. A group of bacteria mostly found in the intestines of man and other warm-blooded

animals. The group includes species mainly associated with animals (*Streptococcus bovis, S. avium* and *S. equinus*), other species with a wider distribution (for example, *S. faecalis* and *S. faecium*, which occur in both man and other animals), as well as two biotypes (*S. faecalis* var. *liquefaciens* and an atypical *S. faecalis* that hydrolyzes starch) that appear to be ubiquitous, occuring in both polluted and unpolluted environments and indistinguishable from the true faecal streptococci under routine detection. Aside from the problem of nonfaecal strains, faecal streptococci have major advantages as faecal indicators. They do not multiply in water, are enumerated by a single step membrane filter procedure at 37 °C, a temperature readily attained in small field laboratories. They are less prone to regrowth, and generally survive somewhat longer than faecal coliforms (Feachem *et al*, 1983; PHLS, 1983) due to their resistance to environmental stress and may, thus be better indicators of excreted bacterial pathogens (that have little regrowth tendency) and excreted viruses (that survive for longer than FC in cool waters). Faecal streptococci are rarely found in apparently unpolluted environment.

1.2.3.3 *Clostridium perfringens.* This is the most important member of the group of anaerobic sulphite-reducing clostridia (PHLS, 1983). It is a gram-positive, spore forming bacteria which is exclusively faecal in origin and is pathogenic causing gas gangrene and food poisoning. In normal faeces, it seldom exceeds 10^4 organisms per gram (PHLS, 1983). Because it is spore-forming it can persist for long periods outside the intestine and may be used as an indicator of occasional or intermittent pollution, or of previous pollution of waters in which neither FC or faecal streptococci can be demonstrated. As such it is of much less significance in terms of immediate or direct risks to health. It is more resistant than both FC and faecal streptococci to antagonistic substances such as chlorine (Rippey and Watkins, 1992). However, its long

persistence is a disadvantage because residual, dormant populations may not reflect the true degree of pathogenic contamination.

1.2.3.4 *Pseudomonas aeruginosa.* An opportunistic human pathogen that causes infection in wounds (especially burns) and also ear and urinary tract infections, meningitis, respiratory infections and other conditions (Feachem *et al.*, 1983). *P. aeruginosa* is a gram-negative, aerobic, non-sporulating rod normally occuring at low concentrations of about 50 organisms per gram in the faeces of a small proportion (3-15%) of healthy people. It probably does not grow in the intestine of healthy people and organisms isolated in faeces may be survivors of ingested bacteria. *P. aeruginosa* occurs widely in nature as a free-living organism and has the ability to multiply in water containing suitable nutrients and, therefore, has little usefulness in studies of faecal contamination.

1.2.3.5 Bifidobacteria and other anaerobic bacteria. Bifidobacteria are gram-positive, non-sporulating, anaerobic organisms that occur in the intestines of man and other animals. The most common species found in man are *Bifidobacterium adolescentis* and *B. longum* (Feachem *et al.*, 1983). Bifidobacteria have previously been proposed as indicator organisms for use in tropical waters because the lactose fermenting species are exclusively faecal in origin. They therefore overcome the principle disadvantage of faecal coliform counts on tropical samples - that such samples may contain a significant proportion of strains that can ferment lactose and produce indole at 44 °C but do not derive from faeces (Cabelli, 1978). However, there is insufficient data on their extraintestinal ecology to know whether or not use of all or some of them as indicators will be practicable. Current techniques for their detection and enumeration are rather complex for routine use.

1.2.4 Validity of Faecal Coliforms as Indicators of other Bacterial Pathogens.

From the time of defecation, the concentration of all pathogens usually declines from the death or loss of infectivity of a proportion of the organisms. Viruses and protozoa will always decrease in numbers (since they need a host to reproduce) but bacteria may multiply if they find themselves in a nutrient rich environment with a minimum of competition from other microorganisms.

Faecal coliforms are most suited to being a reliable indicator organisms for the die-off (and survival) of pathogenic bacteria in wastewater since the die-off rates of FC and enteropathogens are reportedly similar (Bartone *et al*, 1985 and Yanez, 1984). FC can be reasonably expected to mimic those organisms which they resemble. Studies with DNA have found *E. coli* to be almost identical to shigellae and very similar to salmonellae (Jones, 1988). Vibrio spp., although classified in the same subgroup of eubacteria as above, have been shown to differ substantially from *E. coli* (Koh *et al.* 1994 and Colwell *et al,* 1985). Campylobacter spp. are believed to be very different indeed, possibly forming their own group within the eubacteria (Thompson *et al.* 1988). Thus, it may be expected that FC are a good indicator of salmonellae and shigellae, but not necessarily for Vibrio spp. or Campylobacters. Experimental evidence seems to support this. The rate of reduction of FC in WSP is similar to that of salmonellae (Davis and Gloyna, 1972; Yanez, 1984; Curtis, 1986) but is lower than that of campylobacters (Curtis, 1986) and inversely related to that of non-01 *V. cholerae* (Lesne *et al.*, 1991). Kott and Betzer (1972) reported good removal of cultured 01 *V. cholerae* from a model WSP (a 70 litre aquarium), apparently contradicting the work of Lesne *et al.* (1991). Lesne and co-workers reported that algal density was highly and positively correlated with non-01 *V. cholerae* numbers in pilot scale schemes.

Carter *et al.* (1987) did not find any apparent correlation (qualitative or quantitative) between *Campylobacter jejuni* numbers and any of the microbiological and physical parameters studied which included FC, FS, total heterotrophic bacteria, pH, temperature and conductivity. From the available literature it would appear that FC die off should not be considered a valid indicator of die off of 01 *V. cholerae* or Campylobacter spp.

There are little data on the usefulness of faecal coliforms as indicators of viral quality but it would appear that they are unsuitable (Rao *et al.,* 1981; Demmillac and Baron, 1982). In a waste stabilization pond system with a retention time of more than 20 days final effluent will be free from both pathogenic protozoa and helminths but in other treatment systems the situation is more complicated and faecal coliforms are inappropriate as indicators of helminth eggs (Feachem *et al,* 1983).

1.2.5 Tropical versus Temperate Climate.

There are many data (mainly from North America) on the relationship between the survival of bacterial pathogens and indicators in sewage treatment processes in temperate climates, but somewhat less data from tropical countries. The use of indicator organisms, such as faecal coliforms, to predict the likely density of, say, salmonellae in a tropical sewage effluent may be difficult. In contrast, reasonable estimates are possible (Feachem *et al.,* 1983) in a temperate climate effluent since extensive literature has been published on this theme . This makes the establishment of a faecal coliform standard for most tropical sewage effluents a more difficult process. Because engineers design, for example, maturation pond systems on the basis of FC removal to the desired standard, this state of scientific uncertainty can lead to either overdesign (with a consequent increased cost) or underdesign (with a consequent increased health risk).

1.3 The Use of Waste Stabilization Ponds to Treat Wastewater.

1.3.1 Waste Stabilization Ponds.

Waste stabilization ponds are the most economic method of treating wastewater wherever land costs are relatively low. The process is a relatively simple one, not requiring skilled or semi-skilled labour that the more traditional treatment systems do but the quality of effluent produced is of sufficiently high quality for use in unrestricted irrigation. This makes them ideal for use in less developed countries where water is frequently in short supply.

Waste stabilization ponds are basically shallow, large area, man-made basins, usually rectangular, designed specifically to treat wastes through long retention times, by the processes of natural purification involving the bacteria and algae present in the water. They may be considered as the bottom of the list of the methods by which man attempts to accelerate the natural processes of purification and stabilization. They are simple, partially controlled systems which do, however, represent a complex interaction of physical and biochemical processes which must be understood in order to be facilitated. There is surprisingly little, beyond making available an adequate area, that the designer can do to control the processes involved and, once created, there is even less that the operator can do to modify or improve the situation. Arthur (1982) described the three major treatment processes in WSP:

(a) the reservoir effect - which expresses the dilution capacity of a pond and enables it to absorb both organic and hydraulic shock loadings,

(b) primary sedimentation, responsible for the removal of settleable solids to the sludge layer, particularly in ponds receiving raw wastewater,

(c) biodegradation of organic compounds either by aerobic oxidation or anaerobic digestion.

Waste stabilization ponds have been used for over 100 years but it is only since the 1940's that the scientific criteria the design has become defined (WHO/EMRO, 1979). By 1975 ponds were being used in more than 38 countries including Australia (Parker, 1962), Israel (Watson, 1962), South Africa (Coetzee and Fourie, 1965), India (Parhad and Rao,1974), Brazil (Mara and Silva, 1979), the USA (Middlebrooks *et al.*, 1982), Peru (Pearson *et al.*, 1987d) and Kenya (Hunt and Westernberg, 1964). In Europe, France has over 2000 plants (Racault *et al.*, 1995), Portugal has more than 50 with a further 50 planned to be completed by the end of 1993 (Costa *et al.*, 1991) and Germany has more than 2000 operational plants (Bucksteeg, K., 1987). Many different types of ponds have been developed for different situations as the understanding of operating mechanisms has increased. These include ponds for the treatment of factory wastes, ponds used as an advanced treatment stage and ponds which serve small communities.

1.3.2 Advantages of Waste Stabilization Ponds

The advantages of waste stabilization ponds can be summarised as follows:

(a) Simplicity to construct, operate and maintain. Construction consists mainly of earth-moving plus minimal other civil works. Operation and maintenance is usually restricted to routine tasks such as bank care, removal of floating scum and vegetation and keeping inlets and outlets clear (Mara, 1976). Thus labour need not be skilled. However, Yhdego (1989, 1992) has reported problems with WSPs built more than 20 years ago in Tanzania. Many of the ponds are either not working at all or performing poorly. Yhdego states the reasons for this as being:

(i) standard design formulas were adopted without considering local environmental factors resulting in ponds designed without proper care, (ii) lack of maintenance and desludging adversely affecting pond performance and resulting in high mosquito breeding and

(iii) scarcity of funds and skilled operational supervision.

The result is ponds badly polluting the receiving waters. Yhdego (1992) concludes that describing ponds as appropriate technology for developing countries is dangerous since know-how and management factors must be considered, however it is important to note that alternative wastewater treatment processes would necessitate more care and maintenance and would be expected to perform poorer than the WSPs.

(b) Low cost since there is no need for expensive mechanical equipment, such as aerators, and no energy requirement. The likelihood of mechanical failure and the need for spare parts, which can be difficult and expensive to obtain in developing countries, is minimal. The cost advantages are analysed by Arthur (1982) who found that of four different treatment processes designed to produce the same effluent quality waste stabilization ponds were much cheaper to construct and operate than trickling filters, aerated lagoons and oxidation ditches. Gloyna and Tischler (1979) compared the power requirements of various methods of treating 1,000 m^3 of wastewater to the same effluent BOD5 : 724 kWh with an activated sludge plant, 615 kWh with an aerated lagoon and 87 kWh with a rotating biological contractor.

(c) High efficiency. BOD removals in a series of well designed ponds are more than 90%. Faecal coliform removal can be 5 log_{10} units (99.999%). This is better than the 99% removal that activated sludge plants achieve if operating well. Table 1.3 shows a comparison of excreted pathogen removal between WSP and conventional treatment processes. Lime sedimentation is known to remove pathogens equally well but the capital and running costs are similar to an activated sludge plant with chlorination (Gambrill, 1989).

(d) WSP are also extremely resistant to hydraulic and organic shock loads due to their long retention times.

Table 1.3 Removals of Excreted Pathogens achieved by WSP and Conventional Processes.

Excreted Pathogen	Removal WSP	Removal Conventional
Bacteria	up to 6 log units[a]	1 - 2 log units
Viruses	up to 4 log units	1 - 2 log units
Protozoan cysts	100%	90 - 99%
Helminth eggs	100%	90 - 99%

[a] 1 log unit = 90% removal; 2 log units = 99% ; 3 log units = 99.9% etc

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

(e) They can effectively cope with high levels of heavy metals, up to 60 mg l^{-1} (Moshe, 1972) and so can treat industrial wastes which would be too toxic for other treatment processes.

(f) They can be designed so that the degree of treatment is easily altered.

(g) The method of construction is such that the land is easily reclaimable.

The principle disadvantage of waste stabilization ponds is their requirement for relatively flat land, especially in or near large cities where prices may be high as land has possible alternative uses in agriculture and housing. Whatever the design procedure used, the area of land required is proportional to the population served. The treatment of wastewater from 1,000 people should require about 35 m^2 for an activated sludge works, 210 m^2 for a trickling filter, 2,000 m^2 for a pond system in the tropics with no cold season and up to 50,000 m^2 for a pond system where there would be ice cover for part of the year (Hawkes, 1983). Usual pond requirements are 5 to 10 m^2 per

person, depending on the required effluent quality and the ambient temperature (Mara, 1987).

Table 1.4 compares the advantages of waste stabilization ponds with other treatment systems.

1.4 Types of Waste Stabilization Ponds and their function

Stabilization ponds are capable of dealing with the whole spectrum of wastewaters containing biodegradable organic material, from weak domestic wastes to inordinately strong industrial wastes, and also to provide for them the whole range of treatment processes which would be characterised, in conventional treatment, as being from preliminary to tertiary and including anaerobic digestion. To achieve this treatment stabilization ponds are normally subdivided into three types. These are anaerobic ponds, facultative ponds, and maturation (or fully aerobic) ponds. There are, in addition, a few special pond types - seepage and nightsoil ponds, partially aerated facultative ponds, effluent storage reservoirs, macrophyte ponds and high-rate algal ponds designed to fulfill the appreciably different function of producing algae so that it may be harvested and used as high-protein animal food. Each type of pond has different objectives and, therefore, different design and operating criteria. Anaerobic and facultative ponds are primarily designed to remove BOD whereas the function of maturation ponds is the removal of excreted pathogens although some BOD removal occurs in maturation ponds and some pathogen removal in anaerobic and facultative ponds.

Table 1.4 Advantages and Disadvantages of Various Sewage Treatment Systems

Criteria	Package Plant	Activated Sludge Plant	Trickling Filter	Extended Aeration Plant	Oxidation Ditch	Aerated Lagoon System	Waste Stabilization Pond system (including anaerobic units)	Waste Stabilization Pond system (excluding anaerobic units)
BOD5 Removal	**	**	**	**	***	***	***	***
FC Removal	*	*	*	**	**	***	***	***
SS Removal	**	***	***	***	***	**	**	**
Helminth Removal	*	**	*	*	**	**	***	***
Virus Removal	*	**	*	**	**	***	***	***
Ancillary Use Possibilities	*	*	*	*	*	***	***	***
Effluent Reuse Possibilities	*a	*a	*a	**	**	***	***	***
Simple and Cheap Construction	*	*	*	*	**	**	***	***
Simple Operation	*	*	**	*	**	*	***	***
Land Requirements	***	***	***	***	***	**	**	*
Maintenance Costs	*	*	**	*	*	*	***	***
Energy Demand	*	*	**	*	*	*	***	***
Minimization of sludge for Removal	*	**b	**b	**b	*	**	***	***

Key *** good ** fair *poor

a - these effluents frequently have high ammonia levels (>5 mg/l) and faecal bacteria concentrations and are usually not suitable for irrigation or pisciculture without tertiary treatment

b - Assumes provision of sludge digesters

Source: Arthur (1982)

1.4.1 Arrangements of ponds

Although WSP may be used singly, this is considered to be bad practice, and they are usually built as a series of treatment lagoons. The final effluent quality depends largely on the size and number of maturation ponds. Good designs incorporate a facultative pond and 2 or more maturation ponds. For strong wastes, those with a BOD > 400 mg l^{-1}, the use of anaerobic pretreatment ponds minimizes land requirements of the whole system although some designers are wary of including anaerobic ponds due to odour release. Where larger flows are to be treated it is advantageous to construct several series, in parallel, to facilitate maintenance, desludging, expansion etc. In this way waste stabilization pond systems are relatively flexible and can be rearranged or enlarged to suit changing conditions.

1.4.2 Anaerobic ponds

Often used to treat strong industrial wastewaters. Otherwise they are normally the first in a series of ponds and are designed to reduce the concentration of biodegradable organic material primarily as as result of the removal of the suspended solids. Anaerobic ponds are completely devoid of oxygen and contain no algae. Settleable organic material in the waste accumulates in the bottom of the pond as a sludge layer. This material is biologically active and it has a high oxygen demand and rapidly becomes a site for anaerobic microbiological degradation and stabilization much in the style of the old Imhoff tanks (Ellis, 1983).

It was early established that the sludge layer has an important influence on the biological activity of anaerobic ponds (Parker (1950) and Parker and Skerry (1968)) as shown by the data in Table 1.5. The more intimately the sludge solids are brought into contact with the liquid phase, the higher the performance. This is because the methane producing bacteria are much more

Table 1.5 Influence of Sludge layer on Performance of Anaerobic Lagoons.

Period	BOD mg l^{-1} Raw Sewage	Lagoon A	Lagoon B
April 9 - May 21	407	231	133
May 21 - Sept 17	407	291	254
Sept 17 - Dec 3	484	205	138
Dec 3 - Dec 24	448	157	145

Lagoon A desludged before experiment
Lagoon B Sludge Layer = 16"

source: Parker (1979)

biochemically active and multiply more rapidly when associated with solid surfaces (Barker, 1956). Pathogen removal in anaerobic ponds depends largely on temperature and can vary from 60% at 12 °C to 93% at 25 °C (Arthur, 1982).

1.4.2.1 BOD Removal in Anaerobic Ponds.

The performance of anaerobic ponds is highly temperature dependent. Arthur (1982) reports a 45-70% reduction in BOD5 at temperatures of 12 - 25 °C. respectively with a retention time of 2 days. Mara *et al* (1983) reported a mean annual BOD removal of 75% in an optimally loaded pond with a retention time of 0.8 d at 25-27 °C. Effective digestion in an anaerobic pond occurs in the absence of molecular oxygen and combines two processes:

Organic acid formation:

The basic process by which stabilization of organic material occurs is due to the ability of a variety of saprophytic organisms, such as *Pseudomas, Flavobacterium, Alcaligenes, Eschericlia* and *Aerobacter,* to oxidise proteins,

carbohydrates and fats to fatty acids (Parker 1979), mainly acetic, propionic and butyric (Brockett 1976) with the formation of new bacterial cells:

$$5(CH_2O)x \xrightarrow[\text{producers}]{\text{acid}} (CH_2O)x + 2CH_3COOH + \text{energy} \quad (1.1)$$

The acid formed is neutralised by the bicarbonate buffer present in solution:

$$2CH_3COOH + 2NH_4HCO_3 \xrightarrow[\text{producers}]{\text{methane}} 2CH_3COONH_4 + 2H_2O) + 2CO_2 \quad (1.2)$$

Methane production

The organic acids are utilised by methanogenic archaebacteria, such as Methanobacterium, methanosarcina and Methanococcus, to produce methane:

$$4CH_3COOH + 8H \longrightarrow 5CH_4 + 3CO_2 + 2H_2O \quad (1.3)$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \quad (1.4)$$

The second step is rate limiting since the methanogens are sensitive to pH changes, acid conditions and temperature (consequently temperatures of >15 °C, pH > 7.0, volatile acids <3000 mg l^{-1} and alkalinity of >2000 mg l^{-1} (Parker, 1979; Nyns, 1986) are recommended for the successful operation of an anaerobic WSP) and also heavy metals, detergents and sulphides (Middlebrooks *et al.*, 1982). However, data showing minimal sludge accumulation in WSPs in Peru at water temperatures below 15 °C (Pearson, 1987d) suggest that methanogenesis can occur efficiently at lower temperatures.

The reduction of sulphur to H_2S also occurs in anaerobic WSP and such ponds have been unpopular due to odour release. Fortunately, odour control can be achieved by manipulation of the loads. Good pathogen removal is usually

observed in anaerobic ponds (Oragui *et al.*, 1987) but this appears to be largely independent of retention time and load (Silva, 1982) and may be attributed to the sedimentation of solids-associated pathogens in raw sewage.

1.4.2.2 Design of Anaerobic Ponds

Anaerobic ponds are currently most frequently designed on the basis of volumetric organic loadings (λv, g $m^{-3}d^{-1}$), which is given by:

$$\lambda v = Li\ Q/Va \qquad (1.5)$$

where Li = influent BOD, mg l^{-1} (= g m^{-3})

Q = flow, $m^{-3}d^{-1}$

Va = anaerobic pond volume, m^3

The permissible design value of λv increases with temperature. Results obtained in a study of Kenyan ponds by Mara *et al.* (1990) indicate that the recommendations made by Mara and Pearson (1986) and given in Table 1.6 are acceptable.

Table 1.6 Design Values of Permissible Volumetric Loading on and BOD Removal in Anaerobic Ponds

Mean Monthly Temperature (°C)	Volumetric BOD5 Loading (g m^{-3} d^{-1})	BOD5 Removal (%)
<10	100	40
10-20	20 T - 100[a]	2 T + 20[a]
>20	300	60

[a] T = Temperature

Source: Mara and Pearson (1986)

Generally, the values of λv are restricted to between 100 and 300 mg $m^{-3}d^{-1}$, since values of <100 prevent the development of completely anaerobic conditions and values of >400 cause unacceptable levels of odour release due to the anaerobic reduction of sulphates.

If the BOD cannot be measured it may be estimated from the following equation:

$$Li = 1000\ B/q \qquad (1.6)$$

where Li = wastewater BOD, mg l^{-1}

B = BOD contribution, g $caput^{-1}$ d^{-1}

q = wastewater flow, l $caput^{-1}$ d^{-1}

Values of B vary but a suitable design value would be 40 g per caput per day.

Once a value of λv has been selected the pond volume can be calculated from equation 1.1 and the mean hydraulic retention time (θa, d) may be determined by:

$$\theta a = Va / Q \qquad (1.7)$$

For effective solids removal in a conventional sedimentation tank a retention period of between 2 to 6 hours is acceptable. A pond, however, is not an efficient settlement unit and it is necessary to increase this retention time to 12 hours. In addition, it is expected that the pond continue to function until at least half the volume is filled with sludge. The 12 hr is, therefore, doubled to 24 hr. Mara and Silva (1979) recommend retention times not exceeding 24 hr for climates where the pond temperature is > 25 °C. Because the ponds are theoretically devoid of oxygen (due to the high organic loads they receive), there is, theoretically, no limit to the depth of an anaerobic pond. However, a

depth of 4 m is usually optimal from the point of view of the economics of construction (Pearson, H.W., personal communication). Depths of less than 2.5 m should not be used if possible as they leave little depth for sludge accumulation, although still shallower depths may be necessary due to local soil and ground conditions. Deep ponds have a more uniform temperature due to the decreasing effects of fluctuating surface layer temperatures with increasing depth. A more uniform temperature ensures better fermentation. Deeper ponds are also better mixed because they provide a greater bubble path length for upwelling methane gas produced in the settled sludge (Oswald 1968) although Pearson, Mara and Mills (1988) state that excessive mixing in anaerobic ponds is likely to increase odour release and may reduce sludge digestion by inhibiting methanogenesis. Once the retention time and the pond volume are known the pond depth may then be selected and suitable dimensions calculated.

1.4.3 Facultative Ponds

Facultative ponds can be used to receive raw sewage (a primary facultative pond) or they may follow an anaerobic pond or some other form of pretreatment (a secondary facultative pond). They receive lower loadings and have longer retention times than anaerobic ponds, typically from 4 or 5 days to over 40 days. Their function is the removal of organic material and solids and they are distinguished by the presence of an aerobic zone overlying an anaerobic one . The lower layer of a facultative pond behaves in a manner similar to an anaerobic pond with the settlement of solids forming a sludge layer which undergoes degradation and methanogenesis. The lower layer probably also feeds the upper layer with soluble organics such as fatty acids, and sulphide and ammonia where they may effect the ecology of the algae. The upper layer is dominated by algae, which are often present at higher concentrations (1000 - 3000$\mu g\ l^{-1}$) than is usual in other lagoons

(Trousellier *et al* ., 1986; Silva, 1982). The algae aerate the upper zone of the lagoon via photosynthetic oxygen production and the oxygen they produce is consumed by the facultative aerobic bacteria which are able to oxidise organic matter rapidly, efficiently and completely.

Some important environmental fluctuations occur in the water column throughout the diurnal cycle:

(i) the depth of the aerobic top layer varies as a consequence of the rate of algal photosynthesis, and therefore, light penetration and intensity (Sless, 1973). During periods of intense photosynthetic activity oxygen concentrations may reach supersaturation levels. This will coincide with a high photosynthetic demand for carbon dioxide by the algae such that it exceeds that produced by bacterial respiration. This causes a drop in the dissolved CO_2 concentration in the water causing an increase in the pH. At night, in the absence of algal photosynthesis, oxygen levels decrease and the pond may become anaerobic with just a narrow zone, a few mm deep, just below the surface, remaining aerobic (Pearson, 1987). During this time the pH drops towards neutral and becomes homogeneous throughout the pond depth.

(ii) As a result of intense solar radiation, thermal stratification occurs in the water column during daylight hours. High temperatures (30-35 ℃) (Mara, 1977) or more (Ellis, 1983), can be measured above the thermocline (30-50 cm below the pond surface). This causes the upper layers to become less dense and so restricts mixing and its effects (favourable or not). Thermal stratification, which can be overcome by wind action (Marais, 1974; Mara, 1976; 1977; Silva, 1982; Ellis, 1983; James, 1987), causes the non-motile, non-buoyant algae to sink to the lower region of the pond where, due to low light intensity, they demand oxygen rather than produce it. The situation is worsened at the hours of maximum solar radiation by the motile algae which

form a dense band shading the lower layers and so further cooling them. A reduction in the retention time of the pond may be a consequence of this thermal stratification due to influent short circuiting across the pond surface between the inlet and outlets. During the night the surface layers normally lose heat more rapidly than the deeper layers and, therefore, become more dense and convectional mixing can occur.

(iii) Variations of free sulphide concentrations may be observed throughout the day (Mara and Pearson, 1986). Maximum and minimum concentrations occur at dawn in the bulk of the pond and within the upper layers during late afternoon, respectively.

1.4.3.1 BOD Removal in Facultative Ponds

In primary facultative ponds BOD removal is carried out through primary sedimentation and biochemical activity, whereas the role played by sedimentation in secondary facultative ponds is minimal. BOD removal through sedimentation is estimated to be about 30% (Mara, 1976). Figure 1.1 shows the pathways of BOD removal in a facultative waste stabilization pond. Pearson (1987) reported BOD removal in primary facultative ponds as in the range of 60-80%. Pescod and Mara (1988) show that BOD removal is related to the applied BOD surface loading and is usually between 70 and 80%. Silva (1982) found a mean removal of about 75% of the surface BOD loading in ponds with a retention time of between 6.3 and 18.9 days in North-east Brazil. Arthur (1982) reported ranges of 75-84% for ponds with retention times of 7-15 days. The principle biological method for BOD reduction is methane fermentation. Oswald (1968) found that up to 70% of the applied BOD is lost from facultative ponds in the gaseous form (although this would be a mixture of methane and CO_2). Although digestion is highly temperature dependent, pond BOD often appears insensitive to seasonal variations in temperature. Marais (1966) states that this is due to the buffering capacity of

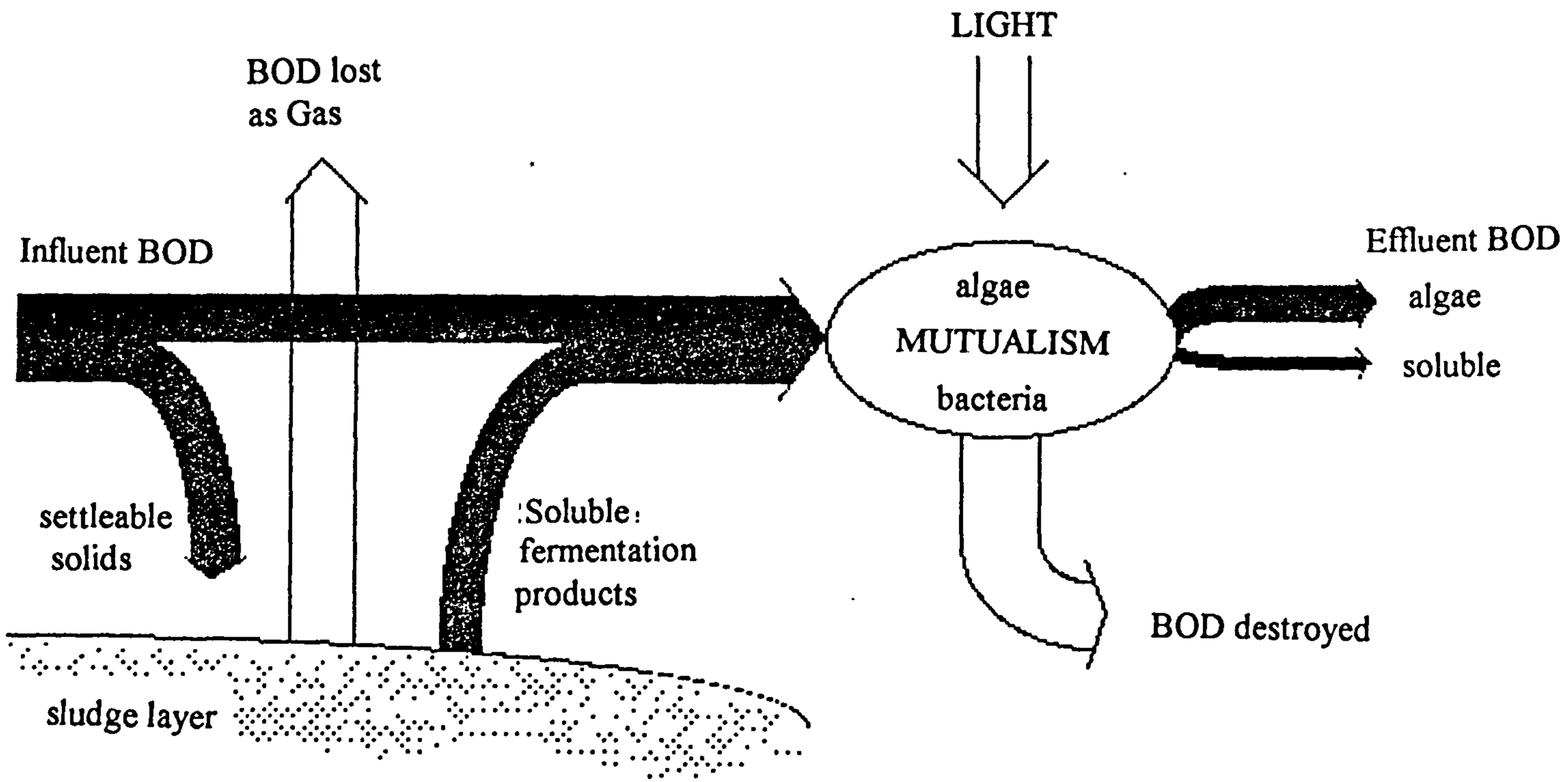


Figure 1.1 Pathways of BOD Removal in Facultative Ponds

the sludge. An equilibrium is established between the BOD settling as sludge and the BOD being released as soluble organics from the sludge. This is a temperature dependent equilibrium, the lower the temperature, the higher the sludge accumulation. In winter, the degradation of BOD in the pond will be lower, but no increase in pond BOD will be noted because the BOD released from the sludge will also be lower. There will, therefore, be a sludge build up in colder weather whilst conversely, in summer, when the degradation is faster and the BOD release to the pond greater, the sludge layer will decrease.

1.4.3.2 Design of Facultative Ponds

Facultative ponds are designed on the basis of surface BOD loadings since the surface area of the pond is critical. The extent of aerobic activity is dependent on the penetration of light and by the amount of illumination received by, and hence photosynthesis in, the pond. The depth of a facultative pond is critical, too shallow and an anaerobic layer is not formed and rooted vegetation may also emerge providing a habitat for mosquito breeding. Too deep and the pond becomes predominantly anaerobic, possibly causing odour problems and the pond becomes less resistant to shock loadings. Thus, facultative ponds are usually 1 to 2 m deep (Mara, 1976; Mara and Pearson, 1986; Pearson, 1987), typically 1.5 m (Ellis, 1983; Pescod and Mara, 1988). Because they are so shallow, facultative ponds occupy fairly large areas and, since anaerobic ponds are designed on the basis of volumetric loading, land may be saved if an anaerobic pond is incorporated into the pond series.

There are numerous methods applied to facultative pond design but none can describe all the processes which take place within a pond:

(a) First Order Kinetics (Marais and Shaw, 1961).

This is a complete mix approach which relies upon three assumptions:

(i) The stabilization of the biodegradable organic material present follows first-order kinetics.

(ii) The contents of the pond are instantaneously and completely mixed.

(iii) There is no seepage or evaporation from the lagoon.

The area (A, m^3) of the pond at mid-depth under a continuous flow process is obtained from:

$$A = [(Li/Le) - 1]. Q/ D.k_1 \quad (1.8)$$

where Q = daily mean flow rate ($m^3 d^{-1}$)
D = depth (m)
Li = influent BOD5 ($mg l^{-1}$)
Le = effluent BOD5 ($mg l^{-1}$)
k_1 = first order rate constant for BOD_5 removal (d^{-1})

Different k_1 values have been suggested by different authors (Ellis, 1983 and Ellis and Rodrigues, 1993). Mara (1976) suggested correcting k1 values for temperature using the equation:

$$k_{1(T)} = 0.3\ (1.05)^{(T-20)} \quad (1.9)$$

where T = temperature (°C)

Silva (1982) found that the constant k1 increases as surface BOD loading increases or as hydraulic retention time decreases.

(b) Thirumurthi method (Thirumurthi, 1969) suggests that for a rational approach plug or complete mixing cannot be assumed thus, a method based on the Wehner - Wilhelm equation for first order BOD_5 removal in dispersed flow reactors. This method utilises a dispersion number (d) to describe the hydraulic regime where d varies from zero (plug flow) to infinity (complete mixing). Lumbers (1979) doubted that d of WSP could be more than 1, Ellis (1983) gives a range of 0.25 - 1.0, Mara and Pearson (1986) suggest values of 0.2 - 2.0, Arceivala (1983) 0.2 - 4.0. The utilisation of this method and other dispersed flow or combined methods according to Ferrara and Harleman

(1981) presents two problems. Firstly, the evaluation of the dispersion number based on tracer studies and, secondly, a dispersion obtained through tracer studies applies only to the given pond and to the prevailing conditions during that investigation. There is no guarantee that a dispersion number value may apply to hydraulic or climatic conditions other than those under which it was determined. Polprasert *et al.* (1985) proposed an equation to estimate d for use at the design stage:

$$d = (0.184\,[HRT.\,v\,\{W + 2D\}]^{0.489}(W)^{1.511} / (L.D)^{1.489}) \qquad (1.10)$$

where HRT = hydraulic retention time (d)
v = kinematic viscosity (m^2s^{-1})
W = pond width (m)
D = pond depth (m)
L = pond length (m)

This equation, however, was based on pilot scale studies and the validity needs to be established.

Mara and Pearson (1986) suggest the design of ponds could be carried out by a combination of Wehner & Wilhelm and Polprasert & Battarai:

$$Le/Li = \{4a.\exp(1/2d)\} / \{(1+a)^2\exp(a/2d) - (1-a)^2\exp(-a/2d)\} \qquad (1.11)$$

where $a = (1+4k1.HRT.d)^{1/2}$

k_1 = first order constant for BOD removal.

Dispersed flow methods are a good design approach, in principle, to ponds which are known to have non-ideal flow but they have many practical difficulties. Thirumurthi (1969) considered plug-flow might be assumed as an approximation to dispersed flow procedures but Ferrara and Harleman (1981) suggest complete mixing thus the correct rational alternative method for dispersed flow procedures continues under dispute and, hence, the adoption of an empirical design method would seem to be more sensible.

(c) McGarry and Pescod empirical procedure (McGarry and Pescod, 1970) is based on the operational data of 143 primary facultative ponds all over the world. It is the most widely adopted method wherever local experience is limited. This method relates maximum surface organic loading (SL BOD_{max}, Kg BOD_5 $ha^{-1}d^{-1}$) that could be applied to primary facultative ponds before becoming anaerobic, to the mean air temperature (°C) of the coldest month of the year:

$$SL\ BOD_{max} = 60.3\ (1.099)^{T} \qquad (1.12)$$

Mara (1976) suggested introducing a safety factor of 1.5 for primary facultative ponds giving a linear relationship between SL BOD_{max} and temperature:

$$SL\ BOD_{max} = 20T - 120 \qquad (1.13)$$

Arthur (1982) using data from primary facultative ponds suggested an alternative:

$$SL\ BOD_{max} = 20T - 60 \qquad (1.14)$$

The procedure of McGarry and Pescod has been supported by many workers in warm climates (WHO, 1987) and reportedly gives a reasonable design under most circumstances (Pearson, 1987).

This procedure is also suitable for secondary facultative ponds but should be adapted to correct maximum areal BOD loading rates since 30% of BOD is removed by sedimentation:

$$SL\ BOD_{max} = 0.7\ (20T - 60) \qquad (1.15)$$

(d) Indian approximate procedure is based on ponds operated in India and is also known as Arceivalas equation (WHO, 1987). It relates permissible loadings to be applied to a primary facultative pond to latitude.

$$SL\ BOD_{max} = 375 - 6.25\ L \qquad (1.16)$$

where L = latitude (°)

Lumbers (1979) does not recommend this method for use outside India.

(e) Global environmental design basis (Gloyna, 1971). This method is based on utilising both organic loading rates and hydraulic retention time determined from facultative ponds in different environmental conditions (Lumbers, 1979; Silva, 1982).

(f) Gloyna empirical formula (Gloyna, 1976). This method uses an Arrhenius style equation developed from results obtained with a number of laboratory scale ponds and pilot scale ponds:

$$V = (3.5 \times 10^{-5}).\ N.\ q.\ Li.\ \theta^{(35-T)}\ f.\ f' \qquad (1.17)$$

where
- V = volume of the pond (m^3)
- N = population
- q = waste per caput ($l^{-1}\ d^{-1}$)
- Li = influent BOD ($mg\ l^{-1}$)
- θ = coefficient of temperature activity
- T = mean water temperature of coldest month (°C)
- f = algal toxicity factor
- f' = sulphur O2 demand factor

This is an updated version of the Gloyna and Herman equation:

$$HRT\ (35)/\ HRT\ (T) = q^{(35-T)} \qquad (1.18)$$

where
- HRT(T) = hydraulic retention time needed for 90% BOD removal at temperature T °C
- HRT(35) = hydraulic retention time at 35 °C, considered as the maximum temperature for algal activity, recommended to be 3.5 days.

This empirical formula is commonly used in the USA.

(g) Solar radiation method (Oswald, 1963; Siddiqi and Handa, 1971).

Based on the work of Oswald and assumes that all the oxygen required for the aerobic stabilization processes in a pond is produced by photosynthesis and that photosynthesis requires both algae and solar radiation and, with limits, the stronger the solar radiation the more photosynthesis. The practical difficulties associated with this method have been discussed by Silva (1982).

Mara (1976) recommended the first four of these methods for general use in hot climates. The simplest of the above methods relate permissible surface loading rates to minimum monthly average temperature. Arthur (1982) recommends the use of the McGarry and Pescod empirical procedure. Recent work in Kenya (Mara *et al*, 1990) found that ponds receiving loads higher than those permitted by equation 1.14 were operating reasonably well in terms of BOD removal. The area (A, m^2) of the pond may then be calculated using:

$$A = 10\,LiQ/\,\lambda s \qquad (1.19)$$

where λs = surface BOD loading (kg ha^{-1} d^{-1})

Some authors (Mara and Pearson, 1970) suggest that since secondary facultative ponds lack a sludge layer they have the loading capacity of only 70% of a primary facultative pond but Sheikh and Beck (1987) thought it possible that primary and secondary facultative ponds under similar loadings could provide similar BOD removals.

1.4.4 Maturation Ponds

Maturation ponds receive the effluents from facultative ponds (or other maturation ponds). Their primary function is pathogen removal although further degradation of organic material also takes place (Mara and Pearson, 1986). They are completely aerobic and contain a lower algal biomass than

facultative ponds, though there is usually a wider variety of species. They are designed on the basis of retention time needed for FC removal and generally a series of maturation ponds is more efficient than a single pond having the same area (Marais, 1966). Completely aerobic maturation ponds can be up to three metres deep, but they are usually the same depth, or shallower than, the preceeding facultative pond (Mara, 1976). They are rich in dissolved oxygen as a consequence of two main causes:

(i) they are less turbid than other ponds, light can penetrate deeper and oxygen is produced photosynthetically by algae and cyanobacteria;
(ii) their organic loading rates are lower than those applied to facultative ponds, therefore, less oxygen is utilised in the degradation of organic material.

Variations in dissolved oxygen levels occur during the daily cycle due to thermal and algal stratification as discussed in section 1.4.3. but higher pH values are attained in maturation ponds, often 9.5 or higher (Pearson *et al.* 1987b). Even at night the pH is maintained at around 8.5. Mechanisms of pathogen removal are similar in facultative and maturation ponds; these are discussed in section 1.7. BOD removal in maturation ponds is complicated by the production of algae which can cause an increase in the BOD and suspended solids in the effluent.

1.4.4.1 BOD Removal in Maturation Ponds

Little work has been reported on BOD removal kinetics in maturation ponds but it is known that it is very much slower than in anaerobic and facultative ponds. Marais and Shaw (1961) calculated that, assuming an influent of less than 75 mg l^{-1}, two maturation ponds in series, each with a retention time of 7 days are required to produce an effluent with a BOD of lees than 25 mg l^{-1}.

The situation is complicated by the production of algae and this is discussed further in section 1.5.1.

1.4.4.2 Design of Maturation Ponds

Maturation ponds are designed primarily for the removal of faecal bacteria. The method described by Marais (1974) is generally used and is well supported in the literature (Mara, 1976; Lumbers, 1979; Arthur, 1982; Feachem *et al.*, 1983; Ellis, 1983; Mara and Pearson, 1986; Pearson *et al.* 1987c; Pescod and Mara, 1988; Mara and Caincross, 1989). This assumes that faecal coliform removal can be modelled by first order kinetics in a completely mixed reactor. The equation for a single pond is:

$$Ne = Ni / (1 + kT\theta) \qquad (1.20)$$

where
Ne = number FC per 100 ml effluent
Ni = number FC per 100 ml influent
kT = first order rate constant for FC removal (d^{-1})
θ = retention time (d)

Marais showed that removal is more efficient with a greater number of ponds in series for the same total retention time.

Equation 1.20 can be adapted for a series of ponds:

$$Ne = Ni / (1 + kT\theta a)(1 + kT\theta f)(1 + kT\theta m)^n \qquad (1.21)$$

where θ is the mean hydraulic retention time in days, the subscripts a,f and m refer to anaerobic, facultative and maturation ponds respectively and n is the number of maturation ponds.

The value of Ni is either known from measurement or can be conservatively estimated as 1×10^8/100 ml and Ne is usually stipulated by the local

regulatory body. Marais (1974) gives the following equation for the variation of k_T with temperature:

$$k_T = 2.6\,(1.19)^{T-20} \qquad (1.22)$$

k_T, therefore, changes by 19% for every 1 °C change in temperature (Mara *et al.*, 1991). Marais observed that this relationship is valid in the range 5-21 °C for a mixed and aerobic or facultative pond. Above 21 °C, if no form of mixing is provided, thermal stratification tends to form during daylight hours and most of the pond bulk turns anaerobic causing k_T to decrease.

At the design stage equation 1.21 has two unknowns, θ_m and n. The recommended minimum value of θ_m is 3 days to avoid hydraulic short circuiting (Marais, 1974) and it is not usual for θ_m to be greater than θ_f. Thus, θ_m may be calculated for various values of n and a suitable combination of θ_m and n chosen bearing in mind that the surface loading on the first maturation pond must be less than that of the preceeding facultative (Mara *et al.*, 1992). The larger the value of n, the smaller the value of θ_m and, hence, the land area requirements will be minimal. The design equation 1.22 does, however, have a number of limitations (Mills *et al.*, 1992). Firstly, the assumption of complete mixing is often invalid and a different hydraulic regime, dispersed flow, dominates. The contents of a dispersed flow reactor are not homogeneous and the effluent quality will reflect the amount of time it takes for water to flow from the inlet to the outlet. Secondly, the relationship between the pond environment and the first order removal coefficient is unlikely to be reflected by air temperature alone. Silva (1982) found that the value of k_T in equation 1.22 depended on surface BOD loading and decreased with increasing loadings indicating that the survival of faecal coliforms may be enhanced as conditions become progressively more anaerobic. Thirdly, when used to

predict FC effluent numbers, the Marais equation adopts a kT value equal for all ponds in a series but it is assumed that die-off rates in maturation ponds are considerably faster in maturation ponds than in anaerobic or facultative ponds so the validity of using the same kT values is questionable (Mills *et al.*, 1992). Cairncross and Feachem (1983) produced an alternative design equation in which the kT value in the anaerobic pond was assumed to be half that in the facultative and maturation ponds. However, subsequent work by Mills *et al.* (1992) in Kenya has shown that removal rates in anaerobic ponds may be as good as, or even better than, in the following ponds. It appears that the inherent overdesign of the Marais equation may well account for its success.

1.5 Effluent Standards

Effluent standards are generally expressed by regulatory bodies in terms of, for example:

(i) organic matter, commonly BOD but increasingly COD

(ii) suspended solids

(iii) nitrogen (total N, ammonia and oxidised N)

(iv) total phosphorus

(v) numbers of faecal coliform bacteria

(vi) numbers of human intestinal nematode eggs (*Ascaris lumbricoides, Trichuris trichiura* and the human hookworms)

(vii) numbers of human trematode eggs (*Schistosoma* spp.)

In many developing countries effluent standards in use are based on the United Kingdom Royal Commission standard (20 mg l^{-1} BOD5 and 30 mg l^{-1} SS) which is inappropriate in since (i) the BOD5 and suspended solids of pond effluents depend largely on algal concentrations and are not, therefore, a

measure of the degree to which the sewage has been treated and (ii) where discharged into a watercourse, water from which is likely to be extracted for domestic use, or where the final effluent is likely to be used for irrigation or fish culture, a standard that includes bacteriological parameters is required.

Effluent standards should depend on intended end use. IRCWD (1985) concluded that previously used guidelines, such as those recommended by Arthur (1982), were overly conservative and restrict appropriate project development thereby encouraging unregulated human use.

1.5.1 Algal Concentration and Effluent Standards

Algae are nearly invariably associated with the discharge from stabilization ponds and it is quite common that the effluent is a bright pea-green colour because of the algal mass it contains (Konig, 1984). The production of algae within the pond represents the reverse of the process which is normally considered to be that of waste stabilization. Waste stabilization is a process of biological oxidation by which the large putrescible organic molecules of pollution (proteins, peptides, carbohydrates, greases, etc.) are turned ultimately into simple stable inorganic molecules such as carbon dioxide, sulphates, phosphates, nitrates, and water. The production of algae starts with the simple inorganic and finishes, with the assistance of solar radiation, as complex organics. This creates two problems as regards effluent standards:

(i) High algal concentrations make it difficult for WSP effluents to meet total suspended solids (TSS) limitations;

(ii) High algal concentrations can add very greatly to the content of biodegradable organic matter, BOD, in the effluent.

The concentration of the algae in the effluent can increase the BOD5 of the discharge by a factor of four, or of six or even higher (Ellis 1983). In addition,

the acceptably low suspended solids content of most pond system effluents can leap up to in excess of 100 mg l^{-1} or even 200 mg l^{-1} as the result of algal blooms. In certain circumstances, however, the presence of high algal concentrations in pond system effluents could be beneficial and there is a good argument for relaxing the suspended solids and BOD standards for effluents in the case of waste stabilization ponds. Bartsch (1961) stated that BOD is almost meaningless as an effluent standard when it involves algal laden samples. This is because:

(i) algal cells are not immediately biodegradable and may disperse over a wide area before contributing to the oxygen demand;

(ii) algae may result in a net oxygen input to the water course;

(iii) in favourable conditions the algae may be able to multiply and out-compete any toxic algae, such as blue-greens, present;

(iv) discharge of algae may be advantageous, in some cases increasing the productivity of the receiving water, for example, in the case of shell-fisheries.

There are numerous techniques for the removal of algae from effluents, these include:

(i) Coagulation and flocculation using alum and acid, magnesium hydroxide and lime chitosan, chlorine and auto flocculation.

(ii) Sand filtration - rapid sand filters, slow sand filters and intermittent slow sand filters.

(iii) Pond operational techniques - variable level discharge and limited period discharge.

(iv) Biological methods - water hyacinth and duckweeds.

(v) Dissolved air flotation

(vi) Horizontal flow rock filters.

(vii) Land application.

It is generally agreed that due to the high costs of employing algal removal, which are contrary to the concept of use of WSP, such methods should not be used.

The algae, therefore, should be regarded as "ultimate" BOD rather than as a organic load on the receiving water body. Blue-green algae appear to cause more of a problem then flagellates or green algae but they are only found in very small numbers around the edges of the ponds (Pearson, 1987).

Gloyna and Tischler (1981) recommended that the EPA should make WSP a special case with regard to effluent TSS, because removal of algae is expensive and eliminates the advantage of low operation costs of WSP systems. Indeed, in the USA waste stabilization pond effluents can have a BOD of up to 45 mg l^{-1} (EPA, 1977), in France up to 40 mg l^{-1} (on filtered samples) (Circulaire Interministérielle, 1980), and in Germany an allowance is made on the basis that 100 μg chlorophyll-*a* is equivalent to 3 mg BOD (Bucksteeg, 1987).

1.6 Microbiology of Waste Stabilization Ponds

Microorganisms found in water and wastewater fall into four general groups: viruses, procaryotic organisms, eucaryotic organisms and simple invertebrates. Higher life forms are also present in the system but are of little importance.

1.6.1 Bacteria

A procaryotic group, bacteria are the organisms of principal interest since a number of species are pathogenic. A WSP environment is so diverse in terms of its constituent microhabitats that most aquatic bacterial groups will be

represented and implicated, either directly or indirectly, in the overall treatment process (Curtis, 1990). The bacteria present in the aerobic conditions of facultative ponds are essentially those saprophytes present in the original sewage. These include *Beggiatoa alba, Sphaerotilus natans, Alicaligenes, Achromobacter, Flavobacteria, Pseudomonas,* and *Zoogloea* spp. (EPA, 1983). In their studies on laboratory scale WSP, Gann *et al.* (1968) showed the bacterial flora to consist mainly of saprophytic gram-negative rod-shaped bacteria. The most numerous was *Achromobacter,* followed by *Pseudomonas* and then *Flavobacteria.* Bacteria of the genus *Bacillus* were also present. These results compared favourably with field counts. Purple sulphur bacteria are found in the anaerobic layer, normally at a specific depth, in a thin layer, where light and nutritional conditions are optimum (Houghton and Mara, 1992).

Pathogenic bacteria present in wastewater and WSP have been discussed in section 1.2.2.

1.6.2 Algae

Eucaryotic organisms, algal speciation in WSP varies considerably depending on pond type and organic loading and algae are good, rapid indicators of pond performance. Commonly they belong to the divisions of Chlorophyta, Euglenophyta and Chrysophyta, but Pearson (1987) reports that the dominant genera are usually members of the first two divisions. *Euglena* and *Chlorella* are particularly common. Cyanobacteria may also be involved in important processes in waste stabilization ponds such as anoxygenic photosynthesis using sulphide as an electron donor under anaerobic conditions. Palmer (1969) reported 21 genera of algae in one pond on a single day and 83 genera during a two year study of three ponds in Indiana, USA. Table 1.7 shows examples of algal genera present in tropical WSP.

Table 1.7 **Examples of Algal Genera Present in Tropical Waste Stabilization Ponds**

Genus	POND	
	Facultative	Maturation
Euglena	+	+
Phacus	+	+
Chlamydomonas	+	+
Chlorogonium	+	+
Pyrobotrys	+	+
Eudorina	+	+
Pandorina	+	+
Scenedesmus	-	+
Volvox	+	+
Dictyosphaerium	-	+
Oocystis	-	+
Cyclotella	-	+
Ankistrodesmus	-	+
Chlorella	+	+
Micractinium	-	+
Rhodomonas	-	+
Coelastrum	-	+
Navicula	-	+
Cryptomonas	+	+
Oscillatorio	+	+
Anabaena	-	+
Spiralina	-	+
Selenastrum	-	+
Carteria	+	+
Coelastrum	-	+

+ = present - = absent

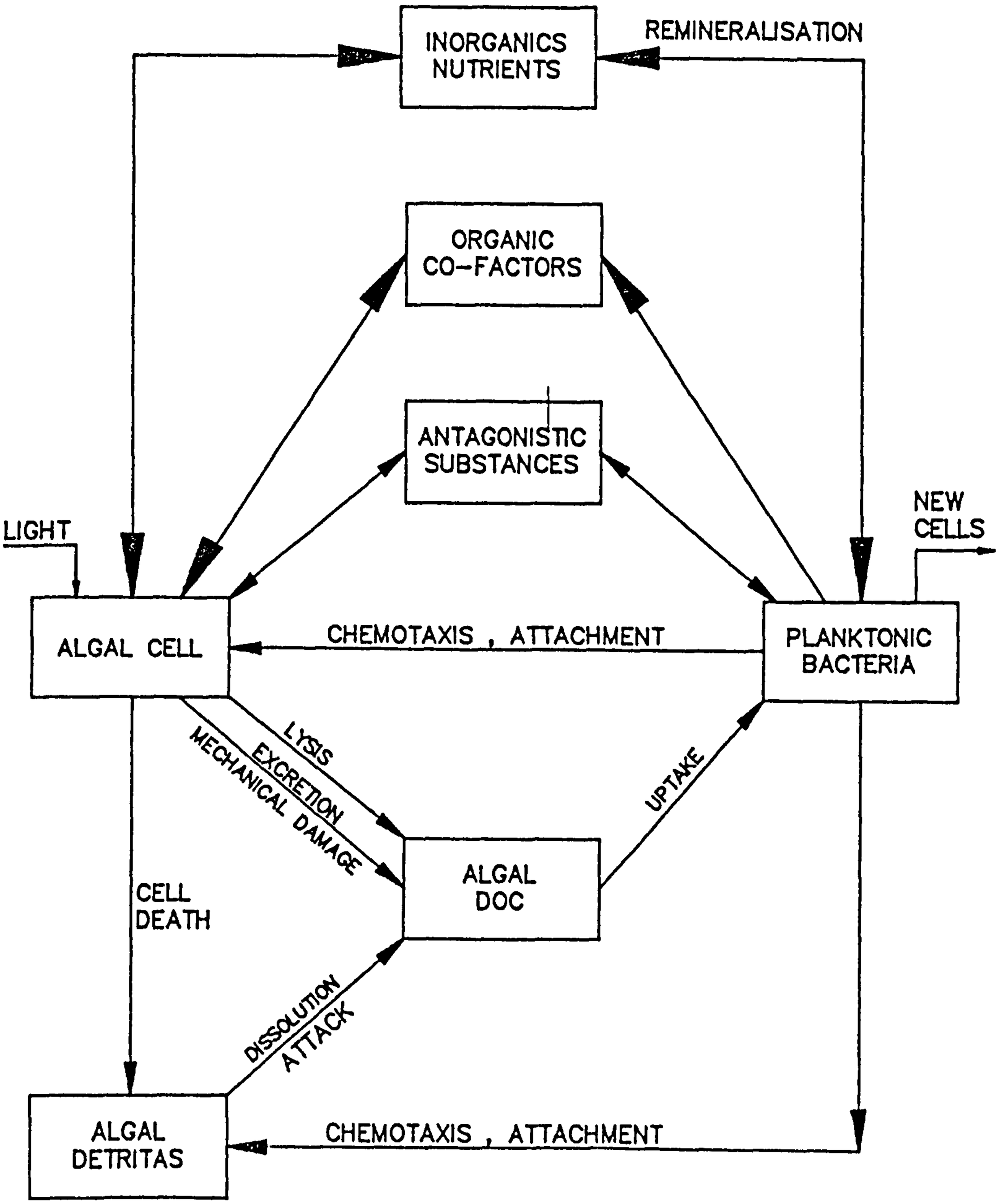
Source: Mara and Pearson (1986)

The organic loading applied to WSP has been found to be a fundamental factor controlling algal diversity. According to Palmer (1969), the organic pollution tends to influence the algal flora far more than other factors such as light intensity, pH, dissolved oxygen, hardness, temperature and other types of pollutants. Konig (1984) identified 16 genera in maturation ponds with organic loadings of less than 100 kg BOD5 ha^{-1} d^{-1}. This decreased to about 9 when the organic loadings were increased to more than 100 kg BOD5 ha^{-1} d^{-1}. Two secondary facultative ponds with organic loadings of 116 and 375 kg BOD5 ha^{-1} d^{-1}. had 10 and 5 genera respectively. Konig (1984) also found that flagellates such as *Euglena* and *Chlamydomonas* dominate with high loadings and they may exist almost as a monoculture in highly loaded facultative ponds. Over-loading can sometimes be recognised by a change in the colour from green to purple as purple sulphur bacteria (members of the Chromatiaceae) predominate. Under-loaded ponds are often those found with the most diverse algal populations. The greater clarity of maturation ponds favours non-motile algae in general and algae tends to be distributed evenly throughout the water column (Konig, 1984). The non-motile *Chlorella* often dominates in maturation ponds and it is thought that this could be due to its resistance to ammonia toxicity (Pearson *et al.*, 1987). In deeper lagoons, where the pH is lower and the amount of ammonia present in its more toxic unionised form is also, therefore, lower, motile algae predominate (de Oliveira, 1990).

1.6.3 Relationship between Algae and Bacteria

The relationship between the microalgae and bacteria is frequently explained, somewhat simplistically, in terms of a mutual association. This produces carbon dioxide and releases inorganic nutrients which in turn are utilised by the algae to grow and photosynthesize (Figure 1.2). Efficient operation of algal treatment systems depends on the establishment of an equilibrium

Figure 1.2 A Simplified Diagram of the Mutual Association between Algae and Bacteria.



The large headed arrows denote the predominant direction of a process.

Adapted from Cole (1982)

between the overall oxygen production by the algae and its consumption by the bacteria and it is generally considered that an optimum biomass ratio for bacteria to algae is in the range 1-2 : 3 (Pearson *et al.*, 1987b). Bacterial metabolism may, however, modify the environment to the detriment of algal growth. If the supply of organic matter is sufficient, aerobic respiration can use up the supply of DO. Under anaerobic conditions most algal taxa would be inhibited by the lack of oxygen (Cole, 1982). Oxygen depletion during daylight hours is unusual in the photic zone, however, consumption of oxygen at night in extremely eutrophic environments could pose a problem for the algae. Bacterial metabolism may also lower the pH both through production of organic acids and through oxidation of NH_4, H_2S etc. There may be some competition between algae and bacteria for nutrients if there is a limited supply of some essential inorganic nutrient, such as N or P, and bacterial uptake of these nutrients could have a temporary impact on algal growth. Some bacterial products are known to stimulate algae (Cole, 1982). The major source of vitamin B_{12} in open waters is probably synthesis by heterotrophic bacteria (Cole, 1982). Similarly, algae may stimulate bacteria and it is likely that the production of planktonic bacteria is dependent upon the production of planktonic algae in many systems (Pearson *et al.*, 1987b). Inhibition of bacterial growth by algae does occur and antibiotics have been isolated from some species of algae, eg. Chlorella, (Pratt *et al.* 1944; Hoppe *et al.* 1979). Other researchers have shown that many algal taxa release substances inhibitory to bacteria (Gupta and Shrivastava, 1965).

1.6.4 Viruses

Viruses are obligate parasites which simply survive outside their host without increasing or decreasing their numbers. Little work has been done on viruses in ponds but they are important when considering pathogen removal from WSPs since the necessary infective dose may be assumed to be

as little as 1 - 10 organisms (Butler *et al.*, 1982).. It is known that over 100 different viruses are faecally excreted by man (Butler *et al.*, 1982) and the diseases they cause range from the trivial to serious and even fatal. The concentration of viruses in raw sewage varies and whereas figures from Israel are high, concentrations in USA are 100 times lower (Irving, 1982). Wastewater may contain human enteric viruses, animal viruses from household pets, effluent of farms and abattoirs and in storm water. Studies show that a large population with a young age structure and poor living conditions tend to produce wastewater with high enteric viral counts (Irving, 1982). There will also be climatic, seasonal and diurnal variations with increased load whenever an outbreak of infection due to a virus excreted in faeces occurs. The types of virus in wastewater reflect those currently circulating in the contributing population (Sellwood *et al.*, 1981). Poliovirus types 1, 2 and 3, both wild and vaccine strains, have been reported in domestic wastes, some of the coxsackie A viruses, all of the coxsackie B and many echoviruses have been detected. Reovirus types 1, 2 and 3, adenovirus, rotavirus and hepatitis A have also been detected. If appropriate techniques were available other viruses would also be expected to be found in wastewater, since epidemiological evidence suggests that Norwalk agent (WHO, 1980; Murphy *et al.*, 1979) and at least one agent causing non A, non B hepatitis (Khuroo, 1980) can be spread via the water route.

1.6.5 Intestinal Parasites and Protozoa

(i) Protozoa. The protozoa are a group of generally motile organisms in most cases predatory, often feeding on bacteria. There are three main groups: amoebae, ciliates and flagellates. The amoeba *Entamoeba histolytica* is an important pathogen causing amoebic dysentery and the flagellate *Giardia lamblia,* which is worldwide in its distribution, can produce a variety of

intestinal symptoms, most frequently in a protracted intermittent diarrhoea. The coccidians *Cryptosporidium* are intracellular parasites causing acute self-limiting gastroenteritis in immunocompetent people for which, at present, there is no effective treatment. The ciliates are also important, being common in sewage treatment works, where they consume considerable numbers of bacteria.

(ii) Helminths. The three main groups of parasitic worms are nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes). The roundworms include *Ancylostoma* and *Necator*, two species of hookworm and *Ascaris lumbricoides* . Three genera of tapeworm are parasites of the human gut. These are *Diphyllobothrium*, *Taenia* and *Hymenolepis*. Intestinal flukes of man include *Fasciola hepatica* (which infects the bile ducts), *Heterophyes heterophyes*, *Metagonimus yokogawai* and *Gastrodiscoides hominis*. Infection by hookworms is often symptomless, but when it does produce illness it is frequently as anaemia and its resulting weakness, debility and other consequences. In areas of high transmission, heavy worm burdens can be built up in early childhood and there may be retardation of mental and physical development (Feachem *et al.*, 1977). *A. lumbricoides* infects the small intestine of man and when symptoms appear they are frequently pneumonitis with cough, fever, breathing difficulties, pain and sometimes blood stained sputum. Heavy burdens may cause digestive disorders, nausea, abdominal pain, vomiting, restlessness and disturbed sleep (Ayres, 1992). Tapeworms may cause abdominal pain, loss of appetite and dizziness. The flukes are generally regarded as of only minor public health importance.

Waste stabilization ponds are able to eliminate helminth eggs completely and reliably.

1.7 Pathogen Removal in Waste Stabilization Ponds

The ability of WSP to remove pathogens depends on the biological and physical nature of the organism in question. Pathogens may be divided into three groups; helminths and protozoa, viruses and bacteria. It has frequently been noted that different types of ponds remove pathogens at different rates (Silva, 1982; de Oliveira, 1990; Curtis, 1986).

1.7.1 Helminths and Protozoa

Helminths eggs and protozoa cysts are heavier than water and so removal is by sedimentation. Their settling velocities are quite high (for example, 3.4 x 10^{-4} m s^{-1} in the case of *Ascaris lumbricoides*) (Shephard, 1977) and consequently removal takes place in the anaerobic and facultative ponds. Mara and Silva (1986) state that a minimum of 2 ponds with a total retention time of 20 days will ensure removal of helminths. Hookworm larvae may survive for up to 16 days in aerobic ponds but have not been reported in pond effluents with a retention time of > 20 days (Ayres, 1992). Schistosome eggs will settle out in anaerobic ponds. In a facultative pond they will either settle or hatch into miracidia, which will either die or infect an intermediate snail host if the correct species is present in the pond (in general ponds are not suitable environments for snail vectors) (Ayres, 1992). Grimason *et al.* (1993) studied the removal of *Cryptosporidium* oocysts and *Giardia* cysts in 12 Kenyan ponds systems with retention days of 15-62 days. The concentration in the raw sewage was 0-73 oocysts l^{-1} and 0-6200 cysts l^{-1} and no oocysts or cysts were detected in the final effluents.

Recent work by Ayres *et al.* (1992) has made it possible to design WSP for helminth removal which may be necessary if the effluent were to be used for irrigation.

1.7.2 Viruses

The mechanism of virus removal in WSP is not clear and it appears that different viruses may have differing abilities to survive the sewage treatment process (Clarke *et al.*, 1961; Russell *et al.*, 1962). Farrah *et al.* (1978) and Gerba *et al.* (1980) attributed these differing abilities to different adsorptive behaviour dependent on net electron negativity in a particular environment. Funderburg *et al.* (1978) found a good correlation between poliovirus and high pH and chlorophyll *a*. Generally it is considered that removal is by adsorption on to settleable solids. Following studies at three sites in India, Rao *et al.* (1981) suggested that other factors involved could include prolonged exposure to temperatures between 20 and 40 °C, high ammonia concentrations, algae-bacterial virucidal activity and sludge activity in the anaerobic zone of the pond. Oragui *et al.* (1987) observed excellent removal of rotavirus and enterovirus from anaerobic ponds but do not make any suggestions as to the mechanism of the virus removal. Feachem *et al.* (1983) found a 1-2 log unit reduction per 5 days in ponds at temperatures > 25 °C. High pH, adsorption and sunlight are factors which may have a role in virus removal from WSP but it is clear that further research is needed. Another important factor in considering virus removal is the original concentration and the ease with which they can be demonstrated in a particular detection procedure. The viruses found in the highest number before treatment will most likely be present in the effluent.

Primary treatment, by settling and retention, can only be expected to remove 0-50% of the initial load. However, much of this influent virus is in embedded in solids and, therefore, may not be included in the measurement of initial virus concentration, but removed as settling takes place (Berg, 1973; Wellings *et al.*, 1976) so that the actual reduction may be greater than supposed.

Secondary treatment is more effective and can remove 90-99%. The well-established processes of trickling filtration, activated sludge and oxidation/stabilization pond treatment are all capable of producing effluent with much reduced viral concentrations. Temperature, ultra-violet light, salinity and algal and bacterial virucidal effects have a major influence on virus reduction in oxidation/stabilization ponds. Rao *et al.* (1981) reported on two full scale and two pilot waste stabilization ponds in India. With a retention time of 17.2 days 88-99% of the viruses were removed. In Israel (Katzenelson and Kedmi, 1979) the virus content of stabilization pond effluents have varied from 17-58 PFU l^{-1} after 5 days storage and 28-572 PFU l^{-1} after 20 days (Shuval, 1970). In the USA and average of 3.4 PFU l^{-1} was found after 18 days retention and 0.6 PFU l^{-1} after 98 days (Vithalbhai *et al.*, 1982).

1.7.3 Bacteria

Most of the published work on the removal of bacteria from WSP is confined to FC, FS and Salmonellae. The validity of this approach has been discussed in section 1.2.4 . Faecal bacteria are mainly removed in facultative and especially maturation ponds, although there is some removal in anaerobic ponds. A considerable number of articles have been published since Caldwell (1946) drew attention to the excellent FC removal by WSP. Table 1.8 summarises the proposed factors involved in FC removal.

As the literature reveals, the die-off of bacteria in WSP depends on environmental and climatological parameters and these factors are discussed below:

1.7.3.1 Temperature

In many of the coliform die-off studies, it is stated that the first-order coliform die-off constant is temperature dependent (Bowles, *et al.*, 1979; Ferrara and Harleman, 1980; Klock, 1971; Mancini, 1978; Marais, 1974) and, therefore,

Table 1.8 **Publications Proposing the Factors Responsible for FC Removal from WSPs.**

Feature Controlling Removal	Reference
Algal derived antibiotics	Caldwell, 1946
Aggregation and Sedimentation	Gannon *et al*, 1983
Bacteriophage attack	Ellis, 1983; Verstraete & Voets, 1976
Predation	Fenchel, 1980; Chamberlain & Mitchall, 1978
Protozoal predation	Ellis, 1983
Algal species	Parker, 1962; Davis and Gloyna, 1972
High pH	Parhad & Rao, 1974; Hirn *et al*, 1980
Dissolved oxygen	Hane *et al*, 1964; Feachem *et al*, 1983
Redox potential	Klock, 1971
Algal toxins/Antibacterial substances	Davis & Gloyna, 1972; Hoppe *et al*, 1979
Temperature	Pearson *et al*, 1987
Nutrient limitations	Klock, 1971
Solar radiation	Oswald & Gotaas, 1955; Moeller & Calkins, 1980; Mancini, 1978; Curtis, 1990
Starvation	Smallhorst *et al*, 1953; Wu and Klein, 1976

almost no other data was made available to compare the die-off rate constants of different studies. Moeller and Calkins (1980) found no temperature-related reductions in coliform densities over a temperature range of <10 °C to >25 °C and have stated that the first-order decay constant is light intensity dependent. Pearson *et al.* (1987c) found that temperature accelerates bacterial die-off and presumed that this be due to to increase in metabolic activity and, thus, susceptability to toxic substances. Temperature will also accelerate substrate utilisation and thus the onset of starvation conditions. Chamberlain and Mitchall (1978) in reviewing the literature on coliform removal rates in lakes and streams found widely varying t90 values (the time taken for a 90% reduction in numbers) at a particular temperature. Die-off in facultative ponds with reductions as high as 95% at 25 °C have been reported by Arthur (1982).

1.7.3.2 Solar radiation

Toms *et al.* (1975) reported that the main mechanism is the exposure to daylight. In a study on three maturation ponds with the same hydraulic retention time Silva (1982) found that values of k_T varied with pond M1 = 2.93 d^{-1}, M2 = 8.81 d^{-1} and M3 = 2.99 d^{-1}. Silva suggested that the high rate for the constant in M2 was due to the effect of a dense algal scum on the surface of the pond. This would reduce the amount of solar radiation penetrating into the water. Moeller and Calkins (1980) concluded that UV light (particularly its germicidal component UV-B (280 - 320 nm)) is responsible for the removal of FC from WSP and they detected UV-B at depths of up to 0.4 m. These authors determined that FC survival can be less than 0.00002% within the first 10 cm of the surface of a pond, 5% within the second 10 cm layer and 100% below 30 cm and that die-off may be reduced by more than 2 orders of magnitude on cloudy days. More recent work carried out by Curtis (1990) has since showed that UV-B can hardly penetrate the water

column of WSPs. Work carried out in Tanzanian ponds by Mayo (1989) demonstrated the importance of light in coliform removal when FC removal was found to vary with depth in clear bottles but not opaque bottles but the authors did not measure or control pH so this difference may have been partly due to a rise in pH. Smallman (1986) undertook investigations into FC removal by suspending pond water in dialysis bags at different depths in anaerobic, facultative and maturation ponds. Removal in the anaerobic pond was negligible and in the facultative and maturation ponds an increased rate of removal was not always observed. Curtis (1990) concluded that light kills FC in WSP principally by photosensitisation that interacts synergistically with elevated pH but that the removal is dependant on the presence of oxygen in a process of photo-oxidation probably because the toxic forms of oxygen damage the inner membrane of FC. Polprasert *et al.* (1983) gave the die-off constant as a function of temperature, light intensity, algal concentration and organic loading. However, the effect of the last two factors seems insignificant as compared to the first two. Generally WSP are very turbid and bactericidal light is known not to penetrate the water as well as other wavelengths of sunlight (Baker and Smith, 1982) making the understanding of the role of sunlight in bacterial removal more complicated.

1.7.3.3 pH

High pH values may develop in ponds due to intense algal photosynthesis. A synergistic effect involving light and pH is suggested by Pearson *et al.* (1987) after they found that pH was a major factor causing coliform die-off in ponds in Loures, Portugal. In vertical transects they found that the greatest FC removal was at the points where pH approached or exceeded 9.0 i.e. in the top 40 - 50 cm of the pond. Parhad and Rao (1974) also concluded that the role of pH in FC die-off is important following investigations in which *E. coli* populations decreased in the presence of pH levels >9.2. Many ponds

function well without ever reaching the high pH which Pearson *et al.* (1987) and Parhad and Rao (1974) state as being the critical level. Hirn *et al.* (1980) found the die-off in summer greater than that at other times of the year in Finland and concluded that this was a pH effect except where *Clostridium perfringens* was concerned. Fernandez *et al.* (1992) found that pH values close to 9, as a single factor do not play an essential role in the die-off of FC bacteria. It has been suggested (Trousellier *et al.*, 1986) that pH may effect bacteria in two ways - internally, by the ionization of the membrane constituents and inactivation of enzymes, and externally, by the modification of ionic disassociation and solubilities with a consequent reduction of the cells absorbtion capacities. Other possible effects include the inability of the bacteria to maintain its' internal pH (of 7.6 - 7.8 in the case of *E. coli*) or the inability to maintain a H^+ gradient over the cytoplasmic membrane.

1.7.3.4 Dissolved Oxygen

Intense algal photosynthesis also results in high levels of dissolved oxygen usually in the surface layers. These supersaturation levels have been reported to accelerate coliform deaths (Feachem *et al.*, 1983) but Pearson *et al.* (1987) found that high dissolved oxygen levels alone had little effect on the rate of FC die-off in waste stabilization ponds.

1.7.3.5 Predation

It is known that protozoa are able to consume bacteria sufficiently quickly to effect significantly their numbers (Fenchel, 1980) and that salmonellae are eaten more quickly than other bacteria (Mallory *et al.*, 1983). This difference has been attributed to the relatively low growth rates of enteric bacteria in the environment. Any pathogenic bacteria unable to grow due to low nutrient availability would be expected to be removed more quickly. Protozoans have been found in WSP but to date there has been no attempt to discover the

influence they have on bacteria removal in WSP. Chamberlain & Mitchall (1978) also note predation as one of the mechanisms involved in bacterial removal.

1.7.3.6 Starvation

Considering that die-off is faster in maturation ponds several authors (Smallhorst *et al.*, 1953; Towne *et al.*, 1957; Klock, 1971; Wu and Klein, 1976) have suggested that nutrient concentration and competition for nutrients are important factors in determining die-off rates. As purification progresses through a series of ponds, the numbers of bacteria are reduced and this may be related to a reduction in organic carbon. Legendre *et al.* (1984) found that the aerobic heterotrophs at the front end of a lagoon were controlled by the influent nutrient concentration but in subsequent zones organisms which are commonly used as pollution indicators were selectively replaced by organisms which could adapt more easily to the nutrient limiting environment. Silva (1982) applied the Marais equations to primary facultative ponds and found that k_T increased as the organic loading decreases. This would suggest a starvation factor. James (1987) also attributes bacterial die-off to starvation. The bacteria must maintain a hydrogen gradient over its cytoplasmic membrane if it is to survive low nutrient conditions. To do this various proteins are induced by the cell and this changes the efficiency of the cell at obtaining nutrients at low concentrations and and makes the cell more resistant to starvation. The energy and 'raw materials' for this are supplied from the intracellular pools of glycogen, protein and ribosomal RNA. These are rapidly used up in the first few hours of starvation conditions and further survival depends on drawing on endogenous reserves until the hydrogen gradient can no longer be maintained or all of the ribosomal RNA is metabolized and proteins can no longer be produced.

1.7.3.7 Other Mechanisms

Enteric bacteria, and probably some viruses, have structures known as adhesins which help the organism colonise the gut (De Graaf and Mooi, 1986). These adhesins normally bind specifically to one of the various sugars in the mucous membrane of the gut lining but it is possible that some bacteria use these adhesins to bind to the sugars in algal mucilages and are, therefore, removed with the algae by, for example, sedimentation. Tamplin *et al.* (1990) demonstrated this ability with *V. cholerae* attached to algae.

Gannon *et al.* (1983) concluded from their work that aggregation and sedimentation played an important role in FC die-off. Bacteriophage attack is listed by Ellis (1983). Algae may have a direct effect on bacterial numbers through the action of algal toxins and antibacterial substances (Davis & Gloyna, 1972; Hoppe *et al.*, 1979). However, Toms *et al.*, (1975) state that there is no evidence to suggest that algae release a toxin lethal to bacteria. Humic substances, ubiquitous in sewage and WSP, absorb sunlight and pass the energy to oxygen leading to the formation of toxic forms of oxygen (singlet oxygen, hydrogen peroxide and probably superoxide and hydroxyl radicals). These can damage and kill FC in pond water (Curtis, 1992a).

There is a general agreement that bacterial die-off is greater during periods of intense algal activity (Patil *et al.*, 1975) but whether this is due to direct toxic effects or indirect effects such as elevated pH and dissolved oxygen has not been established.

Despite the amount of literature published, the die-off mechanisms in WSP remain somewhat hazy. It would seem that no one factor can be wholly responsible for the removal of faecal coliforms but this may be because there is no single factor involved in the reduction of FC numbers. There is good

evidence that pH and light, acting separately or through some sort of interaction, are responsible for the FC removal in WSP. Troussellier *et al.* (1986) hypothesized a complex web of factors involved in the removal of FCs from stabilisation ponds. They found that light, pH and algal concentration had high correlations with the reduction in FC when analysed using a technique known as causal pathway analysis (an extension of multiple regression analysis). There is one thing that is known for sure, the mechanism of bacterial die-off in waste stabilization ponds is an area not fully understood as yet and the actions and interactions of the proposed mechanisms need to be studied further to clarify this situation.

1.8 Influence of Pond Shape and Depth

Pond efficiency is a function of biochemical transformations and hydraulic transport and also environmental and climatological factors such as wind, rain, sunlight intensity etc. No model has yet been found which can describe accurately all these variables affecting WSPs (Curtis, 1992b).

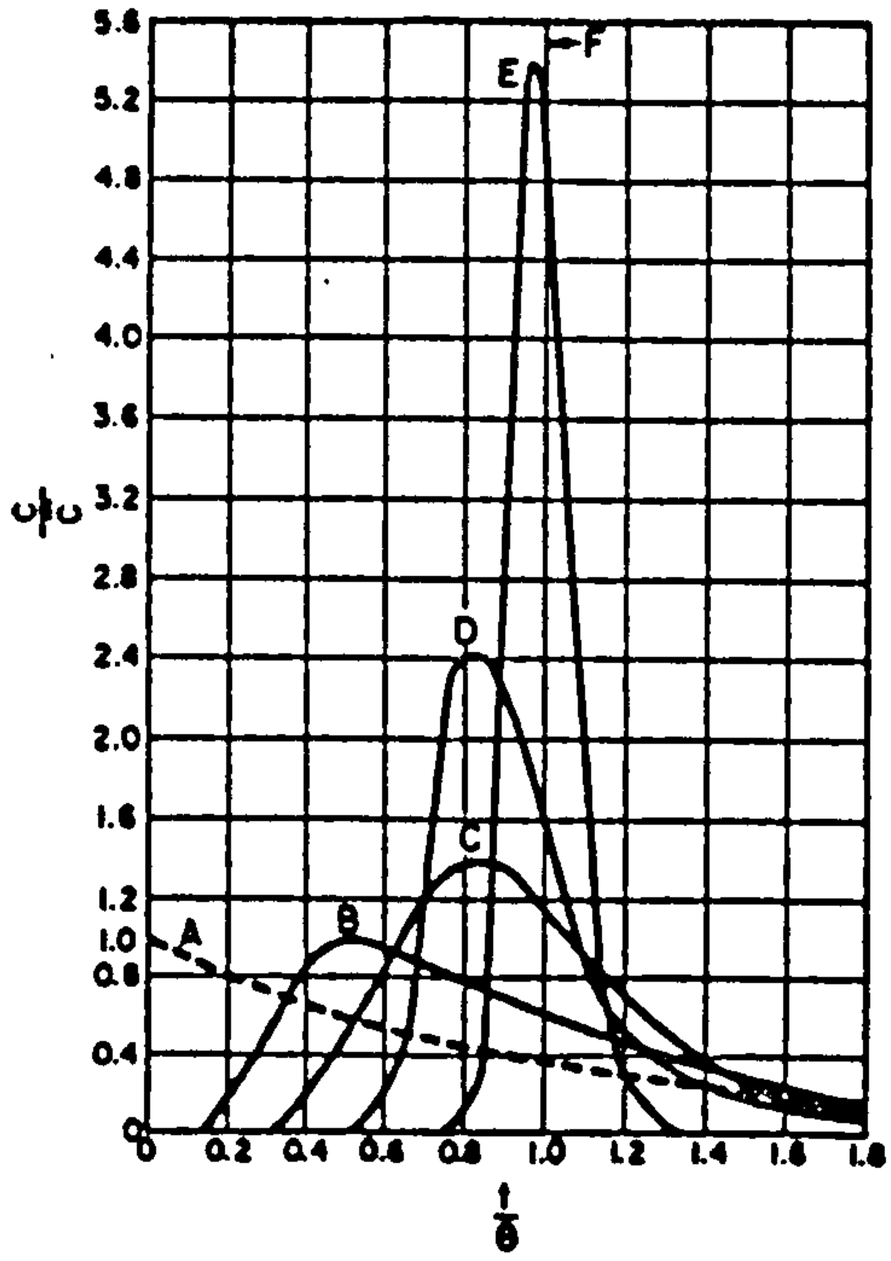
There are two design approaches for WSPs - empirical, based on experimentally derived data, which does not allow extrapolation of pond performance data to all locations and environmental conditions but is a safer method, and rational, a theoretical approach which has not yet produced a model for general applicability (Marecos do Monte, 1985). Hydraulic flow of WSP may be assumed to be either complete mixing, plug flow (both describing ideal conditions) and dispersed flow (non-ideal or real-flow conditions) (Marecos do Monte and Mara, 1987). The importance of mixing characteristics, i.e., short-circuiting, stability and dispersion, in terms of wastewater treatment efficiencies were first recognised almost 50 years ago

(Camp, 1945). Figure 1.3 shows the difference between these hydraulic flow conditions. There have been attempts to model WSP performance in a comprehensive way through a combined or finite stage model which represents the mixing processes in a pond by a network of combinations of completely mixed, short-circuiting plug flow and dead flow stages. However, finite stage models require many input parameters for which values may not be available and also require fairly sophisticated computer analysis for their solution and so are not generally suitable for design purposes.

1.8.1 Shape

Pond shape affects the dispersion number, resistance to flow, retention time, volume of dead zones and the extent of short-circuiting (Agunwamba *et al.*, 1992). As the length to breadth ratio decreases the dispersion number increases. It is claimed that the dispersion number, in turn, affects the pond performance and if it is over-estimated, then pond efficiency is under-rated and the pond will not be optimally utilised; if it is under-estimated, the pond may be over-loaded. Flow resistance is complicated by the shape effect and secondary circulation (affected by the breadth to depth ratio). As the length to breadth and breadth to depth ratios increase so do the volumes of dead zones (Mangelson and Watters, 1972) thus a deep, long rectangular pond will have the largest dead zones and retention times will be reduced accordingly and short-circuiting is more likely to occur.. Dead zones reduce the effective retention time of ponds by reducing the capacity. This results in the wastewater not staying in the pond long enough to be adequately treated. Inclusion of pond dispersion characteristics into design equations could yield better prediction results because they would account for a ponds hydraulic phenomena i.e. pond shape, flow velocity, short-circuiting and in- and outlet

Figure 1.3 Dimentionless Plot of Dispersion Illustrating Different Hydraulic Conditions in Ponds



Curve A is the theoretical curve for ideal dispersion - completely mixed

Curves B, C, D and E show non-ideal dispersion - partially mixed conditions

Line F shows what would take place in an idealized pond with flow velocity the same throughout - plug-flow.

$\bar{C}$ = Concentration obtained if tracer was mixed instantaneously and uniformly throughout the pond.

C = Actual concentration of tracer

θ = Retention time of the pond

t = Actual time a certain tracer concentration appears at outlet

Source: Polprasert, Bhattarai and Kiran (1985)

devices. However, use of dispersed flow models does not account for the existence of dead zones which reduce the active volume of ponds having low length to breadth ratios.

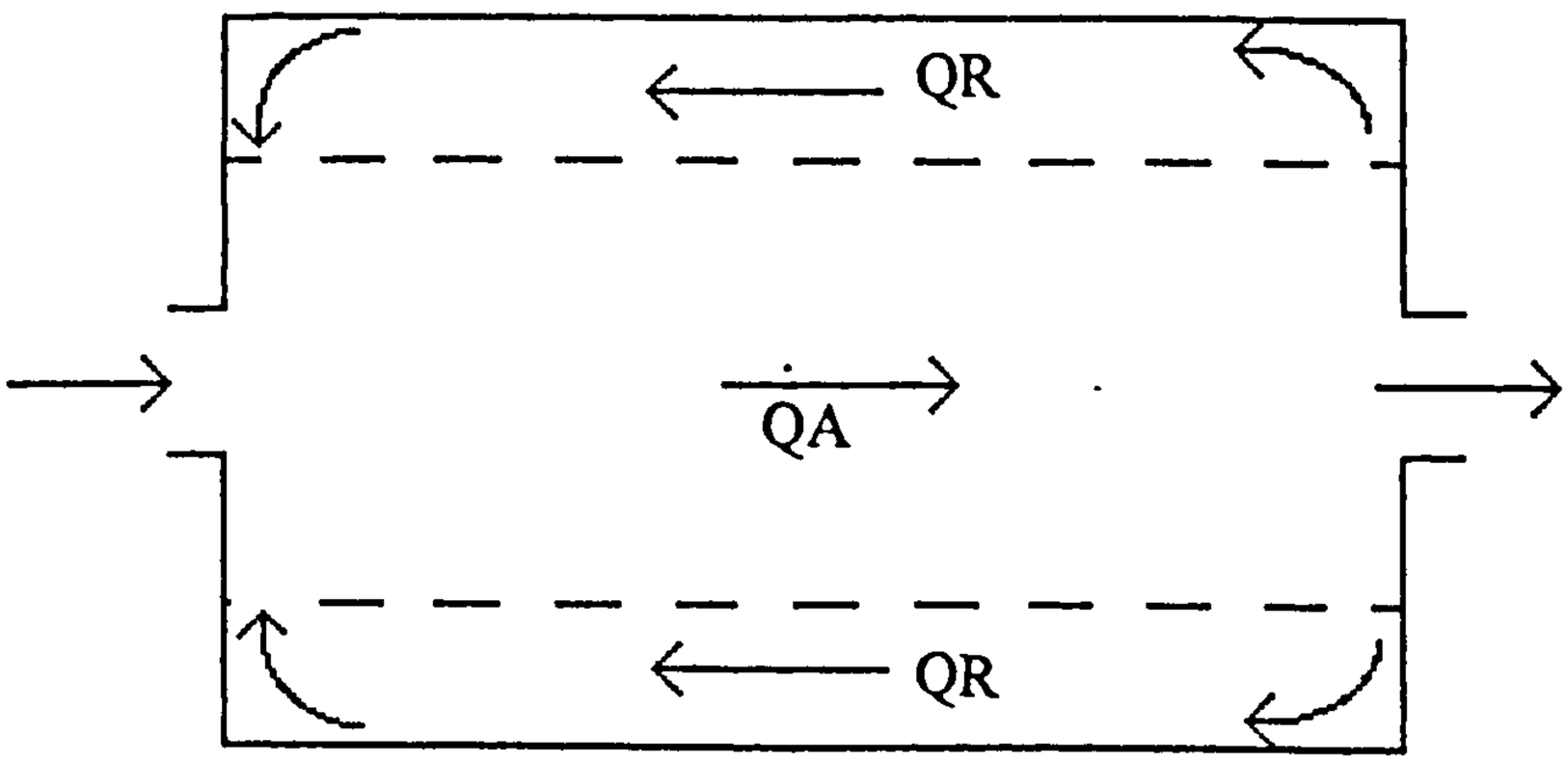
The dispersed flow method of describing the hydraulic regime in WSPs is supported by several authors including Thirumurthi (1974), Uhlmann *et al* (1983) and Polprasert and Bhattarai (1985). Tracer studies are used to determine dispersion numbers. However, Agunwamba (1991a) notes there is still no very accurate method of estimating the dispersion number, d, from these studies. There are other problems associated with tracer studies including the fact that they are tedious, time consuming and expensive. The tracers used (such as NaCl and fluorescent dyes) often possess dispersive characteristics quite different from the wastewater pollutants they are supposed to represent (Agunwamba, 1992a). Tracers are non-settleable and conservative and, thus, the determined values of d are bound to be very approximate. Also, the results obtained from a tracer study apply only to the given pond under the conditions during which the study was conducted. There can be no guarantee that the parameter values apply under different hydraulic or climatic conditions. Another problem identified by Agunwamba is that the time period between tracer release and measurement of tracer concentration. The dispersion number, d, is time-dependent within the convective period and time induced within the diffusive period of dispersion. During the convective period, movement of the tracer is primarily influenced by its initial convective velocity and so it does not follow the Taylor one-dimensional diffusion equation. But the diffusive period, which follows the convective period, satisfies the one-dimensional equation. Since the tracer clouds behave differently during these two periods the d value determined will also differ. Agunwamba calculated the distance from source (L) of the convective period (using data from various authors) and found it to be greater than the length of the ponds in all cases. Thus, in experimental

conditions it is impossible to take measurements within the diffusive period and values of d calculated using the one-dimensional equations from tracer readings taken in the convective period will be inaccurate.

Agunwamba (1992a) proposed a new method for determining the dispersion number without the use of tracer studies using the number of coliform bacteria with distance. This method, however, does not solve one of the greatest problems with tracer studies, namely that the dispersion number cannot be calculated for ponds not yet constructed.

Ferrara and Harleman (1980) used model simulations to indicate the efficiencies of various pond geometries and to investigate whether simple models such as completely mixed or plug flow could be effectively utilized in an overall model of dynamic water quality in ponds. Most ponds without mechanical mixing do not reach completely mixed conditions. The hydraulic pattern in a pond, proposed by Ferrara and Harleman, is shown in Figure 1.4. The model shows an active zone, the centre portion where the flow proceeds from the inlet to the outlet, and a return zone, the side portions where the water flows back from the outlet end of the pond to the inlet end. This return flow provides recirculation of material and dilution of the influent. The combined effect of the active and return flow zones will result in peak concentrations at the outlet before the theoretical retention time, the exact time will depend on the relative sizes of each zone. Ferrara and Harleman (1980) concluded that treatment efficiency predicted by a completely mixed model could provide a reasonable approximation to the hydraulic transport processes in ponds. Polprasert and Bhattarai (1983) disagreed with this conclusion and state that a completely mixed model is only true if the active zone is approximately half of the total pond volume. They suggest a partially mixed or dispersed flow equation to be used in pond design. Arceivala (1983) suggests that it would be possible to design ponds and lagoons more

Figure 1.4 Model of Hydraulic Pattern in a Pond According to Ferrara and Harleman (1981)



QA = Active Zone

QR = Rerun Flow Zone

economically if the geometry of the pond is manipulated to give the desired dispersion condition. If process requirements demand it, it should be possible to design ponds for complete mixing or plug flow. If a particular wastewater is best treated under completely mixed conditions, eg. an industrial waste with fluctuating flows or relatively toxic constituents (or both), the pond geometry should be consciously designed to promote good mixing by adopting a square or rectangular shape with low length to breadth ratio (less than 4). The unit can then be designed using the complete mixing model. If wastewater would benefit from plug flow type conditions (as with domestic sewage) geometry should be manipulated to give high L:W ratio (more than 4-8) either through elongated configuration or by the use of around the edge type baffles so the dispersion number is reduced as much as possible. Yhdego (1990) states that the predicted FC removal efficiency can be reached only if the system approaches plug flow conditions which requires a high length to breadth ratio. More recently, the PAHO Environmental Health Program defined a mathematical model by analyzing data from studies and evaluations of stabilization pond behaviour and found that in tropical areas the necessary area and volume of a pond could be minimized by using elongated facultative ponds with length to breadth ratios of at least 15:1 (Saenz, 1992).

Mangelson and Watters (1972) investigated the hydraulic flow characteristics of WSP in Utah, USA using tracer studies utilising rhodamine and found that as the length to breadth ratio increases conditions for plug flow are closer to being satisfied and, therefore, the efficiency of wastewater treatment increases. They noted that many factors influence the accuracy of the pond data. These include environmental conditions, such as wind, temperature, composition and density of wastewater, radiation, evaporation and others, inaccurate and discontinuous sampling, unsteady flow in ponds and flow

measurement errors. Experimental variability will also be introduced due to random behaviour of flow turbulence. Overall Mangelson and Watters (1972) found that the effects of shape, depth and other factors investigated, including inlet and outlet devices, were not as large as expected.

Historically, the question of pond geometry has always been under discussion. In 1960 the Ten State Standards for sewage works in such states as Alaska, Colorado, Georgia and South Dakota (Shindala and Murphy, 1969) called for the shape of ponds to be such that there were not narrow or elongated portions. Acceptable shapes were round, square or rectangular with a length to breadth ratio of less than 3:1. Texas standards called for lagoons to be of a shape that ensures even distribution of loads. Shindala and Murphy (1969) studied various sizes and geometries of lagoons and concluded that load distribution is influenced by shape and rectangular ponds enhance better distribution.

It can be seen that considerable work has been published concerning the influence of pond shape on the efficiency of WSPs and, although there is some disagreement as to whether ponds should be assumed to be completely mixed, partially mixed or of plug flow, for the purposes of design models there is a general consensus of opinion that efficiency is increased as the length to breadth ratio is increased and plug flow conditions approximated. This is reflected in the ponds in operation around the world. The most common pond shape is rectangular, although there is much variation in the length to breadth ratios. At Meze in southern France, ponds are curved to fit the site and have length to breadth ratios of approximately 8:1 for the primary pond and 4-5:1 for the following two ponds in the system. In contrast, WSPs serving Sesimbra in Portugal have much smaller length to breadth ratios with a 3:1 ratio in the secondary pond (which is further

reduced by two baffles). The shape of a pond and the situation of inlets and outlets are clearly the factors which minimalise hydraulic short-circuiting (Mara and Pearson, 1987b). It is generally thought that anaerobic and primary facultative ponds should be rectangular with length to breadth ratios of 2-3 to 1 (with inlets and outlets placed at opposite ends of the pond) so as to avoid sludge banks forming near the inlet (Mara and Pearson, 1987b). The optimal shape for secondary facultative and maturation ponds is also generally taken to be rectangular with a high length to breadth ratio (up to 10 to 1) so that they approximate plug flow conditions (Mara and Pearson, 1987b). Ponds may be curved if necessary or for aesthetic reasons. Inlet and outlet positions are best situated along the ponds longest dimension (diagonally).

1.8.2 Depth

The depth of a pond may have one of two effects on the bacterial die-off in that pond:

(i) If sunlight is taken to be an important factor in bacterial die-off, an increased depth would provide an area of the pond in which sunlight penetration would be minimal and probably not sufficient to cause death. Die-off would occur in the upper layers of the pond but below a critical depth (which could be calculated from the depth of penetration of bacteriocidal light) the bacteria would have an escape from the light. This would be further complicated by other factors responsible for bacterial die-off. Algal photosynthesis occurs in the top photic layer of the pond and would cause an increase in pH and dissolved oxygen but would reduce light penetration. Thus increased depth would provide a refuge for the bacteria from these effects and would have a diluting effect on the bacteriocidal efficiency of the pond. In the case of secondary facultative ponds this would not be so much of a problem since these ponds are succeeded by maturation ponds and

the main function of the secondary facultative ponds is to reduce the organic content of the wastewater. Motile bacteria would have an advantage in this situation since they have the ability to seek out a position in the water column which is most favourable for their survival. Wind-mixing would minimise the availability of refuges by breaking down the algal and thermal stratification. This mixing would be advantageous in a deep maturation pond since it would not allow the escape of bacteria from the surface layers of the pond where bacteriocidal activity is at its highest. Sless (1973) suggested that thermal stratification leads to short-circuiting and reduces efficiency with the possible loss of oxygen at supersaturation levels. However, in shallower ponds thermal stratification is not as marked as in deeper ponds (due to the affects of surface mixing), and since the retention time of a shallower pond is usually shorter, short-circuiting should not present a problem. The shape of a pond is probably a greater influence on short-circuiting than the depth and degree of stratification.

(ii) Increasing the depth of a pond can be a design strategy to increase retention time where land area is limited or where it is perceived that retention time is a key criterior controlling treatment efficiency. Increasing the retention time may (a) increase the die-off of bacteria due to nutrient-limiting conditions or, (b) increase the time available in a particular pond for predation, aggregation, sedimentation and other mechanisms of bacterial removal.

Much research has been performed on the performance of deep ponds (de Oliveira, 1990; Del Riquelme, 1989) but not comparisons of ponds of different depths under the same environmental conditions. A commonly held idea is that the minimum depth which a pond may have is that at which rooted vegetation cannot survive (when the light penetrating into the water falls below 1% of the value at the surface net photosynthesis and, therefore, plant

growth cannot occur). The maximum depth of a pond depends on what type of pond it is but is usually dependant on the degree of oxygenation of the water. Normal design depths of anaerobic ponds are usually between 2.5 and 4 m and facultative and maturation ponds are usually deeper than 1 m to avoid rooted vegetation, with its consequent hazard of mosquito and snail breeding. To facilitate wind mixing the pond is normally constructed with its longest dimension lying in the direction of the prevailing wind. If the wind is seasonally variable wind direction in the hot season is used since this is when the effects of thermal stratification are at their greatest.

Sarikaya *et al.* (1987) investigated the effect of depth (0.5 - 1.5 m) on bacterial die-off and found that the depth averaged die-off rate was inversely proportional to pond depth. This would seem logical since bacterial die-off is effected by solar radiation and solar radiation is subject to attenuation so bacterial die-off is faster in shallow ponds than in deeper ponds (Toms *et al.*, 1975). There is little hard evidence that vertical mixing improves treatment efficiency (Mara and Pearson, 1987) and allowing stratification to occur may improve pathogen removal in maturation ponds and possibly BOD removal in facultative ponds. Sless (1974) compared ponds exposed to wind mixing with sheltered ponds and found that thermal stratification is undesirable since it leads to short-circuiting and reduces the efficiency of coliform removal and since deep ponds more frequently undergo thermal stratification than shallow ponds this indicates that pond efficiency would be improved by shallower ponds. Although mixing reduces short-circuiting, it also reduces the penetration of bactericidal light by increasing the amount of total suspended solids. Short-circuiting may be reduced in other ways such as the design and positioning of inlet and outlets and the use of baffles and scum guards.

Bacterial die-off is largely restricted to the surface layers of ponds where pH and light penetration are sufficient to have a bactericidal synergistic effect on the rate of die-off and a stratified water column will aid the development of a high pH zone. In facultative ponds stratification could be even more beneficial since it would compliment the development of the facultative zone below which low levels of light and oxygen would be prejudicial to bacteria. Thus, designing ponds to stratify rather than be well mixed could improve the microbiological quality of the effluent. In maturation ponds, however, increasing depth merely increases the zone of bacteriocidal inactivity and a mixed system dilutes the benefits derived from the presence of a high pH zone and, therefore, may be expected to decrease the quality of the final effluent.

Trends for building deeper and deeper ponds with a view to reducing land requirements would appear to be fruitless where maturation ponds are concerned. In keeping maturation ponds shallow the retention times may also be reduced without any deleterious effects on the effluent quality and may offset the apparent increased land requirement. Pearson, Mara and Mills (1988) recommend that in order to maximise the amount of time pathogens are in a position in the water column in which they are subject to bacteriocidal influences, such as light and high pH, maturation ponds should be a maximum of 1 m deep.

1.9 Introduction to Current work

Stabilization ponds are far more complex than their physical exteriors suggest and despite their apparent simplicity they represent a complexity of interactions between biological, physical, chemical and environmental factors.

Current methods of pond design are based on organic loading and temperature but this does not take into account any effects due to depth, pond shape or baffling. Pond designers often assume the same value for the first order rate constant for FC removal in anaerobic, facultative and maturation ponds when this may not, in fact, be the case. Furthermore, the most commonly used design equation produced by Marais (1974) does not attempt to reflect individually all the multitude of different factors, complex interactions, reactions and influences which combine or interact to produce the final effluent in a pond, but incorporates them all in a temperature-dependent rate constant whose solution is often taken as trial and error since the equation contains two unknowns, namely, the number of ponds and the size of each.

It is possible that a purely empirically derived formulae for pond design can reflect the operations of some ponds, designed with certain common factors such as standard depth, minimum length, acceptable organic loadings. The rational approaches based on fundamental interactions and relationships, are unlikely to represent the complexity of pond symbiosis unless all the relevant factors are included. It is, perhaps, doubtful that all the factors affecting pond operation are known or at least whether their importance is fully appreciated. Indeed, it is important to distinguish between the physical design of a reactor and the process design, in which a reactor is designed with respect to its' biology with a view to efficient operation. The process design must be translated into a physical design which is specific to the available site. In the past the process design has been used to calculate pond areas, retention times etc using the methods outlined in section 1.4 and the physical design has then followed the traditional ideas of pond size, shape and depth, fitting the lagoons into the land available as best as possible.

The aim of this research project was to determine the effects, in particular on the efficiency of pathogen removal, of pond shape and depth using two experimental waste stabilization pond systems,. Facultative and maturation ponds of different depths and length to breadth ratios were investigated to enable comparisons of pond performance. These ponds represent different hydraulic flow regimes under the classification used by Arceivala (1983). The two different WSP systems operating using identical wastewater could be compared to see the effect of retention time and the number of ponds in a series.

Several points were to be addressed:

(i) Whether the removal of pathogens is effected by the geometry (and, therefore, the flow regime) of a particular pond. Flow regimes may be described as either predominantly plug flow (ie a high length to breadth ratio) or completely mixed (ie a low length to breadth ratio). Several authors (Ferrara and Harleman, 1980; Mangelson and Watters, 1972 and Mara and Pearson, 1987b) have expressed concern over the potential for short-circuiting in ponds with low length to breadth ratios whereas other authors (Agunwamba *et al,* 1992) are concerned over the volume of dead spaces in ponds with a high length to breadth ratio.

(ii) Whether stratification effects pond efficiency as questioned by Sless (1974) and Mara and Pearson (1987).

(iii) Using the results obtained, it was hoped to be able to refine existing design processes to encompass additional parameters thought to influence pond performance but not taken into account in the conventional design formulas and to try and resolve the controversy surrounding the optimum depth and shape of a waste stabilization pond.

(iii) The arrival of a cholera pandemic in N.E. Brazil in 1991 allowed the opportunity of studying the efficiency of Waste Stabilization Ponds in removing this organism after some reports (Anon, 1992 and Lesne *et al*, 1991) that WSPs may have little effect on removal of the vibrios.

(iv) In addition, it was intended to review the situation of using faecal coliforms as indicators for the removal of pathogens (especially *Vibrio cholerae*, since it is rarely possible to study such an important pathogen outside of the laboratory) throughout a system of waste stabilization ponds in a tropical environment.

Chapter 2 Experimental Materials and Methods

2.1 Pilot Systems - Introduction

Two pilot systems were built at Caatingueira, near Campina Grande, N. E. Brazil (latitude 7º 13' 11"S and longitude 35º 52' 31" W, 550 m above m.s.l.). The ponds were built adjacent to the Municipal Sewage treatment works owned by CAGEPA (Compania de água e esgoto de Paraiba). The construction was completed in September 1991. The laboratory facilities used were situated at EXTRABES, 12 km from Caatingueira, which is part of the Departamento de Engenharia Civil of the Universidade Federal da Paraiba. The ponds were constructed in brick with vertical sides, the walls and bottom were rendered with a 25 mm layer of cement to ensure water tightness.

2.2 Description of Pilot Systems

The two highly experimental systems comprised of the following ponds:

a) Innovative system - two 1 day anaerobic ponds in parallel (A9 and A10), five facultative ponds in parallel (F21 - F25), one primary maturation pond (M15) followed by five parallel secondary maturation ponds (M16 - M20) followed, finally, by four tertiary maturation ponds (M21 - M24). At a later date three rock filters (FB2 - FB4) were added to the system to run parallel with the secondary maturation ponds. The rock filters differed in the size of rock. FB2 was filled with rock of a 38 mm mean size, FB3 25 mm and FB4 19 mm.

b) 10 pond series - one 1 day anaerobic pond (A11), one facultative pond (F26) followed by a series of eight maturation ponds (M25 - M32) each with a hydraulic retention time of 2 days.

Flow rates, retention times and loadings are shown in Table 2.1. Pond dimensions are shown in Table 2.2. Figure 2.1 shows the physical arrangement of the two systems and a cross section of the ponds. Figures 2.2 to 2.7 show the two pond systems photographically.

2.3 Sewage Pumping

Raw sewage was collected from the trunk sewer feeding the municipal sewage works. Water was pumped 24 hours a day to a constant level tank inside the pump house using a 1.2 hp, 3380 rpm submersible pump (Dynapac Equipamentos Industriais Ltda, São Paulo, Brazil. A heavy duty variable speed peristaltic pump (model HRSV, Watson-Marlow, England) then supplied water to the anaerobic pond A11 and a NETZSCH pump, model NE30A (Pomerode, Brazil) fed the other two anaerobic ponds, A9 and A10, to give loadings of between 195 and 197 kg BOD_5 m^{-3} d^{-1}. Influent flow rates were checked monthly and adjusted as necessary.

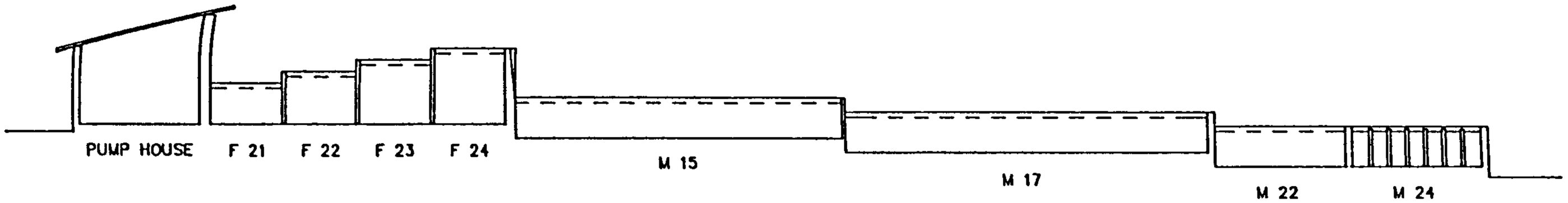
F21 - F25 received the mixed effluents from A9 and A10 which were separated by the use of V notches. The effluents from F21 - F25 flowed into M15 and M16 - M20 received the effluent from this pond once again using V notches. M21 - M24 received the mixed effluents from M16, M17 and M18. The rock filters received the effluent of ponds M15. The two anaerobic ponds were run in parallel for several months to establish

Table 2.1 **Flow rates, Retention times and Loadings of the Ponds at Caatingueira**

POND	FLOW RATE $m^3\ d^{-1}$	RETENTION TIME, d^{-1}	LOADINGS SURFACE $Kg\ ha^{-1}\ d^{-1}$	LOADINGS VOLUMETRIC $g\ m^3\ d^{-1}$
INNOVATIVE SYSTEM:				
A9	20.0	1.0	4876	195
A10	20.0	1.0	4876	195
F21	8.0	3.0	247	25
F22	8.0	4.0	247	19
F23	8.0	5.0	247	15
F24	8.0	6.0	247	12
F25	8.0	6.0	246	12
M15	40.0	3.8	72	7
M16	5.0	7.0	24	3
M17	5.0	5.0	24	4
M18	5.0	3.0	24	6
M19	5.0	3.0	24	6
M20	5.0	1.0	70	18
M21	3.75	5.0	29	5
M22	3.75	5.0	29	5
M23	3.75	5.0	29	5
M24	3.75	5.0	34	6
FB2	5.0	1.0	190	19
FB3	5.0	1.0	190	19
FB4	5.0	1.0	190	19
10 POND SERIES:				
A11	3.24	1.0	2955	197
F26	3.24	2.0	465	31
M25	3.24	2.0	218	15
M26	3.24	2.0	135	9
M27	3.24	2.0	98	7
M28	3.24	2.0	68	5
M29	3.24	2.0	53	4
M30	3.24	2.0	60	4
M31	3.24	2.0	75	5
M32	3.24	2.0	68	5

Table 2.2 **Dimensions of the Ponds of the two Ponds Systems**

SYSTEM	POND	DIMENSIONS (m) LENGTH	WIDTH	DEPTH	AREA (m^2)	VOLUME (m^3)
Innovative	A9	4.90	1.65	2.50	8.08	20.21
System	A10	4.90	1.65	2.50	8.08	20.21
	F21	12.00	2.00	1.00	24.00	24.00
	F22	12.00	2.00	1.33	24.00	31.92
	F23	12.00	2.00	1.67	24.00	40.08
	F24	12.00	2.00	2.00	24.00	48.00
	F25	4.90	4.90	2.00	24.01	48.02
	M15	17.35	8.80	1.00	152.68	152.68
	M16	10.40	3.75	0.90	39.00	35.10
	M17	10.40	3.75	0.64	39.00	24.96
	M18	10.40	3.75	0.39	39.00	15.21
	M19	10.40	3.75	0.39	39.00	15.21
	M20	10.40	1.30	0.39	13.52	5.27
	M21	8.45	3.70	0.60	31.26	18.75
	M22	8.45	3.70	0.60	31.26	18.75
	M23	8.45	3.70	0.60	31.26	18.75
	M24	8.45	3.70	0.60	26.03	15.61
	FB2	5.00	1.00	1.00	5.00	5.00
	FB3	5.00	1.00	1.00	5.00	5.00
	FB4	5.00	1.00	1.00	5.00	5.00
10 Pond	A11	1.80	1.20	1.50	2.16	3.24
System	F26	3.60	1.20	1.50	4.32	6.48
	M25	3.60	1.20	1.50	4.32	6.48
	M26	3.60	1.20	1.50	4.32	6.48
	M27	3.60	1.20	1.50	4.32	6.48
	M28	3.60	1.20	1.50	4.32	6.48
	M29	3.60	1.20	1.50	4.32	6.48
	M30	3.60	1.20	1.50	4.32	6.48
	M31	3.60	1.20	1.50	4.32	6.48
	M32	3.60	1.20	1.50	4.32	6.48



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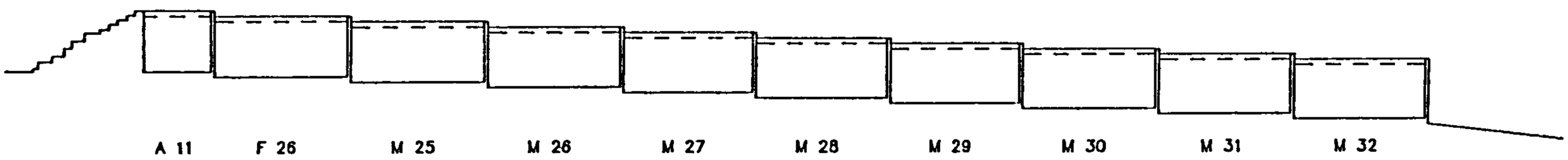

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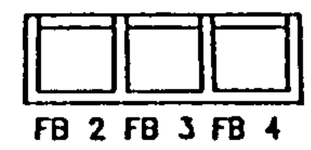

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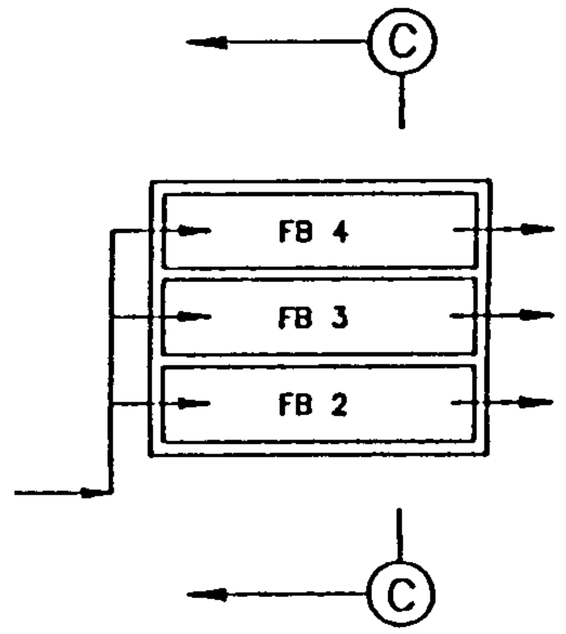


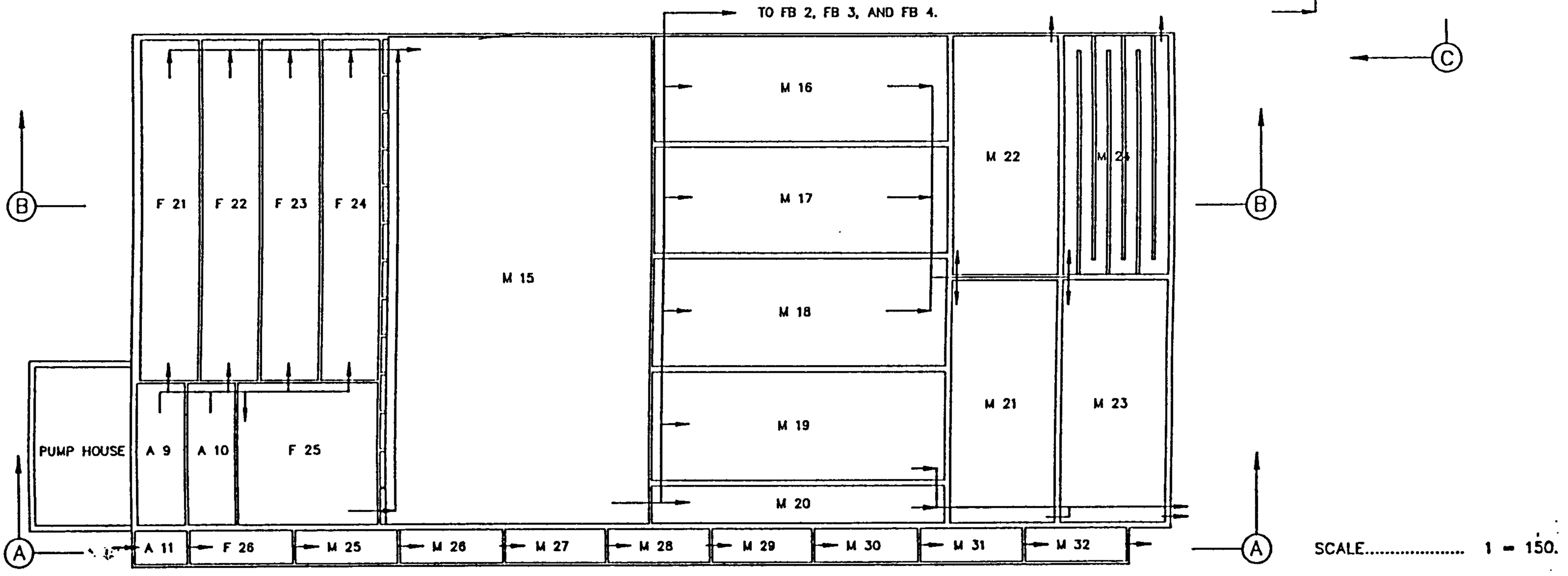


Figure 2.1 Layout of Experimental Waste Stabilization Pond Complex at Caatingueira, N.E. Brazil, Including Pond Coding

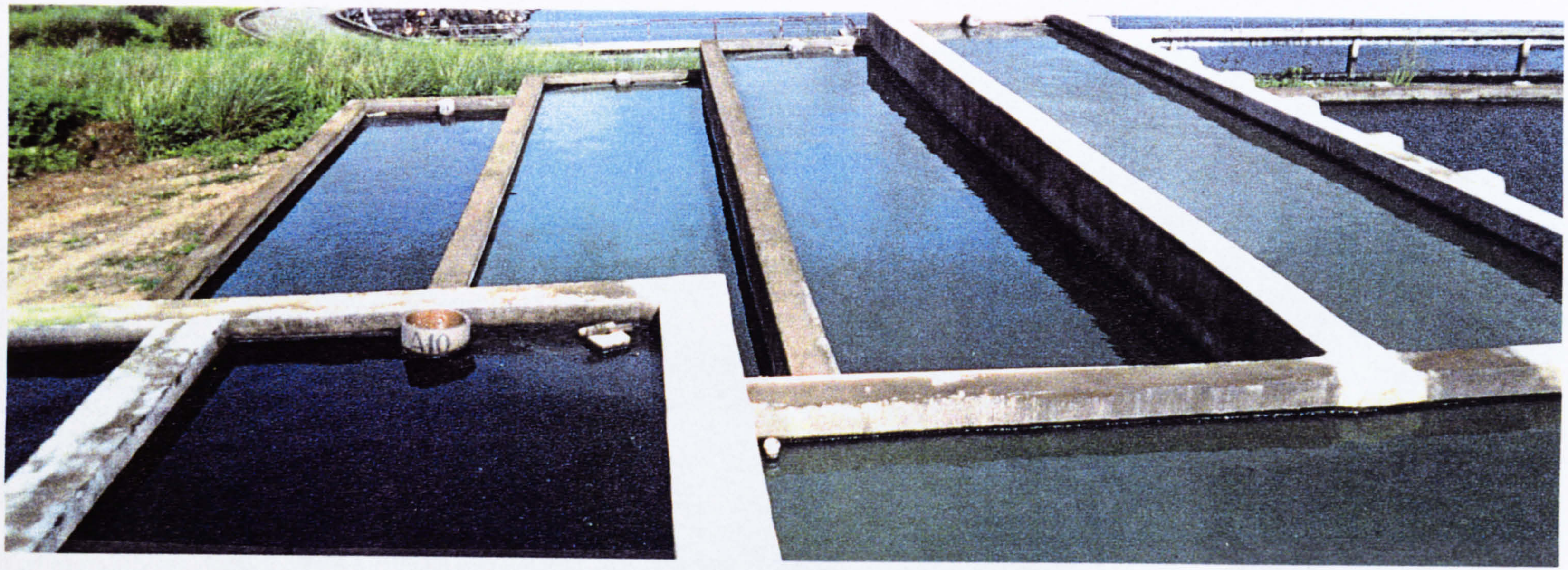


Figure 2.2 Anaerobic (bottom laft) and secondary facultative ponds at Caatingueira

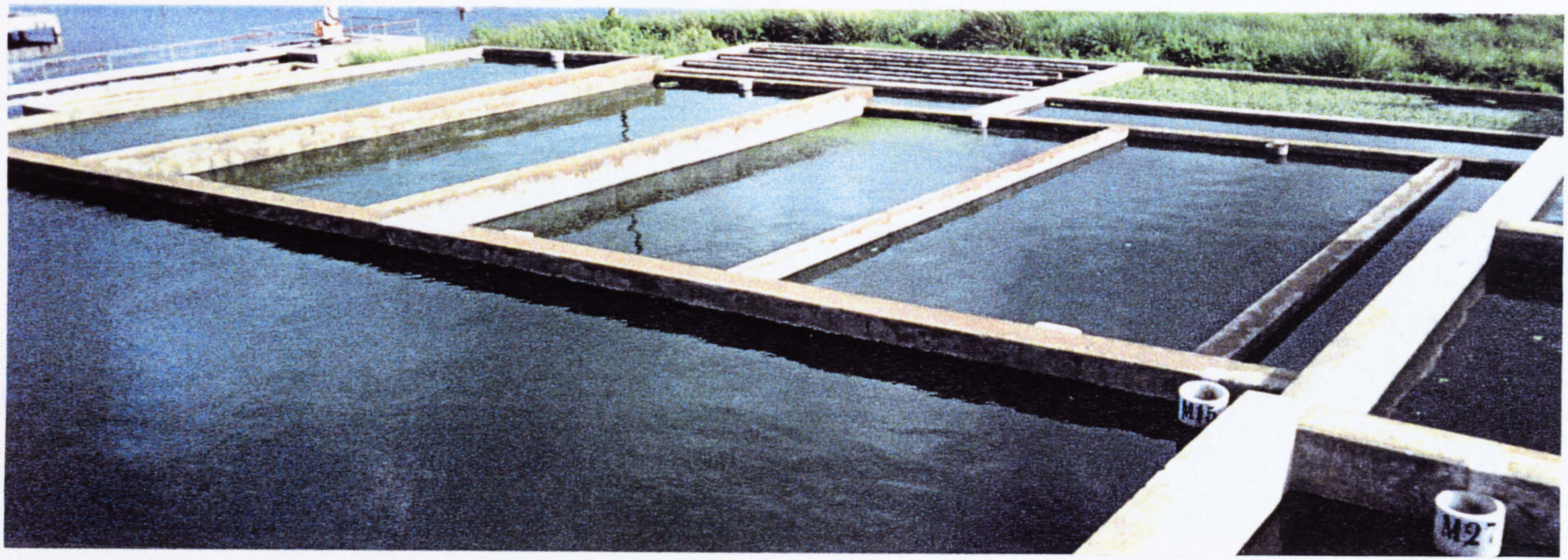


Figure 2.3 Primary (foreground) and secondary maturation ponds at Caatingueira

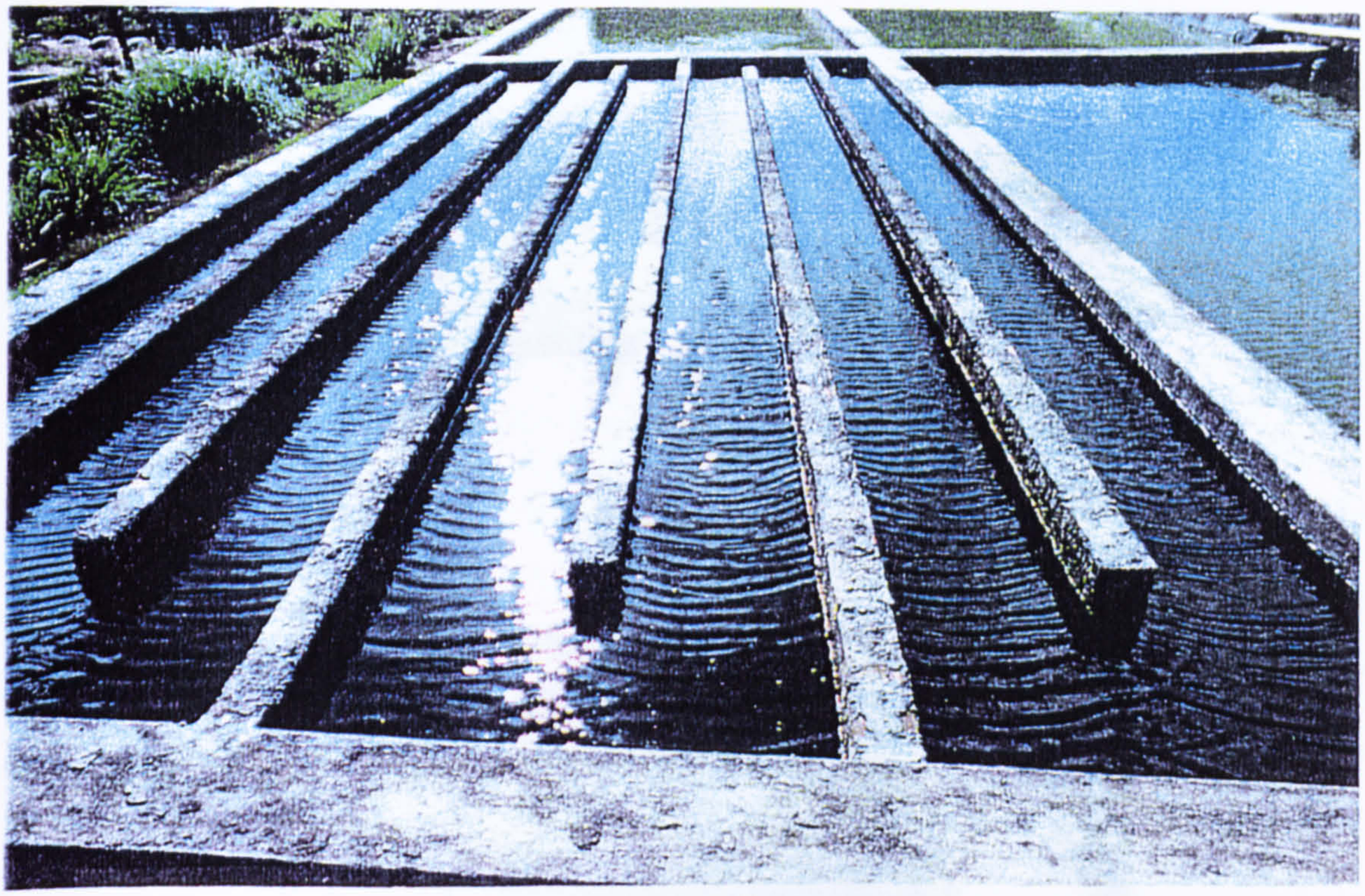


Figure 2.5 Tertiary Maturation Pond M24

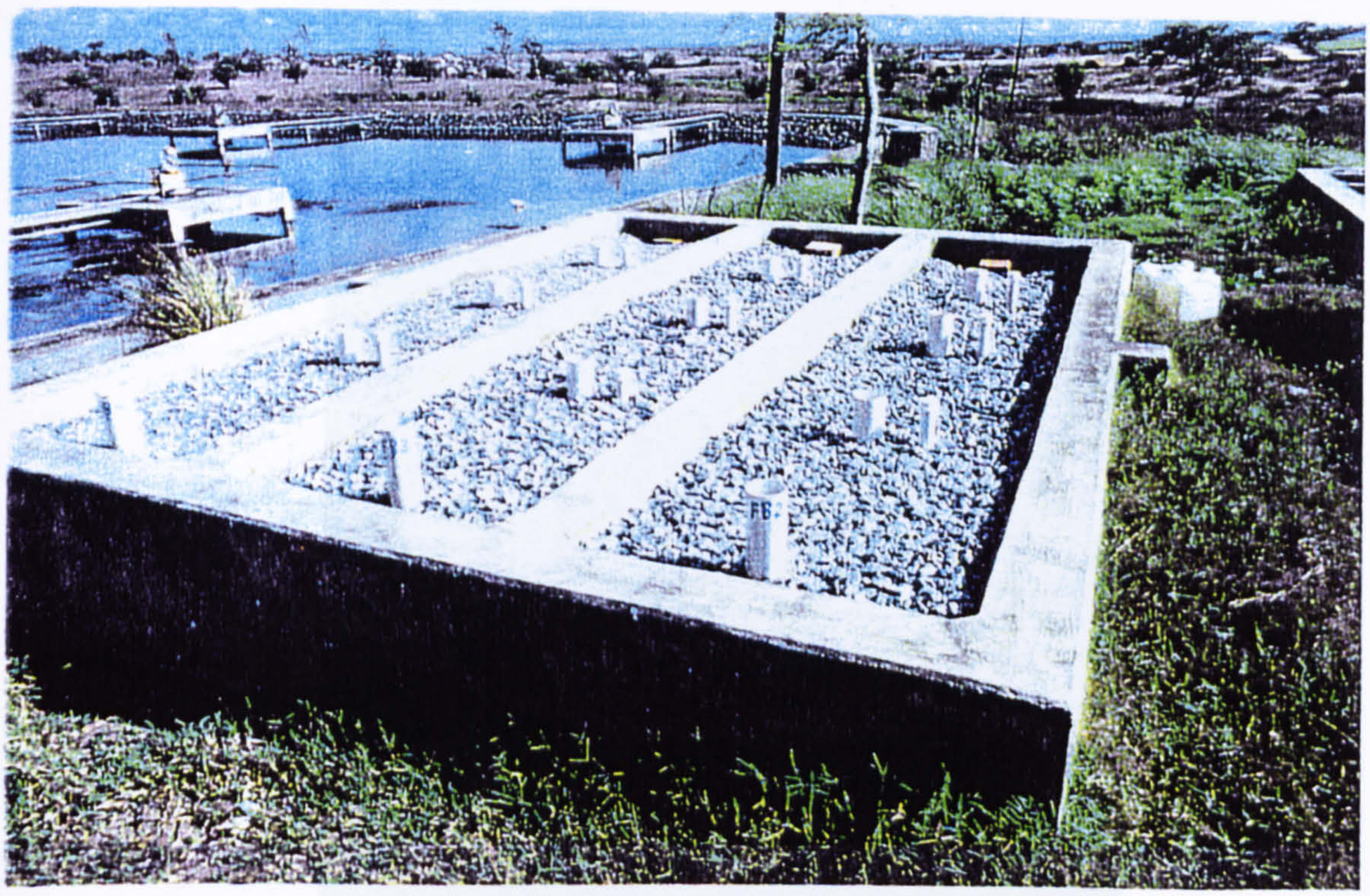


Figure 2.6 Experimental Rock Filters

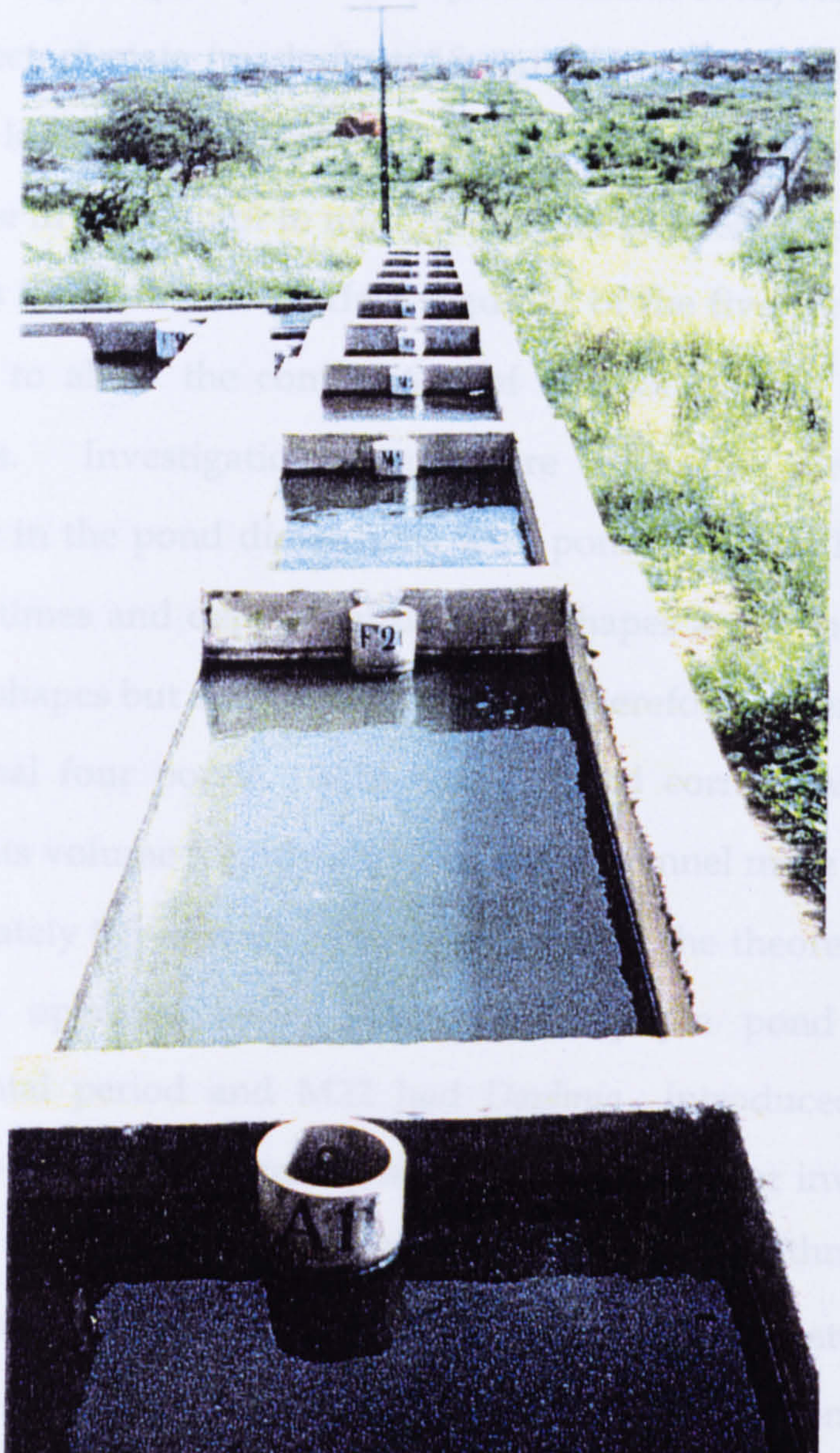


Figure 2.7 System XVII - Series of Ten WSP at Caatingueira

whether they were producing effluents of essentially the same helminthological quality and then (in December 1992) A10 was fitted with an "egg deflector" plate (made from 15 mm PVC sheet, dimensions: 0.9 m wide and 1 m long; and angled at 17.5° to the vertical) immediately below the outlet pipe in an attempt to minimise the carry over of helminth eggs buoyed up by gas flotation. The surface loadings of the five facultative ponds were the same to allow the comparison of the ponds with the same operating conditions. Investigations here were primarily directed towards the difference in the pond dimensions with ponds F24 and F25 having identical retention times and depths but different shapes and ponds F21 - F24 having the same shapes but different depths and, therefore, different retention times. Of the final four ponds, M24 had a round corner baffle system built in reducing its volume slightly and forming a channel more than 50 m long and approximately 0.5 m wide. This also reduced the theoretical retention time. M23 was operated as a floating macrophyte pond for some of the experimental period and M22 had *Daphnia* introduced in an attempt to induce a population of grazing macroinvertebrates for investigations into the reduction of total suspended solids and BOD_5. The three rock filters were also constructed for comparison with the secondary maturation ponds with respect to algal and bacterial removal efficiency. All pond inter-connections utilized 100 mm PVC piping and effluent take off points were fitted with scum guards. The lower end of the inlet pipes was situated 50 cm above the bottom of the pond. The final effluent of both systems was discharged into the nearby municipal treatment plant.

2.4 Performance Evaluation of the Experimental Systems

Both systems were operated from September 1991 to March 1993 under the estimated hydraulic and organic loading rates given in Table 2.1. Sampling

was carried out once weekly on each system. Due to limitations of glassware and media the samples were analysed once weekly for all physico-chemical parameters, Faecal coliforms, *Faecal streptococci* and *Clostridium perfringens* and once fortnightly for *Salmonella, Vibrio cholera* and *Campylobacter*. Analysis for rotavirus was carried out less frequently due to the length of time involved in the preparation of samples and the inhibitory cost of materials. Analyses involved all the technicians at EXTRABES.

2.4.1 Sampling Procedure

Waste stabilization ponds are neither spatially nor temporally homogeneous, so the site and time of sampling is important. Pearson *et al.* (1987a) describe methods of sampling and analysis for the evaluation of waste stabilization pond performance, which are outlined here.

Pearson *et al.* (1987a) stated that analysis for organic material and solids content should be performed on 24 hour flow weighted composite samples of raw wastewater and pond effluents due to strong diurnal variations in content. Samples should be taken every one to three hours along with flow measurements, stored at 4 °C and analysed within 30 hours. Analyses for faecal indicator organisms should be performed within six hours of collection on grab samples taken when pond contents are most homogeneous (i.e. before or at dawn). pH readings should be taken on grab samples of raw wastewater and pond effluents at 8 am to 10 am and 3 pm to 5 pm.

Pearson and Konig (1986) found that algal concentrations of pond effluent are not representative of the pond population due to stratification of the motile species. Pearson (1987e) recommended the use of column samplers to obtain a mixed vertical cross section of a WSP. Three to five samples should be taken from different parts of the pond, using a platform or small boat if

necessary, then pooled for microscopic examination and chlorophyll-*a* analysis.

Several in pond investigations were performed at the beginning of the experimental period to determine the most appropriate sampling regime. Both grab and column samples were taken hourly from selected ponds over a 24 hour period and analysed for various bacteria and physico-chemical parameters and it was found that there were no significant differences in the sample qualities except for chlorophyll-*a* and it was thus decided to take column samples from the ponds in innovative system with additional effluent samples from ponds A9 and A10 (to evaluate the efficiency of the deflector) and rock filters FB2- FB4. Samples from the 10 pond series were taken on alternate weeks as column sample and effluent grab sample. This was due to the fact that part of the wider project horizons was to compare sampling methods. Grab samples were taken at 8 am.

The plexiglass column sampler consisted of three transparent UPVC sections which screwed together to form a 3 m long water tight tube with a diameter of approximately 100 cm. In the shallower ponds 1 or 2 of the sections were removed to make handling easier. Samples were taken by slowly lowering the column vertically into the water, with the cap open. When the column touched the bottom it was raised 50-100 mm, to avoid including the bottom sediment, the cap shut and the column withdrawn and emptied into a bucket. Several samples were taken around the outlet of the ponds and mixed in a bucket from which a 20 litre sample was taken. Separate samples were taken for D.O., sulphide and bacteriology. The grab effluent samples from the 10 pond series were taken using a simple siphoning system. The DO and sulphide samples were collected first ensuring there was no air introduced whilst filling up the bottles. Samples for microbiological analysis were

collected separately in sterile bottles. All samples were then transported back to the laboratories at EXTRABES for analyses. Composite samples of raw wastewater were collected over a 24 hour period for analysis by a Sirco Control Ltd (UK) autosampler model SM0008/24A.

2.4.2 Parameters Studied

The routine sampling programme included analysis for the following parameters:

(a) pH was measured using a Pye Unicam model PW 9418 pH meter with a combined pH electrode (Pye Ingold model 4013E07) and a temperature compensator probe (Pye Unicam PT 100)

(b) Dissolved Oxygen (DO), BOD_5 and Temperature (Temp) were measured using a Yellow Springs Instrument (YSI) model 54A DO meter with a YSI model 5720A dissolved oxygen probe. 5 day biochemical oxygen demand was determined using the standard BOD bottle dilution procedure described in APHA (1989). Filtered BOD_5 was performed on samples filtered through Whatman GF/C paper. Maximum and minimum mid-depth in pond temperatures were recorded daily.

(c) Chemical Oxygen Demand (COD) was measured using the closed reflux titrimetric method. Corning glassware digestion tubes were used in conjunction with a Grant BT4 Block Thermostat. Filtered COD was performed on samples filtered through Whatman GF/C paper.

(d) Sulphide and Sulphate were measured using the methylene blue and the turbimetric methods respectively (APHA, 1992)

(e) Nitrogen

(i) Nitrate was determined using the colorimetric chromotrophic acid method (APHA 1980)

(ii) Ammonia was measured using the nesslerization method following distillation of the samples. Spectrophotometric readings at 450 nm were obtained with a LKB Biochrom Ultraspec model 4053.

(f) Total Phosphorus and Soluble Orthophosphates were determined using the colorimetric ascorbic acid method. Colorimetry was carried out on filtered samples to obtain soluble orthophosphate concentrations and on digested samples (persulphate digestion) to determine total phosphorus.

(g) Alkalinity was measured by the potentiometric titration method. The pH meter was used to detect the end point of 4.5.

(h) Chloride was measured using the argometric method (APHA, 1992).

(i) Solids. Analyses were performed using methods described in APHA (1989).

(j) Faecal coliforms (FC), Faecal streptococci (FS) and *Clostridium perfringens*. Viable counts were made using the standard membrane filtration technique (APHA 1989; PHLS 1983). Millipore Corporation polycarbonate filter holders were used with Millipore membranes (47 mm, 0.45 µm, gridded) and absorbent pads (1 mm thick). Filtration was carried out using quarter strength Ringers solution prepared from Oxoid tablets. Membrane Lauryl Sulphate Broth (Oxoid) was utilised and incubation was for 18 - 24 hours at 44 °C. Pre-incubation at 30 °C (as recommended by Davenport *et al.* 1976) was not possible due to restrictions of incubator facilities. FS numbers were

quantified using KF Streptococcus agar (Oxoid) with incubation at 44 °C for 48 hours. Clostridium numbers were determined using Shahidi-Ferguson Perfringens agar (SFP) (Oxoid code CM 587) plus perfringens selective supplement (SR 93) containing Kanamycin sulphate and Polymyxin B. This was then incubated at 37 °C in an anaerobic jar with a gas generating kit BR 38 to produce 90% hydrogen and 10% carbon dioxide.

(k) *Salmonella*. numbers were quantified using the 5 tube Most Probable Number (MPN) technique (APHA Standard Methods 1989) adapted as discussed in Oragui *et al* (1993). Rappaport Vassiliadis enrichment broth (Oxoid code CM 669) was utilised at suitable dilutions and was incubated at 37 °C for 18 - 24 hours. The samples were then sub-cultured onto xylose lysine deoxycholate agar (XLD) (Oxoid) and incubated for a further 24 hours at 37°C. Serology (slide agglutination test) was then carried out with Salmonella H and O anteserum. Where direct serology was not possible colonies were subcultured onto MacConkeys agar and Lysine Iron Agar (LIA), incubated at 37 °C for 18 - 24 hours and confirmed by serology.

(l) *Campylobacter*. The 5 tube MPN method utilising Nutrient Broth No. 2 (Oxoid) with added growth supplement (SR 84), Preston (or occasionally Skirrow) selective supplements (SR 117 (or SR 69)) and lysed horse blood (SR 48) was performed. Serial dilutions were prepared and incubated for 24 hours at 37 °C. Samples were then subcultured onto either blood agar base no. 2 (CM 271) or Colombia agar base (CM 331) both with added Skirrow selective supplements (SR 69) or blood free agar base (CM 739) plus cefoperazone selective supplement (SR 125) and incubated in a low oxygen (5-6%) environment (produced by gas generating kit BR 56 in Oxoid anaerobic jars (HP 11 or BBL) with an active catalyst (BR 42) for 48 hours at 37 °C. Confirmation was by the oxidase and catalase tests. Further confirmation

was by sub-culturing and incubating aerobically. Since some Campylobacters (notably *C. intestinalis*) do not grow at 42 °C incubation was carried out at 37°C.

(m) *Vibrio cholera* 01 (El Tor). The 5 tube MPN method comprising enrichment in either Alkaline Peptone Water (pH 8.6) or Salt Colistin Broth followed by confirmation on TCBS agar (Oxoid code CM 333) and serology. Plates with many *Vibrio cholera* type colonies giving a negative result were subcultured onto Nutrient Agar or MacConkey agar and identified using API.

(n) Rotavirus. Ultrafiltration was used to concentrate viruses from samples of raw wastewater and lagoons according to the method of Oragui *et al.* (1989). Samples were first clarified by passing through Consler filters of 1 - 10 µm pore size and concentration was achieved by using an amicon CH2 concentrator in conjunction with a single hollow fibre cartridge (type HIP100-20) or a spiral fibre cartridge (type SIY 100) with nominal cut-off of 100,000 mol. wt. (Amicon Ltd., Upper Mill, Stonehouse, UK.). During concentration the restriction valve was adjusted so that the apparatus was run at about quarter speed with an inlet pressure of 5 - 10 psi. The volume of the concentrates ranged from 25-120 ml. Concentrates were stored at 4 °C until detoxified and assayed. Detoxification was carried out using polyacrylamide or dextran and decontaminated with penicillin and streptomycin. The indirect immunofluorescent technique was used to enumerate rotaviruses.

(o) Chlorophyll-*a* (Chl-a). All algae contain this photosynthetically active pigment and due to differences in algae size a measurement of chlorophyll-a is a more appropriate measure of algal biomass than is algal numbers. Chl-a

was estimated using methanol as the extractant by the method published by Jones (1979) and given in appendix A.1 .

(p) Ascaris lumbricoides and other helminths and protozoans. Ascaris was determined using the method devised by Ayres (1992). Analyses was also carried for *Tricocephelus trichurus, Ancylostimideos Larvae, Schitisoma, Hymenolepes nana, Enterobius vermiculares, Entamoeba hystolytica, Giardia lamblia, Iodamoeba, Ancylostimideos* eggs and *Endolymax nana* using methods described by Silva (1982). A bench centrifuge (Damon model HN-S) was used for compacting the organisms after preserving the samples with formaldehyde.

(q) Algal species. Microscopic algae identification and counting were carried out by using an Improved Naubauer Haemocytometer Chamber with an Olympus microscope (model FHT 202027).

Meteorological data was obtained from EMBRAPA 2 km from Extrabes.

2.5 In-Pond Studies

In pond diurnal variation investigations started in November 1992. These were intended to provide information on the changes in temperature, pH, DO, Chlorophyll-*a*, light, algae species and chemical parameters throughout the water column during the daily cycle, from 8 am one day to 8 am the next day.

2.5.1 Sampling procedure

Experiments were performed twice a week between November and January 1993 and once weekly between January and March 1993. Sample collection

was carried out at different depths near the effluent of the pond at 4 hour intervals and temperature, DO and light readings were obtained every 2 hours. The sample collection depths are shown in Table 2.3. Sampling was limited to four depths for physico-chemistry, five depths for *Salmonella* and seven depths for FC and chlorophyll-*a*. These restrictions were due to the constraints of laboratory space and equipment. Light measurements were taken at the surface and every 5 cm to a depth of 50 cm and then every 10 cm up to a depth were no more light was detectable, oxygen readings were similarly taken with measurements taken at 1 cm and 5 cm deep and then at 5 cm intervals until 50 cm deep and then at 10 cm intervals until no more oxygen was detectable or the bottom of the pond was reached. Light was recorded as both photosynthetically active radiation (PAR) and lux. The depths at which temperatures were recorded is shown in Table 2.4.

Samples from various depths were obtained by pumping using a ten channel Watson-Marlow model MHRE peristaltic pump fitted with 5 mm flexible silicone rubber tubing. This tubing was mounted on a second aluminium pole and immersed in the pond. Samples for sulphide analysis were collected separately and all samples were refrigerated at 4 °C until analysis was performed. Filtration for FC and chlorophyll-*a* analysis was performed on site, as was alkalinity and pH. Samples were also distributed into the dilution bottles for Salmonella MPN on site and were incubated at 37°C until transported to EXTRABES. Figure 2.8 shows the equipment used for the in-pond studies.

A depth profile was performed simultaneously on ponds F21 - F25, M15, M16 and M23. Parameters studied were light and D.O. On ponds F21 and F24 samples were taken at various depths (see Table 2.5) and analysed for pH, FC.

Table 2.3 **Sampling Depths used for In-Pond Depth Profile Studies**

DATE	POND	DEPTH (cm)[a]						
		1	2	3	4	5	6	7
29/11/92	F24	4	36	66	96	126	156	179
2/12/92	F25	4	36	66	96	126	156	179
6/12/92	M15	5	15	30	45	60	75	90
9/12/92	M16	5	15	25	40	50	65	80
13/12/92	M20	5		20		35		
16/12/92	M23	5		25		50		
10/ 2/93	F24	6	33	65	93	123	154	181
17/ 2/93	M31	6	25	45	65	85	105	127
10/ 3/93	F21	6	17	32	47	62	72	84
17/ 3/93	M26	3	25	45	62	88	103	128
24/ 3/93	F25	4	29	65	98	124	149	175

[a] Depths 1,3,5,7 analysed for all parameters
Depths 1,2,3,5,7 analysed for Salmonella
All depths analysed for FC and Chlorophyll-*a*

Table 2.4 **Depths (cm) used for Temperature Measurements during In-Pond Studies**

Pond					
F24 29/11/92	F25 2/12/92	M15 6/12/92	M16 9/12/92	M20 13/12/92	M23 16/12/92
+10	+10	+10	+10	+10	+10
1	1	1	1	1	1
5	5	5	5	5	5
15	15	10	10	10	10
25	25	15	15	15	15
35	35	20	20	20	20
45	45	25	25	25	25
60	60	30	30	30	30
80	66	35	40	38	35
110	80	40	50		40
150	96	45	57		45
180	110	60	65		50
	126	75	80		58
	156	90	90		
	180	100			

Pond				
F24 10/2/93	M31 17/2/93	F21 10/3/93	M26 17/3/93	F25 24/3/93
+10	+10	+10	+10	+10
1	1	1	1	1
6	6	6	6	6
20	15	10	15	20
33	25	16	25	31
50	35	23	35	50
65	42	30	45	67
80	55	36	55	86
93	65	45	65	100
123	85	52	85	125
154	103	70	100	150
181	125	80	125	175
195	139	92	147	187

Figure 2.8 Sampling Equipment used During the In-pond Profiles

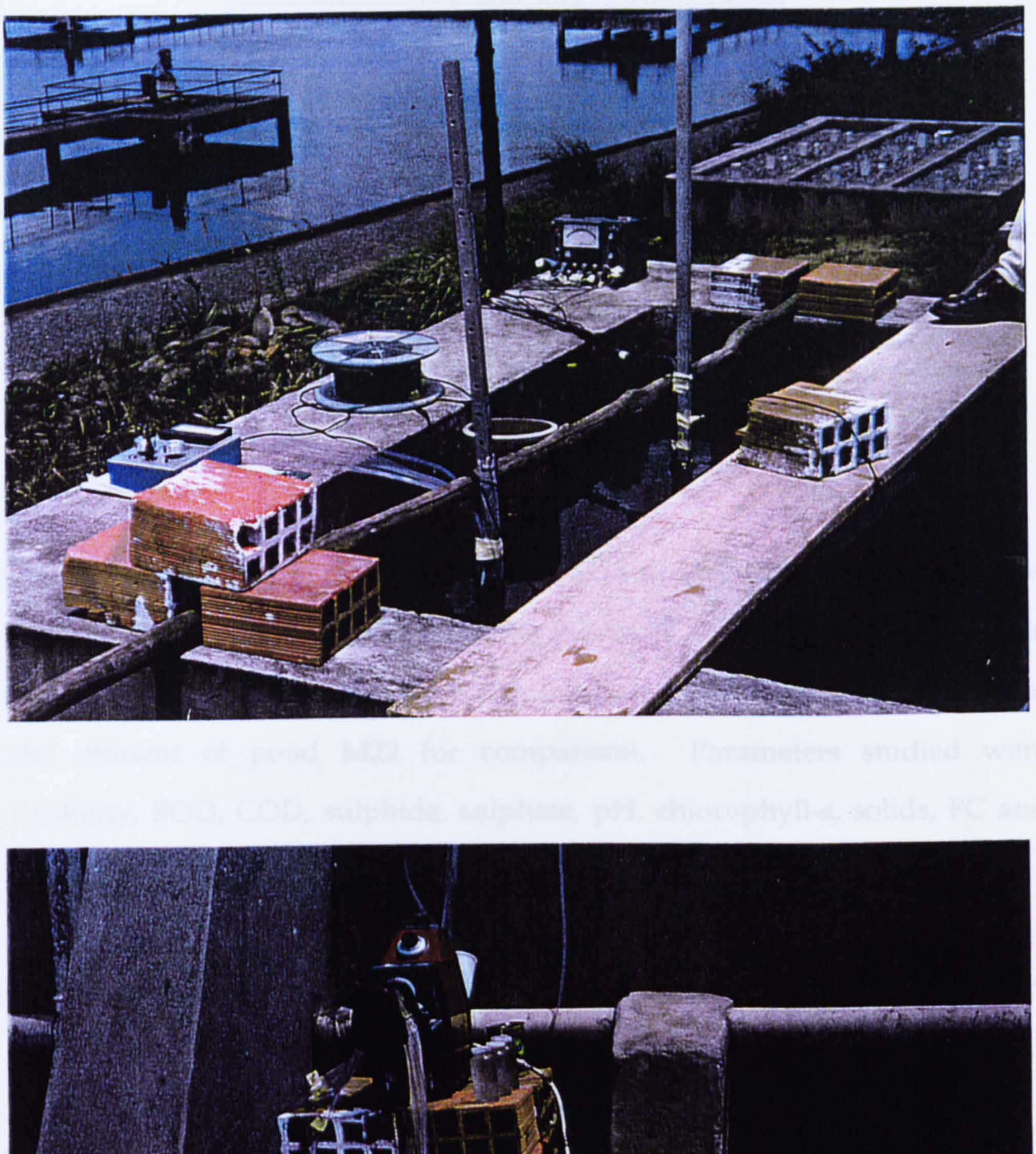


Table 2.5 **Sampling Depths (cm) used for In-Pond Depth Profile Studies of Ponds F21 and F24**

F21	F24
12	4
26	14
55	24
71	50
90	74
	103
	150
	179

A linear profile was performed on pond M24 to investigate the effect of the chicane. Figure 2.9 shows the sampling points. Column samples were taken at 8 am and 3 pm. Samples were also taken at these times near the influent and effluent of pond M22 for comparison. Parameters studied were alkalinity, BOD, COD, sulphide, sulphate, pH, chlorophyll-*a*, solids, FC and *Salmonella*.

The sample collection equipment, telethermometer, light and oxygen meters were all protected under a cardboard box covered with plastic sheeting.

2.5.2 Parameters Studied

The in-pond studies included analysis for the following parameters:

(a) pH, Alkalinity, BOD, COD, ammonia, nitrate, sulphide, sulphate, total P, soluble P, Chloride, solids, chlorophyll-*a*, FC and *Salmonella* were all measured according to the methods outlined in section 2.4.2.

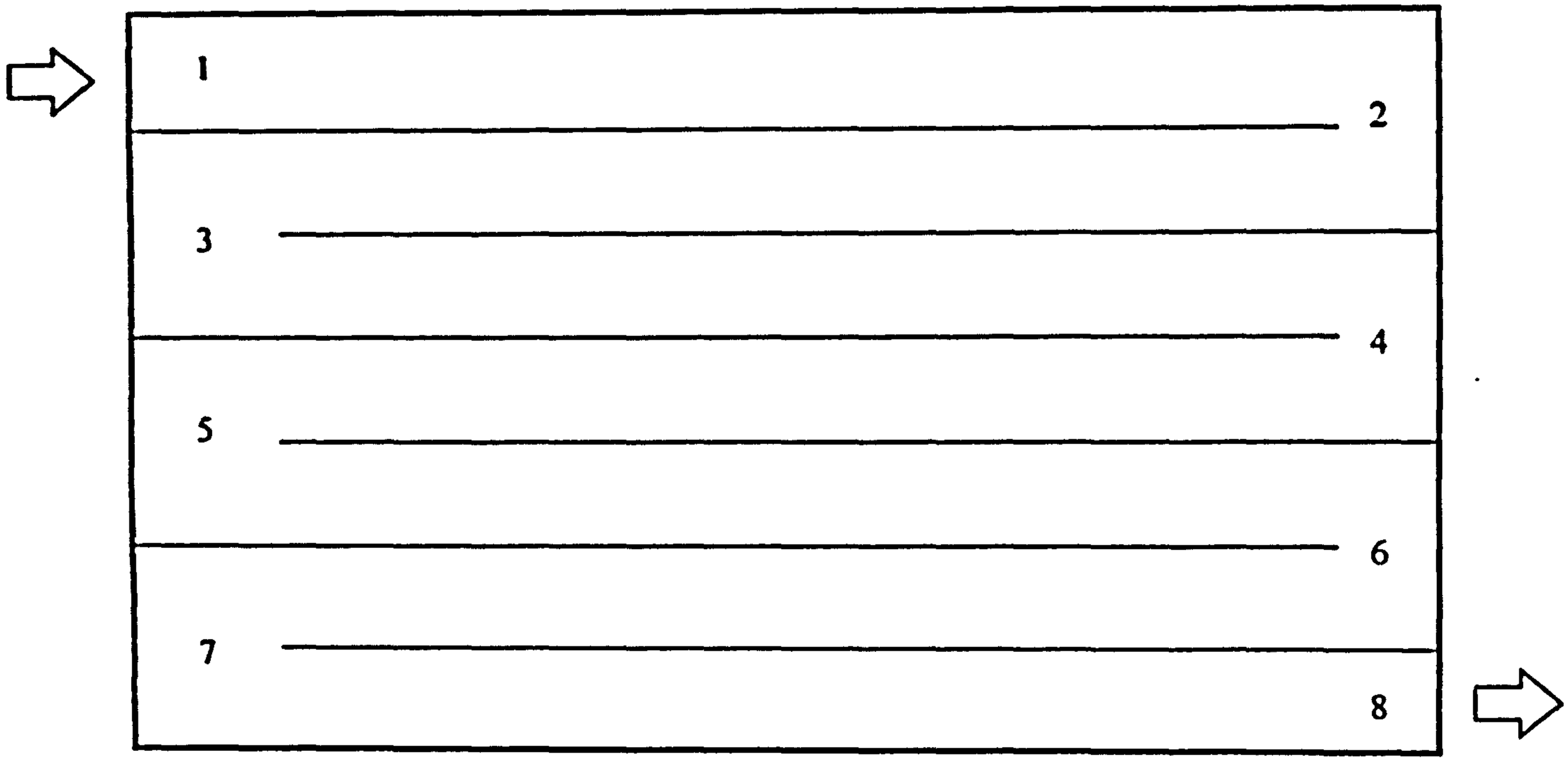


Figure 2.9 Sampling Regime of Pond M24

(b) Dissolved Oxygen (DO) was measured using a YSI 5739 submersible probe with a YSI 5795A submersible stirrer and a YSI model 54A DO meter with a battery pack, YSI 5492A.

(c) Light was measured using a Crump Quantum radiometer/photometer control unit (SN 550) in conjunction with an atmospheric quantum sensor (SN 555) probe at the surface. An underwater quatum sensor (SN 552) with an underwater cable and spider (SN 504A) were used to obtain light readings throughout the water column.

(d) Temperature profiles were obtained using a set of YSI model 401 thermistor probes, mounted on a 3 m length of 25 mm hollow section aluminium rod, connected to a YSI model 44 TD telethermometer.

2.6 Meteorological data

Meteorological data was obtained from EMBRAPA (2 Km from EXTRABES, 10 Km from Caatingueira) on the days in-pond profiles were performed. The readings were taken at 9 am on the following instruments:

(a) Maximum, minimum, wet and dry bulb thermometers;

(b) Campbell-Stokes tropical sunshine recorder (Wilhelm Lambrecht KG Gottingen type 1603);

(c) Gunn-Bellani solar radiation integrator (Baird & Tatlock, England);

(d) Anemometer;

(e) evaporation tank;

(f) rain gauge.

Chapter 3 Experimental Results - Performance Evaluation

3.1 Introduction

The monitoring of the innovative system and the 10 pond system started at the beginning of August and the end of October 1991 respectively and finished at the end of March 1993. During the first two to three months the ponds were undergoing a period of stabilization as is normal with new ponds or ponds which have undergone a change in organic and hydraulic loading and, thus, the results obtained were variable during this initial period. As a consequence, these data were not included in the calculations to obtain mean performance for the various parameters. The large degree of variation was especially true for the maturation ponds M25 - M32 and M15 - M24 since the preceeding ponds have combined retention times of three to eight days and so stabilization of these maturation ponds could only be achieved after stabilization of the preceeding ponds.

The innovative system was investigated intensively because of its highly experimental design and greater flexibility in terms of the number of ponds, total retention times and pond geometry that could be considered. Different combinations of the ponds give total retention times of between 13.8 days and 22.8 days. In the innovative system, there are four facultative ponds and four maturation ponds with identical shapes but different depths whose results shall be considered. Direct comparison is complicated by the fact that an increase in the depth of a pond is normally accompanied by an increase in the retention time (except in situations were a deeper pond is constructed in order to reduce land requirements and a pond with a smaller surface area built). Since the mechanisms of bacterial die-off in ponds have still not been

fully elucidated, differentiating between the affects of increased depth and increased retention time can be difficult. For example, an increase in retention time will increase the time a bacteria spends in a high competition, low nutrient environment and the resultant increased die-off, due to starvation, will have no correlation with an increase in depth. This factor of increased retention time can be eliminated by considering bacterial die-off in terms of a removal rate constant (k_T) whose calculation takes into account the retention time of a pond. Expressing pathogen die-off as the percentage of the influent number which has been removed is deceptive since, unlike physico-chemical parameters where a removal of 90% may be considered excellent, a 4-6 log removal of pathogens (99.99-99.9999%) is necessary to minimise health risks. Although percentage removals have been stated in the results, the k_T value and the log removals are more appropriate for making comparisons between ponds.

The depth of the pond will affect the degree of mixing which occurs in the ponds and, through this mixing, the oxygen regime of the pond will also be affected. It is not unreasonable to suggest that shallow ponds are generally better mixed (often due to wind action) and the shallower a pond is, the less thermal stratification will occur (in-pond profile studies given in chapter 4 support this idea since the deeper ponds were found to have larger ranges of temperature throughout their depths). In the case of facultative ponds the depth is critical since a very shallow pond will not develop an anaerobic bottom layer, whereas a deep pond may become anaerobic for most of its depth. In the case of maturation ponds, which are designed to be completely aerobic, a deep (for example, more than 1.5 m deep) pond may develop an anaerobic water column of significant depth. It should be noted, however, that the degree of oxygenation is also dependant on the organic loading of the pond.

The shape of a pond may affect its functioning by altering the predominant hydraulic regime. To investigate the influence of pond size and shape on bacterial die-off two secondary facultative ponds (F24 and F25), three secondary maturation ponds (M18, M19 and M20) and three tertiary maturation ponds (M21, M22 and M24) from the innovative system were used and the anaerobic ponds from both systems were also compared. These ponds differ in their length to breadth ratios (defined as the distance between a ponds' inlet and outlet). It is preferable to use ponds with the same depths and retention times for this type of investigation, but this was not possible with secondary maturation ponds due to the constraints of the project as a whole. M20, a secondary maturation pond with a different length to breadth ratio to the other secondary maturation lagoons, may be used to give an indication of the efficiency of a pond with a higher length to breadth ratio.

The length to breadth ratios of the ponds in the innovative system are as follows;

A9 and A10	3:1
F21 to F24	6:1
F25	1:1
M15	1.83:1
M16 to M19	2.8:1
M20	8:1
M21 to M23	2.28:1
M24	>140:1

Using the classification suggested by Arceivala (1983) ponds with length to breadth ratios of <4 may be assumed to approximate completely mixed conditions and ponds with a length to breadth ratio of >8 approximate plug flow. Those with a ratio of between 4 and 8 will have dispersed flow somewhere between completely mixed and plug flow. Using this criterion

the facultative ponds F21-F24 have dispersed flow and F25 differs since it may be assumed to be completely mixed. Ponds M16 to M19 and M21 to M23 may also be taken to approximate completely mixed conditions and ponds M20 and M24 are probably better described as approaching plug flow. These predominant hydraulic conditions are considered important in the design of ponds. The conflict of ideas as to whether plug-flow is better than completely mixed or vice-versa is further complicated by the function for which the pond is meant and the biological, physical and chemical interactions occurring within them. For BOD removal in anaerobic and facultative ponds complete mixing is preferable to maintain a uniform pH and temperature throughout the pond column and so encourage methane fermentation. However, the heterogeneous microbial populations contained in these ponds utilise a wide range of different substrates and also require different, and sometimes, highly specific and conflicting environmental conditions for optimal growth and survival. In contrast to the very stable, anaerobic conditions necessary for methanogenesis to take place, microbial oxidation of soluble organics is dependant upon the maintenance of aerobic conditions and would, perhaps, benefit from a degree of stratification, for example, if the surface layers underwent gentle mixing which may facilitate beneficial oxygen and substrate circulation without the loss of anaerobic conditions and disturbance of the redox potential further down the water column which would inhibit methanogenesis (Pearson *et al.*, 1988).

Maturation ponds may be better designed to approach plug-flow conditions allowing stratification to develop since this provides the environmental conditions conducive to pathogen die-off such as higher temperature, pH etc. It is preferable that these ponds be shallow rather than deep, since the increased depth merely increases the zone of bacteriocidal inactivity and in a

mixed system dilutes the benefits derived from the presence of the high pH zone. This inevitably decreases the quality of the final effluent.

The design of WSP is heavily reliant on these theories regarding optimal size, shape and depth and on equations based on time and temperature functions to determine loading rates and land area requirements and to predict the effluent quality (Marais and Shaw, 1961; McGarry and Pescod, 1970; Gloyna, 1971; Mara, 1976; Arthur, 1983). The physical design of the ponds, from which these equations have been derived, has remained unaltered for many years leading to consistent loading rates and predictions of effluent quality under different environmental conditions. This has done little to advance design with a view to improve treatment efficiency and reduce capital costs by lower land requirements. It has even been suggested (Pearson *et al.*, 1988) that this approach has led to the refinement, but perpetuation, of well tried designs to the detriment of design advancement, since the data collected are a function of the original design concept.

The aim of this investigation was to clarify the importance of waste stabilization pond shape and depth in the removal of pathogens from wastewater and to review the accuracy of the Marais equation (Marais, 1974) which is most frequently used in the design of ponds, principally, maturation ponds.

3.1.1 Statistical Analysis

Statistical analysis of the results was performed on the Minitab package. Geometric means and the corresponding standard deviations were calculated for all the bacteria and rotaviruses investigated since this compensates for the logarithmic type growth pattern. Arithmetic means were calculated for the physico-chemical data. Bartlett's box test was used to establish if the data were homogeneous and heterogeneous data were $\log_{10}$ transformed, to

induce normality, before a oneway analysis of variance was carried out. If the log transformed data were found to be significantly different and continued heterogeneous the Kruskal-Wallis and Moods median non-parametric tests were used to confirm the significance of the result. Simple regression analysis was applied to the raw data to establish any relationships between the variables.

3.1.2 Climatic Influences

The bacterial and viral die-off in both systems was not found to have any significant variations attributable to seasonal affects. This is because of the minimal seasonal climatic changes which occurred during the experimental period of 1991 to 1993 and the lack of a wet season in 1992.

3.2 BOD Removal from the Innovative System

Mean BOD values for both systems are given in Table 3.1 and the corresponding k_T values are presented in Table 3.2.

BOD removal in WSPs is generally assumed to take place mainly in the primary ponds of a system, in this case the anaerobic ponds and the secondary facultative ponds, and the processes responsible for this removal have been described in sections 1.4.2.1 and 1.4.3.1.

3.2.1 Anaerobic Ponds

Mean BOD removal in these ponds was 62% which is around the expected figure of 60% given in Table 1.6 for the design temperature of the Caatingueira ponds (25 °C).

Table 3.1 Mean BOD, Chlorophyll-*a* and Total Suspended Solids of Ponds in Both Systems

Pond	BOD mg/l		Chlorophyll-*a*		TSS
	Unfiltered	Filtered	µg/l	mg/m^2	mg/
INNOVATIVE SYSTEM:					
RSC	197 (65)	61 (23)	-	-	280 (85
A9	73 (60)	33 (11)	-	-	121 (107
A10	75 (46)	-	-	-	442 (320
F21	32 (23)	13 (7)	435 (255)	435	81 (35)
F22	29 (19)	-	543 (437)	722	77 (29)
F23	28 (20)	-	392 (264)	655	67 (35)
F24	24 (20)	11 (6)	358 (200)	716	63 (28)
F25	25 (18)	-	353 (221)	706	69 (42)
M15	19 (13)	8 (4)	369 (247)	369	53 (32)
M16	22 (10)	6 (3)	497 (451)	447	74 (31)
M17	21 (10)	7 (4)	612 (500)	392	73 (30)
M18	25 (12)	6 (4)	901 (615)	351	115 (71)
M19	26 (13)	-	925 (591)	361	118 (47)
M20	25 (22)	-	517 (379)	201	103 (73)
M21	24 (12)	15 (11)	658 (421)	395	101 (44)
M22	26 (14)	5 (3)	547 (365)	328	89 (41)
M23	20 (13)	6 (4)	480 (332)	288	94 (44)
M24	17 (9)	5 (4)	663 (388)	398	107 (69)
10 POND SERIES COLUMN:					
A11	62 (27)	39 (21)	-	-	124 (119
F26	29 (14)	14 (7)	252 (128)	378	84 (99)
M25	18 (12)	8 (5)	196 (139)	294	46 (35)
M26	10 (8)	6 (5)	137 (91)	206	36 (18)
M27	9 (7)	5 (4)	169 (81)	227	33 (22)
M28	7 (4)	4 (3)	169 (132)	254	35 (21)
M29	8 (5)	5 (2)	180 (108)	270	39 (30)
M30	10 (6)	6 (3)	211 (120)	317	27 (12)
M31	9 (5)	5 (2)	191 (92)	287	38 (27)
M32	11 (5)	5 (3)	230(103)	345	37 (14)
10 POND SERIES EFFLUENT:					
A11	74 (48)	47 (14)	-	-	85 (60)
F26	56 (39)	17 (7)	226 (221)	339	49 (16)
M25	45 (37)	9 (4)	223 (250)	335	42 (27)
M26	38 (32)	6 (3)	148 (145)	222	41 (25)
M27	31 (30)	5 (3)	157 (179)	236	41 (35)
M28	26 (25)	6 (3)	270 (321)	405	44 (37)
M29	24 (24)	5 (3)	269 (272)	404	41 (31)
M30	16 (16)	5 (3)	301 (390)	452	35 (27)
M31	13 (13)	4 (2)	233 (243)	350	36 (12)
M32	11 (8)	6 (4)	205 (193)	308	43 (28)

Standard Deviation values are given in parenthesis
NOTE: RSC = Raw sewage, A = Anaerobic, F = Facultative, M = Maturation

Table 3.2 First Order Removal Rate Constants (k_T) for BOD_5 in the Ponds of Both Systems

Pond	K_T Value
INNOVATIVE SYSTEM:	
A9	1.70
A10	1.63
F21	0.44
F22	0.39
F23	0.33
F24	0.35
F25	0.33
M15	0.12
M16	-0.02
M17	-0.02
M18	-0.08
M19	-0.09
M20	-0.24
M21	-0.01
M22	-0.03
M23	0.03
M24	0.03
10 POND SERIES COLUMN:	
A11	2.18
F26	0.59
M25	0.29
M26	0.39
M27	0.10
M28	0.08
M29	-0.03
M30	-0.12
M31	0.06
M32	-0.11
10 POND SERIES EFFLUENT:	
A11	1.65
F26	0.16
M25	0.13
M26	0.08
M27	0.13
M28	0.08
M29	0.05
M30	0.25
M31	0.10
M32	0.11

NOTE: A = Anaerobic, F = Facultative, M = Maturation

3.2.2 Secondary facultative Ponds

BOD removal in the secondary facultative ponds was in the range 57% (F21) to 62% (F24) which is as expected since the figure for BOD removal in facultative ponds stated in the literature (usually 60-80%, Pearson, 1987) is for primary facultative ponds and secondary facultative ponds may be expected to remove up to 30% less than this. This is because of the role sedimentation and methane fermentation play in BOD removal in primary facultative ponds which takes place on a much reduced scale in secondary facultative ponds (Mara *et al,*. 1992). F25 showed no difference to the other secondary facultative ponds with respect to its efficiency in removing BOD.

3.2.3 Maturation Ponds

BOD removal in the primary maturation pond was 24% which is around the figure normally expected in a primary maturation pond. The reduction in BOD in maturation ponds is usually balanced by the increase in algae present (when compared to anaerobic and facultative ponds) which exert a demand for oxygen. This has been discussed in section 1.5.1.

There was an increase in the BOD of the water in the secondary maturation ponds which then remained constant through the tertiary ponds. This may be attributed to the algal biomass present in these ponds. The ponds M18, M19 and M20 had higher suspended solids and chlorophyll-*a* concentrations than the other two secondary maturation ponds and these ponds had corresponding increases in BOD5. M23, the pond which had been operating as a macrophyte pond for a period of approximately 5 months in 1992 had a lower mean concentration of chlorophyll-*a* due to the shading affect of the macrophytes.

3.3 BOD Removal from the 10 Pond Series

The overall BOD removal achieved in the ponds of this system was 94% with most of the reduction (more than 60%) occurring in the anaerobic pond as would be expected. The first four ponds were responsible for reducing the mean unfiltered BOD5 to 10 mg/l (38 mg/l in the effluent grab sample) and the remaining ponds did little to further reduce this.

3.4 Removal of Nutrients and other Chemical Constituents from the Innovative System

A further important role of any sewage treatment system is the removal of nutrients such as nitrogen and phosphorus to prevent eutrophication of the receiving waters. The mechanisms for nitrogen/ammonia removal in ponds are:

(i) gaseous ammonia removal (volatilization) where the pH is greater than 7,

(ii) ammonia assimilation into algal biomass (in facultative and maturation ponds),

(iii) biological nitrification coupled to denitrification (Middlebrooks *et al.*, 1982).

Total nitrogen removal in WSP systems can be 80% and ammonia reduction can be as high as 95% (Mara *et al,*. 1992). The removal of nitrogen from the two pond systems at Caatingueira has benn found to be efficient with removal rates in the maturation ponds being the highest and both secondary and tertiary maturation ponds performing similarly (Silva *et al.*, 1995).

Phosphorus removal occurs due to sedimentation (as P in the algal biomass) and precipitation coupled to mineralization and resolubilization. With both nitrogen and phosphorus, the non-biodegradable fraction of the algal cells remain in the sediments and, thus, the best way of maximising the nitrogen and phosphorus removal would be to increase the number of maturation ponds, so that progressively more of these nutrients become immobilized in the sediments.

The subject of nutrient removal from WSP is a complex one and a whole thesis could easily be devoted to this subject alone. In this case, the removal of nutrients from the ponds has been considered in the context of its' association with the reduction of pathogen numbers throughout the system.

Tables 3.3 and 3.4 show the mean values for various chemical parameters measured in the ponds of both systems.

It was found that 50% of the organic nitrogen was removed in the anaerobic ponds and the remaining ponds all had nitrogen concentrations of less than 10 mg N/l, except for the secondary maturation ponds M18 (12.4 mg N/l), M19 (12.1 mg N/l) and M20 (13.2 mg N/l), which had relatively high numbers of microcrustaceans, and the tertiary maturation pond M21 (10.8 mg N/l), which acted as a microcrustacean grazing pond for part of the experimental period.

There is a negligible increase in the amount of ammonia present in the anaerobic ponds to that present in the raw sewage which indicates that the biodegradation of organic compounds, such as amino acids, and urea hydrolysis, by urease, probably occurred during the transport of the

Table 3.3 Mean Values for Nutrients and other Chemical Constituents Measured

POND	Ammonia mg N/l	Nitrate mg N/l	Total Phos mg P/l	Ortho-Phos mg P/l	COD mg/l
			INNOVATIVE SYSTEM:		
RSC	33.3 (37.6)	0.47 (0.29)	5.5 (1.4)	2.2 (1.0)	528 (126)
A9	33.6 (6.6)	0.41 (0.27)	5.1 (1.5)	3.6 (1.0)	274 (167)
A10	33.8 (6.2)	0.41 (0.26)	5.5 (1.8)	3.7 (1.1)	345 (236)
F21	29.9 (7.9)	0.49 (0.30)	5.4 (1.1)	3.8 (1.0)	205 (61)
F22	28.8 (8.3)	0.51 (0.31)	5.3 (1.0)	3.6 (0.9)	211 (106)
F23	28.8 (7.7)	0.52 (0.32)	5.1 (1.1)	3.7 (1.5)	183 (55)
F24	28.4 (7.3)	0.51 (0.31)	5.1 (1.0)	3.2 (1.2)	171 (45)
F25	27.6 (8.5)	0.47 (0.28)	5.2 (1.0)	3.3 (1.2)	179 (51)
M15	17.0 (10.1)	0.59 (0.34)	4.8 (1.2)	2.9 (1.2)	159 (46)
M16	5.5 (6.3)	0.53 (0.32)	3.6 (1.6)	1.8 (1.0)	188 (65)
M17	5.4 (5.4)	0.52 (0.35)	3.8 (1.7)	1.9 (1.3)	191 (48)
M18	6.2 (6.3)	0.51 (0.34)	4.5 (1.5)	1.9 (1.1)	228 (75)
M19	6.2 (6.6)	0.48 (0.30)	4.9 (2.0)	1.8 (1.0)	232 (65)
M20	14.1 (10.3)	0.51 (0.28)	5.7 (1.5)	2.9 (1.3)	216 (99)
M21	1.8 (4.3)	0.48 (0.35)	3.0 (1.5)	1.1 (0.8)	219 (51)
M22	1.3 (4.2)	0.48 (0.35)	2.5 (1.3)	0.9 (0.8)	209 (47)
M23	1.4 (3.9)	0.51 (0.34)	3.1 (1.5)	1.6 (1.4)	201 (56)
M24	1.0 (3.6)	0.45 (0.30)	3.1 (1.1)	1.3 (1.1)	196 (65)
			10 POND SERIES COLUMN:		
A11	34.1 (8.9)	0.48 (0.31)	6.0 (2.5)	3.5 (0.8)	377 (30)
F26	34.6 (8.0)	0.49 (0.26)	5.2 (1.5)	2.8 (1.1)	178 (25)
M25	31.5 (9.5)	0.53 (0.25)	5.0 (1.7)	2.3 (1.2)	132 (28)
M26	24.3 (6.9)	0.67 (0.40)	4.7 (1.9)	2.4 (1.3)	109 (32)
M27	19.3 (9.2)	0.67 (0.41)	4.8 (1.9)	2.6 (1.1)	105 (39)
M28	19.7 (12.7)	0.72 (0.38)	5.1 (1.7)	3.4 (0.9)	103 (46)
M29	15.7 (12.2)	0.85 (0.52)	4.9 (1.9)	3.4 (1.1)	108 (55)
M30	12.3 (11.2)	0.98 (0.68)	4.7 (2.4)	3.3 (1.4)	107 (48)
M31	9.3 (9.5)	0.93 (0.59)	4.6 (2.5)	3.2 (1.8)	106 (38)
M32	6.3 (7.4)	0.86 (0.56)	4.4 (2.6)	3.0 (1.8)	101 (32)
			10 POND SERIES EFFLUENT:		
A11	36.4 (6.7)	0.32 (0.28)	4.9 (1.4)	3.7 (1.1)	232 (100)
F26	36.8 (6.0)	0.34 (0.29)	5.1 (1.0)	3.8 (1.0)	176 (61)
M25	35.6 (5.6)	0.39 (0.33)	5.0 (1.1)	3.6 (1.1)	161 (60)
M26	34.1 (7.5)	0.43 (0.36)	5.0 (1.0)	3.5 (1.0)	146 (55)
M27	31.1 (9.4)	0.44 (0.36)	4.9 (1.0)	3.6 (1.1)	142 (64)
M28	28.0 (10.8)	0.46 (0.44)	4.8 (1.1)	3.6 (1.1)	131 (62)
M29	25.1 (11.7)	0.46 (0.40)	4.6 (1.2)	3.6 (1.2)	130 (58)
M30	22.5 (12.3)	0.49 (0.47)	4.5 (1.4)	3.7 (1.4)	117 (46)
M31	19.9 (12.2)	0.53 (0.46)	4.3 (1.5)	3.5 (1.5)	108 (43)
M32	17.2 (11.6)	0.53 (0.46)	4.1 (1.5)	3.3 (1.4)	98 (35)

Standard Deviation values are given in parenthesis

NOTE: RSC = Raw sewage, A = Anaerobic, F = Facultative, M = Maturation

Table 3.4 Mean Values for Nutrients and other Chemical Constituents Measured

POND	Alkalinity	Sulphide mgS/l	pH	Chloride mg Cl/l
		INNOVATIVE SYSTEM:		
RSC	311 (30)	0.59 (0.34)	7.3 (0.3)	360 (86)
A9	368 (30)	11.95 (4.28)	7.2 (0.3)	365 (58)
A10	371 (31)	12.29 (5.25)	7.2 (0.3)	353 (61)
F21	362 (30)	1.06 (1.28)	7.5 (0.3)	347 (28)
F22	359 (28)	1.10 (1.64)	7.5 (0.3)	367 (49)
F23	366 (29)	1.02 (1.27)	7.5 (0.3)	352 (28)
F24	366 (28)	0.81 (1.26)	7.6 (0.3)	343 (43)
F25	363 (29)	0.83 (1.22)	7.6 (0.3)	338 (39)
M15	320 (43)	0.04 (0.08)	8.0 (0.4)	369 (35)
M16	279 (48)	0.02 (0.02)	8.7 (0.6)	382 (43)
M17	273 (48)	0.01 (0.02)	8.7 (0.6)	378 (40)
M18	273 (54)	0.02 (0.03)	8.6 (0.5)	382 (58)
M19	271 (55)	0.01 (0.02)	8.7 (0.6)	376 (41)
M20	314 (42)	0.02 (0.03)	8.0 (0.3)	365 (41)
M21	248 (46)	0.10 (0.32)	9.1 (0.4)	384 (52)
M22	245 (43)	0.07 (0.30)	9.2 (0.4)	408 (52)
M23	249 (47)	0.12 (0.51)	8.7 (0.8)	417 (66)
M24	240 (46)	0.10 (0.33)	9.1 (0.4)	394 (35)
		10 POND SERIES COLUMN:		
A11	377 (30)	11.31 (8.78)	7.3 (0.2)	354 (29)
F26	377 (25)	1.75 (2.69)	7.5 (0.2)	364 (45)
M25	367 (28)	0.27 (0.54)	7.7 (0.2)	354 (30)
M26	342 (32)	0.09 (0.18)	7.7 (0.2)	335 (18)
M27	322 (39)	0.02 (0.07)	7.8 (0.2)	341 (21)
M28	325 (46)	0.02 (0.03)	7.8 (0.2)	363 (54)
M29	320 (55)	0.03 (0.08)	7.9 (0.4)	370 (39)
M30	308 (48)	0.01 (0.02)	8.1 (0.4)	374 (41)
M31	296 (38)	0.00 (0.01)	8.2 (0.4)	392 (53)
M32	293 (32)	0.00 (0.01)	8.3 (0.5)	384 (42)
		10 POND SERIES EFFLUENT:		
A11	366 (51)	9.04 (3.92)	7.1 (0.2)	344 (42)
F26	377 (45)	5.45 (5.26)	7.3 (0.2)	364 (74)
M25	384 (49)	3.77 (5.41)	7.5 (0.2)	359 (65)
M26	379 (56)	2.74 (4.27)	7.6 (0.2)	351 (52)
M27	370 (60)	2.19 (3.78)	7.7 (0.2)	252 (127)
M28	369 (62)	1.51 (2.67)	7.7 (0.2)	365 (40)
M29	362 (64)	0.89 (2.09)	7.8 (0.2)	346 (47)
M30	352 (68)	0.58 (1.77)	7.9 (0.3)	363 (37)
M31	349 (72)	0.35 (1.17)	7.9 (0.3)	357 (39)
M32	340 (74)	0.06 (0.22)	8.0 (0.4)	355 (35)

Standard Deviation values are given in parenthesis
NOTE: RSC = Raw sewage, A = Anaerobic, F = Facultative, M = Maturation

wastewater in the sewer. In the secondary facultative and maturation ponds there is a reduction in the amount of ammonia as it is incorporated into new algal biomass. This is especially true in the case of the secondary maturation ponds. M20, which has a higher organic load than the other secondary maturation ponds, has more ammonia than ponds M16 to M19, and has a correspondingly lower algal concentration as shown by the chlorophyll-*a* levels.

The physico-chemical parameters studied showed a gradual reduction through the system of lagoons and BOD, COD, alkalinity, ammonia, sulphide,total phosphorus and soluble ortho-phosphate concentrations were all reduced. pH and chlorophyll-*a* concentrations showed an increase and chloride concentrations throughout the system were constant.

It was observed that the different depths of the secondary facultative ponds and secondary maturation ponds have very little affect on BOD, COD, TSS, Chl-*a* and the other nutrient parameters studied (eg. nitrate, nitrite, ammonia, sulphate, sulphide, total phosphorus and soluble ortho-phosphate) since all the secondary facultative pond show similar amounts of these and no significant differences were found in the quantities present in the reactors.

3.5 Removal of Nutrients and other Chemical Parameters from the 10 Pond Series

As was the case with the innovative system the main physico-chemical parameters in the 10 pond series showed a gradual reduction throughout the system. BOD, COD, alkalinity, ammonia, sulphide, total phosphorus and soluble ortho-phosphate concentrations were all reduced. pH, chlorophyll-*a*

and nitrate increased throughout the system and chloride, as in the innovative system, remained relatively constant.

The first four ponds of this system, as well as showing a good reduction in BOD, were also the site for the majority of the reductions in nutrients and succeeding ponds had a much more limited affect. Given that the retention time of the individual ponds and the series as a whole is considerably shorter than is normally found in a system of WSPs, the physico-chemical results are excellent meeting the maximum permissible BOD and SS concentrations stipulated by the EU Directive on Urban Wastewater (Council of the European Communities, 1991) of a filtered BOD5 of less than 25 mg/l and a suspended solids concentration of less than 150 mg/l after just two ponds, the anaerobic and the facultative ponds (based on the column samples). and with a retention time of just 3 days.

3.6 Pathogen Removal in the Innovative System

Tables 3.5 and 3.6 show the mean (geometric) number, standard deviation (population) and number of samples taken of bacteria present at the outlet of each pond studied and the k_T first order rate constant values. An analysis of variance showing the significant differences between the numbers of organisms in each pond is given in Tablees 3.7a and 3.7b.

Mechanisms of pathogen removal have been discussed in section 1.7.

Mean temperatures were 23°C in the anaerobic ponds and 25°C in the others. Typically neutral pH mean values and dissolved oxygen conc-entrations of less than 1mg/l were detected in the anaerobic ponds. Both pH and dissolved

Table 3.5 Geometric Mean, Standard Deviation and Number of Samples of Bacteria at the Effluent Removal Point of the Ponds in the Innovative System

Pond	F.C.	F.S.	C.P.	SAL	CAMP	V.C.	R.V.
RS	2.66×10^{7}	3.92×10^{6}	2.07×10^{4}	329.2	18.2	3.25	6.70×10^{4}
	1.7×10^{7} (103)	1.7×10^{6} (93)	1.1×10^{5} (55)	2.2×10^{3} (45)	96.3 (20)	5.2 (27)	1.9×10^{5} (15)
A9	7.06×10^{6}	1.14×10^{6}	1.32×10^{4}	361.5	7.7	2.63	1.71×10^{4}
	4.3×10^{6} (51)	2.4×10^{5} (46)	5.4×10^{4} (23)	9.2×10^{2} (25)	20.4 (11)	6.1 (16)	1.7×10^{4} (13)
A10	7.15×10^{6}	9.50×10^{5}	1.60×10^{4}	365.6	10.8	1.95	1.78×10^{4}
	6.1×10^{6} (50)	2.3×10^{5} (45)	6.9×10^{4} (27)	9.2×10^{2} (22)	17.8 (11)	2.8 (17)	1.4×10^{4} (10)
F21	1.08×10^{6}	9.72×10^{4}	3.90×10^{3}	29.9	3.6	1.25	8.21×10^{3}
	1.5×10^{6} (48)	2.5×10^{5} (41)	1.2×10^{4} (25)	9.5×10^{1} (25)	9.5 (12)	2.0 (18)	1.1×10^{4} (12)
F22	9.22×10^{5}	6.10×10^{4}	4.31×10^{3}	30.9	5.5	1.19	1.02×10^{4}
	1.3×10^{6} (48)	1.2×10^{5} (44)	1.2×10^{4} (23)	1.2×10^{2} (24)	11.7 (12)	1.6 (18)	9.5×10^{3} (11)
F23	9.15×10^{5}	6.88×10^{4}	2.77×10^{3}	19.7	3.4	1.17	6.92×10^{3}
	1.5×10^{6} (49)	1.4×10^{5} (44)	8.7×10^{3} (25)	1.2×10^{2} (26)	6.9 (12)	1.7 (18)	7.0×10^{3} (12)
F24	7.76×10^{5}	5.00×10^{4}	2.18×10^{3}	14.5	2.8	1.06	5.50×10^{3}
	1.3×10^{6} (48)	8.1×10^{4} (44)	8.3×10^{3} (31)	44 (22)	3.6 (12)	1.1 (18)	9.5×10^{3} (12)
F25	8.90×10^{5}	5.78×10^{4}	2.84×10^{3}	23.0	3.7	1.08	5.50×10^{3}
	1.4×10^{6} (44)	8.9×10^{4} (45)	1.5×10^{4} (26)	1.2×10^{2} (22)	8.5 (12)	1.3 (18)	5.0×10^{3} (13)
M15	2.36×10^{4}	2.77×10^{3}	2.95×10^{2}	3.2	1.7	0	5.37×10^{3}
	9.1×10^{4} (40)	7.4×10^{3} (40)	1.5×10^{3} (27)	36 (24)	5.2 (11)	- (11)	3.6×10^{3} (10)
M16	5.45×10^{2}	7.28×10^{2}	1.72×10^{2}	1.1	0	0	1.25×10^{3}
	2.4×10^{3} (40)	5.0×10^{3} (37)	2.2×10^{3} (27)	1.1 (25)	0.3 (10)	- (10)	1.1×10^{3} (10)
M17	6.81×10^{2}	8.33×10^{2}	2.92×10^{2}	1.2	1.1	0	1.90×10^{3}
	2.5×10^{3} (41)	2.1×10^{3} (36)	2.0×10^{3} (24)	1.2 (25)	1.1 (11)	- (11)	1.1×10^{3} (8)
M18	7.58×10^{2}	1.44×10^{3}	2.17×10^{2}	1.4	0	0	2.36×10^{3}
	5.8×10^{3} (46)	2.8×10^{3} (38)	1.3×10^{3} (25)	3.8 (25)	0.3 (11)	- (11)	1.5×10^{3} (10)
M19	6.30×10^{2}	9.17×10^{2}	2.46×10^{2}	1.2	1.1	0	2.34×10^{3}
	3.9×10^{3} (44)	2.5×10^{3} (35)	9.8×10^{2} (25)	1.2 (23)	1.1 (10)	- (10)	1.3×10^{2} (9)
M20	1.60×10^{3}	9.44×10^{2}	2.54×10^{2}	1.4	1.2	0	1.79×10^{3}
	1.6×10^{4} (47)	1.6×10^{3} (37)	3.1×10^{3} (26)	1.9 (24)	1.4 (9)	- (11)	2.3×10^{3} (8)
M21	3.49×10^{1}	7.37×10^{2}	7.21×10^{1}	1.0	0	0	15.1
	1.6×10^{2} (40)	5.1×10^{3} (35)	1.1×10^{3} (24)	1.1 (25)	- (10)	- (11)	2.7×10^{2} (10)
M22	4.17×10^{1}	3.79×10^{2}	9.12×10^{1}	1.0	0	0	2.6
	1.4×10^{2} (44)	1.3×10^{3} (33)	8.5×10^{2} (22)	1.0 (28)	- (10)	- (11)	87 (11)
M23	8.30×10^{1}	3.51×10^{2}	8.92×10^{1}	1.4	0	0	0
	3.7×10^{2} (42)	8.2×10^{2} (35)	7.5×10^{2} (25)	4.2 (25)	- (10)	- (11)	- (12)
M24	1.92×10^{1}	4.03×10^{2}	1.21×10^{2}	1.1	0	0	0
	8.2×10^{1} (39)	1.0×10^{3} (35)	4.6×10^{2} (21)	1.1 (28)	- (10)	- (11)	- (12)

Mean Numbers
Standard Deviation - Population
(Number of Samples)

F.C. - Faecal coliforms
F.S. - *Faecal streptococci*
C.P. - *Clostridium perfringens*
SAL - *Salmonella*
CAMP - *Campylobacter*
V.C. - *Vibrio cholerae*
R.V. - Rotaviruses

Table 3.6 k_T Values (First Order Rate Constant for Bacterial Removal) of Ponds in the Innovative System

Pond	F.C.	F.S.	C.P.	SAL	CAMP	V.C.	R.V.
A9	2.76	2.44	0.57	-0.09	1.36	0.24	2.92
A10	2.72	3.13	0.29	-0.10	0.69	0.89	2.76
F21	1.86	3.25	0.91	4.03	0.53	0.31	0.38
F22	1.68	4.03	0.60	2.69	0.17	0.25	0.18
F23	1.35	3.05	0.85	3.49	0.34	0.21	0.30
F24	1.36	3.32	0.95	4.01	0.39	0.18	0.36
F25	1.16	2.85	0.69	2.47	0.26	-	0.46
M15	9.96	6.10	2.59	1.68	0.33	-	0.08
M16	6.04	0.40	0.10	0.27	-	-	0.47
M17	6.73	0.47	0.002	0.33	0.11	-	0.37
M18	10.04	0.31	0.12	0.56	-	-	0.43
M19	12.15	0.67	0.07	0.56	0.18	-	0.43
M20	13.75	1.93	0.16	1.29	0.42	-	2.00
M21	3.59	0.06	0.46	0.04	-	-	25.30
M22	2.97	0.31	0.32	0.04	-	-	148.10
M23	1.39	0.35	0.33	-0.05	-	-	-
M24	7.96	0.34	0.23	0.03	-	-	-

F.C. - Faecal coliforms CAMP - *Campylobacter* C.P. - *Clostridium perfringens* F.S. - *Faecal streptococci*
V.C. - *Vibrio cholerae* R.V. - Rotaviruses SAL - *Salmonella*

NOTE: RSC = Raw sewage, A = Anaerobic, F = Facultative, M = Maturation

Figure 3.1a Significant Differences (ANOVA) between the Number of Organisms in the Ponds of the Innovative System

Faecal coliforms

	A9	A10	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
A9		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A10	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F21	ND	ND		-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F22	ND	ND	-		-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F23	ND	ND	-	-		-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F24	ND	ND	-	-	-		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F25	ND	ND	-	-	-	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M15	ND	ND	ND	ND	ND	ND	ND		-	-	-	-	-	-	-	-	-
M16	ND	ND	ND	ND	ND	ND	ND	S		-	-	-	-	S	**S**	S	S
M17	ND	ND	ND	ND	ND	ND	ND	S	-		-	-	S	S	**S**	S	S
M18	ND	ND	ND	ND	ND	ND	ND	-	-	-		-	-	S	**S**	S	S
M19	ND	ND	ND	ND	ND	ND	ND	S	-	-	-		-	S	**S**	S	S
M20	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-		**S**	**S**	S	S
M21	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-		-	-	-
M22	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-	-		-	-
M23	ND	ND	ND	ND	ND	ND	ND	S	-	-	S	S	S	S	-		-
M24	ND	ND	ND	ND	ND	ND	ND	S	-	-	S	S	S	-	-	-	

Faecal streptococci

Salmonella

	A9	A10	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
A9		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A10	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F21	ND	ND		-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F22	ND	ND	-		-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F23	ND	ND	-	-		-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F24	ND	ND	-	-	-		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F25	ND	ND	-	-	-	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M15	ND	ND	ND	ND	ND	ND	ND		**S**	**S**	**S**	**S**	**S***	**S**	**S**	**S**	**S**
M16	ND	ND	ND	ND	ND	ND	ND	-		-	-	-	**S***	**S**	-	-	-
M17	ND	ND	ND	ND	ND	ND	ND	-	-		-	-	**S**	**S**	**S**	-	-
M18	ND	ND	ND	ND	ND	ND	ND	-	-	-		-	-	-	-	-	-
M19	ND	ND	ND	ND	ND	ND	ND	-	-	-	-		-	**S**	-	-	-
M20	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-		S	-	-	-
M21	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	-		-	**S**	-
M22	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	-	-		S	-
M23	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	-	-	-		-
M24	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	-	-	-	-	

Clostridium perfringens

KEY
- ND No ANOVA done
- \- ANOVA showed the numbers of organisms in the ponds were not significantly different
- S ANOVA showed the numbers of organisms in the ponds were significantly different (Homogeneous)
- **S** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was confirmed by non-parametric tests
- **S*** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was not, however, confirmed by non-parametric tests

A= anaerobic
F = facultative
M= maturation

Figure 3.1b Significant Differences (ANOVA) between the Number of Organisms in the Ponds of the Innovative System

Campylobacter

Rotaviruses	A9	A10	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
A9		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A10	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F21	ND	ND		-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F22	ND	ND	-		-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F23	ND	ND	-	-		-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F24	ND	ND	-	S	-		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F25	ND	ND	-	S	-	-		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M15	ND	ND	ND	ND	ND	ND	ND		-	-	-	-	-	-	-	-	-
M16	ND	ND	ND	ND	ND	ND	ND	S		-	-	-	-	-	-	-	-
M17	ND	ND	ND	ND	ND	ND	ND	S	-		-	-	-	-	-	-	-
M18	ND	ND	ND	ND	ND	ND	ND	S	-	-		-	-	-	-	-	-
M19	ND	ND	ND	ND	ND	ND	ND	S	-	-	-		-	-	-	-	-
M20	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-		-	-	-	-
M21	ND	ND	ND	ND	ND	ND	ND	**S**	**S**	**S**	**S**	**S**	-		ND	-	ND
M22	ND	ND	ND	ND	ND	ND	ND	**S**	**S**	**S**	**S**	**S**	S			-	ND
M23	ND	ND	ND	ND	ND	ND	ND	**S**	S	**S**	S	S	S				-
M24	ND	ND	ND	ND	ND	ND	ND	**S**	**S**	**S**	**S**	**S**	S				

Vibrio cholerae

	A9	A10	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
A9		-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A10			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F21				-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F22					-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F23						-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F24							-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F25								ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M15									ND	ND	ND	-	ND	ND	ND	ND	ND
M16										ND	ND	-	ND	ND	ND	ND	ND
M17											ND	-	ND	ND	ND	ND	ND
M18												-	ND	ND	ND	ND	ND
M19													ND	ND	ND	ND	ND
M20														ND	ND	ND	ND
M21															ND	ND	ND
M22																ND	ND
M23																	ND
M24																	

KEY
- ND No ANOVA done
- \- ANOVA showed the numbers of organisms in the ponds were not significantly different
- S ANOVA showed the numbers of organisms in the ponds were significantly different (Homogeneous)
- **S** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was confirmed by non-parametric tests
- **S*** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was not, however, confirmed by non-parametric tests

A= anaerobic
F = facultative
M= maturation

oxygen concentration increased throughout the system, as the organic loadings decreased, reaching, in the effluents of the tertiary maturation ponds, levels of between pH 8.7-9.2 and 9.5-10.6 mg O2/l.

It should be noted that when considering the die-off of *Campylobacter, Salmonella* and *Vibrio cholerae,* it must be taken into account that the number of organisms entering the pond systems was very low and the rate constants have been calculated from very small mean numbers, for example, of between 1.06 and 3.25 organisms per 100 ml for VC. These low numbers obviously introduce an element of inaccuracy since there is a greater chance of low or no detection. A result of zero can only be considered as being "none detected" rather than "none present" and this became more apparent during the in-pond studies when, at certain times of the day, some organisms (in particular *Salmonella*) which had not previously been detected in over a year of performance evaluation were found.

3.6.1 Anaerobic Ponds.

The anaerobic ponds in this system are identical in geometry but a note on pathogen removal is included here for completeness. Designed for BOD removal anaerobic ponds are devoid of oxygen. They are normally highly turbid (therefore minimal light penetration) with a pH of around 7.5. Bacterial removal in these ponds is generally considered to be by sedimentation since other removal mechanisms, such as starvation, high pH and solar radiation, do not apply. They are not expected to remove bacterial pathogens as well as facultative and, especially, maturation ponds.

Both A9 and A10 showed similar results, and thus good evidence of reproducibility, for bacterial removal. Mean FC and FS removals in the anaerobic ponds were 73% and 73.5% (0.57 log units). *Salmonella* mean

numbers show an apparent increase by 9.8% and 11.1% respectively in ponds A9 and A10 although the large standard deviations mean that the number of *Salmonella* present in the anaerobic ponds is not significantly higher than that of the raw sewage. Mean removal of *Clostridium perfringens* was 29.5% (0.1 log units) and *Campylobacter* die-off was 49.5%. A mean of 33% of *Vibrio cholerae* were removed in ponds A9 and A10.

The results show the environment of an anaerobic pond is one which is generally prejudicial to bacterial survival. The increase in *Salmonella* seen in the anaerobic ponds is similar to that observed by Soler *et al.* (1995) in ponds in Spain. It would seem unlikely that the *Salmonella* are able to increase in number in the anaerobic ponds and this apparent increase may be due to low detection of these pathogens in the raw sewage. The reasons for inhibition in the raw sewage are not known (and this is an possible area for further research to establish whether sewage spiked with *Salmonella* shows inhibition in a similar way) but this leads to an underestimation of numbers present and of the true rate of removal.

Rotavirus enumeration was chosen since it gives an indication of viral die-off in WSP and because they are known to be the cause of acute gastroenteritis in adults (Keswick *et al.*, 1982) and diarrhoea and vomiting in children (Brandt *et al.*, 1983). Virus removal in anaerobic ponds was reported to be minimal by Irving (1982) but Rao *et al.* (1981) found a viral die-off of 96% in single ponds with retention times of 2.7 days. The results presented here show a mean percentage removal of 74% (equivalent to a log reduction of 0.6 units) supporting the latter of these two authors. The wide variation in virus removal in WSP in different geographical areas and by different authors may, perhaps, be due to different field and experimental conditions and sampling

and enumeration methods (Rao *et al.* , 1981) since no standard method has been adopted as yet.

Although the primary function of anaerobic ponds is BOD removal, reports in the literature state that mean faecal bacterial die-offs of up to 93%, i.e. more than 1 log reductions, are attainable (Arthur, 1982).

3.6.2 Secondary Facultative Ponds.

Secondary facultative ponds are also designed primarily for BOD reduction but pathogen removal also occurs in these ponds to the extent that a mean of 83% (0.97 log units) of FC which entered the pond were removed, although the large standard deviations mean that the numbers of FC in the anaerobic and secondary facultative ponds were not significantly different. Since they receive the effluent of the anaerobic ponds, these ponds are considerably less turbid, but have a large biomass of algae and so are generally well oxygenated, especially at the surface since thermal stratification often limits mixing within the pond and the algae present in the pond tend to be present in a band 20-30 cms thick which adjusts its position in the upper photic zone (frequently to a depth of between 40 and 65 cm, see section 4.2.3) of the pond largely in response to light and nutrient concentrations, but are more dispersed during the hours of darkness when they are thought to enter the sediments (Pearson *et al.*, 1987b)

The five facultative ponds show similar log removals of FC with values of 0.82 (F21) to 0.96 (F24) with no significant differences in the number of FC in the different ponds. The square pond, F25, showed comparable removal of FC with the elongated pond of the same depth and retention time.

FS die-off in the facultative ponds was consistently better than that of FC with log reductions in the range of 1.03 (F21) to 1.32 (F24) The differences in

numbers of FS in the various secondary facultative ponds were found not to be significant. Once again, the square pond showed comparable removal to that of the elongated pond.

The removal of *Salmonella* in the secondary facultative ponds was better than in the anaerobic ponds and was comparable with that of FS with log removals of between 1.08 (F21) and 1.40 (F24). The least die-off occurred in the two shallowest ponds F21 and F22 (1 m and 1.33 m deep respectively) and the greatest die-off followed the pattern of the other organisms in being F24, the deepest rectangular pond (2 m deep). The square pond, F25, was once again observed to remove a comparable amount of *Salmonella* as the elongated pond with the same depth. The differences in the numbers of *Salmonella* in the various ponds were not significant. Overall, the mean removal of *Salmonella* in the secondary facultative ponds was 93.8% (equivalent to a log removal of 1.3 units).

The low number of *Campylobacter* species in the raw sewage, in this case, makes log reduction an unsuitable measure of die-off since a 1-2 log reduction would be sufficient to ensure mean *Campylobacter* numbers of less than 1 per 100 ml in the pond effluent. These low numbers are probably because of the limited quantity of the citys' wastewater collected in the sewerage system, especially from the poor areas of the city. Stelzer and Jacob (1991) reported *Campylobacter* levels in raw sewage in Germany to be 10^3 per 100 ml but these authors suggest that the nature of *Campylobacter* (it is microaerophilic and survives better at low temperatures) may be that lower numbers could be expected in tropical climates. Since *Campylobacter* is not an organism normally included in performance evaluation, especially in developing countries where the anaerobic jars and gas packs needed to carry

out the enumeration procedure are inhibitively expensive, there is a lack of published data concerning normal sewage concentrations.

The removal of *Campylobacter* varied with means of between 60% in F22 and 70% in F24 following a similar pattern seen with the other pathogens studied. None of the treatments gave significantly different numbers.

Clostridium perfringens showed the same pattern of removal with a range of log reductions of 0.53 (F22) to 0.82 (F24) but with no significant differences once again. The removal rate of *Clostridium perfringens* in the secondary facultative ponds is higher than that in the anaerobic ponds as the respective k_T values show.

Rotavirus removal in these ponds is less than in the anaerobic ponds, F22 once again removing the least number (0.23 log units removed). However, the greatest rotavirus removal was observed in pond F25 with a 0.58 log reduction in numbers. The number of rotavirus in pond F22 was significantly higher than those in ponds F24 and F25 indicating a possible depth affect. Once again, the large standard deviations involved mean that F21 did not also show significantly different numbers than the two deepest ponds.

Vibrio cholerae removal was greatest in F24 with 54% of the influent vibrios dying. The other secondary facultative ponds had mean removals of between 45% (F21) and 53% (F25). Once again, the very low numbers of VC in the raw sewage make the expression of the results in terms of log reductions unsuitable. The numbers of VC in the various secondary facultative ponds were not significantly different.

In terms of the actual numbers of bacteria removed in the secondary facultative ponds studied the different depths had very little influence.

Ponds F21 to F24 had very similar log removals in terms of FC, FS, *Salmonella, Campylobacter, Vibrio cholerae* and *Clostridium perfringens*. Rotavirus was different in that significantly more viruses were found in ponds F24 and F25 than in pond F22. These differences may be because of different predominant hydraulic regimes of the ponds since a shallow rectangular pond would be expected to have large dead zones (Mangelson and Watters, 1972) and both the deeper rectangular pond and the square pond may be assumed to be better mixed. The results may indicate that completely mixed conditions are more conducive to rotavirus removal. This could be due to a higher settleable solids content through resuspension as a consequence of mixing (although the recorded settleable solids contents of these particular ponds were not significantly different), and the ability of rotaviruses to adhere to solids, including algae, and be subsequently removed by sedimentation. Unfortunately, no clear conclusions are possible due to the lack of significance between the results.

The shapes of the deepest ponds also had little influence and there were no significant differences between any of the measured parameters in ponds F24 and F25. Mara and Pearson (1987b) have suggested that ponds with length to breadth ratios of more than 2-3:1 may encourage the formation of sludge banks near the inlet and the results of a white towel test performed on all of the ponds at three sites along their lengths supported these findings (Table 3.8).

In the squarer ponds (F25 and F26) the sludge was distributed more or less evenly over the bottom of the pond but the more elongated ponds F21-F24 had more build up of sludge near the inlet of the pond.

Table 3.8 Towel Test Performed on the Facultative ponds to show Sludge Distribution.

POND	INLET	MIDDLE	OUTLET
F21	.15 m	.13 m	.11 m
F22	.35 m	.11 m	.11 m
F23	.35 m	.13 m	.11 m
F24	.39 m	.18 m	.13 m
F25	.42 m	.31 m	.31 m
F26	.45 m	.40 m	.30 m

3.6.3 Primary Maturation Ponds

In all cases, except rotavirus, the large primary maturation pond (M15) showed pathogen reductions of an equal or greater magnitude than seen in the preceeding ponds although more than 90% of the organisms of all the species investigated, except *Campylobacter* and *V. cholerae*, had been removed by this stage in the treatment process. It was expected to find better log reductions and removal rate constants in the maturation ponds because these ponds are designed primarily for the disinfection of wastewater. The mean FC, FS and *Clostridium perfringens* log reductions were 1.59, 1.38 and 1.04 respectively, representing larger reductions than those recorded in the secondary facultative ponds. *Salmonella* removal, however, was poorer with a die-off of 0.87 log units, less than in the preceeding secondary facultative ponds. The same was true for *Campylobacter* and rotavirus with mean log reductions of 0.35 and 0.12 respectively. The amount of algae in the primary maturation pond was somewhat less than that in the preceeding ponds (369 mg/m^2 compared to a mean of 647 mg/m^2 in the secondary facultative ponds) but the total suspended solids and settleable solids concentrations were not significantly different to those in the secondary facultative ponds

and, thus, the lower rotavirus removal could not be considered due to less sedimentation taking place.

No *V. cholerae* was detected at the effluent take off point of M15, from which samples were collected, but this is not surprising since there was a mean of less than 1 organism entering the pond from the effluents of F21 to F25.

3.6.4 Secondary Maturation Ponds

The effluent of pond M15 was split by V-notches and flowed into the secondary maturation ponds, M16 to M20, and the rock filters. Since the influent quality was common to all the secondary ponds any differences in the effluent quality between these ponds would be due to the treatment received in that individual pond.

Log removal of FC in the secondary maturation ponds is comparable to that in M15 with a mean of 1.49. FS removal appears to be less efficient than in the primary maturation pond with a mean log reduction of 0.47 much poorer than the 1.38 log reduction recorded in M15. CP removal was very poor compared to that in M15 and in four of the five secondary maturation ponds a log reduction of less than 0.1 was observed. *Salmonella* and *Campylobacter* similarly showed log reductions less than those in M15 but the very small numbers of influent organisms may distort this method of measurement. The numbers of *Salmonella* present in the secondary maturation ponds was significantly different to the number detected in M15. Rotavirus removal in ponds M16 to M20 was also significantly better than in M15 and comparable with the reductions seen in the secondary facultative ponds.

FC removals in the secondary maturation ponds were very similar and the lowest log reduction was that of pond M20, the shallow pond with the larger

length to breadth ratio but short retention time. Table 3.7 shows the significant differences in the numbers of FC present in the ponds and it can be seen that the number of FC in M20 is significantly different to that in M17.

Numbers of FS were similar in all ponds and there were no significant differences in the secondary maturation ponds.

CP removal in the secondary maturation ponds was similar in all the ponds with a range of 0.004 (M17) and 0.23 (M16) mean log removal. This is poor when compared to the 1.04 log removal achieved in M15. However, there were no significant differences between the different secondary maturation ponds or between the primary and secondary maturation ponds in terms of numbers of CP present in the ponds but the differences in removal are reflected in the k_T values as discussed below.

Significant differences in the numbers of *Salmonella* in the secondary maturation ponds were found between M16 and M20 and between M17 and M20.

The numbers of *Campylobacter* and rotaviruses present in the secondary maturation ponds were significantly different to those present in M15, but there were no differences between the various secondary maturation lagoons.

The reductions of *Salmonella* and *Campylobacter* are difficult to comment upon because of the very few influent organisms and, similarly, there were no *Vibrio cholerae* detected in the influent of the secondary maturation ponds.

Ponds M18 and M19 are identical in their shapes and depths, and thus retention times, and showed no significant differences in the numbers of

pathogens present. This illustrates how predictable WSP performance can be and that the behaviour of the system is reproducible. M20, although a much narrower pond with a shorter retention time of just 1 day, removed pathogens well, in all cases being comparable to the other secondary maturation ponds. The k_T values of M20 (discussed in section 3.8) show how efficient it was and these rates are higher than for the other secondary maturation ponds in the cases of all the pathogens.

3.6.5 Tertiary Maturation Ponds

The tertiary maturation ponds were originally included in the design of the pond system to act as polishing ponds and during several short periods of the whole experimental time the ponds underwent separate experiments investigating algal removal techniques. Pond M23 underwent a period from November 1991 to March 1992 as a floating macrophyte pond and several times pond M21 had macroinvertebrates introduced to investigate the affects of grazing on the algal population. Hence the results of these ponds are complicated by these other influences. During the time that M23 was acting as a macrophyte pond the numbers of bacteria, especially *Salmonella*, were higher than in the other periods suggesting that these organisms were either transferred into the pond with the plants (even though the roots and leaves were thoroughly washed with clean running water before introduction into the pond) or the pond was providing a habitat conducive to the survival and reproduction of the bacteria.

Generally, pathogen removal throughout the system was good with total removals between 99.664% (*Salmonella*) and 99.99983 (FC) which corresponds to a 2 to 6 log removal.

3.7 Pathogen Removal in the 10 Pond Series

Results of the performance evaluation of this system are shown in Tables 3.9 and 3.10. Significant differences between the numbers of pathogens present in the ponds are show in Table 3.11.

The sampling of the ponds in the series of 10 ponds differed from that of the innovative system in that two methods were used to be compared as part of the wider project horizons. Thus, both column sampling and effluent sampling were undertaken and the results from each are given. Effluent sampling has been used for monitoring WSP systems but there is evidence that the diurnal variations, not accounted for in grab effluent samples, may be better represented by taking column samples (Pearson *et al.*, 1987). Table 3.11 shows that significant differences in the results from the two sampling methods were found in only two cases, FC numbers in samples taken from M27 and M28. Statistical analysis was carried out on the results from the grab samples because of the larger sample number. As discussed in chapter 2, the ponds, except the anaerobic pond, A11, are identical having the same geometries, volumes and retention times.

The results for FC show that removal was in the ranges of -0.83 to 1.00 orders of magnitude for samples taken by the column method and -0.06 to 0.81 for effluent samples, with overall reductions of 4.5 and 5.0 $\log_{10}$ units respectively. The greatest removal occurred in the first four maturation ponds with the anaerobic, secondary facultative and the last four maturation ponds removing less FC and, in the case of the last maturation pond, the number of FC increased (with respect to the number in the previous pond).

Table 3.8 Geometric Mean, Standard Deviation and Number of Samples of Bacteria in the Ponds in the 10 Pond Series

Pond	F.C.	F.S.	C.P.	SAL	CAMP	V.C.	R.V.
			COLUMN:				
RS	2.66×10^{7} 1.7×10^{7} (103)	3.92×10^{6} 1.7×10^{6} (93)	2.07×10^{4} 1.1×10^{5} (55)	329.2 2.2×10^{3} (45)	18.2 96.3 (20)	3.25 5.2 (27)	6.70×10^{4} 1.9×10^{5} (15)
A11	7.44×10^{6} 4.0×10^{6} (21)	1.04×10^{6} 7.9×10^{5} (20)	6.87×10^{3} 3.6×10^{4} (14)	51.3 321 (10)	10.4 2.9 (3)	1.43 2.6 (5)	3.75×10^{4} 2.7×10^{4} (5)
F26	3.24×10^{6} 2.6×10^{6} (20)	3.07×10^{5} 7.7×10^{5} (20)	2.55×10^{3} 1.4×10^{4} (15)	37.0 340 (10)	7.1 5.3 (3)	1.35 2.3 (6)	2.18×10^{4} 2.8×10^{4} (5)
M25	5.37×10^{5} 8.8×10^{5} (19)	6.00×10^{4} 7.4×10^{5} (19)	1.00×10^{3} 2.5×10^{3} (14)	17.2 28 (10)	5.4 110 (3)	1.0 1.0 (6)	1.41×10^{4} 1.4×10^{4} (5)
M26	1.04×10^{5} 5.3×10^{5} (19)	8.27×10^{3} 1.9×10^{4} (16)	4.07×10^{2} 2.4×10^{3} (16)	3.9 10 (9)	3.4 1.6 (3)	0 - (5)	9.17×10^{3} 4.7×10^{3} (5)
M27	1.04×10^{4} 8.0×10^{4} (19)	1.73×10^{3} 2.4×10^{3} (18)	2.30×10^{2} 7.5×10^{2} (15)	1.4 1.7 (9)	2.3 3.1 (3)	0 - (5)	6.71×10^{3} 2.2×10^{3} (4)
M28	1.03×10^{3} 1.9×10^{4} (20)	4.94×10^{2} 4.5×10^{3} (17)	1.34×10^{2} 3.8×10^{2} (16)	1.2 1.9 (8)	1.0 1.0 (3)	0 - (5)	2.43×10^{3} 1.1×10^{3} (4)
M29	5.13×10^{2} 4.5×10^{3} (20)	4.25×10^{2} 2.0×10^{3} (17)	4.95×10^{1} 2.3×10^{2} (15)	1.0 0.9 (10)	0 - (3)	0 - (5)	2.14×10^{3} 9.0×10^{2} (5)
M30	1.90×10^{2} 5.3×10^{2} (21)	6.57×10^{2} 1.8×10^{4} (18)	3.82×10^{1} 60 (15)	0 - (10)	0 - (3)	0 - (5)	6.92×10^{2} 4.0×10^{2} (5)
M31	1.15×10^{2} 3.1×10^{2} (20)	3.36×10^{2} 1.9×10^{3} (17)	5.04×10^{1} 5.1×10^{2} (13)	0 - (10)	0 - (3)	0 - (5)	2.53×10^{1} 1.7×10^{2} (5)
M32	7.78×10^{2} 9.0×10^{3} (18)	4.49×10^{2} 2.5×10^{3} (17)	4.50×10^{1} 4.0×10^{2} (14)	0 - (9)	0 - (3)	0 - (5)	0 - (5)
			EFFLUENT:				
A11	6.35×10^{6} 2.5×10^{6} (41)	1.02×10^{6} 1.7×10^{6} (33)	4.06×10^{3} 1.2×10^{4} (22)	58.3 248 (17)	15.85 7.5 (6)	2.14 4.8 (5)	3.02×10^{4} 2.6×10^{4} (5)
F26	2.10×10^{6} 1.8×10^{6} (43)	1.65×10^{6} 2.3×10^{5} (33)	2.18×10^{3} 7.5×10^{3} (20)	81.3 190 (17)	11.40 71.3 (6)	1.32 1.5 (4)	1.02×10^{4} 1.1×10^{4} (4)
M25	7.31×10^{5} 7.6×10^{5} (41)	7.66×10^{4} 1.7×10^{5} (33)	1.52×10^{3} 5.5×10^{3} (21)	34.6 168 (17)	1.76 1.8 (6)	1.0 1.0 (4)	9.55×10^{3} 7.5×10^{3} (5)
M26	1.96×10^{5} 4.1×10^{5} (43)	2.16×10^{4} 7.5×10^{4} (32)	9.85×10^{2} 6.6×10^{3} (21)	13.7 49 (17)	2.56 11.6 (6)	1.3 1.4 (6)	6.03×10^{3} 5.2×10^{3} (5)
M27	4.50×10^{4} 2.9×10^{5} (41)	1.01×10^{4} 5.4×10^{4} (32)	4.73×10^{2} 4.0×10^{3} (22)	6.9 23 (17)	1.62 1.5 (6)	1.0 0.9 (6)	5.89×10^{3} 1.6×10^{3} (4)
M28	6.90×10^{3} 1.0×10^{5} (44)	2.59×10^{3} 2.3×10^{4} (32)	2.79×10^{2} 1.9×10^{3} (25)	3.2 8.4 (18)	1.62 1.5 (6)	1.0 1.0 (7)	2.63×10^{3} 2.9×10^{3} (5)
M29	2.47×10^{3} 3.0×10^{4} (36)	1.49×10^{3} 9.1×10^{3} (32)	1.64×10^{2} 7.3×10^{2} (21)	1.8 3.6 (17)	1.44 1.6 (6)	0 - (6)	1.23×10^{3} 1.6×10^{3} (4)
M30	6.70×10^{2} 1.7×10^{4} (41)	9.93×10^{2} 5.6×10^{3} (30)	1.34×10^{2} 7.7×10^{2} (21)	1.3 2.8 (17)	1.62 2.5 (6)	0 - (6)	1.86×10^{2} 7.7×10^{2} (5)
M31	1.96×10^{2} 6.6×10^{3} (41)	3.77×10^{2} 2.4×10^{3} (26)	9.29×10^{1} 5.6×10^{2} (21)	1.2 2.3 (17)	1.20 1.4 (6)	0 - (6)	1.32×10^{1} 5.6×10^{2} (5)
M32	2.25×10^{2} 2.2×10^{3} (38)	2.42×10^{2} 1.7×10^{3} (28)	6.64×10^{1} 3.6×10^{2} (21)	1.3 4.5 (17)	0 - (6)	0 - (6)	4.57 6.5×10^{2} (5)

Mean Numbers
Standard Deviation Population
(Number of Samples)

F C - Faecal coliforms
F S - Faecal streptococci
C.P - *Clostridium perfringens*
SAL - Salmonella

CAMP - *Campylobacter*
V C. - *Vibrio cholerae*
R V - Rotaviruses

Table 3.9 K_T Values (First Order Rate Constant for Bacterial Removal) of the Ponds in the 10 Pond Series

Pond	FC	FS	CP	SAL	CAMP	VC	R.V.
				COLUMN:			
A11	2.60	2.80	2.00	5.40	0.80	1.30	0.80
F26	0.60	1.20	0.80	0.20	0.20	0.02	0.40
M25	2.50	2.10	0.80	0.60	0.20	0.20	0.30
M26	2.10	3.10	0.70	1.70	0.30	-	0.30
M27	4.50	1.90	0.40	0.90	0.20	-	0.20
M28	4.50	1.30	0.40	0.10	0.70	-	0
M29	0.50	0.10	0.90	0.10	-	-	0.10
M30	0.90	-0.20	0.10	-	-	-	1.10
M31	0.30	0.50	-0.10	-	-	-	13.20
M32	-0.40	-0.10	0.10	-	-	-	-
				EFFLUENT:			
A11	3.20	2.80	4.10	4.70	0.10	0.30	0.60
F26	1.00	-0.20	0.40	-0.10	0.20	0.30	1.00
M25	0.90	10.30	0.20	0.70	2.70	0.20	0.03
M26	1.40	1.30	0.30	0.80	-0.20	-0.10	0.30
M27	1.70	0.60	0.50	0.50	0.30	0.20	0.01
M28	2.80	1.50	0.30	0.60	0	0	0.60
M29	0.90	0.40	0.40	0.40	0.10	-	0.60
M30	1.30	0.30	0.10	0.20	-0.10	-	2.80
M31	1.20	0.80	0.20	0.04	0.20	-	6.50
M32	-0.10	0.30	0.20	-0.04	-	-	0.90

FC - Faecal coliforms
FS - *Faecal streptococci*
CP - *Clostridium perfringens*
SAL - *Salmonella*
CAMP - *Campylobacter*
VC - *Vibrio cholerae*
RV - Rotaviruses

Figure 3.2 Significant Differences (ANOVA) between the Number of Organisms in the Ponds of System XVII - Effluent Sample

Faecal coliforms

Salmonella

	A11	F26	M25	M26	M27	M28	M29	M30	M31	M32
A11		S	**S**	S	**S**	**S**	**S**	**S**	**S**	**S**
F26	-		S	**S**	-	**S**	-	**S**	-	-
M25	-	-		**S**	-	**S**	-	**S**	-	-
M26	S	-	-		**S**	-	-	-	-	-
M27	-	-	-	-		S	S	S	S	S
M28	-	-	-	-	-		S	S	S	S
M29	S	-	-	-	-	-		**S***	S	S
M30	S	-	-	-	-	-	-		-	-
M31	S	-	-	-	-	-	-	-		-
M32	-	-	-	-	-	-	-	-	-	

Rotaviruses

	A11	F26	M25	M26	M27	M28	M29	M30	M31	M32
A11		-	-	S	-	S	S	**S**	**S**	-
F26			-	-	S	**S**	**S**	**S**	**S**	**S**
M25				-	-	-	S	**S**	**S**	**S**
M26					-	-	-	-	S	S
M27						-	**S**	-	**S**	**S**
M28							-	-	**S**	-
M29								-	-	-
M30									-	-
M31										-
M32										

KEY
- ND No ANOVA done
- \- ANOVA showed the numbers of organisms in the ponds were not significantly different
- S ANOVA showed the numbers of organisms in the ponds were significantly different (Homogeneous)
- **S** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was confirmed by non-parametric tests
- **S*** ANOVA showed the numbers of organisms in the ponds were significantly different (Heterogeneous). This was not, however, confirmed by non-parametric tests

A= anaerobic
F = facultative
M= maturation

Table 3.10 Significant Differences between Two Sampling Methods, Column and Effluent, on the Ponds of the 10 Pond Series for Various Bacteria.

POND	FAECAL COLIFORMS	SALMONELLA	ROTAVIRUS
A11	NS	NS	NS
F26	NS	NS	NS
M25	NS	NS	NS
M26	NS	NS	NS
M27	S	NS	NS
M28	S	NS	NS
M29	NS	NS	NS
M30	NS	NS	NS
M31	NS	NS	NS
M32	NS	NS	NS

NS = NOT SIGNIFICANT
S = SIGNIFICANT

The removal of FS followed a similar pattern to that of FC with the first four maturation ponds having log reductions of 0.54 to 0.86 (column) and 0.33 to 1.33 (effluent) compared to the remaining four maturation lagoons which showed reductions of -0.19 to 0.29 (column) and 0.18 to 0.42 (effluent). The anaerobic pond removed a greater number of FS than any of the final four maturation ponds and, although the secondary facultative pond showed significantly different results for the numbers of FS in the column and effluent samples, the column sample showed that there had been a reduction of 0.53 log units which is also a greater removal than in the final maturation ponds. There was an increase in the number of FS present in pond M30.

CP removal was greatest in the anaerobic, secondary facultative pond and primary maturation ponds with the following maturation ponds all showing lower log reductions and there was, once again, an increase in CP numbers in M31.

Salmonella, Campylobacter and *Vibrio cholerae* were eliminated from the system by the seventh, sixth and fourth pond respectively (column samples). However, effluent samples detected both *Salmonella* and rotaviruses in the ponds in which column samples had not detected the presence of these organisms. *Campylobacter* and *Vibrio cholerae*, not detected in any pond after M28 and M25 respectively with the column sampler, were found in all ponds up to M30 and M28 respectively when grab samples of the effluents were taken. It is not clear why these organisms were detected in the effluents and not in the column samples but it may be due to a micro-habitat around the outlet suited to bacterial survival possibly caused by the (rather unsuccessful) scum guards. Floating matter and material attached to the outlet and the scum guard was frequently noted and this may provide a refuge for the bacteria.

The log reduction in rotaviruses was greatest in the final maturation ponds as had also been observed in the ponds of the innovative system and in the two final ponds of this series of ten there were reductions of up to 1.44 units (column, 1.15 effluent sample). When column samples were taken, rotaviruses were not detected in the final pond of the series, M32, but it is known that these organisms were present as they were detected in effluent grab samples.

3.8 Efficiency of Faecal Coliform Removal - k_T Values

The k_T values were calculated using equation 1.20 derived by Marais (1974) and given in section 1.4.4.2 using the actual geometric mean influent and effluent numbers of each organism. In order to establish whether the k_T values differed significantly between ponds, bimonthly values were calculated for FC and an one-way analysis of variance was performed after logarithmic transformation when appropriate. Table 3.13 shows the significant differences between the k_T values for Faecal coliforms and *Faecal streptococci* in the ponds of the innovative system.

The low influent numbers of some of the pathogens investigated, especially *Salmonella, Campylobacter,* and *Vibrio cholerae,* mean that it is difficult to comment on the removal efficiency of the ponds with respect to these organisms.

3.8.1 k_T values in the secondary facultative ponds.

A simple regression performed on the overall k_T values of FC in the secondary facultative ponds gave a regression coefficient of 0.9 indicating a strong correlation of FC with the depth of the pond. However, when an

Figure 3.3 Significant Differences (ANOVA) between the Kt Value of Organisms in the Ponds of the Innovative System

Faecal coliforms

Faecal streptococci	A9	A10	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
A9		-	-	-	S	S	S	**S**	ND	ND	ND	ND	ND	ND	ND	ND	ND
A10	-		-	-	S	-	S	S	ND	ND	ND	ND	ND	ND	ND	ND	ND
F21	-	-		-	-	-	-	S	-	-	-	-	-	ND	ND	ND	ND
F22	-	-	-		-	-	-	S	-	-	-	-	-	ND	ND	ND	ND
F23	-	-	-	-		-	-	S	-	-	-	-	-	ND	ND	ND	ND
F24	-	-	-	-	-		-	**S**	-	-	-	-	-	ND	ND	ND	ND
F25	-	-	-	-	-	-		**S**	-	-	-	-	-	ND	ND	ND	ND
M15	-	-	-	-	-	-	-		**S***	-	-	-	-	-	S	-	-
M16	ND	ND	S	S	S	S	S	S		-	-	-	-	-	-	-	-
M17	ND	ND	S	S	S	S	S	S	-		-	-	-	-	-	**S***	-
M18	ND	ND	S	S	S	S	S	S	-	-		-	-	-	-	-	-
M19	ND	ND	S	S	S	S	S	S	-	-	-		-	-	-	-	-
M20	ND	ND	S	S	S	S	S	S	-	-	-	-		S	-	-	-
M21	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-		-	-	-
M22	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-	-		-	-
M23	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-	-	-		-
M24	ND	ND	ND	ND	ND	ND	ND	S	-	-	-	-	-	-	-	-	

KEY
- ND No ANOVA done
- \- ANOVA showed the Kt values of organisms in the ponds were not significantly different
- S ANOVA showed the Kt values of organisms in the ponds were significantly different (Homogeneous)
- **S** ANOVA showed the Kt values of organisms in the ponds were significantly different (Heterogeneous). This was confirmed by non-parametric tests
- **S*** ANOVA showed the Kt values of organisms in the ponds were significantly different (Heterogeneous). This was not, however, confirmed by non-parametric tests

A= anaerobic
F = facultative
M= maturation

analysis of variance was performed on the same data it showed that although there is a correlation between depth and the k_T values for FCs, the differences between the k_T values are not significant and all the ponds are, therefore, equally efficient at removing FC. This indicates that the depth and shape of this type of pond do not appear to influence the rate at which pathogens are removed.

3.8.2 k_T values in the primary maturation pond.

The k_T values for FC were significantly higher in M15 than in the secondary facultative ponds indicating that the primary maturation pond is more efficient at removing FC than are the secondary facultative ponds.

3.8.3 k_T values in the secondary maturation ponds.

The overall k_T values for FC are inversely proportional to the depths of the ponds as shown in Table 3.6 with a coefficient of correlation of 0.9. Once again, however, if the bimonthly k_T values are plotted this coefficient is reduced to 0.4 suggesting no correlation between the constants for pathogen removal and depth. k_T values of FC for the secondary maturations ponds were all significantly higher than those of the secondary facultative ponds, as was expected since maturation ponds are designed to primarily remove pathogens.

3.8.4 k_T values in the tertiary maturation ponds.

Die-off of FC in the tertiary ponds was not as good as the preceeding secondary maturation ponds as shown by the lower k_T values. This could be because of the smaller influent numbers but it is possible that the group of organisms known as faecal coliforms may be composed of different species some of which being more resistant to those mechanisms of removal which are normally considered to be responsible for the die-off of pathogenic

bacteria in WSP. It is possible that the most susceptible organisms of the FC population had been removed in the lagoons up to and including the secondary maturation ponds and the poor k_T values in the tertiary ponds are due to the residual population being *ipso facto* - the more hardy survivors of the original population. This would need to be investigated by carrying out identification of the faecal coliform organisms present in each pond. Of the tertiary ponds, the pond with the built-in chicane (M24) was the most efficient, shown by the largest k_T value but the results for this pond were not significantly different to those of the other tertiary ponds.

3.8.5 k_T values in the 10 pond Series

The first order rate constants for the removal of the different pathogens confirm that the first four maturation ponds in the series are the most efficient. This is especially apparent in the results obtained with the column sampler.

The differences in the k_T values of the ponds in this system are not due to differences in geometry or retention time since all the ponds are identical. Simple regression of the influent numbers of FC and the k_T value, and also of the areal load and the k_T value both gave a coefficients of correlation of 0.1 showing that the k_T values were not due to differences in the organic loading or in the number of influent organisms.

Chapter 4 Experimental Results - In-Pond Profile Studies

4.1 Introduction

Biological processes are essential components of most wastewater treatment systems including stabilization ponds and in order to understand the functioning of ponds and the degree of influence of geometry these processes must be investigated. From October 1992 to March 1993 studies on pond profiles based upon 24 hour in-pond experiments were carried out on various ponds of each system. The purpose of these experiments was to investigate the processes occurring in the ponds and to characterise the changes throughout the whole diurnal cycle in terms of light, pH, temperature, dissolved oxygen concentration, BOD, chlorophyll-*a*, total suspended solids, faecal coliforms and *Salmonella*. Analysis for various other physico-chemical parameters was undertaken on some of the profiles and this included COD, alkalinity, sulphide, sulphate, total phosphorus, ortho-phosphate and chloride. In total eleven profiles were performed on the dates given in Table 4.1. The meteorological parameters recorded for each day are given in Table 4.2. The profiles were performed in the hottest time of the year in N.E. Brazil and, consequently, most of the experiments were performed on sunny days. Some days were more overcast than others and this has been indicated in Table 4.2. The equipment used for gathering samples has shown in Figure 2.8.

No profile was carried out on the anaerobic ponds since these ponds are assumed to be fully mixed and previous work in N.E. Brazil (de Oliveira, 1990) has shown that there are minimal fluctuations in the physico-chemical and bacterial concentrations throughout the 24 hour cycle.

Table 4.1 24 Hour Profiles carried out between November 1992 and March 1993.

DATE	SYSTEM	POND
29.11.92	XVI	F24
2.12.92	XVI	F25
6.12.92	XVI	M15
9.12.92	XV1	M16
13.12.92	XVI	M20
16.12.92	XVI	M23
3. 2.93	XVI	M24 Linear + M22 infl & effl
10. 2.93	XVI	F24
17. 2.93	XVII	M31
10. 3.93	XVI	F21
17. 3.93	XVII	M26
24. 3.93	XVI	F25
31. 3.93	XVI	All Facultative Ponds + M15, M16, M23

Table 4.2 Meteorological data recorded on the day of each profile

DATE	RAINFALL mm	MAX AIR TEMP °C	COMMENTS
29.11.92	0	31	Clear all day
2.12.92	0	29.5	Overcast
6.12.92	0	30	Overcast
9.12.92	0	30	Cloud early am
13.12.92	0	37	Clear all day
16.12.92	0	34.5	Clear all day
3. 2.93	0	31	Clear all day
10. 2.93	0	31	Clear all day
17. 2.93	0	32	Intermittent cloud
10. 3.93	0	32	Clear all day
17. 3.93	trace	30	Cloudy, drizzle
24. 3.93	0	31	Cloud cover pm
31. 3.93	0	26.5	Intermittent cloud

In addition to the eleven profiles carried out on individual ponds there were a further two profiles performed. Firstly, a horizontal profile on M24, the pond with the built-in chicane. This was aimed at understanding the changes occuring along the length of a pond with a high length to breadth ratio, which may be assumed to be under a regime of plug flow. Finally, a profile was carried out on all of the secondary facultative ponds and selected maturation ponds to observe the differences in pH, dissolved oxygen concentration, temperature and light in different types of ponds and, within those types, the affects of geometry on one particular day.

4.2 Experimental Results and Discussion

4.2.1 Presentation of Results

The results are presented in Appendix iii. Figures A3/1 to A3/11 are based on the arithmetic mean values calculated on raw data of pH, temperature, D.O., chlorophyll-*a* and light. FC and *Salmonella* results are given as geometric means. From an engineering point the net effect of changes in the parameters measured over a 24 hour period over the depth of the pond was considered important, thus the results have been presented graphically as means over time and depth. Presenting the data in this way is not ideal since samples taken at the same time but different depths and samples taken at the same depth but different times are not in any way replicate samples. However it was considered that this type of presentation made the various parameters easily comparable. Due to the nature of these means statistical analysis could not be carried out. Standard deviations of such 'means' would be very large especially of those samples taken at the surface of the ponds and those taken during daylight hours when the pond undergoes stratification and the range of values of many of the parameters studied is large. 3-D

graphs showing a typical diurnal pattern of each of the parameters studied are given in the text with the discussion of the appropriate parameter. Variations in the recorded parameters are discussed below.

4.2.2 Temperature

In all the ponds studied a daily cycle of stratification and mixing was observed but the degree of this stratification depended on the particular meteorological conditions of the day. Figure 4.1 shows a typical temperature profile observed in the ponds at Caatingueira. On very hot, sunny days the ponds showed marked stratification between 10 am and 4 pm with typical peak ranges of 5 °C frequently occurring between 12.00 and 14.00 hours. The maximum recorded range was 9 °C at 14.00 hours in M20 over a range in depth of less than 40 cm. More overcast days showed a smaller temperature gradient, typically of 1 °C. The highest gradients occurred within the top 25 cm of the water column and below this the temperature tended to be more or less constant. The maximum temperature recorded at a 1 cm depth was 31 °C and occurred in M20 on 13.12.92 at 14.00 hours and the minimum temperature recorded at this depth was 21.5 °C in pond M23 (16.12.92) at 4.00 hours. These were also the highest and lowest water temperatures recorded in any of the profile studies.

The water temperature at the bottom of the ponds was constant in most cases, with a maximum variation of 3 °C (M20 13.12.92) but more usual variation throughout the 24 hour cycle was of 1 to 2 °C.

The stratification cycle can be seen to start at 8.00 hours, when the water temperature throughout the water column was almost uniform, with perhaps the top 5 cm of the pond being a maximum of 1 °C warmer than the rest of the water column, but lower than the air temperature. By 10.00 hours the degree

Figure 4.1 Typical Diurnal Profile of the Temperature in the Caatingueira WSPs

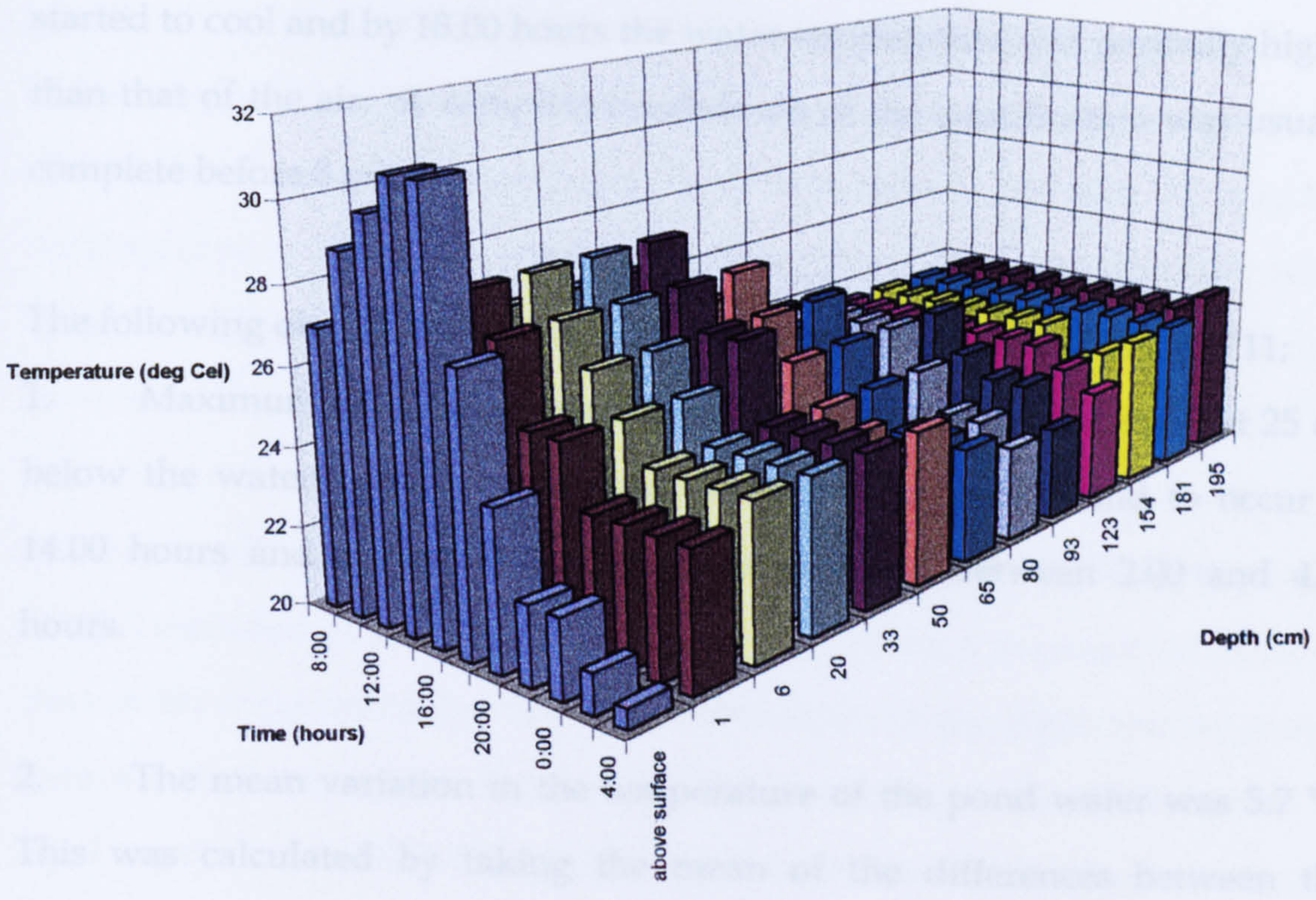
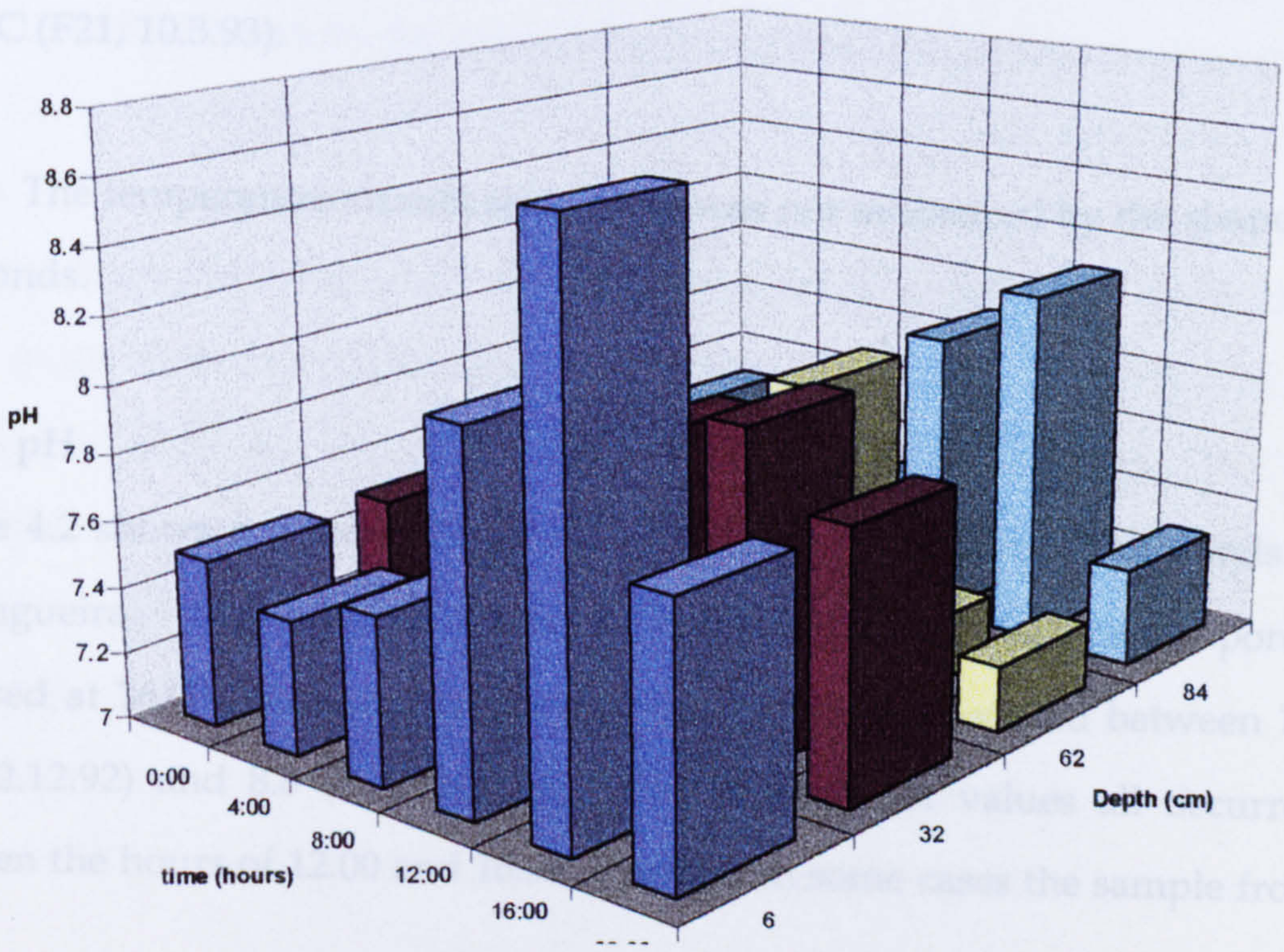


Figure 4.2 Typical Diurnal Profile of the pH in the Caatingueira WSPs



of thermal stratification was marked and this increased to a maximum usually occurring at 14.00 hours. From 16.00 hours the surface layers of the pond started to cool and by 18.00 hours the water temperature was normally higher than that of the air. A complete breakdown of the stratification was usually complete before 8 pm.

The following observations have been made from Figures A3/1 to A3/11;

1. Maximum temperature variations occurred throughout the first 25 cm below the water surface. Maximum temperatures were found to occur at 14.00 hours and minimum temperatures occurred between 2.00 and 4.00 hours.

2. The mean variation in the temperature of the pond water was 5.7 °C. This was calculated by taking the mean of the differences between the maximum and minimum temperatures recorded in the ponds over the whole pond depth.

3. Mean water temperatures varied between 23.6 °C (F25, 2.12.92) and 25.4 °C (F21, 10.3.93).

4. The temperature stratification cycle was not influenced by the shape of the ponds.

4.2.3 pH

Figure 4.2 shows a typical pH profile of the secondary facultative ponds at Caatingueira. Maximum pH values in the secondary facultative ponds occurred at 16.00 hours at a depth of 4 cm. The values varied between 7.9 (F25, 2.12.92) and 8.8 (F24, 10.2.92). The highest pH values all occurred between the hours of 12.00 and 16.00 although in some cases the sample from

20.00 hours also had a high pH in the aerobic part of the pond. The minimum pHs recorded in the secondary facultative ponds occurred at 4.00 hours. The profile study performed on F25 on 2.12.92 gave pH, dissolved oxygen and chlorophyll-*a* results lower than expected although the meteorological data shows that it was a hot, sunny day. The differences in pH and DO may be a consequence of the low concentration of algae in the pond that day which may have been because of unusually high numbers of grazing macro-invertebrates which were observed in this pond at the time of the profile.

Maximum pH values in the maturation ponds occurred in M23 with a value of 10.5 recorded at 16.00 hours. This pond acted as a macrophyte pond for part of the experimental period but examination of the algal species present before and after this period shows that there was no permanent change to the algal population in M23. The performance evaluation results show mean algal concentrations in M23 to be lower than those of the other tertiary maturation ponds. However, on this particular day the concentrations were higher, with a range between 520 and 580 µg/l which may account for the high pH value recorded. The pH of the maturation ponds was frequently high between 12.00 and 16.00 hours in the top 5 cm of water but also at depths of up to 50 cm. The minimum recorded pH (7.7) occurred in ponds M26 and M20 at 4.00 hours.

In general, the pH values recorded in the maturation ponds were higher than those in the secondary facultative ponds which is probably a reflection of the quantity of algae present elevating the pH by the consumption of CO_2. However, the range of pH values recorded in the secondary facultative ponds was greater than those recorded for the maturation ponds. Excluding the results for F25 obtained on 2.12.92 (see above) the mean pH range in the secondary facultative ponds was 1.25 compared to 1.03 for the maturation ponds of the same system. The maturation ponds of the 10 pond series

showed much lower pH ranges (mean of 0.6). Normally, it may be expected to find a wider range of pHs in facultative ponds since they have a bottom zone in which little pH change occurs, but they also contain an upper zone of higher algal concentration which will undergo an increase in pH during the day due to photosynthesis and a decrease during the night due to respiration. Maturation ponds are usually shallower, more stable and less turbid than secondary facultative ponds and, thus, the range of pHs is usually smaller.

Maximum pH values would be expected to occur in the surface layers of a pond due to the concentration of algal photosynthetic activity in this region of the water column and minimum values would be expected at deeper levels and also during the night at all positions in the water column as the algal stratification disperses.

The pH values in the secondary facultative pond were constant throughout the water column at 8.00 hours with a gradient of only 0.1 pH units. The daily cycle then continued and between 8.00 and 12.00 hours the overall pH of the pond increased but the top 5 cm showed the greatest increase in relation to the rest of the pond. This situation is maintained throughout the day and by 20.00 hours ponds F21 and F24 still show high pHs in the aerobic, photic zone of the pond. F25, however, showed that the gradient of pH had dispersed between the samples taken at 16.00 hours and 20.00 hours. At 20.00 hours the pond is homogeneous with a mean pH of 7.7. The water then remains in this state until around 8.00 hours when the daily cycle then beings again.

The diurnal cycle of pH variations in the maturation ponds was similar to that of the secondary facultative ponds except that homogeneity appeared to occur earlier in the day. The very shallow secondary and tertiary maturation ponds, M20 and M23, showed less stratification since the whole pond column

is maintained in the photic zone and, therefore, photosynthetic activity was high in all of the water column. M23 illustrates this well since the minimum and maximum pHs recorded were 9.5 and 10.5 respectively. The deeper maturation pond, M16, shows a wider variation in pH due to the affects photosynthesis and respiration have on the oxygen regime of a pond.

The following observations have been made from Figures A3/1 to A3/11;

1. Maximum pH values frequently corresponded with peaks in the concentration of chlorophyll-*a* in the water. Thus the maximum concentrations occurred between 12.00 and 16.00 hours and were in the top 30 to 40 cm of the water.

2. Minimum pH values were found at the bottom of the ponds and once again corresponded with the minimum concentrations of chlorophyll-*a*.

3. The observed variations in pH in the secondary facultative ponds were associated with those of dissolved oxygen and increases and decreases in pH corresponded to increases and decreases in DO. To a lesser extent the pH values were also associated with the changes in chlorophyll-*a* seen in the ponds. The variations of pH in the maturation ponds also correspond to the variations in DO although the correlation is not as good as with the secondary facultative ponds.

4.2.4 Dissolved Oxygen

Figure 4.3 shows a typical DO profile. The maximum dissolved oxygen concentrations varied widely throughout the experiments conducted. On the sunniest days, for example 17.2.93, the water was supersaturated with oxygen with readings of more than 20 mg O2/l occurring to a depth of 45 cm in readings taken at 14.00 and 16.00 hours. This supersaturation occurred in all the profile studies, except F25 (2.12.92) and M26 (17.3.93), in one or more of

the samples and up to depths of 50 cm (F24, 10.2.93). The profile performed on pond M26 was a more overcast day and this is reflected in the DO, light and temperature readings obtained.

Higher maximum dissolved oxygen concentrations were found during experiments in which chlorophyll-*a* concentrations were high and lower ones coincided with lower chlorophyll-*a* levels. However, a direct relationship between the concentrations of DO and chlorophyll-*a* was not consistently observed as regression coefficients, which ranged from r=-0.09 to r=0.67 for secondary facultative ponds and from r=0.08 to 0.83 for maturation ponds, show. Many of the maximum concentrations of chlorophyll-*a* took place in the mornings or at midday and the majority of the peaks of DO were at 14.00 hours. This implies that the algae may stratify earlier in the day and that it takes several hours for the DO concentrations to build up.

The depth in the secondary facultative ponds where the concentration of DO in the water suddenly decreases (the oxypause) varied from being at the water surface, early in the day, to a maximum of 55 cm at 14.00 and 16.00 hours (F24, 10.2.93). In the other secondary facultative ponds it was recorded at a maximum depth of 20 to 25 cm. During the daily cycle it followed a pattern such that before 10.00 hours it was close to the water surface. It then became gradually deeper until it was at its lowest position at 16.00 hours following which it decreased and was normally at the surface again by 20.00 hours. Therefore, the majority of the water column of the secondary facultative ponds was anaerobic during the night. In maturation ponds the oxypause behaved as in the secondary facultative ponds except that the water column of the maturation ponds was usually well aerated throughout the whole daily cycle, with minimum DO concentrations higher than those of the secondary facultative ponds. When the DO concentration did fall below 1 mg

Figure 4.3 Typical Diurnal Profile of the Dissolved Oxygen in the Caatingueira WSPs

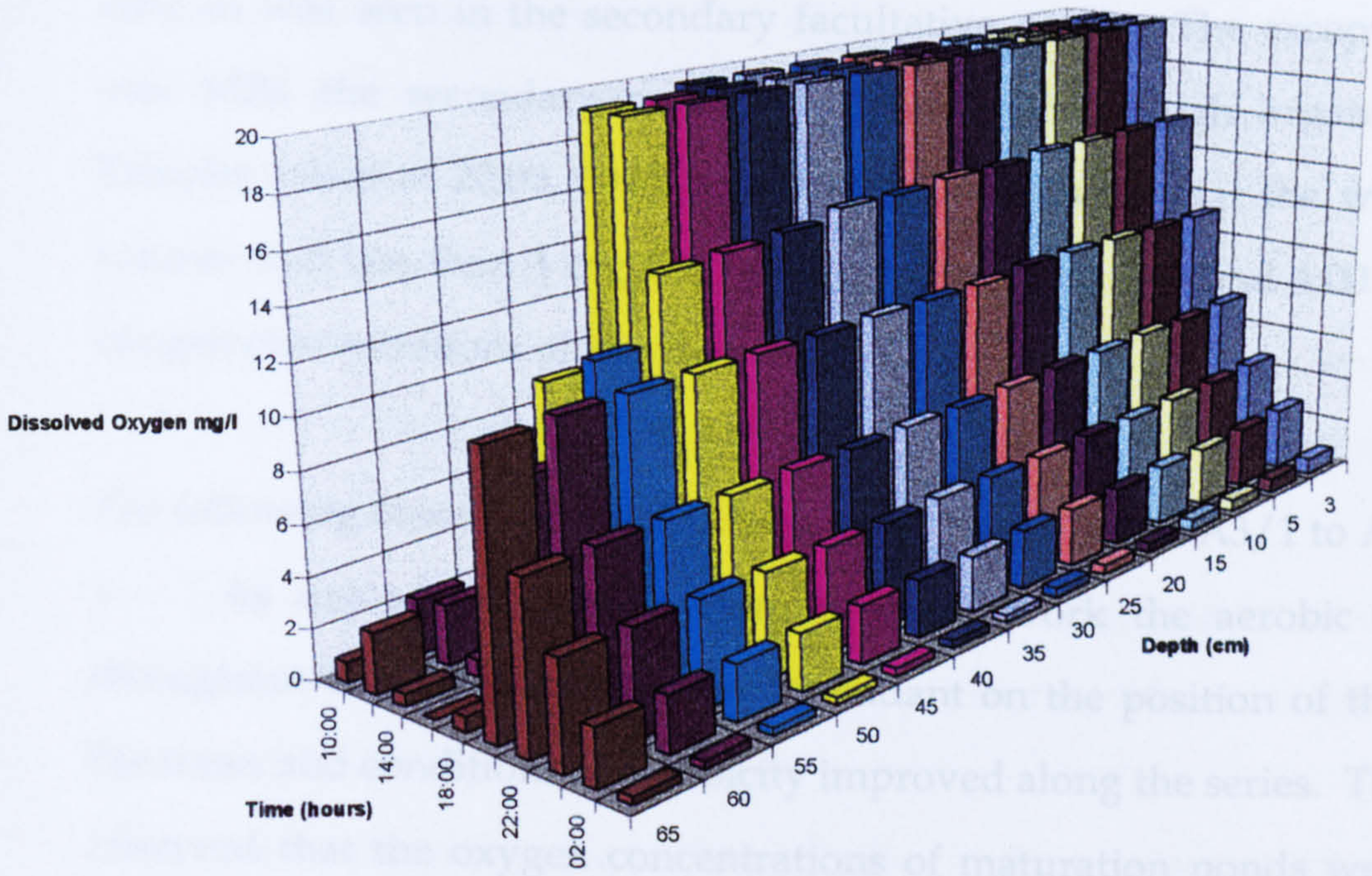
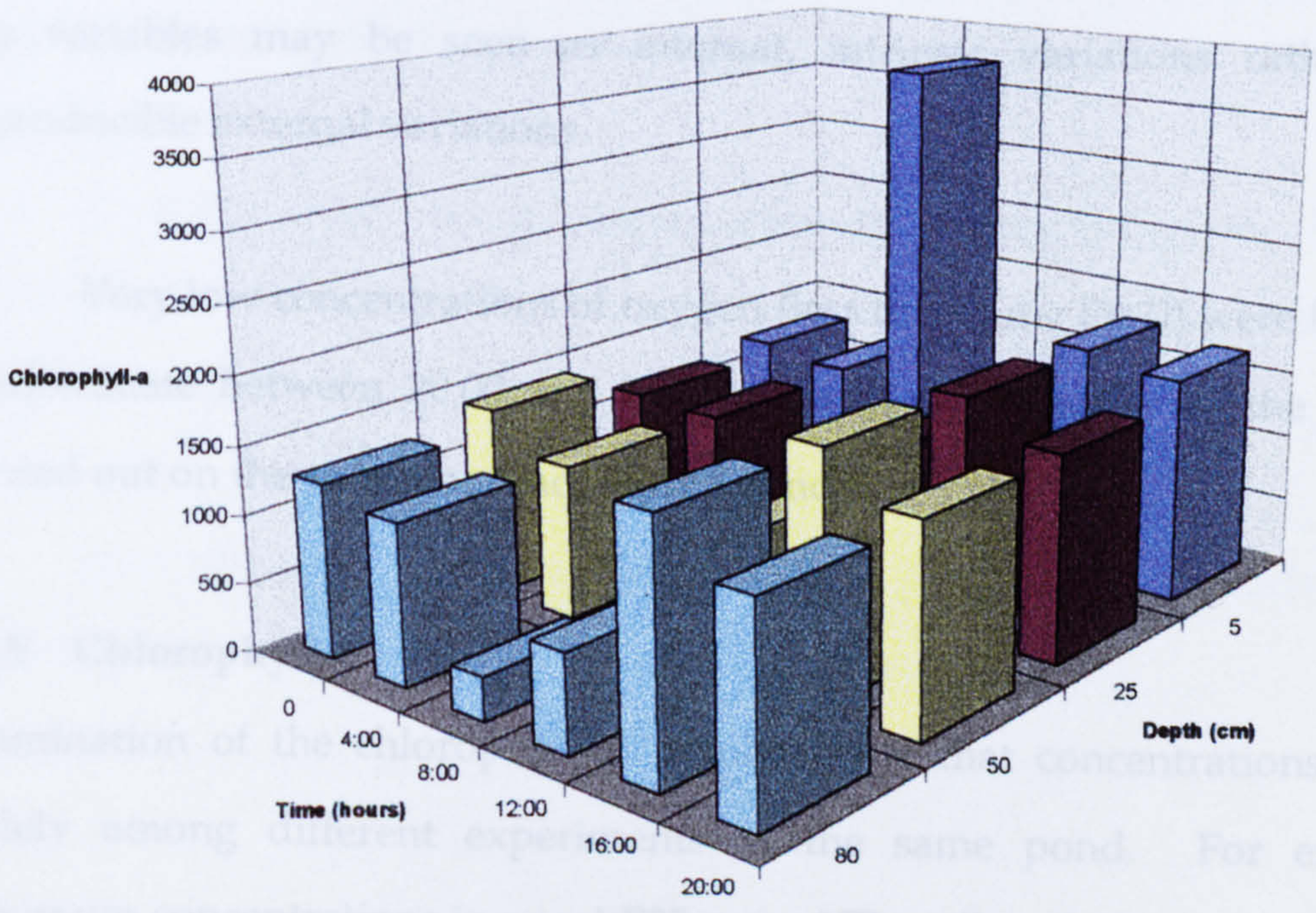


Figure 4.4 Typical Diurnal Profile of the Concentration of Chlorophyll-*a* in the Caatingueira WSPs



O2/l it was not maintained at this low level for more than a few hours. Thus, the maturation ponds did not undergo long periods of time in an anaerobic state as was seen in the secondary facultative ponds. The exception to this was M20, the secondary maturation pond with a high organic loading. Samples taken at 22.00, 00.00 and 8.00 hours showed that the whole water column had less than 4 mg O2/l and samples from 2.00 and 4.00 hours had oxygen concentrations of less than 1 mg O2/l.

The following observations have been made from Figures A3/1 to A3/11;

1. As one would predict from previous work the aerobic conditions throughout the water column are dependant on the position of the pond in the series and conditions of aerobicity improved along the series. Thus, it was observed that the oxygen concentrations of maturation ponds were greater than those of secondary facultative. This is in agreement with previous profile studies carried out in N.E. Brazil (de Oliveira, 1990). The degree of aeration also depended on the climatic conditions of the particular day as profiles performed on the same pond but on different days show. Indeed, it may be seen that repeated profiles on particular ponds are not replicates since the variables may be seen as internal, intrinsic variations rather than reproducible external variations.

2. Very low concentrations of oxygen (less than 1 mg O2/l) were found to predominate between 20.00 and 8.00 hours in the majority of the profiles carried out on the secondary facultative ponds.

4.2.5 Chlorophyll-*a*

Examination of the chlorophyll-*a* results show that concentrations varied widely among different experiments in the same pond. For example, maximum concentrations in pond F25 were 177 µg/l and 428 µg/l on 2.12.92

and 24.3.93 respectively. These maximum concentrations did not occur at the same time or depth once again illustrating that repeat profiles cannot be assumed to be replicates. The highest mean concentrations were not associated with the weather conditions of that day, for example, 13.12.92 was the warmest day and a profile was carried out on M20. The chlorophyll-a concentration in this pond on this day was 466 μg/l, significantly less than the 1320 μg/l recorded in M16 on 9.12.92, a day on which there was complete cloud cover in the early morning and intermittent cover throughout the day.

The daily cycles exhibited by the algae are more complicated than those of the physical and chemical parameters discussed so far. However, some typical variations can be identified;

1. Mean concentrations of chlorophyll-*a* in the ponds varied considerably with time, depth and pond. Mean overall concentrations (obtained by summing the results for all the samples taken all the different depths) gave the results give in Table 4.3.

2. In general, secondary facultative ponds were not observed to contain significantly less algae than the maturation ponds studied.

3. At 0.00 and 4.00 hours concentrations of chlorophyll-*a* were low and homogeneous throughout the water column due to the breakdown in algal stratification at night.

4. High chlorophyll-*a* concentrations were predominantly reached until 16.00 hours and were primarily in the upper layers.

Table 4.3 Mean Chlorophyll-*a* Concentrations (and Standard Deviations) in the Profile Studies

POND	CHLOROPHYLL-*a*, µg/l	CHLOROPHYLL-*a*, mg/m^2
F21	434 (414)	434 (414)
F24	485 (537)	970 (1074)
F25	90 (35)	180 (70)
F25	148 (92)	297 (184)
M15	244 (135)	244 (135)
M16	1320 (664)	1188 (598)
M20	192 (107)	75 (42)
M23	561 (50)	337 (30)
M26	225 (91)	225 (91)
M31	227 (95)	227 (95)

5. The distribution of chlorophyll-*a* in the water column during the daily cycle occurred according to a pattern. At 8.00 hours the levels of chlorophyll-*a* were low and uniform. By 12.00 hours the concentration of chlorophyll-*a* in the whole water column had increased with highest concentrations being found in the top layers of the water column. The peak chlorophyll-*a* concentrations were observed during the period 12.00 to 16.00 hours, frequently with the highest concentrations occurring in the top 30 cm. By 20.00 hours the algae had moved out of the surface layers and are dispersed more evenly in the water column. Until early morning, they remain uniform in the water column although, occasionally there is a higher concentration at the very bottom of the pond during the night. This would support the theory that algae stratify during the day but are more dispersed during the hours of darkness when they may enter the sediments (Pearson *et al.*, 1987b) although very low concentrations of DO at the bottom of the ponds may influence the position of the algae in the water column. This diurnal pattern may be

illustrated by two ponds F21 and M15. F21 shows that the mean concentration of chlorophyll-*a* during the night at depths greater than 30 cm is 535 µg/l and in shallower depths is 266 µg/l. M15 shows that during daylight hours there are mean chlorophyll-*a* concentrations of 784 µg/l and 199 µg/l in depths of less than and more than 30 cm respectively.

4.2.6 Total Suspended Solids and BOD5

Figures 4.5 and 4.6 show typical profiles of TSS and BOD observed in the facultative ponds at Caatingueira. The maximum concentrations of total suspended solids and BOD5 frequently coincided, for example F24 (both profiles), F25 (2.12.92), M20, M23 and M31. These maximum values were most frequently recorded in the top 4 to 6 cm of the water column in daylight hours and so may be attributed to the presence of algae. Minimum BOD5 concentrations were observed to occur mostly at the bottom of the ponds at 12.00 and 16.00 hours. In general, the concentrations of BOD5 in the hours of darkness was more uniform throughout the water column as the algal population would be. Minimum TSS concentrations were also found to occur at the bottom of the ponds (the lowest sampling point was 5 to 10 cm above the bottom of the pond).in the samples taken at 8.00, 12.00 and 16.00 hours.

From Figures A3/1 to A3/11 the following observations can be made;

1. The mean diurnal variations in BOD5 and TSS frequently coincided with each other and with the variations in chlorophyll-*a*. The algae present in the water column was an obvious component of both the suspended solids and the BOD5 concentrations.

Figure 4.5 Typical Diurnal Profile of the Total Suspended Solids in the Caatingueira WSPs

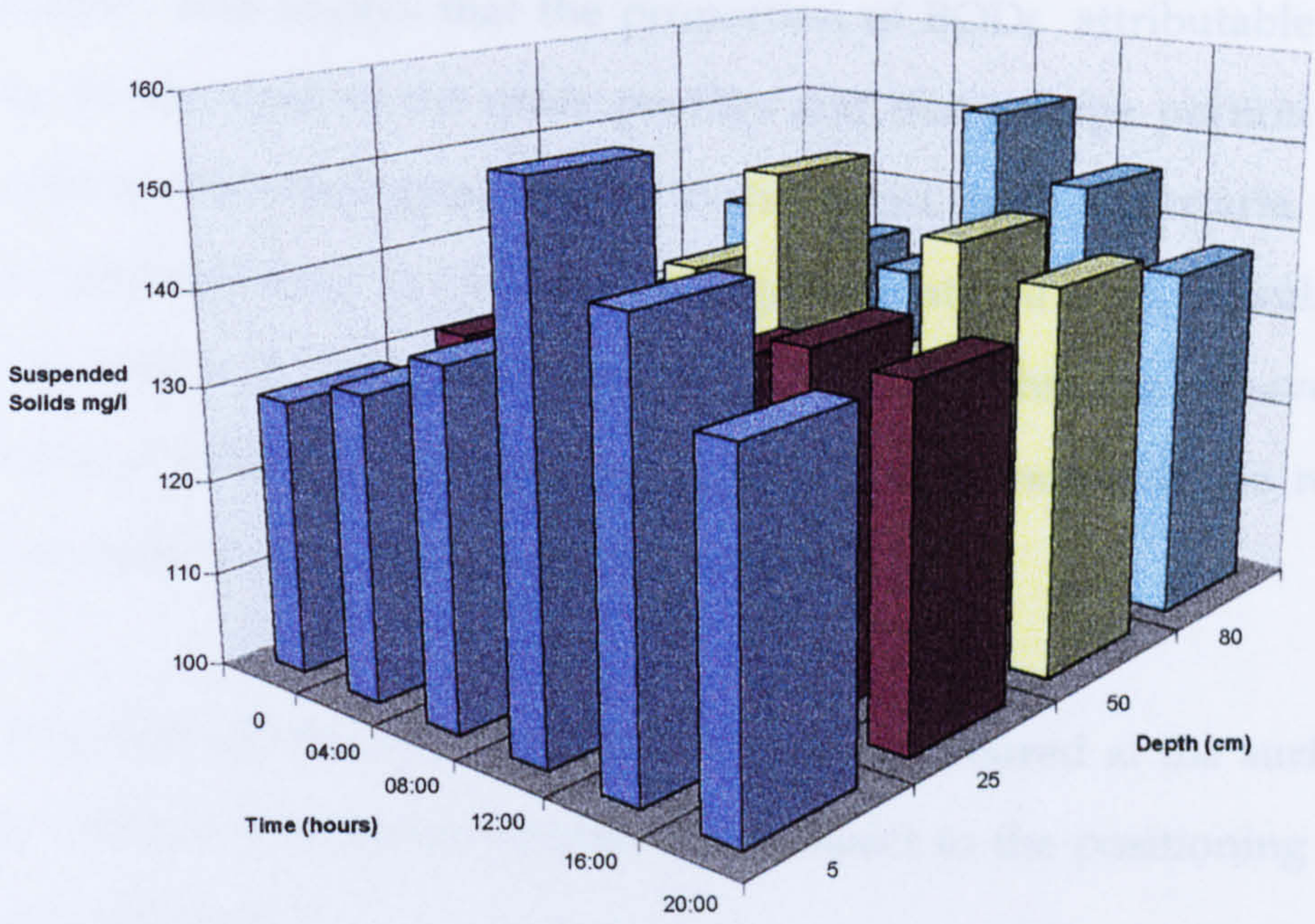
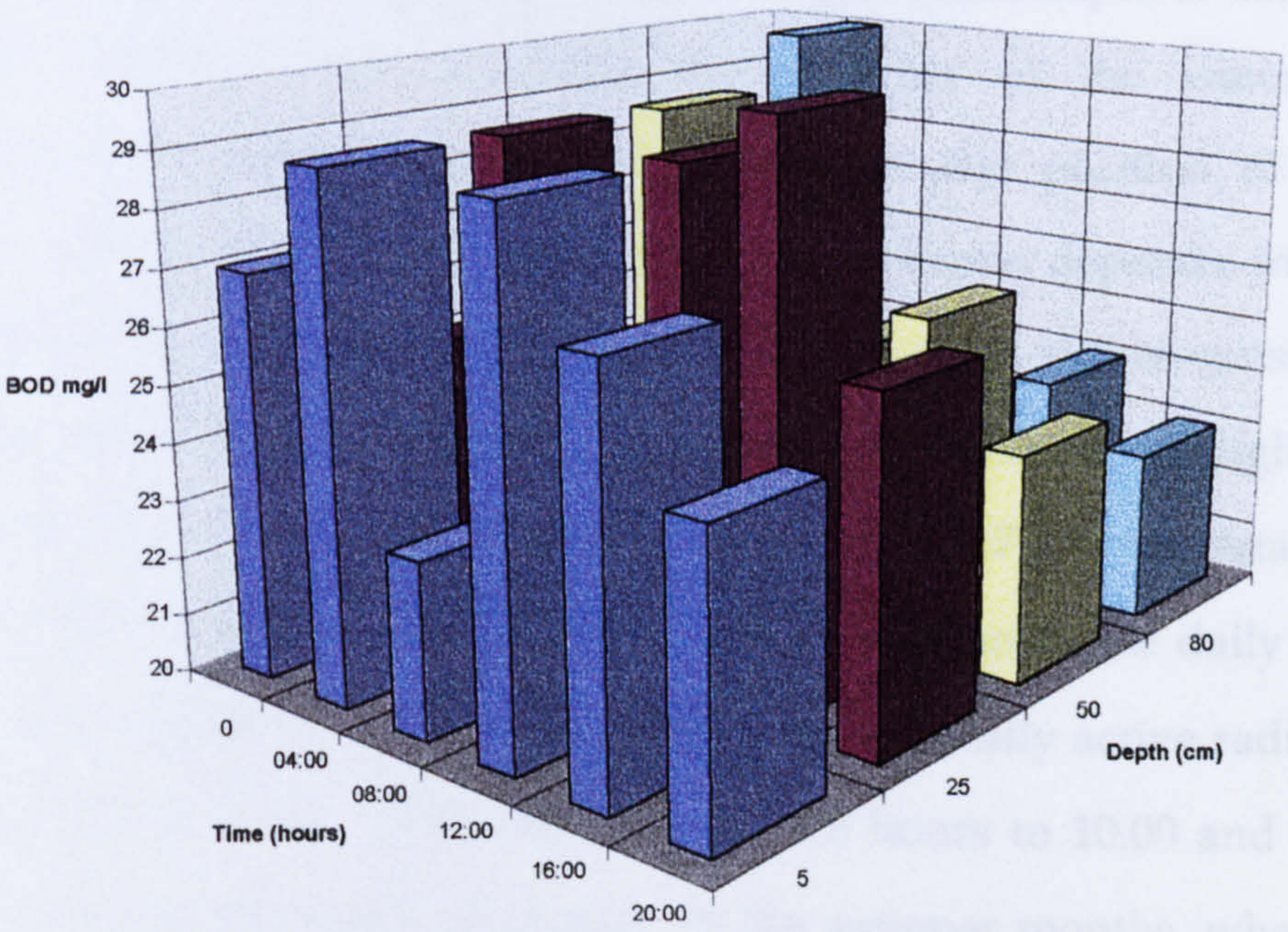


Figure 4.6 Typical Diurnal Profile of the Concentration of BOD in the Caatingueira WSPs



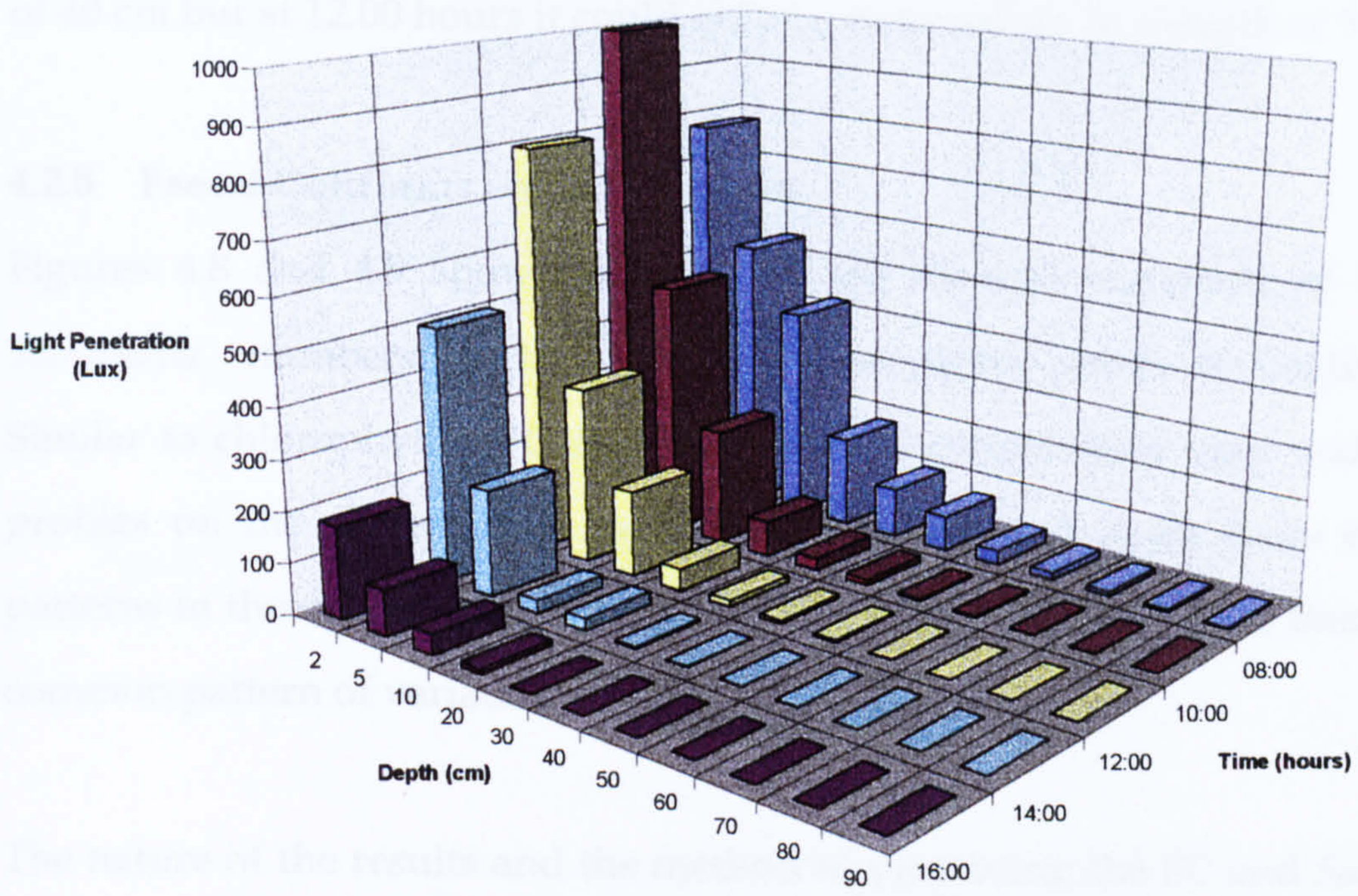
2. In the cases in which the results for BOD_5, TSS and chlorophyll-*a* did not appear to be correlated the concentrations of TSS and chlorophyll-*a* alone are correlated. This implys that the proportion of BOD_5 attributable to the algae may be less than in the other profiles and that a large portion of the BOD_5 is due to other biological activity in that pond (such as bacteria, micro- and macro-invertebrates, oxidation of inorganic material such as sulphides and ferrous iron and nitrogenous oxygen demand). This could have been made clearer if filtered BOD_5 had been performed to establish the relative amount of soluble BOD_5 compared to the total BOD_5.

3. The greatest BOD_5 concentrations frequently occured at the surface of the ponds which is a factor to consider with respect to the positioning of the effluent take-off point.

4.2.7 Light Intensity

Light falling on the surface on the pond depends on the climatic conditions of that particular day. The attenuation of light throughout the depth of the pond depends on various factors including the turbidity of the water, the concentration of suspended solids and the degree and position of algal stratification. The position of the algae in the water column depends, in turn, on the amount of light penetrating the water since the algae will be constantly re-positioning themselves to receive the optimum amount of light for photosynthesis. A typical light profile is given in Figure 4.7 and the data from the other profiles are given in appendix ii. Figure 4.7 shows the daily cycle and, as would be expected, the amount of photosynthetically active radiation (PAR) at the surface of the water increases from 8 hours to 10.00 and often 12.00 hours and then gradually decreases. In the summer months, when the profiles were performed, sunset falls between 16.00 and 17.00 hours. The amount of PAR detected decreases with depth and at midday, when the light

Figure 4.7 Typical Diurnal Profile of the Light Penetration in the Caatingueira WSPs



intensity may be assumed to be at its greatest, PAR at the surface is in the region of 1000 μE m^{-2} s^{-2} but has been reduced to less than 10 μE m^{-2} s^{-2} at a depth of 30 cm. The affect of algal stratification can be seen since at 8.00 hours, in many cases, PAR may be detected at a greater depth than later in the day when the light intensity at the surface is greater. This is illustrated by F21 (10.3.93) when the PAR at 8.00 and 12.00 hours is 550 μE m^{-2} s^{-2} and 1000 μE m^{-2} s^{-2} respectively and at a depth of 15 cm the PAR was 185 μE m^{-2} s^{-2} and 110 μE m^{-2} s^{-2} respectively. In this case, at 8.00 hours PAR was detected to a depth of 40 cm but at 12.00 hours it could only be detected up to a depth of 30 cm.

4.2.8 Faecal Coliforms and Salmonella.

Figures 4.8 and 4.9 show examples of the diurnal variations of FC and *Salmonella* numbers in the secondary maturation ponds at Caatingueira. Similar to chlorophyll-*a*, FC and *Salmonella* measurements vary widely and profiles on the same ponds carried out on different dates show different patterns in the daily cycles of FC and *Salmonella*. In several cases there was a common pattern of variation.

The nature of the results and the method of presenting the FC and *Salmonella* concentrations in the profile investigations means that statistical analyses was not possible. In order to gain an insight into the variations in concentration of these pathogens over the experimental period means were taken for each sample time (using the results of all the sample depths) and for each sample depth (using the results of all the sample times). The resulting standard deviations (particularly for those samples taken in daylight hours) were correspondingly large.

Figure 4.8 Typical Diurnal Profile of the Numbers of Faecal coliforms in the Caatingueira WSPs

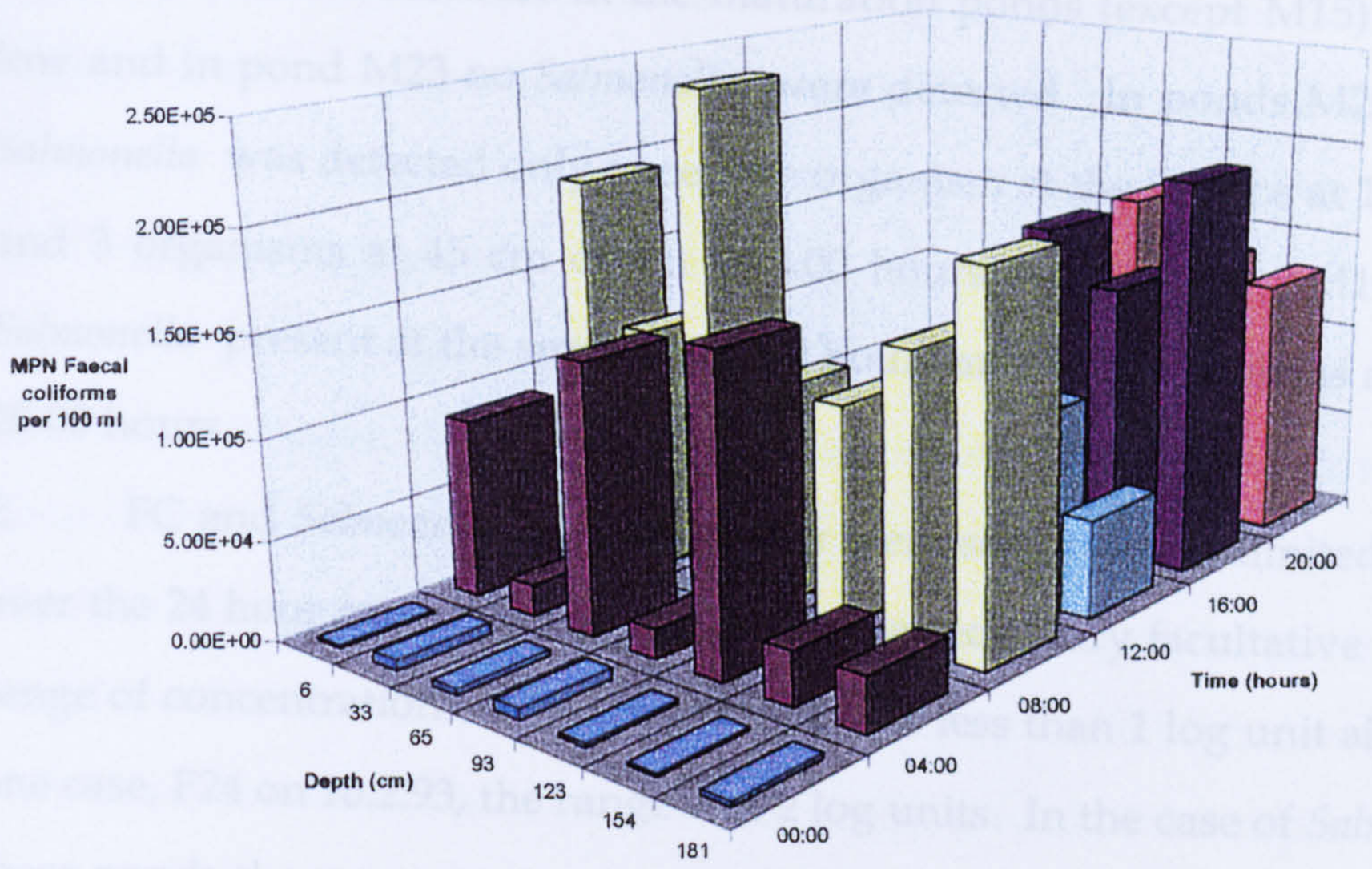
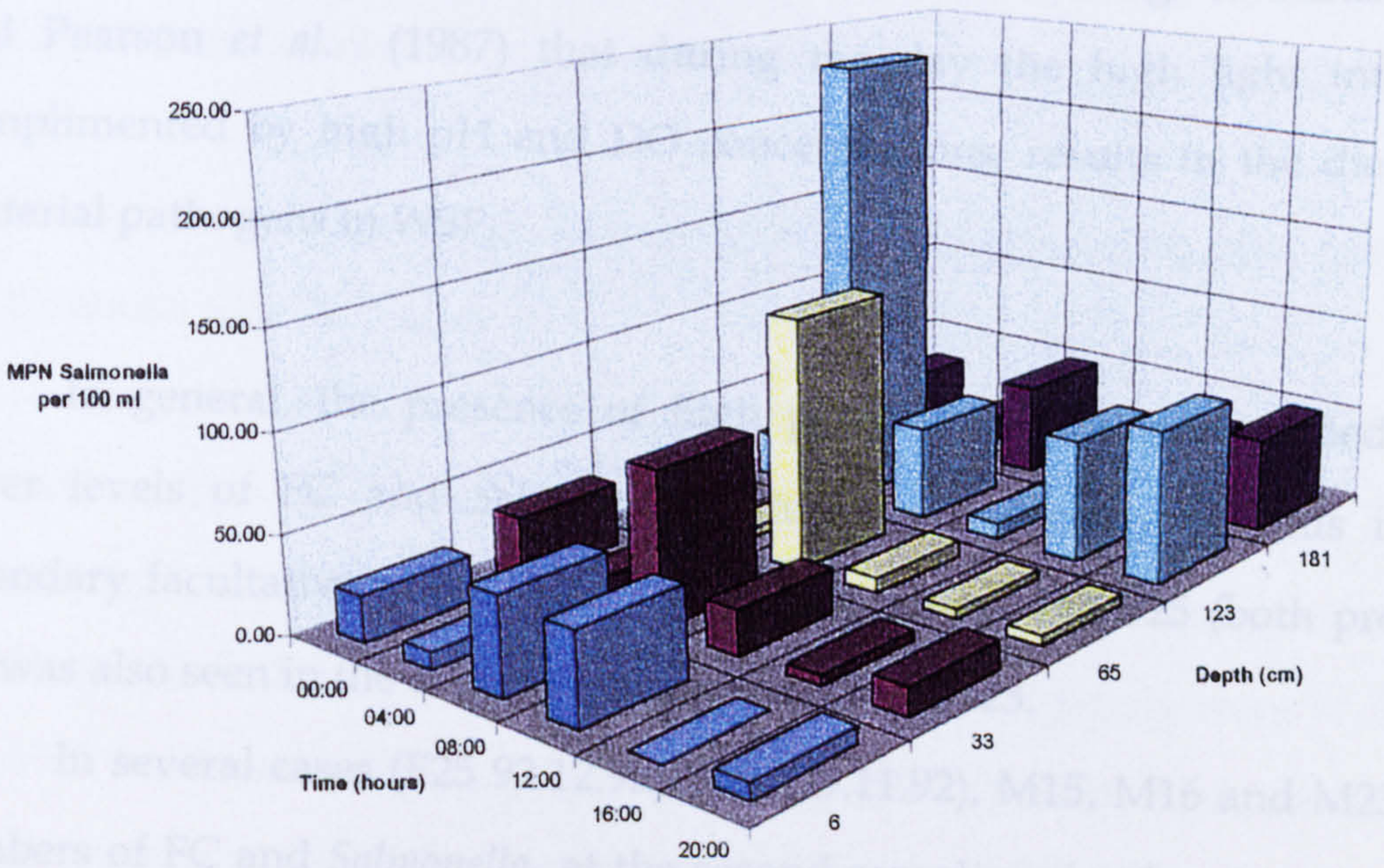


Figure 4.9 Typical Diurnal Profile of the Numbers of Salmonella in the Caatingueira WSPs



From Figures A3/1 to A3/11 the following observations may be made;

1. *Salmonella* numbers in the maturation ponds (except M15) were very low and in pond M23 no *Salmonella* were detected. In ponds M20 and M31 *Salmonella* was detected only once, one organism at the surface at 16.00 hours and 3 organisms at 45 cm depth at 8.00 hours respectively. M16 had one *Salmonella* present at the surface at 8.00 hours and two organisms at 25 cm at 20.00 hours.

2. FC and *Salmonella* concentrations were seen to have limited variation over the 24 hour experimental period. In the secondary facultative ponds the range of concentrations of FC, in general, was less than 1 log unit although in one case, F24 on 10.2.93, the range was 2 log units. In the case of *Salmonella* in these ponds the range was in the order of 0.5 log unit. The maturation ponds had slightly larger ranges. However, it was observed that the pathogen numbers were frequently highest at 4.00 and 8.00 hours following which the numbers decreased to a minimum at 16.00 or 20.00 hours and then increased again during the night. This would substantiate the findings of Curtis (1990) and Pearson *et al.* (1987) that during the day the high light intensity, complimented by high pH and DO concentrations, results in the die-off of bacterial pathogens in WSP.

3. In general, the presence of high pH concentrations coincided with lower levels of FC and *Salmonella*. This was especially obvious in the secondary facultative ponds F21, F24, (both profiles) and F25 (both profiles) but was also seen in the tertiary maturation pond, M23.

4. In several cases (F25 92.12.92), F24 (29.11.92), M15, M16 and M23) the numbers of FC and *Salmonella* at the second sampling depth, around 30 cm, were lower than at the surface of the water. It is difficult to equate this with

any of the physico-chemical parameters of the pond since analyses were not carried out at this depth due to logistical constraints. At a depth of 30 - 40 cm the pH and DO concentrations are as high as at the surface but the light intensity is less than at the surface so this apparent better removal cannot be explained by the action of light, pH and DO.

4.3 Linear Profile - M24

As part of the investigation into pond geometries a chicane was built into the tertiary maturation pond, M24, which increased its' length to breadth ratio from 2.28:1 to more than 140:1. Walls to act as baffles were built which also reduced the volume and the theoretical retention time of the pond. Column samples were taken at the inlet ("influent" sample) and outlet of the pond and at the end of each of the chicane walls giving a total of 8 samples. Samples were also taken from the inlet and outlet areas of another tertiary pond, M22, to act as a comparison. Samples were analysed for faecal coliforms, *Salmonella*, pH, alkalinity, BOD, COD, sulphate, sulphide and various solids. Samples were taken twice throughout the day - at 8.00 hours and at 15.00 hours - and the samples were taken back to the main laboratories at EXTRABES and analysed immediately.

From the results, given in Figure A3/12 in appendix iii, the following observations may be made;

1. The concentrations of BOD, COD, chlorophyll-*a*, total suspended solids and pH in the influent of M22 were higher than those of M24. Total phosphorus, sulphate, sulphide, FC and *Salmonella* concentrations were very similar in the influents of the two ponds. Since both ponds were receiving identical wastewater, from ponds M16 to M18, it would be expected that samples taken around the inlet areas of the ponds would be similar. The differences between these samples were in accordance with those results

obtained in the performance evaluation of these ponds. The difference in BOD5 of ponds M22 and M24 in the performance evaluation was also observed in the profile samples. The lower pH found in M24 in the performance evaluation was also seen in the profile but the chlorophyll-*a* concentrations seen in the performance evaluation (higher chlorophyll-*a* in M24) was not observed in the profile. Chlorophyll-*a* levels in the ponds, however, are known to be highly transient.

2. The results for most of the parameters studied gave higher concentrations in the sample taken at 15.00 hours than at 8.00 hours. The exceptions to this were the results for phosphorus, where both forms (total and soluble orthophosphate) were higher earlier in the day, and sulphate, where concentrations in the samples from points 1 to 4 were higher at 8.00 hours but samples from points 5 to 8 were higher at 15.00 hours. As the pH increases phosphorus is known to precipitate giving more soluble orthophosphate during the day. This is often mineralized and resolubilized during the night. From previous studies of the sulphur cycle in WSP systems (Houghton and Mara, 1992) it is known that levels are normally lowest in the morning because of sulphide generation during the night when the concentrations of DO are lowest. During the day, when DO concentrations increase due to the photosynthetic activity of the algae, the sulphide is oxidised to sulphate, either by a chemical reaction involving molecular oxygen, or by sulphur-oxidizing photosynthetic bacteria, thus highest concentrations of sulphate would be expected to occur during the afternoon when the water is more oxygenated. Some sulphur-oxidizing bacteria of the family Chromatiaceae perform anoxygenic photosynthesis by using hydrogen sulphide as the main electron donor (some genera can use elemental sulphur, thiosulphate and sulphite). Other bacteria of the family Chlorobiaceae also perform anaerobic convertion of sulphide to sulphate. These purple and

green sulphur bacteria are reported to frequently occur in ponds especially those with high organic loadings so would perhaps be more likely to be found in anaerobic and secondary facultative ponds than this tertiary maturation pond. The differing levels of sulphate between the sample points in this case may indicate differences in the concentration of DO although this is unlikely since M24 is shallow and well oxygenated throughout its length and depth.

3. Almong the length of the chicane phosphorus levels decreased very slightly (more so in the samples collected at 15.00 hours) and this was also observed in M22. pH was constant and chlorophyll-*a* (expected to remain constant) decreased along the profile except for a peak as described in 3 above. The concentrations of BOD from both the 8.00 and 15.00 hours samples decreased along the profile (except for the peak at sampling point 5 as above). Unfortunately a problem with the equipment used for COD meant that results for sampling numbers 1 to 4 in the 8.00 sample could not be analysed, but from the other results a slight (and insignificant) increase in concentration along the pond length seems evident. The suspended solids also seem to have gradually decreased along the pond length. Finally, in the case of sulphate there was a gradual increase which was also observed in M22 and follows the pattern seen in the performance evaluation.

4. The suspended solids results for sampling point 5 at 15.00 hours shows a sharp peak. There are corresponding peaks in the concentration of COD, BOD and chlorophyll-*a* suggesting this increase in suspended solids to be due to the algae present. It is not known why there should be more algae at this particular sampling point and time.

5. Levels of sulphide in the ponds shows a pattern not observed in any of the other parameters studied in that the concentrations at the most sheltered

end of the pond (points 1,3,5 and 7) was higher than at the other end of the pond. This suggests that this more sheltered end of the pond is more anaerobic and that sulphide generation is correspondingly higher.

6. Faecal coliform levels were higher at 8.00 hours than at 15.00 hours as observed in the other profiles. Along the length of the chicane, the numbers of FC were constant (with the exception of sampling point 5 where the concentration was much lower than found in the rest of the pond) with a range of 1 log unit. *Salmonella* was detected only at the last two sampling points along the pond profile, taken at 8.00 hours. This was somewhat unexpected since, if the pond was under a regime of plug-flow *Salmonella*, if pesent, should be found at or near the influent and that treatment along the pond would then eliminate the organisms. This result may indicate that vertical stratification and layering are more important in pathogen removel than hydraulic plug flow. Samples taken for the performance evaluation programme were also taken at the outlet site and *Salmonella* was detected in 14% of the samples taken over a 17 month period.

The high length to breadth ratio of pond M24 should, theoretically, promote plug flow through the pond and it would be expected to see gradual decreases in the parameters studied along the pond length. As to whether this increases the efficiency of the pond is more difficult to say since the concentrations of the individual parameters studied at the inlet point in M22 and M24 differed, by a substantial amount in some cases. The results from the performance evaluation, of column samples taken at the outlet point of the pond, show no significant differences between ponds M24 and the other tertiary maturation ponds

4.4 Comparative Profile - F21 to F25, M15, M16 and M23

In order to understand better the in-pond differences possibly caused by geometry and depth, a 24 hour depth profile was carried out simultaneously on ponds F21 to F25 and M15, M16 and M23. Due to logistical constraints, samples for pH taken throughout the depth of the ponds could only be taken from two ponds. F21 and F24 were chosen as these represent the two extremes of depths investigated. Dissolved oxygen, temperature and light readings were taken in all of the ponds at 3 hourly intervals throughout the day and night.

4.4.1 Dissolved Oxygen

The dissolved oxygen results show clearly the variations in the degree of oxygenation experienced by the different pond types, and within those types, by the different depths. The graphs in Figure A3/13 confirm that the further along the treatment series the greater the degree of aeration. The secondary facultative ponds are well aerated during the day but during the night (from 20.00 to 5.00 hours) all of these ponds have dissolved oxygen concentrations of less than 0.2 mg/l. In contrast, the maturation ponds studied underwent a much more limited time in these very low concentrations of dissolved oxygen. Two of the ponds studied, F21 and M15, are of the same depth and so show that this state of anaerobicity is not simply a factor of depth and it is probable that the lower organic loadings and the higher concentrations of algae normally found in maturation ponds are responsible for this difference in dissolved oxygen. In all of the ponds the DO gradually built up from low concentrations at 8.00 hours (between 1 and 3 mg/l in the secondary facultative ponds and between 10 and 18 mg/l in the maturation ponds at the surface) to being at its highest between 14.00 and 17.00 hours and then rapidly decreasing to less than 1 mg/l and less than 10 mg/l at the surface for the

secondary facultative and maturation ponds respectively. The different depths and geometries of the secondary facultative ponds did not appear to have any significant effect on the DO regime of the ponds. The oxypause of the shallower ponds was observed to occur 20 - 30 cm from the surface of the water. The deeper ponds, however, had a more variable oxypause which was at a depth of 30 - 40 cm between 8.00 and 1.00 hours but moved to 50 - 60 cm in the 14.00 and 17.00 hour samples.

4.4.2 pH

The pH levels in F21 and F24 were similar (Figure A3/14). The readings taken during the day were found to decrease gradually throughout the depth of the ponds giving the lowest pH readings at the bottom. During the hours of darkness the pH of the surface waters remained constant to a depth of 70 cm in F21 and between 100 and 150 cm in F24, after which the levels dropped to approximately pH 7.0. Thus, it may be seen that the pH regime in ponds of different depths varies in the respect that deeper ponds maintain a high pH to a greater depth than do shallower ponds. Up to a depth of approximately 40 cm both F21 and F24 maintain pHs in similar ranges. Below this the pH in F21 decreases to give a mean of 7.22 at the bottom (90 cm). The pH in F24 remains high up to a depth of 103 cm where the mean pH is 7.84. Below this there is a reduction and the mean pH at the bottom of the pond (180 cm) is 7.14, similar to that at the bottom of F21.

4.4.3 Temperature

The mean temperature of all of the ponds investigated increased towards the middle of the day and subsequently decreased in the early evening and remained constant throughout the night, a pattern following that observed in the other profiles performed. During the hours 20.00 to 8.00 the temperatures of all the ponds were similar with maximum ranges of 1.5 °C through the

water column and ranges of 3 °C between the ponds. Figure A3/15 shows that at 11.00 and 14.00 hours (and to a lesser extent 17.00 hours) thermal stratification was observed in the same way as it had been in the other profiles carried out. During these hours it was frequently observed that the temperature at a depth of 3 cm was less than that at 5 and 10 cm and this is probably due to wind cooling. The thermocline was observed to remain in a more or less constant position in the water column throughout the day but to differ between the secondary facultative ponds and the maturation ponds. In ponds F21 to F25 at 11.00 hours the thermocline occurs between the depths 20 and 40 cm and at 14.00 hours it occurs at a depth of between 20 and 50 cm . In the maturation pond M15, at 11.00, the thermocline was between 50 and 60 cm deep which changed to lie between 40 and 50 cm deep at 14.00 hours. M16 had a thermocline at between 20 and 30 cm at 11.00 and 14.00 hours, and M23s' thermocline was found between the depths of 10 and 20 cm at 11.00 hours and 20 and 30 cm at 14.00 hours. From the results it may be noted that the shallower ponds have higher mean temperatures, as may be expected, since the whole water column is heated by solar radiation. However, there does not appear to be a simple linear relationship between pond depth and thermocline position since the shallow ponds M15 and M23 have thermoclines at depths differing by up to 40 cm and the deeper maturation pond, M16, has a thermocline at an intermediate depth. The thermoclines observed in the secondary maturation ponds appeared to be unaffected by the pond depth.

4.4.4 Light Intensity

The readings for light intensity throughout the pond columns where complicated by the fact that the day on which this profile was performed there was intermittent cloud cover making the levels between the ponds and between the sample times difficult to compare.

4.5 Summary

The results of the profile studies presented in this chapter may be summarised as follows;

1. Deeper ponds undergo more stratification in the respect that the range of values for parameters such as temperature, pH and BOD is greater in deeper ponds than in shallower ones. However, the diurnal fluctuations of the chemical parameters follow the same predictable pattern in all ponds regardless of depth.

2. Thermal and algal stratification occurred in all of the ponds studied but the degree of stratification depended on the depth of the pond (see 1.) and on the climatic conditions of that particular day.

3. Geometry did not appear to have an affect on the dissolved oxygen, pH, temperature and light intensity of the ponds investigated.

4. Faecal coliform and *Salmonella* diurnal variations are less predictable than the variations in chemical parameters.

5. The depths and collection times at which extreme concentrations of pathogen numbers were predominantly observed suggest that light intensity, pH and dissolved oxygen are the factors influencing faecal coliform die-off, as may be expected from previous work (Moeller and Calkins, 1980; Pearson *et al.*, 1987; Curtis, 1990).

6. Chlorophyll-*a* and pH concentrations, during the day, were correlated with typical regression coefficients of 0.8 (eg. F24 29.11.92). Linear regression

did not indicate any correlation between DO and pH and DO and chlorophyll-*a*.

7. In the series of ponds, aerobic conditions improved from one pond to another, along the series.

8. The diurnal variations observed were seen to be intrinsic variations and profiles carried out on the same pond, at different times, could not be assumed to be replicates.

Chapter 5 *Vibrio cholerae* Survival in Waste Stabilisation Ponds

5.1 Introduction

The seventh cholera pandemic arrived in Peru in January 1991, causing more than 200,000 cases and 1500 deaths within six months. The disease quickly spread to Ecuador, Colombia, Brazil, Chile, Mexico, Guatamala and El Salvador. This follows a pattern of dissemination first seen in Africa which, after being cholera free for more than 70 years, saw cholera spread rapidly to 30 of the 46 countries, and these countries reported nearly 90% of the cholera cases in the world in 1990 (Glass *et al.*, 1991). During the present pandemic, cholera has been introduced into nearly 100 countries and while it has become endemic in many of these, it has failed to persist in others. These countries where it has not led to continuing disease problems - Australia, Portugal, Italy, Bahrain and the USA - all have low rates of diarrhoeal disease in children and relatively good sanitary conditions and clean water (Glass *et al.*, 1991). Tamplin and Parodi (1991) have demonstrated the rapid and widespread transmission of *V. cholerae* O1 in Peru emphasising the importance of treating wastewater.

Ventura *et al.* (1992) studied non-O1 *Vibrio cholerae* in lagoons in Peru and found that numbers of *V. cholerae* and reported cases followed a seasonal pattern (more in the summer) and the seasonal cycles of O1-El Tor and non-O1 tend to coincide, a phenomenon also reported by WHO (1980). This would suggest that surveillance of sewage lagoons may allow prediction of the time and duration of *V. cholerae* O1 epidemics. Kott and Betzer (1972) studied the removal of *V. cholerae* in a 70 l laboratory WSP with a retention time of 5 days and found that from an original concentration of 10^3 per 100 ml

all the *V. cholerae* cells died within 24 hours. Lesne *et al.* (1991) studied the removal of non-O1 *V. cholerae* in an experimental WSP system in Morroco and found that removal was seasonal with a 2 $\log_{10}$ (but not significant) removal in the cold season and little removal in the warm season. Another report (Anon, 1992) suggested that WSP may fail to remove *V. cholerae* and that the numbers may even increase since it was suggested that there may be a possible aquatic reservoir for the organism in the cyanobacterium *Anabaena* spp. (Islam *et al.*, 1990) which may be found in WSP. More recent research in Morocco (Mezrioui *et al*, 1995) has found that non-O1 *V. cholerae* numbers were not significantly reduced in numbers by stabilization pond treatment and that the seasonal dynamics of this bacterium were the inverse of those of faecal coliforms, with high levels in hot periods and low levels in cold periods.

Some studies (see Feachem, 1983) have suggested that sewage may provide a permanent culture medium for some strains of *V. cholerae*. A study of a bath house sewerage system in the USSR (Altukhov *et al.*, 1975 cited by Feachem, 1983) found *V. cholerae* in samples taken over a 13 month period although there was no cholera infection in the community and *V. cholerae* was not isolated from the incoming water supply or from samples of human faeces, water, fish and frog that were examined. The source of infection was not identified but it was clear that once infection had taken place, *V. cholerae* maintained itself in the warm (20-25 °C) sewage. Other evidence (Colwell *et al*, 1980) indicates that *V. cholerae* in natural waters is frequently found in association with bottom sediments, zooplankton and plant surfaces which may be important when considering sewage lagoons. A review of the major ecological studies involving *V. cholerae, V. parahaemolyticus* and *V. vulnificus* (West, 1989) indicates that, in general terms, water temperature, concentration of organic material in the water, salinity and the potential for

association with sediments or the surfaces of higher organisms, significantly influence the occurrence and number of these pathogens.

The recent outbreak of cholera in South America has offered an ideal opportunity to study the survival of the causative organism of cholera in waste stabilization ponds.

An investigation was undertaken to study the die-off of *Vibrio cholerae* O1-El Tor in the experimental WSP at Caatingueira.

5.2 Experimental Materials and Methods

5.2.1 Performance Evaluation

V. cholerae was enumerated as part of the performance evaluation. All the ponds in the innovative system were analysed for *V. cholerae* biweekly from April 1992 to the end of November 1992. From November 1992 to March 1993 the effluent of the large primary maturation pond, M15, and the rock filters, FB2, 3 and 4, were monitored weekly in addition to ponds A9, A10, F21 to F25 and raw sewage. The other ponds were not monitored due to logistical and financial considerations. Enumeration was carried out by the MPN technique using Alkaline Peptone Water (APW) and Thiosulphate-Citrate-Bilesalts-Sucrose (TCBS) agar. Cultures which were presumed positive (PPV) were identified by slide agglutination using *V. cholerae* O1 polyvalent anteserum and by API. PPVs which were negative by the slide agglutination test were identified by API. In additon API tests were performed to identify contaminating bacteria present on TCBS plates.

5.2.2 In-Pond Studies.

Three ponds were chosen to be investigated, A9 (an anaerobic pond with a retention time of 1 day), F21 (a 3 day secondary facultative pond) and M15 (a 3.8 day primary maturation pond). In addition raw sewage (RS) and quarter strength ringers solution were investigated. Ringers solution was included to simulate die-off in nutrient limiting conditions. Figure 5.1 shows the buckets in place in the ponds. The procedure was as follows:

(i) Each pond was studied for several weeks leading upto the experimental period to establish background counts of *V. cholerae* and ensure the level of inoculation was significantly higher. The strain of *V. cholerae* used in this study was isolated from raw sewage, seeded into APW (pH 8.4) and subcultured onto TCBS agar. Both media were incubated at 37°C for 10-24 hours. Cultures were identified by slide agglutination and stock cultures were then maintained on Nutrient agar slopes stored at 4°C.

(ii) *V. cholerae* was inoculated into 10 x 1 l bottles of nutrient broth and incubated at 37°C for 24 hrs. The cultures were then centrifuges at 3500 rpm for 15-20 mins to pack the organisms. The supernatant was discarded and the deposit resuspended in Ringers solution. Washing and centrifuging was repeated 3 times to remove any traces of nutrients. Solutions were then pooled and made up to 100 ml in ringers solution and stored at 4°C over night. Plate counts were performed on Nutrient agar and incubated for 24 hrs at 37°C to ensure sufficient organisms were present for inoculation.

(iii) Five litres of effluent from each pond was placed into sterilised plastic buckets which were then suspended into the following ponds:

RS and A9 suspended in pond A9

F21 and Ringers suspended in pond F21

Figure 5.1 Equipment Set-up for the In-Pond Cholera Studies

M15 suspended in pond M15

The buckets were held in position with wooden poles and secured with wire. Limited chemical analyses was performed on the pond effluents.

(iv) The pond effluents were seeded with the washed suspension of *V. cholerae* O1 to give a concentration of between 10^8 and 10^9 organisms per 100 ml. A sample was immediately taken and enumerated.

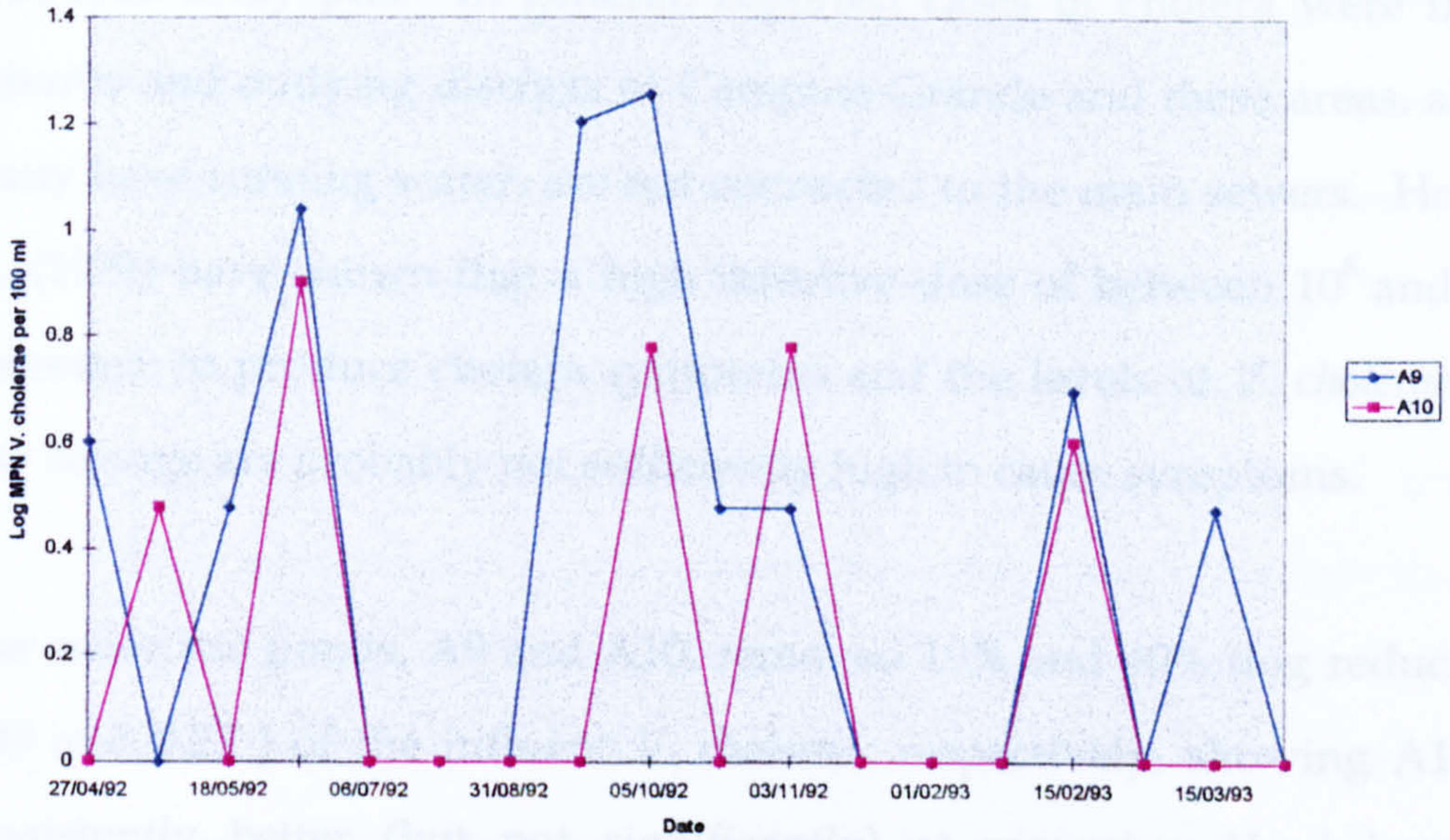
(v) Further samples were withdrawn at intervals and the numbers of *V. cholerae* O1 enumerated by MPN technique using APW and TCBS agar. Plate counts were also performed but these proved to be of limited usefulness due to the large numbers of other Vibrios and contaminating bacteria. Isolates were confirmed by slide agglutination tests using *V. cholerae* O1 polyvalent anteserum.

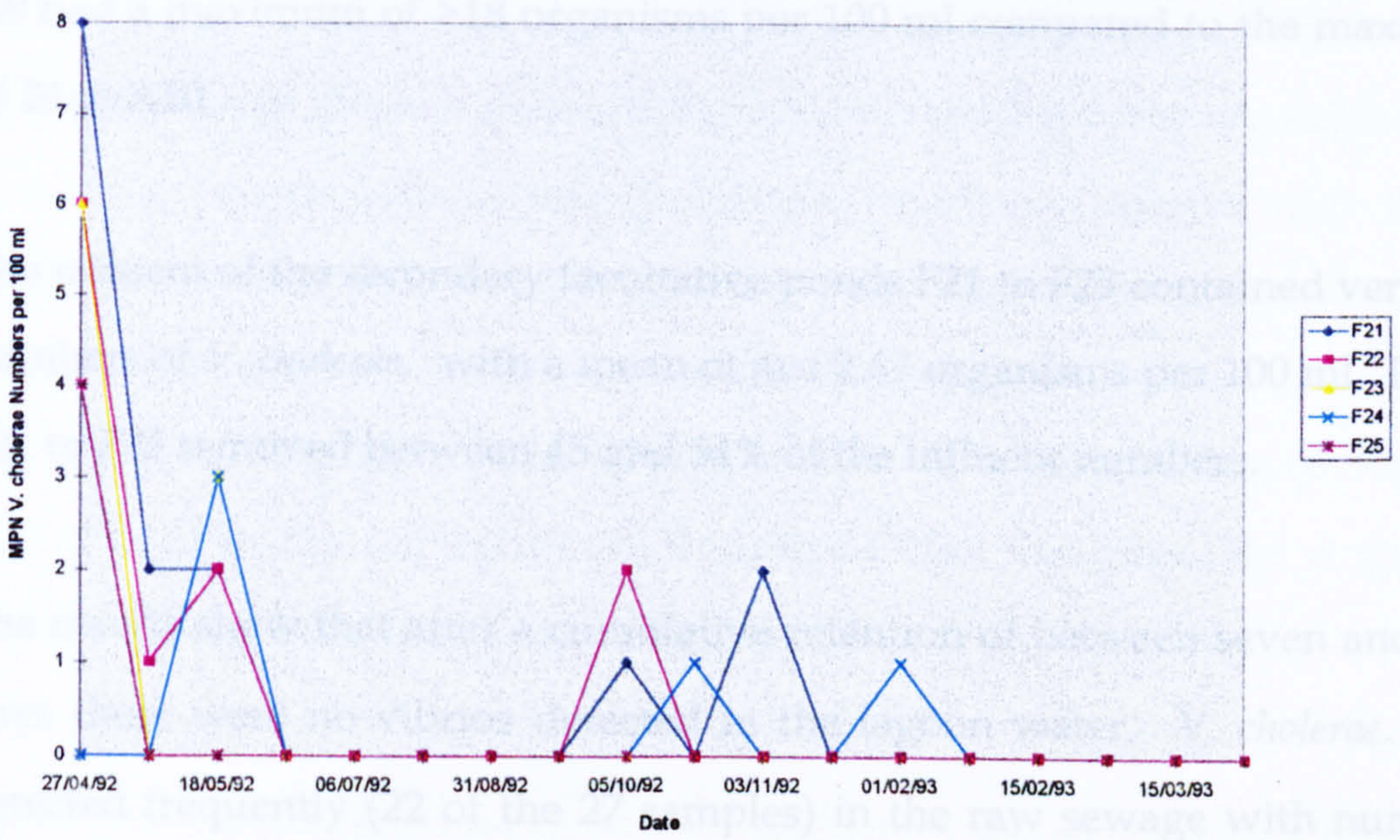
5.3 Experimental Results and Discussion

5.3.1 Performance Evaluation

Results are shown in Figure 5.2 and have been discussed further in chapter 3. The performance evaluation shows that no *V. cholerae* were detected in any of the maturation ponds in the innovative system and was only present up to M25 in the 10 pond series (column samples; vibrios were detected in all ponds up to M29 in the effluent samples). The levels of *V. cholerae* in raw sewage were extremely low with a mean of 3.25 organisms per 100ml. This highlights one of the problems of studying an organism which affects mainly the poor with little or no access to adequate sanitation. There is no doubt that much higher levels of *V. cholerae* were present in the region of Campina

Figure 5.2 *Vibrio cholerae* Numbers in the Anaerobic and Facultative Ponds of the Innovative System - Performance Evaluation Results





Grande but since little of the housing is connected to the main sewers the numbers *V. cholerae* in the wastewater is minimal and it is probable that most of the water infected with the vibrios came from hospitals treating the cases of cholera. Even in the more middle class areas most houses tend to have cess pits/soak away pits. In general, reported cases of cholera were from the suburbs and outlying districts of Campina Grande and these areas, although many have running water, are not connected to the main sewers. Hornick *et al.*, (1971) have shown that a high infective dose of between 10^8 and $1O^{11}$ is necessary to produce cholera symptoms and the levels of *V. cholerae* in the raw sewage are probably not sufficiently high to cause symptoms.

The anaerobic ponds, A9 and A10, removed 19% and 40% (log reductions of 0.09 and 0.22) of the influent *V. cholerae* respectively, showing A10 to be consistently better (but not significantly) at removing *V. cholerae*. The difference between the two anaerobic ponds in real numbers is very small. A9 had a maximum of >18 organisms per 100 ml compared to the maximum of 24 in A10.

The influent of the secondary facultative ponds F21 to F25 contained very low numbers of *V. cholerae*. with a mean of just 2.67 organisms per 100 ml. Ponds F21 to F25 removed between 45 and 54% of the influent numbers.

The results show that after a cumulative retention of between seven and 10.8 days there were no vibrios detected in the lagoon water. *V. cholerae*.. was detected frequently (22 of the 27 samples) in the raw sewage with numbers varying from 1 to 20 organisms per 100 ml (MPN). The presence of *V. cholerae*. in A9 was detected 11 of the 17 times the pond was sampled and in A10 the detection was less with only 6 of the 18 samples being positive.

F21 to F24 had detection frequencies of between 1 and 5 of the 18 samples taken over the experimental period.

In the 10 pond system, A11 removed 56% (0.36 log units) of *V. cholerae* leaving a mean of just 1.43 organisms entering the second pond in the series, F26. After a cumulative retention time of less than 9 days no more vibrios were detected in the effluents of the 10 pond system. The difference in the results of the samples taken by different methods is interesting. The column sampler, which may be expected to give a higher number and detection frequency since it is assumed to be more representative of the pond temporally and spatially, gave a poor detection rate, although the actual numbers of organisms when detected were similar to those from the effluent sample. The ponds up to M25 gave very similar results in terms of both numbers detected and frequency of detection but no *V. cholerae* was detected in the column samples taken from ponds M26 to M28. It has been suggested (Colwell *et al*, 1980; Anon, 1992) that *V. cholerae* may be frequently found in association with phytoplankton and zooplankton and these organisms may provide a haven, even a microhabitat in which the vibrios may thrive. Effluent samples taken from the experimental lagoons were removed from the area between the scum guard and the exit pipe, an area often noted to be thick with floating algae, other organic matter and zooplankton (thought to be feeding on the organics). It is possible that this provided a suitable environment for the vibrios to survive.

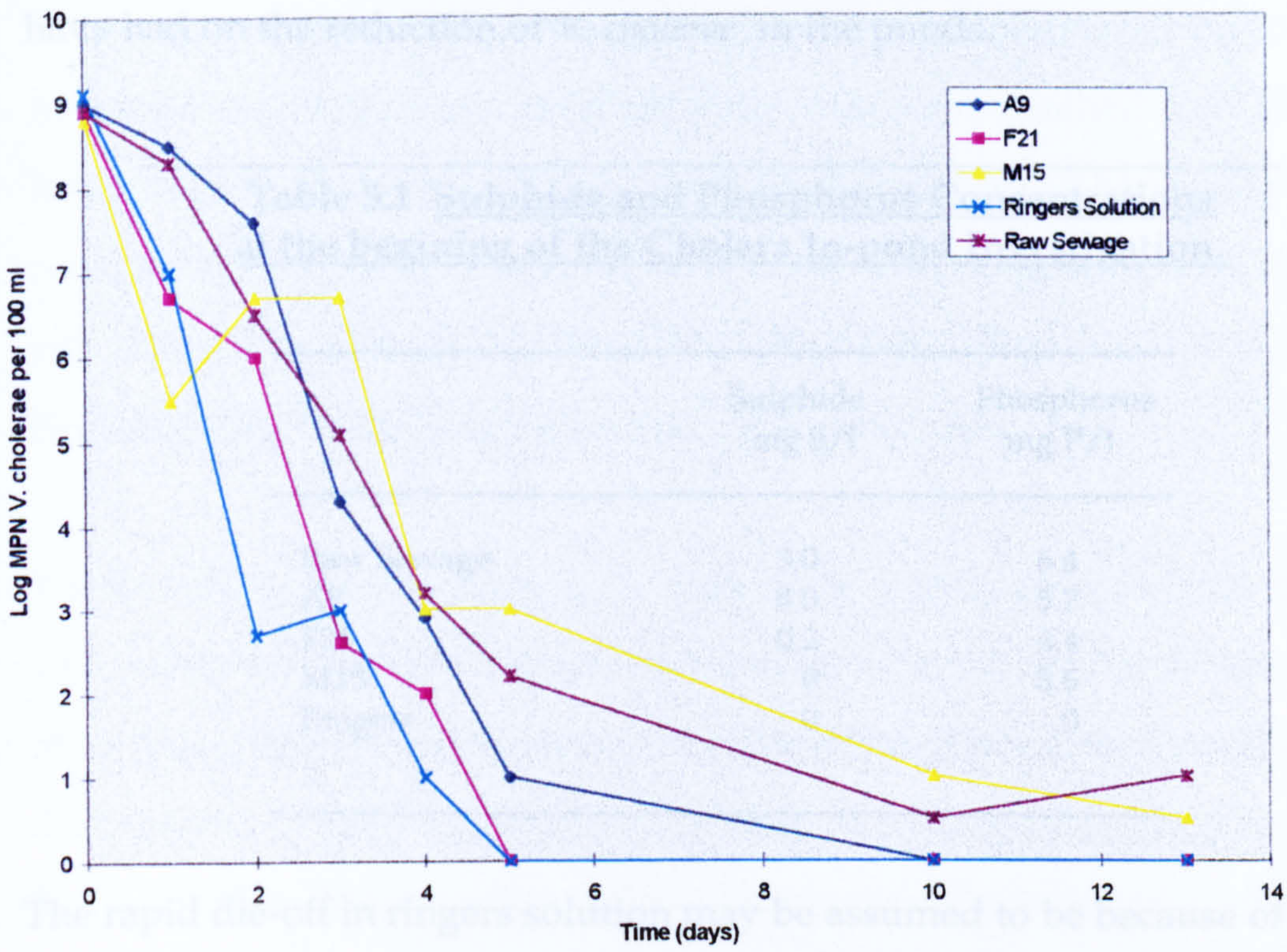
5.3.2 In-pond Studies

The results of the in-pond studies show that after five days 100% of the *V. cholerae* in raw sewage and ringers solution had been removed. Organisms in pond A9 were inactivated after 10 days and ponds F21 and M15 did not completely remove *V. cholerae* up to the time the last sample was taken 13

days after inoculation although the numbers were greatly reduced (Figure 5.3). From the results, *V. cholerae* are able to survive the highly competitive, low oxygen environment of the raw sewage and the anaerobic ponds for sufficient time to pass into the secondary facultative ponds. After 5 days, the vibrios had been reduced by 8 log10 units in pond A9. The effluent from pond F21 also showed a significant reduction in the number of vibrios after 5 days with <100 organisms per 100 ml remaining, consituting a reduction of 99.9998% (a reduction of more than 6 log10 units). M15 showed a reduction of 6 log10 units after 5 days. However, during the last 3 days of the experiment the numbers of vibrios in pond F21 showed a slight increase, from 2 to 7 organisms per 100 ml. Although this is a small (and not significant) increase it demonstrates that the organism *V. cholerae* has the ability to survive in the effluent of a facultative pond and may even reproduce and increase its numbers. Similarly, the survival of *V. cholerae* in the effluent of pond M15 shows that the organism is able to survive the physico-chemical and environmental influences at work in this pond.

It should be noted that, after 24 hours, the water from A9 had a percentage reduction in numbers that appears very good for an anaerobic pond, with 85% of the influent organisms being removed, but is in fact only a 1 log10 reduction. As such, the actual number of *Vibrio cholerae* present was still high with a mean of 2.4×10^8 organisms. Since the usual retention time of anaerobic ponds is only 1 day this indicates that there is a high probability that *V. cholerae* organisms would be present in the effluent of an anaerobic pond and that these may even survive treatment in the secondary facultative ponds since after 4 - 5 days treatment in these ponds organisms were still detected. Further treatment in the maturation ponds may also fail to remove all the organisms since it was observed that even after 13 days in water taken from the pond M15 *V. cholerae* organisms were still detected. However,

Figure 5.3 *Vibrio cholerae* Numbers in the In-pond Studies



looking at the removal over the whole 13 day period we can see that in all cases the initial concentration of *V. cholerae* of between 10^8 and 10^9 organisms was reduced to less than 10^3 after just 4 days and less than 10 after 13 days, a reduction of 8 $\log_{10}$

Sulphide and total phosphorus concentrations in the water of each pond are given in Table 5.1. Since the chemical analysis performed was very limited it is difficult to make comments about the affect the chemical environment may have had on the reduction of *V. cholerae* in the ponds.

Table 5.1 Sulphide and Phosphorus Concentrations at the begining of the Cholera In-pond Investigation.

	Sulphide mg S/l	Phosphorus mg P/l
Raw Sewage	3.0	6.4
A9	8.0	5.7
F21	0.2	4.4
M15	0	3.6
Ringers	0	0

The rapid die-off in ringers solution may be assumed to be because of the lack of nutrients present. In the two effluents which removed the *V. cholerae* fastest, namely raw sewage and A9, the sulphide levels were highest. If the log removals are considered F21 removed organisms as well as the raw sewage and A9 up to the third day when the RS and A9 showed a considerably greater removal than F21. It would seem that the chemical composition of the water did not exert much of an influence on the die-off of *V. cholerae* since sulphide was not detected in M15, the pond which performed best on the first day of the experiment, showing a log removal of 3.4 units.

It must be noted that the environment in which the experiment was performed is somewhat different to the normal environment of the pond in that the organisms were unable to sediment out of the upper layers of the pond and therefore escape any effects of sunlight, pH or increased temperature. Also, the algae in the water tended to settle to the bottom of the bucket making the water less turbid than would be normally expected but this should have lead to a faster rate of die-off than would be expected in a normal pond situation. Although the buckets were immersed up to the rim in the ponds, an increase in temperature may have resulted from the water being heating by solar radiation. These influences could have been reduced considerably by using dialysis bags to suspend the organisms into the ponds and allow free passage of water and electrolytes into and out of the bag. Unfortunately no dialysis bags were available for use.

The technique for enumerating *V. cholerae* is unsatisfactory in that the TCBS agar used does not discriminate between non-O1, O1- El Tor *V. cholerae* and other *Vibrio* species and, therefore, a plate which is presumed positive due to the presence of yellow cholera-like colonies is frequently negative for the O1-El Tor species. Identification of these contaminating bacteria by API showed that in many cases the organism was *Aeromonas hydrophila*. Other organisms identified were *Vibrio cholerae* non O1, *Vibrio fluvialis, Chromobacterium violaceum, Pasteurella multocida, Klebsiella pneumoniae, Citrobacter freundii* and many doubtful or unacceptable profiles but indicating *Flavobacterium* spp., *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp.,

It has been suggested that the cyanobacterium *Anabaena* may act as an aquatic reservoir for *V. cholerae*, but this organism was not found in any of the ponds studied during the experimental period up to March 1993 so it is not possible to comment on this.

Comparison of the efficiency of WSP with respect to other sewage treatment systems is hampered by the fact that most people likely to suffer from cholera are not connected to a sewage main, therefore *V. cholerae* is rarely found in sewage and even then in low concentrations. This coupled with the fact that cholera is very much a disease of developing countries which often lack any sort of wastewater treament and/or the ability (financial and technical) to study the microbiology of sewage, has resulted in a serious lack of published data concerning the die-off of *V. cholerae* in sewage treatment systems other than WSP. Feachem *et al.* (1983) cites two reports of the die-off of *V. cholerae* in wastewater treament systems other than WSP: Flu (1921) studied die-off of *V. cholerae* in five septic tanks of which only one had *V. cholerae* present in the elluent; Kabler (1959) reported a 98% reduction of *V. cholerae* in an activated sludge plant.

Daniel and Lloyd (1980a) studied non-O1 *V. cholerae* in sanitation units in Bangladesh. These units comprised of two tanks in series and removals of 99.8% and 96.4% were found at the two sites studied. Work published by these same authors (1980b) showed that adding small trickling filters to these tanks increased the numbers of *V. cholerae* in the final effluent.

It would seem reasonable to assume, since WSP are more efficient at removing other bacterial pathogens from wastewater than other treatment technologies, that they will similarly be better at removing *V. cholerae*. Indeed, the cumulative kt values calculated for the in-pond experiment, shown in Table 5.2 show how highly efficient these ponds are at removing *V. cholerae*.

5.4 The Importance of Wastewater Treatment in Controlling the Transmission of Cholera

Cholera was and remains a disease of poverty and the living conditions that are associated with poverty. The impact of water supply and sanitation schemes on endemic and epidemic cholera in poor communities is uncertain. Six studies in Bangladesh showed no improvement (Briscoe, 1978 in Feachem, 1983) whereas a study in the Philippines showed a very considerable impact (Azu and Alvaro, 1974 in Feachem, 1983). The potential effects that sanitation may have on a range of enteric and other diseases appears to be far greater than vaccination, for example, which (even if a more protective vaccination were available) is difficult to administer, probably requires readministration, and only protects against a single pathogen (Feachem, 1983).

Cholera has been classically regarded as a waterborne disease but there is evidence (Feachem, 1976) that in some locations of the world, the major route of transmission of cholera may not be waterborne, but may be, for example, due to contaminated foodstuffs or poor home food preparation practices. Glass *et al.* (1991) suggest that where the onset of cholera is prolonged and sporadic transmission is probably not by contamination of the water supply. In households without their own personal water supply it has been found (Swerdlow *et al.*, 1992) that the water in storage containers becomes contaminated from hands and receptacles used to scoop out the water. In the cases of cholera transmission being by some route other than waterborne, it would seem sensible that the effects of sanitation will not be as marked as in a community where the waterborne route is prevalent.

Non-human reservoirs in India have been identified by Sanyal *et al.* (1974) and household cows, goats, dogs and chickens were found to serve as

Table 5.2 Cumulative kT Values for *Vibrio cholerae* in the In-Pond Experiments.

Pond Water	Cumulative Retention Time (days)	Cumulative kT Value
Raw Sewage	1	197
	2	282
	3	5.2×10^5
	4	2.0×10^6
	5	1.6×10^8
Ringers Solution	1	176
	2	1.7×10^6
	3	5.7×10^5
	4	4.2×10^7
	5	3.4×10^8
A9	1	6
	2	24
	3	2.8×10^4
	4	4.2×10^5
	5	4.9×10^7
F21	1	4
	2	123
	3	2.4×10^3
	4	9.9×10^4
	5	1.6×10^6
	10	3.9×10^7
M15	1	1.7×10^3
	2	45
	3	30
	4	1.2×10^5
	5	9.8×10^4
	10	9.8×10^6

possible sources of infection of humans in the community. These animals were found to remain infected for longer periods than humans and the water and sewage of the area and so have a possible role in maintaining the infection in the community.

Evidence that *Vibrio cholerae* and related organisms may occur in surface waters which are not known to be faecally contaminated or in areas where no human infection has been recorded, has been presented by Colwell, Kaper and Joseph (1977). Non-O1 *Vibrio cholerae* was isolated from various parts of Chesapeake Bay in the USA. *Vibrio cholerae,* El Tor and non-O1, were frequently isolated from wells in India in the 1930s and 1940s but their potential pathogenicity was not recognised at that time (Read and Pandit, 1941; Taylor and Ahuja, 1938; both cited by Feachem, 1983). Colwell *et al.* (1980) have summarised *V. cholerae* isolations from various brackish and estuarine environments and this has led them to suggest that *V. cholerae* "is an autochthonous species in the estuarine ecosystem."

There are considerable amounts of data on the survival of *V. cholerae* in water and these have been summarised by Feachem (1983). It is clear that survival can be greatly prolonged in nutrient-rich waters especially if the temperature of those waters is relatively high and the salinity is above 5°/oo. In water with a temperature of less than 10 °C most pathogenic vibrios rapidly disappear from the water column but can persist in sediments. (West, 1989). Under more favourable conditions the vibrios can proliferate and re-emerge in the water and this has been demonstarted with *V. cholerae* (West and Lee, 1982; cited in West, 1989). Pathogenic *Vibrio* species have halophilic properties and occur frequently in water ranging in salinity from 5 °/oo to 30 °/oo so limits their presence to estuarine and inshore coastal areas (West, 1989). Pathogenic vibrios may be isolated from some freshwaters (less than 5

°/oo salinity) where it is possible that a combination of high temperatures and high organic nutrient concentration overcomes the deleterious effect of low salinity. This is applicable to the environment of a sewage lagoon.

Xu *et al.* (1983) and Roszak and Colwell (1987) (both cited in West, 1989) have demonstrated that *V. cholerae* O1 can enter a state of 'dormancy' under unfavourable aquatic environmental conditions and in this state, viable cells remain demonstrable by fluorescent microscopy, but are non-culturable on conventional culture media. Furthermore, these 'viable but non-culturable' toxigenic *V. cholerae* O1 retain their virulence.

In summary, it can be seen that the routes of transmission of cholera in the community are more complex than traditionally believed. In a community suffering from a cholera epidemic it is clearly preferable that the method of wastewater treatment eliminates *V. cholerae* but in situations where transmission is thought to be primarily by alternative non-waterborne routes 100% removal is not, perhaps, necessary. Furthermore, no detection of *V. cholerae* in a water sample is not an assurance that the organisms are not present in the lagoons since they may be found in association with plankton in the pond, or they may be in a state of dormancy in which they are not detected by the usual method of enumeration by MPN. The organisms may even take refuge in the bottom sediments and so escape detection.

5.5 Summary

1. The very low numbers of organisms entering the Caatingueira pond system made it difficult to accurately evaluate their efficiency at removing *Vibrio cholerae*. However, the performance evaluation showed complete

removal from the ponds within 6 days and 2 ponds in series (the innovative system) and 7 days and 4 ponds (the 10 pond system column). Effluent samples from the 10 pond system detected *Vibrio cholerae* upto and including the 7th pond in the series, a cumulative retention time of 13 days.

2. In-pond experiments showed that survival of the treatment process is possible for at least 13 days in facultative and maturation ponds. However, after a maximum of two days, in all cases, the number of organisms present was below the critical level which could cause symptoms to occur. This is due to the very high infective doses necessary.

3. The k_T values calculated for the die-off in these in-pond experiments are very high with values ranging between 10^6 and 10^8 after 5 days retention time. This indicates that WSPs are highly efficient and suitable for the disinfection of wastewater containing *V. cholerae* in higher numbers than those seen in the ponds at Caatingueira.

4. If a pond system was to completely remove *V. cholerae* it would be necessary for the maturation ponds to have a retention time of more than 13 days, which is the time span over which the organism was detected in the in-pond experiments. Whether a treatment system includes an anaerobic pond is not important since the retention time of these ponds is normally just 1 day, a period over which the removal of *V. cholerae* was less then one log unit.

5. The large infective doses require to produce cholera-like symptoms, and the alternative transmission routes available to *V. cholerae,* through poor hygiene and food handling techniques, suggest that complete removal of *V. cholerae* from wastewater is not necessary. In addition, levels in the

environment, for example in water bodies, wells, and animal reservoirs, would probably be sufficient to maintain the infection in the community.

6. Comparison with other wastewater treatment systems is not possible due to the low numbers of *V. cholerae* in sewage and the lack of research in this field.

Chapter 6 General Discussion and Conclusions

6.1 Pathogen Removal

The results given in Chapter 3 showed that by the fourth pond in series in the innovative system and the seventh pond in series in the 10 pond system bacterial pathogens had been removed to a point where the effluent is suitable for unrestricted irrigation (WHO, 1989 - guideline level of less than 1000 FC per 100 ml for unrestricted irrigation). Bacterial removal in the ponds of the 10 pond system was understandably different to that of the innovative system. After five ponds in series, the innovative system had a mean of 32 FC per 100 ml (excluding the macrophyte pond, M23). In contrast, after 5 ponds, the 10 pond system still contained more than 10^4 FC per 100 ml. The difference is probably a combination of loading and retention time. The final effluent of the pond series removed 99.9998% (the innovative system) and 99.996% (the 10 pond system) of FC.

Generally, the removal of all the bacterial pathogens and rotaviruses was not consistently better in either of the pond systems, although direct comparison is difficult due to the different loadings of the systems. Both systems showed poor removal of CP in the anaerobic ponds and while the *Salmonella* spp. present in the anaerobic ponds of the innovative system increased in numbers, in the 10 pond system they were reduced by 84%. If there was inhibition of the *Salmonella* occurring in the anaerobic pond of one series it would be expected to also see this in the other system since the anaerobic ponds of both systems receive identical sewage and have the same retention times.

From the bacteriological results of the ponds in both systems it may be seen that the anaerobic and facultative ponds, although designed principally for BOD removal, performed as well as, and in some cases (for example *Faecal streptococci*, see section 3.6) better than, the maturation ponds in terms of bacterial removal. This phenomenon was also observed by Silva *et al* (1987) in ponds in N.E. Brazil and may indicate that the shape of these ponds is not as important as other factors such as the amount of settleable and suspended solids and the concentration of algae present. The results for BOD, COD, and SS for all the anaerobic ponds were similar which suggests that within the length to breadth ratios of 1.5:1 (A11) and 3:1 (A9 and A10) and depths of 1.5 m (A11) and 2.5 m (A9 and A10), studied in this investigation, anaerobic pond geometry does not affect pond performance.

The data for the secondary facultative ponds also indicate that length to breadth ratios in the range of 1:1 and 6:1 and depths within the range 1 to 2 m and retention times of 2-6 days do not significantly affect pond performance with respect to BOD, COD and SS. In terms of the pathogen removal, the mean numbers of organisms and the corresponding k_T values found in ponds F24 and F25, ponds of the same volume and retention time but different L:B ratios, were not significantly different to those of F25 and there were no significant differences between the k_T values of any of the secondary maturation ponds.

Of the five secondary maturation ponds (M16 - M20) the most efficient with, respect to FC and FS removal, was M20, a shallow pond with a higher BOD surface loading than the other shallow ponds, and which may be considered to have a hydraulic regime approaching plug-flow, a condition seen as conducive to pathogen removal. However, statistical analyses on the bacteriological data showed that the shallower lagoons were not significantly

more efficient at FC removal than the deeper maturation ponds. Figure 3.3 showed few significant differences between the first-order rate constants for FC removal. The other bacterial pathogens and rotaviruses also had high removal rates in M20 compared to the other secondary maturation ponds but low influent numbers mean that the k_T values were not true indications of the pond performance and statistical procedures could not be carried out.

The tertiary maturation ponds were comparable with the secondary facultative ponds at removing FC, although M24, the pond with the built in chicane, had a higher k_T value which may suggest that at this later stage in the treatment process there is some advantage to be gained by having a larger length to breadth ratio, but it is difficult to make any conclusions to this effect because of the low numbers of pathogens in the influent to this pond. M23, the macrophyte pond, performed well at removing BOD but was less efficient in terms of pathogen removal having a lower k_T value than the other tertiary ponds. At this stage in the treatment the BOD is already below the required standards for effluent discharge so the reduction seen here is superfluous and a further reduction in pathogen numbers would be preferable. The results from M23 suggest that the quality of the effluent from a pond covered in floating macrophytes is poor in terms of pathogens, with *Salmonella* being found in greater numbers than in the other tertiary ponds. The tertiary maturation ponds, with the exception of M24, appeared to perform less efficiently than even the deeper secondary maturation ponds but this may reflect the smaller microbial populations and the fact that those organisms still remaining at this stage in the treatment series are more resistant to the ambient conditions.

Rotavirus removal in the tertiary maturation ponds was higher than in the preceeding ponds and the k_T values were much larger than seen for any other

pathogen in any other pond. The values are, however, somewhat divergent but this probably relates to the low number of virus particles and the impact small changes in numbers has on k_T values.

Although the results of the performance monitoring are not conclusive due to the lack of significant differences in the results, they indicate that the shallower maturation ponds with higher length to breadth ratios have larger kT values indicating a more efficient performance. The results presented suggest that there is no advantage in building deeper facultative and maturation ponds, at extra expense, since there is no increase in removal rates, as shown by the kT values of the pathogens in ponds F21-F24. Five out of the seven organisms studied had the highest rate removal constants in the shallowest pond, F21, followed by the deepest secondary facultative pond, F24, with intermediate ponds performing less efficiently. The same pattern was observed in the secondary maturation ponds with the shallower ponds (M18, M19 and M20) having the highest removal rate constants followed by the deepest pond, M16, and the pond with a depth between these ponds performing less efficiently.

The removal rates of BOD5 also indicate that an increase in efficiency does not accompany an increase in depth.

24 hour profiles carried out on the secondary facultative ponds and the secondary maturation ponds did not show the geometry of these ponds to be having any affect on their performance and this supported the findings from the performance evaluation monitoring that within the ranges investigated there was no indication that the shape or depth of the ponds effected the performance.

6.2 Position of Current Research with respect to Previously Published Data

Although there exists a considerable amount of published work dealing with the influence of pond shape and depth on the performance of WSP there has been little rigourous work done on the determination of optimal pond geometries and throughout the world ponds are designed and built according to the prevalent attitudes of the engineers involved. Previous thinking assumes pond geometry is critical to ensure the whole pond volume is active, to minimise dead zones which effectively decreases the active volume of the pond and reduces the retention time, and to minimise short-circuiting. Pond geometry includes not only the shape and depth of a pond but also the relative positions of the inlet and outlet structures (Mara and Pearson, 1987b).

6.2.1 Shape

Traditionally, and well documented, the shape most often employed is rectangular but the ratios of length to breadth vary widely.

Using the classification suggested by Arceivala (1983) the ponds in the two WSP systems investigated here ranged from completely mixed to plug flow. A completely mixed regime is assumed for a pond with a length to breadth ratio of <4 and a plug-flow regime has a L:B >8. Agunwamba (1991) found that greater facultative pond efficiency in terms of effluent quality would be achieved with a plug-flow regime rather than a completely mixed one. Juanico (1991) agreed stating that plug-flow ponds perform better than completely mixed ones for removal of parameters with high removal constants eg bacteria and when the removal constant is lower eg BOD, plug-flow and completely mixed perform similarly. However, as the length to breadth (and breadth to depth) ratios increases so do the volume of dead

zones (Mangelson and Watters, 1972) thus there is a limit to the advantage a large length to breadth ratio conveys. Ferrara and Harleman (1980) found that most ponds do not reach completely mixed conditions so there is always an element of plug flow even in very small length to breadth ratios. Thirumurthi (1969) concluded that stabilization ponds cannot be rationally designed by assuming them as plug-flow or completely mixed flow systems.

In summary:

The results presented herein suggest that increasing the length to breadth ratio does not obviously influence pond performance or improve the quality of the effluent. The significance of designing long rectangular WSP to encourage plug-flow is probably overstated (Forero, 1993).

It would appear that vertical stratification of the water column is more important in determining treatment efficiency than bulk flow along a longitudinal axis (Pearson *et al* , 1988). Ellis (1983) states that smaller ponds are less affected by wind and, therefore, more prone to stratify. Small ponds generally have shorter retention times and a series ponds acting facultatively may be needed and this would also mean an increase in capital costs.

This apparent lack of impact of pond shape on pond performance, within a realistic range, allows more freedom in shaping ponds to make the best use of available land, particularly on awkwardly shaped sites.

6.2.2 Depth

Hosetti and Patil (1986) carried out investigations into the performance of facultative WSP with depths of 1.22 (the depth quoted in the paper as the accepted standard depth for operation of stabilization ponds), 1.83 and 2.44 m. After looking at enzyme activity to determine microbial growth and

activity they concluded that ponds of depths up to 2.44 m should perform to an acceptable level.

Sarikaya and Saatchi, 1987; Sarikaya *et al* , 1987 and Mayo, 1989 report that the deeper the pond, the lower the bacterial die-off rate which is confirmed by the results presented in this thesis since the die-off rate is a function of the retention time and the deeper ponds have a longer retention time. It would be necessary to separate the effects of retention time and depth in order to confirm that this decrease in die-off rate is due to the depth alone. The work of Silva *et al* (1987) in N.E. Brazil investigated this problem and concluded that the critical factor was indeed depth since the shallow ponds produced effluents with up to 2 orders of magnitude fewer faecal coliforms than deeper ones at more or less the same retention time.

In summary:

Increasing pond depth does not appear to significantly improve the physico-chemical or microbiological quality of the effluent and this is important due to two factors:

Firstly, there has been a tendency to increase the depth of ponds in order to increase the retention time in the belief that the microbiological quality of the effluent will be improved. This is because the Marais equation, when used to predict FC numbers in the effluent, will give a lower predicted FC value for a deep pond than for a shallow one receiving the same surface loading. For example, taking a maturation pond such as M16, with a retention time of 7 days and a flow of 5 $m^3 d^{-1}$, an influent FC of 1×10^7 and a k_T of 1, using the Marais equation:

$$\text{Number of effluent FC} = Ni/(1 + kT\theta)$$
$$= 1.25 \times 10^6$$

With a deeper pond receiving the same surface loading the retention time would be increased, for example, to 10 days giving an effluent FC of 9.1×10^{5}.

Secondly, on purely financial grounds deeper ponds are usually more expensive to build than shallow ones due to the costs of excavation.

6.3 Design Implications of Experimental Results

The controversy surrounding optimal pond shape and depth has been discussed in section 1.8 and it is frequently stated that the performance of a pond may be improved by increasing the length to breadth ratios and decreasing the depth to favour a hydraulic regime more approximating plug flow than completely mixed (Mara and Pearson, 1987b). The results presented here, for ranges of depths (secondary facultative ponds of 1 to 2 m, maturation ponds of 0.4 to 1 m) and shapes (length to breadth ratios of 1:1 to 6:1 for the secondary facultative ponds and 2:1 to 2.7:1 for the maturation ponds) which are realistic for many pond sites, suggest that the impact of pond geometry on the quality of the effluent is minimal (aside from the impact on retention time if the volume of a pond is altered).

The lack of significance of the results mean that the design approaches and equations presently in use, with respect to the depth, shape and size of reactors, cannot presently be improved and further investigations would be needed to definitively settle the question of optimal pond geometry. However, it can be seen that designing a pond for BOD and nutrient removal does not limit that pond to just that function and efficient pathogen removal can, and will, occur. Indeed, these two functions are compatible and there is

no trade off necessary since designing for BOD removal is not detrimental to pathogen removal and vice versa.

The BOD results for both pond systems show that, for crude sewage with unfiltered BOD of 197mg/l, after two (innovative system) and four ponds (10 pond series) in series (equivalent to a retention time of 7-9 days) there was no real improvement in the BOD quality of the pond effluents and this is important in that a design engineer is assured that providing a pond system has a retention time of at least 7-9 days and the design loadings are not exceeded the effluent will meet standards regulating BOD emissions regardless of the number of ponds in the series.

The consequences of establishing the effects of pond depth and geometry on the performance of WSPs are far reaching. It allows design engineers more flexibility in shaping ponds to make best use of the land available without compromising the effluent quality achieved and construction costs can be reduced by building shallow ponds which perform as, if not more, efficiently than the deeper ponds.

6.4 Accuracy of the Marais Equation for Predicting Bacteria Numbers

The results obtained from the performance evaluation monitoring show that all the ponds, whether anaerobic, facultative or maturation, have different k_T values for the removal of bacteria. This indicates that the Marais equation frequently used to calculate the expected numbers of FC in the effluent of a pond, which uses k_T values calculated from the mean ambient temperature, is not perfect for predicting the numbers of pathogens in all pond types since the k_T values in anaerobic, facultative and maturation ponds may differ significantly. An alternative design equation produced by Cairncross and

Feachem (1983) attempted to rectify this and the k_T values they used for the anaerobic ponds were half those of the facultative and maturation ponds. However, the k_T values found in the anaerobic ponds during this work were found to be higher than those of the secondary facultative ponds.

Since all of the ponds studied in this investigation were subject to identical environmental conditions the differences in the results are wholly attributable to the treatment received in the lagoons. When using the Marais equation to calculate the final effluent quality (in terms of pathogens) of a system of anaerobic, facultative and maturation ponds it is important to note that the k_T values used in the Marais equation were derived using data solely from maturation ponds. Maturation ponds are generally accepted as being responsible for disinfection and so it might be expected that using this equation would overestimate the true k_T value obtainable for a facultative pond since these pond are designed more for BOD removal than for disinfection. From the results obtained in this study it seems that the equation is conservative in it's estimation of the k_T values of the maturation ponds and so there is a balance between the over-estimation of the k_T value of the secondary facultative ponds and the under estimation of the k_T value of the maturation ponds giving an overall value of effluent pathogen numbers of the series which is a reasonable estimation of the actual numbers present. Table 6.1 shows the predicted numbers of FC in the ponds using the Marais equation and the published k_T values obtained for 25 °C (the mean ambient temperature recorded at the pond site) of 6.2 d^{-1}. These predicted numbers are expressed in two forms; the number of FC in the effluent of that individual pond calculated using the actual influent FC numbers to that pond and the number of FC in the effluent using the raw sewage influent FC value and determining the theoretical Ne value (number of organisms in the effluent) for each pond according to its position along the five pond series.

Table 6.1 **Predicted and Recorded Bacteriological Effluent Quality of the Ponds in the Innovative System**

POND	Predicted N_e based on		Recorded
	Actual N_i*	or N_i of RS**	
A9	3.69×10^6	3.69×10^6	7.06 x 1
A10	3.69×10^6	3.69×10^6	7.15 x 1
F21	3.63×10^5	1.88×10^5	1.08 x 1
F22	2.76×10^5	1.43×10^5	9.22 x 1
F23	2.22×10^5	1.15×10^5	9.15 x 1
F24	1.86×10^5	9.67×10^4	7.76 x 1
F25	1.86×10^5	9.67×10^4	8.90 x 1
M15	3.73×10^4	4.78×10^3	2.36 x 1
M16	5.32×10^2	1.10×10^2	5.45 x 1
M17	7.38×10^2	1.53×10^2	6.81 x 1
M18	1.20×10^3	2.50×10^2	7.58 x 1
M19	1.20×10^3	2.50×10^2	6.30 x 1
M20	3.28×10^3	6.79×10^2	1.60 x 1
M21	2.07×10^1	6.49	3.49 x 1
M22	2.07×10^1	6.49	4.17 x 1
M23	2.07×10^1	6.49	8.30 x 1
M24	2.44×10^1	7.68	1.92 x 1

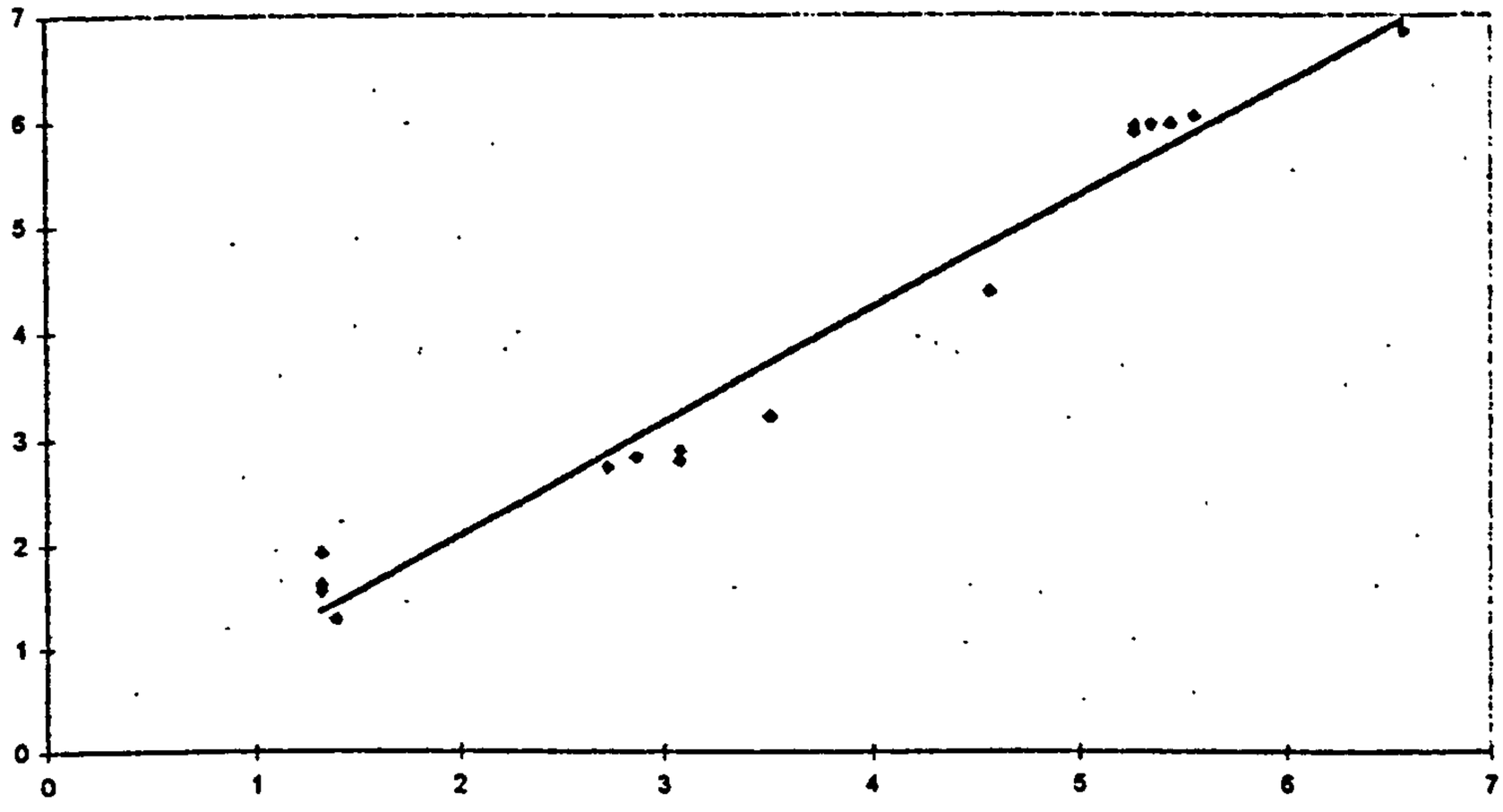
*Actual N_i = Actual number of FC in the influent of that individual pon to calculated the theoretical number in the effluent.

**N_i of RS = Number of FC in the raw sewage used to calculate the theo number in the effluent of the series up to and including that pond.

Although the predicted value for FC numbers can be nearly an order of magnitude lower than the recorded effluent values for the anaerobic and facultative ponds in the series when calculated from FC numbers in raw wastewater, the numbers then converge as the higher than predicted faecal coliform removal rates come into play in the maturation ponds. Despite these differences in experimentally determined and theoretically derived k values, plotting log recorded effluent FC numbers for each pond against log predicted numbers using either the actual influent numbers or extrapolating from raw sewage FC values (Figure 6.1) gave linear regressions with highly significant positive correlations (r^2) of 0.99 and it can be seen that theoretical k_T calculations indicate that at Caatingueira, for an crude sewage of the strength currently received and with an anaerobic pond and facultative ponds operating on similar retention times to those used in the experiments, a series of 4 ponds would be needed to reduce FC numbers to less than 1000 per 100 ml and the actual results show this was appropriate. Thus, predicting effluent bacteriological quality by using the Marais equation to predict effluent FC numbers is acceptable in pond systems comprising of at least three cells (including an anaerobic pond) but is less reliable where only two ponds are in series due to the over-estimation associated with applying the equation to anaerobic and facultative lagoons.

It was observed that, once the influent to a pond contained less than 10^3 FC per 100 ml, using the Marais equation for predicting the numbers of organisms in a pond became more difficult due to the possible presence of more resistant organisms and for which the k_T value of 6.2 d^{-1} used in the equation is not suitable. Table 6.1 illustrates this since the estimated effluent numbers (using the actual recorded influent to the facultative ponds) up to the secondary maturation ponds are a fair approximation to those numbers actually recorded.

Figure 6.1 Actual versus predicted Numbers of Faecal coliforms in the Effluents of the Ponds of the Innovative System



Regression Equation:

Predicted = -68208 + 0.5 Actual

r^2 = 0.99

6.5 BOD Removal

The BOD results obtained using the column sampler show that in the effluent of the primary maturation ponds in both systems similar amounts of BOD have been removed with the mean BOD of ponds M15 (the innovative system) and M25 (the 10 pond system) approximately 18 mg/l in the unfiltered samples and 8 mg/l in the filtered samples. In the innovative system this reduction in BOD was followed by a slight increase, due to the presence of algae in the secondary and tertiary maturation ponds, which was maintained throughout these reactors. The maturation ponds of the 10 pond system did not exhibit such an increase in BOD (the concentration of chlorophyll-*a* was also low with less than 200 µg/l in most of the ponds). In the case of grab samples obtained from the effluent of the ponds of the 10 pond system, the BOD measured throughout the system was higher than that in the column samples, as was the concentration of chlorophyll-*a*. The concentration of BOD in the column samples was constant from M26 to M32. It appears that the maturation ponds of the 10 pond system are able to maintain a low BOD, without the usual increase due to algae, and this may be because the surface loadings on these ponds are higher than those of the maturation ponds of the innovative system. The one pond in the innovative system which has a higher loading, namely M20, has a lower chlorophyll-*a* concentration similar to the ponds of the 10 pond system. Overall, the ponds of the 10 pond system do not contain concentrations of algae as high as those of the innovative system and this could be because of the small surface area to volume ratio of the ponds in the 10 pond system restricting light penetration and, thus, reducing algal productivity. It is unlikely that the levels of suspended solids in the ponds were high enough to restrict light penetration however the position of the ponds of the 10 pond system, relative to the sun, is such that for a large part of the day the pond water is in the shade of its

own walls and this may be an important factor in influencing algal activity in this case.

In both experimental systems, the European Union (EU) pond effluent quality of less than 25 mg filtered BOD_5 per litre and less than 150 mg SS per litre (Council of the European Communities, 1991) was achieved by the second pond in the series, that is after a retention time of 3 days (A11 and F26) in the ten series of ponds and after 4 days (A9/A10 and F21) in the innovative ponds. Although filtered COD was not measured, the BOD infers that the secondary facultative pond effluent would have easily attained the EU quality of less than 125 mg filtered COD per litre.

6.6 Validity of FC as Indicators of *Vibrio cholerae* and other Pathogens from WSP in the Tropics.

In chapter 3 the results showed that there was a positive correlation between the numbers of pathogens in the pond systems since the numbers decline through the series and a simple product-moment correlation shows a strong positive correlation. Tables 3.6 and 3.10 show that the rate of removal of pathogens from the different types of ponds in the two pond systems studied varied considerably and it was found that whereas FC had the highest rate of removal in the maturation ponds of the innovative system, FS were removed fastest in the secondary facultative ponds. Calculating just one k_T value according to the mean influent and mean effluent number of organisms in a pond gives a long term picture of what removal is occurring in a pond but a better picture of seasonal changes in this rate of removal can be gained by calculating several k_T values by using the corresponding influent and effluent numbers over a short period of time. This was carried out by calculating the two-monthly (or bimonthly) k_T values. A product-moment correlation (a

measure of the functional relation of one variable upon the other) was then performed to see if changes in the removal rates occurred similarly for all of the pathogens studied. Using this method it may be possible to determine the suitability of using one of the pathogens as an indicator organism.

Table 6.2 Product-Moment Correlation Coefficients of Faecal Coliforms and *Faecal streptococci* in both WSP Systems

	Faecal Coliforms	Faecal streptococci
The Innovative System		
Faecal streptococci	-0.19	-
Salmonella	-0.34	0.63
Clostridium perfringens	0.08	0.82
Rotaviruses	-0.16	-0.28
The Innovative System Bimonthly		
Faecal streptococci	0.72	-
Salmonella	-0.19	0.15
Clostridium perfringens	0.05	0.20
Rotaviruses	-0.10	-0.40
The 10 Pond System Effluent		
Faecal streptococci	0.03	-
Salmonella	0.72	0.22
Clostridium perfringens	0.67	0.09
Rotaviruses	-0.12	-0.26

Table 6.2 shows the product-moment correlation coefficients for the k_T values for each series and also a correlation between the bimonthly k_T values of the ponds of the innovative system. It was not possible to carry out statistical analysis of the removal rates of *Vibrio cholerae* due to the very low numbers

detected in only a few of the ponds studied and therefore the accuracy of the k_T values obtained. The same may be said for *Campylobacter* and to a lesser degree *Salmonella*.

The above table firstly indicates that when the k_T values for both systems over the whole of the experimental period are compared there is very little indication of any correlation, positive or negative, between the removal rates of the different organisms. Secondly, it is interesting to note that the correlation coefficients calculated from bimonthly k_T values are different from those calculated over the whole sampling period. This shows that, for example, if the whole experimental period is examined there appears to be no correlation between FC and FS removal rates but the bimonthly figures show that the removal rates are more similar than first supposed with a coefficient of 0.72.

From these statistical calculations it can be seen, for example, that the rate of rotavirus removal has no correlation with either FC or FS as might be predicted since the organisms are totally different in nature and behaviour.

Salmonella shows a positive correlation with FC in the 10 pond system but this is based on k_T values over the whole experimental period. In the innovative system, using bimonthly k_T values, this bacterium has no correlation with either FC or FS.

In the innovative system *Clostridium perfringens* shows a positive correlation when the whole period of study is considered but the bimonthly figures do not confirm this. It was not expected to find a correlation between this

organism and FC and FS due to the nature of *Clostridium,* being a spore forming anaerobe.

The use of Faecal coliforms or *Faecal streptococci* as an indicator organism, based on the findings of this work, would be inappropriate. A universal indicator organism does not exist due to the different natures of the pathogens (eg. microaerophilic, halophilic etc) and it may be more appropriate to use BOD as an indication of the pathogenic quality of wastewater. Until a more suitable indicator is found, the coliform will be valuable as an indicator of faecal pollution and treatment performance only.

These findings agree with those of several authors including:
Carter *et al* (1987) in experimental work at two natural ponds in Washington, USA found no obvious relationship (positive or negative) between the densities of any indicator bacteria (total and faecal coliforms and faecal streptococci) and that of *Campylobacter* spp.

Rippey and Watkins (1992) found faecal coliforms were eliminated at a much more rapid rate in chlorinated sewage effluents than *Clostridium perfringens.* These authors also enumerated a Male-Specific Bacteriophage and, on the assumption that the MSB are probably a more reliable indicator of enteric viruses they concluded that coliforms are inappropriate for reflecting viral behaviour.

In early work by Gallagher and Spino (1968) there was little apparent correlation between levels of total or faecal coliforms and *Salmonella.* It was found that *Salmonella* survived longer than the indicator bacteria.
Total coliforms may be non-faecal in origin especially in hot climates, and may multiply so presence may not be relate to the occurrence of faecal

contamination. Faecal indicator bacteria are useful in assessing the safety of drinking water and water for recreational use but when the health aspects of sewage treatment processes are considered it would be advantageous to have a pathogen indicator organism as opposed to a faecal indicator bacterium so that there could be a reliable measure of total pathogen content at the end of the treatment process (Feachem *et al* , 1983).

In contrast some authors have found correlations between indicator bacteria and enteric pathogens. A paper by McFeters *et al,* (1974) reported comparative survival of these organisms in well water and found that *Salmonella, Faecal streptococci* and coliform bacteria have similar survival times and Bartone (1985) found that *E. coli* was a good indicator for *Salmonella* in studies carried out in Peru.

Feachem *et al* (1983) presented a summary of the survival of *Vibrio cholerae* in water and found that t90 values were not greatly lower than those reported for coliforms and may be similar to those reported for other bacterial enteric pathogens. Survival was prolonged in nutrient rich waters and seawater which had been sterilised, thus eliminating competing microorganisms. However, Pandit *et al* (1967; cited in Feachem *et al*, 1983) found survival of El Tor to be 2 to 5 times longer than *E. coli, Pseudomonas* spp. and *Aerobacter* spp.

Hirata *et al* (1993) found that the removal efficiencies of total coliforms were similar to or higher than those of *Clostridium perfringens* in several wastewater treatment processes and they concluded that *C. perfringens* is an effective indicator microorganism for evaluating microorganism removal in wastewater processes.

The routine analysis of pond effluents for pathogenic viruses and bacteria is not yet feasible due to the high financial cost and intensive labour this would entail, bearing in mind WSP systems are looked upon as being ideal for use in developing countries. The choice of suitable pathogen indicator is difficult. Bacteriophages, more specifically coliphages, are a possibility but laboratory techniques may not be suitable for developing countries. Faecal coliforms and/or *Faecal streptococci* are an obvious choice but there is little archive data on the usefulness of either as viral indicators and literature, especially relating to tropical pond effluents, is scarce.

6.7 Conclusions

From the findings of the research reported in this thesis, the following conclusions can be drawn:

1. The performance of, and effluent quality from, secondary facultative ponds was not influenced by the geometry of the pond, within the ranges investigated and based on a crude sewage strength of 197 mg/l BOD (unfiltered). The ranges investigated were depths of 1 to 2 m and length to breadth ratios of 1:1 to 6:1. Increasing the depth of the facultative pond or reducing the length to breadth ratio while maintaining the same surface loading did not significantly improve the quality of the effluent and, in the case of increased depth, resulted in the pond efficiency, as measured by K_T value, being reduced. Traditional techniques of designing secondary facultative ponds on the basis of surface BOD loadings are, therefore, validated and are preferable to other approaches such as Marais and Shaws (1961) method based on first order kinetics or the Thirumurthi method (Thirumurthi, 1969) which employs hydraulic dispersion.

2. The performance of secondary maturation ponds is also not significantly influenced by the geometry of the pond, within the ranges investigated of 0.39 to 1 m and length to breadth ratios of 2.3:1 to 8:1 and with an influent (to the anaerobic ponds) crude sewage BOD of 197 mg/l unfiltered. Increasing the depth, and therefore, the retention time of secondary maturation ponds does not result in an increase in effluent quality.

3. From the kT values of the secondary and tertiary maturation ponds it can be seen that the shallower ponds with higher length to breadth ratios have greater removal rates. This suggests that increasing the depths to achieve an increase in retention time and, therefore, an improvement in the bacteriological quality of the effluent for the same pond area is not a valid strategy. If the ranges of depths investigated had been greater more conclusive results may have been obtained.

4. A pond system with a crude sewage influent similar to that found at Caatingueira (average unfiltered BOD of 197 mg/l) comprising a one day, 2.5 m deep anaerobic pond and a 3 day, 1 m deep facultative pond is sufficient to comply with the European Union effluent requirement of less than 25 mg filtered BOD5 per litre and less than 150 mg suspended solids per litre.

5. In areas where cholera is endemic the inclusion of an anaerobic pond in the system of WSP is essential for the efficient removal of *Vibrio cholerae* O1. A primary facultative pond may be found to have a similar affect on *Vibrio cholerae* removal.

6. The use of faecal coliforms or *Faecal streptococci* as an indicator organism for the removal of other pathogens from a system of WSP is not recommended based on the findings of this research.

6.8 Suggestions for Further Work

1. WSPs of a larger range of depths and geometries would allow further confirmation of the results presented in this thesis.

2. The crude sewage flowing into the ponds at Caatingueira may have been weaker than that found in other locations and experiments on different strengths of sewage would allow further recommendations as to the optimum geometry of facultative and maturation waste stabilization ponds.

3. The WSPs investigated were situated in a tropical climate and the period of time over which the results were gathered was exceptional due to the lack of a wet season. Temperate and a more defined tropical climate may influence the findings of such an investigation due to the affect local weather conditions can have on the performance of WSPs. Wind and rain cause turbulence at the pond surface and can increase evaporation and break down stratification, especially in shallower ponds. Turbulence also reduces the algal photosynthetic activity which in turn affects the DO, pH and ultimately the BOD of a pond. Further work on pond systems in different climates may show that depth and geometry are important factors in those particular conditions.

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Appendix i

Method of Estimation of chlorophyll - *a*

Appendix I Method of Estimation of chlorophyll - *a*

The procedure was as follows:
(i) 10 ml of 0.1% $MgCO_3$ suspension was filtered through a 5 cm diameter Gf-C Whatman filter. This serves to aid retention of the algal cells onto the filter and also maintains an alkaline environment which prevents the transformation of chl a into its degradation products such as phaenophytin.

(ii) a known volume of sample was filter through the filter. The volume filtered was dependant upon the amount of algae present in the sample but was in the region of 25 - 100 ml. Munawar (1982) demonstrated that some phytoplankton may pass through glass fibre filters but the algae filtered in these studies was of a sufficient size for this to be negligible.

(iii) The filters were placed in plastic boiling tubes and were often stored at this point at 4 °C. 10 mls of 90% methanol was added and samples boiled for 2 minutes to aid extraction.

(iv) The samples were then centrifuged at 2500 rps to remove filter debris and the volumes completed to 10 mls with 90% methanol.

(v) The samples were placed in a 1 ml glass cuvette and the absorbance was read at 750 nm and 663 nm.

(vi) Chl a levels were then calculated as follows:

$$\text{Chl a } (\mu g\ l^{-1}) = (A663 - A750) \times 10^6 \times v/VLa$$

where
A663 = absorbance at 663 nm.
A750 = absorbance at 750 nm.
v = solvent extract volume, ml.
V = original solvent volume, ml.
L = spectrophotometer light path, cm.
a = extinction coefficient for chlorophyll -*a* in 90% methanol at 663 nm, $l\ g^{-1}cm^{-1,}$ (= 77).

Appendix ii

Light Intensity (PAR) Readings for the Profiles Performed between November 1992 and March 1993

Appendix II Light Intensity (PAR) Readings for the Profiles Performed between November 1992 and March 1993

All measurements are in $\mu E\ m^{-2}\ s^{-2.}$

DEPTH	08.00hrs	10.00hrs	12.00hrs	14.00hrs	16.00hrs
F24 29.11.92					
+10 cm	1100		450		80*
- 2 cm	700		1100		60
- 5 cm	400		700		80
-10cm	200		300		15
-20 cm	100		20		1
-30 cm	2		10		0
-40 cm	1		0		0
-50 cm	0		0		0
F25 2.12.92					
+10 cm	200	-	50	-	40
- 2 cm	700	-	900	-	250
- 5 cm	400	-	410	-	200
-10 cm	300	-	370	-	120
-20 cm	100	-	130	-	54
-30 cm	40	-	60	-	24
-40 cm	18	-	25	-	12
-50 cm	2	-	4	-	5
-60 cm	1	-	2	-	2
-70 cm	0	-	1	-	1
-80 cm	0	-	0	-	0
M15 6.12.92					
+10 cm	270	420	1100	-	100
- 3 cm	457	240	500	150	90
- 5 cm	150	220	400	140	50
-15 cm	75	210	90	10	11
-30 cm	10	215	10	0	3
-45 cm	0	200	0	0	1
-60 cm	0	50	0	0	0
-75 cm	0	1	0	0	0
M16 9.12.92					
+10 cm	250		60		260
- 2 cm	320		800		200
- 5 cm	100		340		80
-10 cm	50		130		40
-20 cm	12		40		20
-30 cm	3		10		3
-40 cm	1		0		1
-50 cm	0		1		0

DEPTH	08.00hrs	10.00hrs	12.00hrs	14.00hrs	16.00hrs
M20 13.12.92					
+10 cm	540	900	1400	1000	105
- 2 cm	760	1100	1200	840	52
- 5 cm	580	500	900	600	40
-10 cm	380	460	700	500	24
-15 cm	200	400	480	300	13
-20 cm	160	380	400	220	9
-25 cm	110	300	300	120	7
M23 16.12.92					
+10 cm	200	-	1000	-	230
- 2 cm	100	-	300	-	90
- 5 cm	20	-	100	-	20
-10 cm	20	-	40	-	10
-15 cm	8	-	25	-	4
-20 cm	2	-	10	-	1
-30 cm	1	-	2	-	0
-40 cm	0	-	0	-	0
F24 10.2.93					
+10 cm	450	1200	1200	800	420
- 2 cm	360	100*	420	230	80
- 5 cm	280	220	200	110	42
-10 cm	210	110	75	15	18
-20 cm	85	30	15	9	4
-30 cm	46	12	7	1	0
-40 cm	70	5	2	0	0
-50 cm	13	2	2	0	0
-60 cm	7	1	0	0	0
-70 cm	4	1	0	0	0
-80 cm	2	0	0	0	0
M31 17.2.93					
+10 cm	130	1100	1100	420	270
- 2 cm	300	410	380	260	46
- 5 cm	270	350	350	230	30
-10 cm	200	320	250	190	8
-15 cm	180	290	160	165	0
-20 cm	160	255	130	130	0
-30 cm	110	200	54*	16	0
-40 cm	68	110	64	0	0
-50 cm	37	70	18	0	0
-60 cm	17	36	0	0	0
-80 cm	5	3	0	0	0

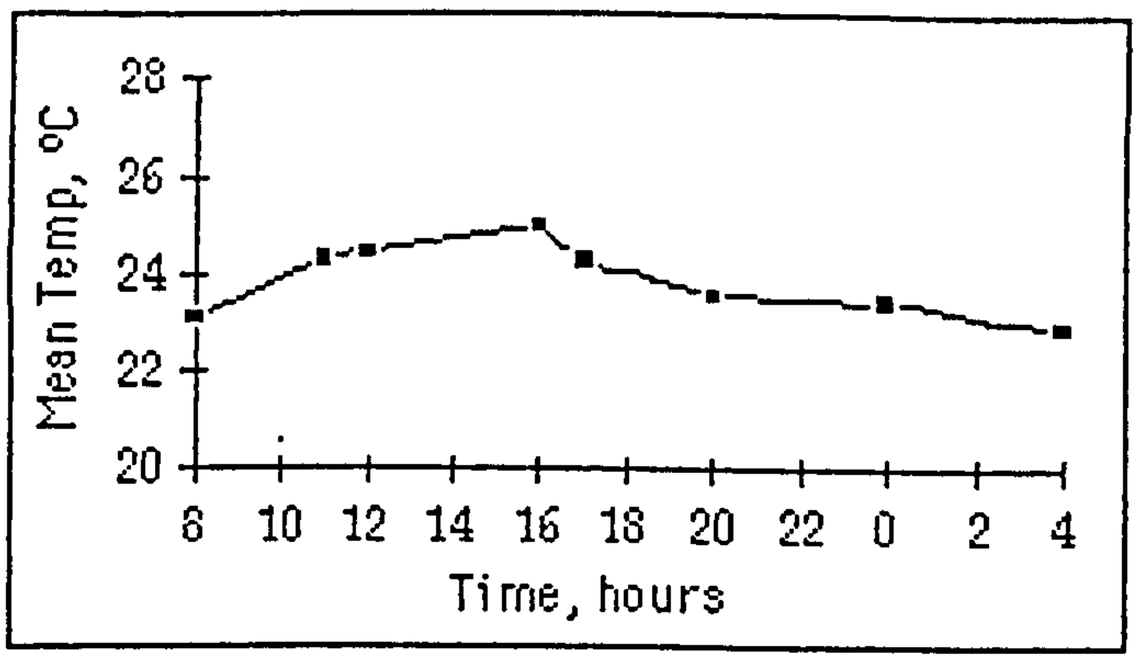
DEPTH	08.00hrs	10.00hrs	12.00hrs	14.00hrs	16.00hrs
F21 10.3.93					
+10 cm	550	1000	1050	890	375
- 2 cm	320	350	420	360	80
- 5 cm	300	250	320	260	50
-10 cm	140	170	220	170	25
-15 cm	185	110	150	130	15
-20 cm	25	12	25	75	7
-30 cm	2	0	0	1	0
F25 24.3.93					
+10 cm	560	1100	1250	250*	180*
- 2 cm	210	400	480	100*	90*
- 5 cm	150	350	380	70*	86*
-10 cm	100	150	300	48*	62*
-20 cm	45	85	170	26*	31*
-30 cm	22	30	75	11*	14*
-40 cm	10	15	24	5*	6*
-50 cm	5	6	12	2*	3*
-60 cm	2	2	5	0*	1*
-70 cm	1	1	2	0*	0*

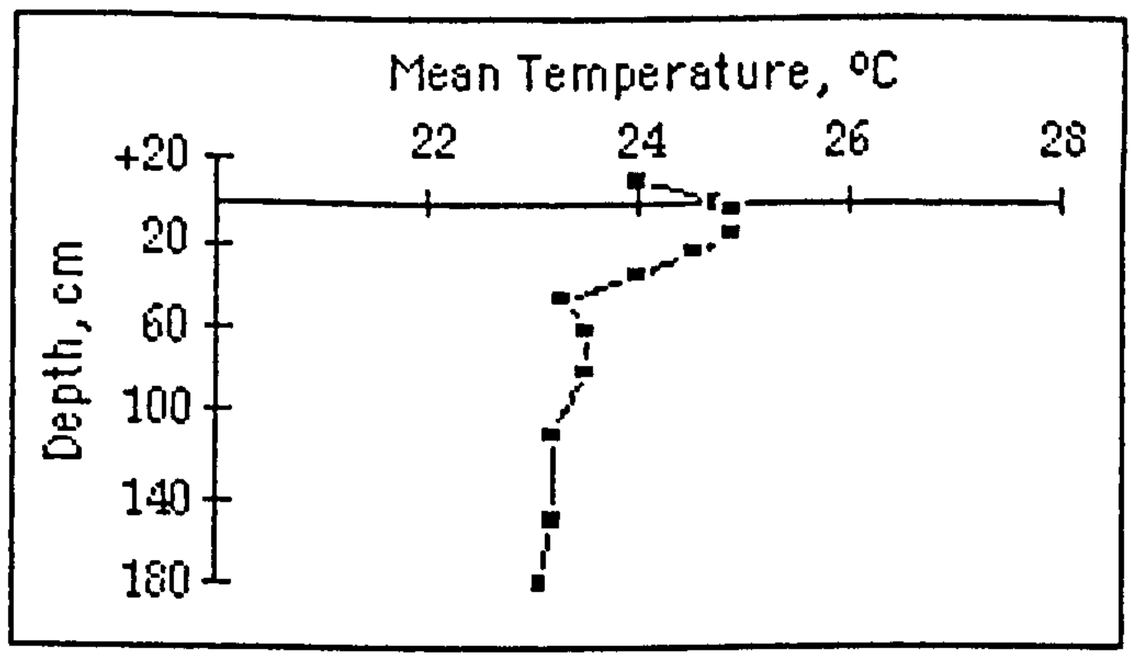
* Cloud Cover

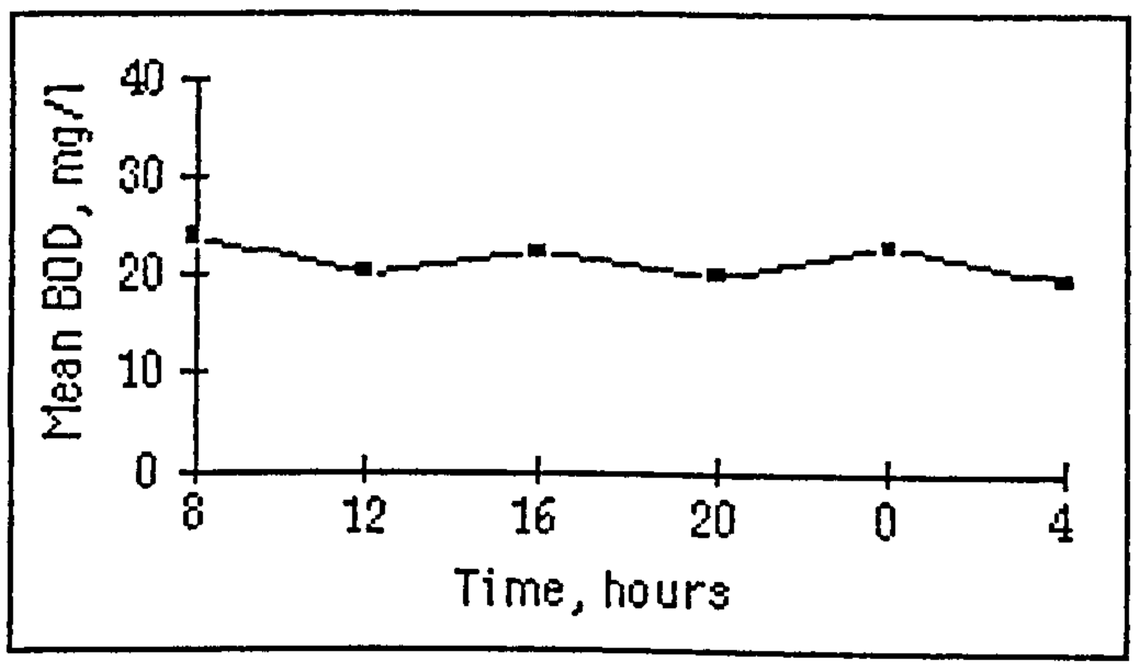
Appendix iii

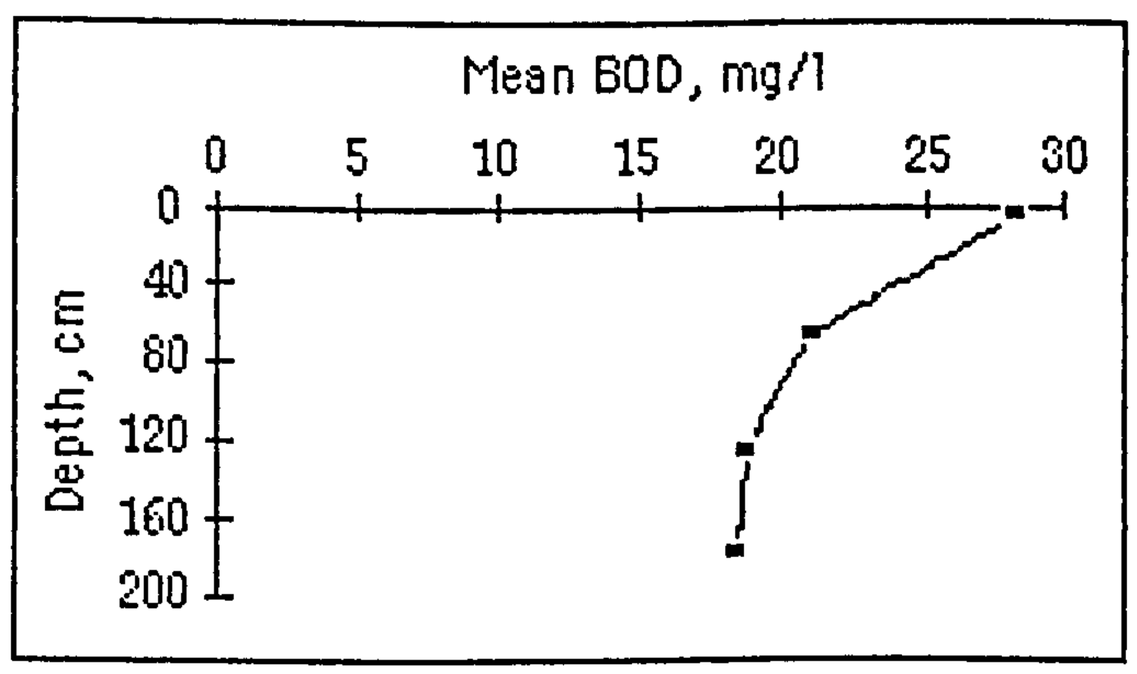
Experimental Results from In-Pond Profile

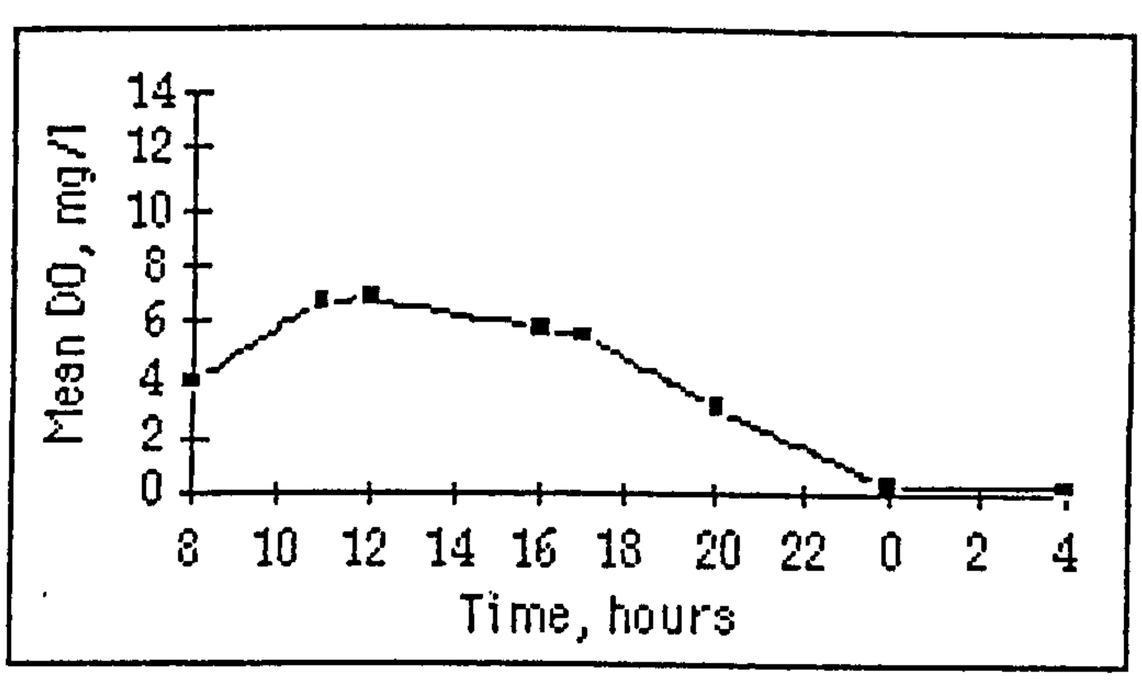
Figure A3/1 **Mean Results of the Profile carried out on F24 on 29.11.92.**

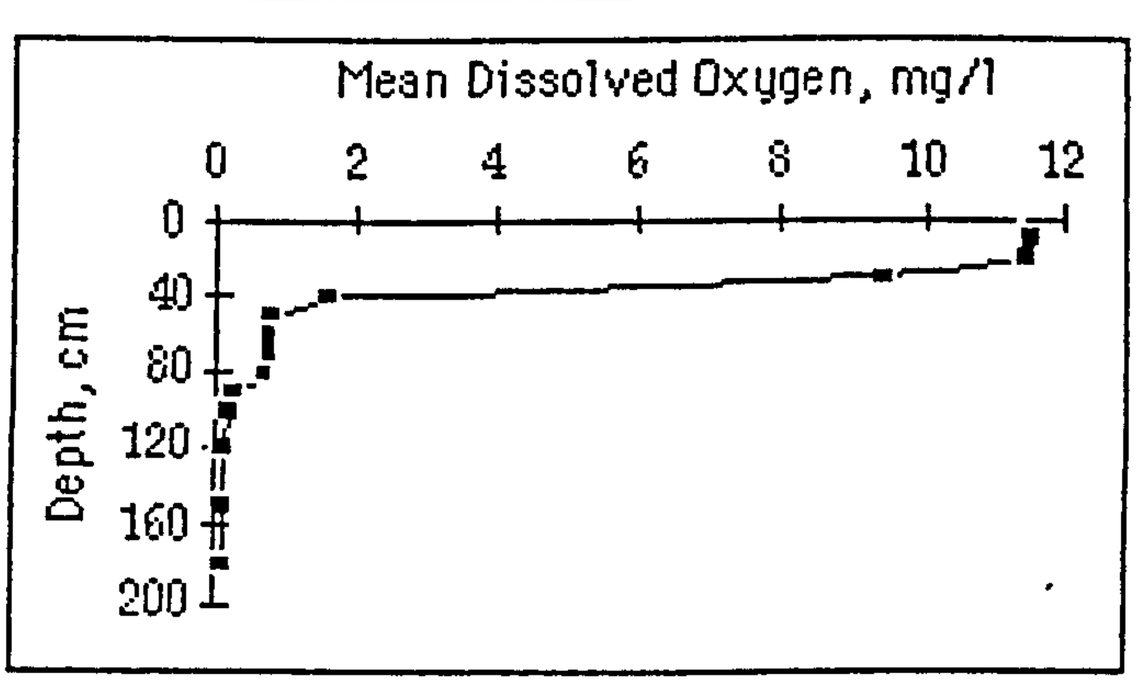


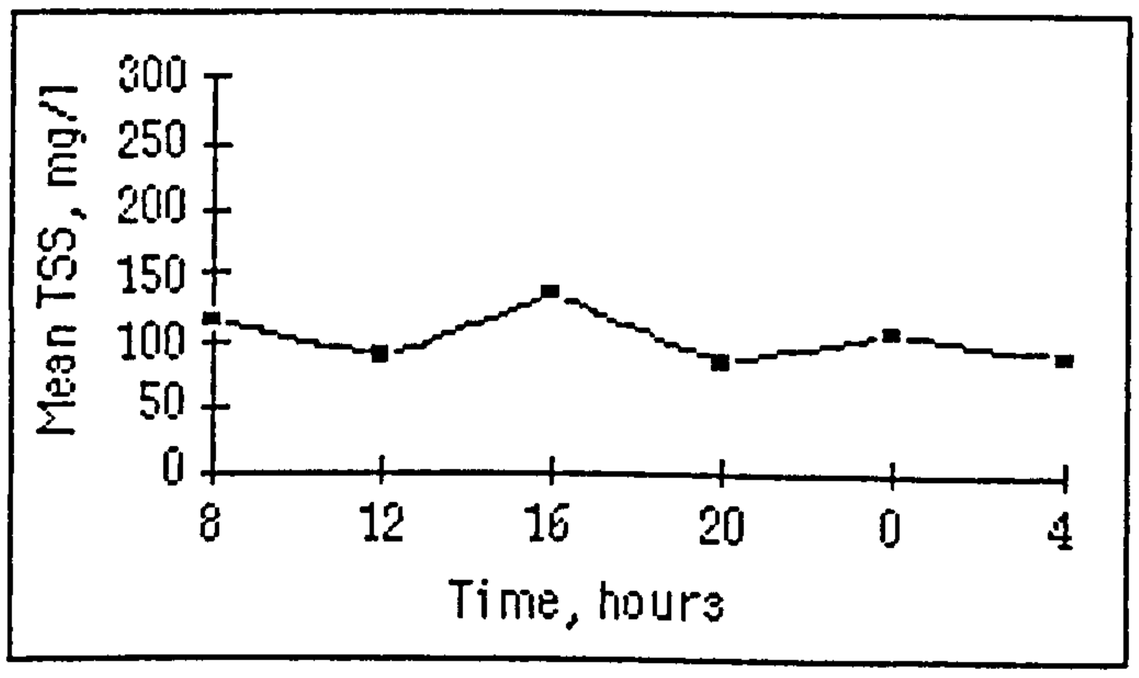












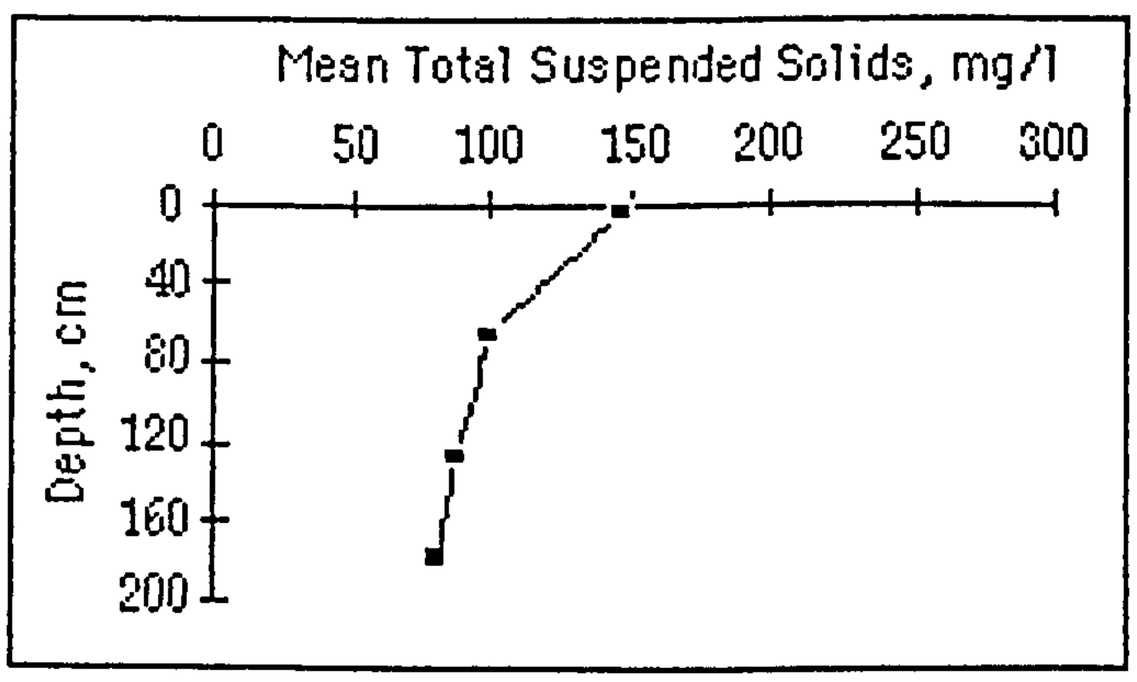
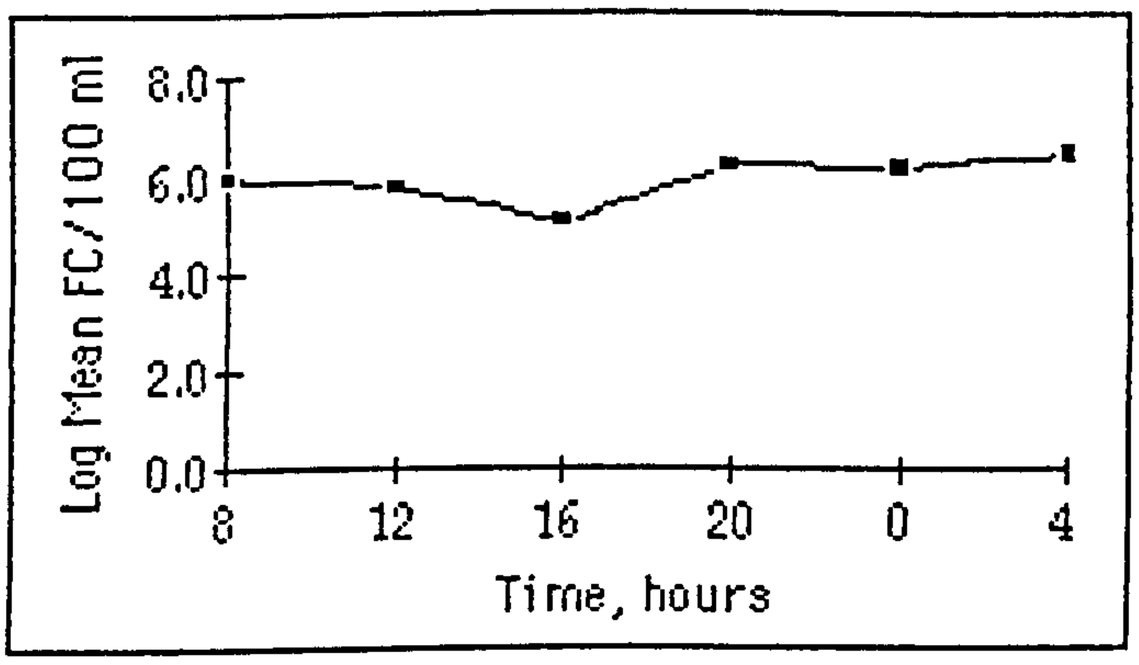
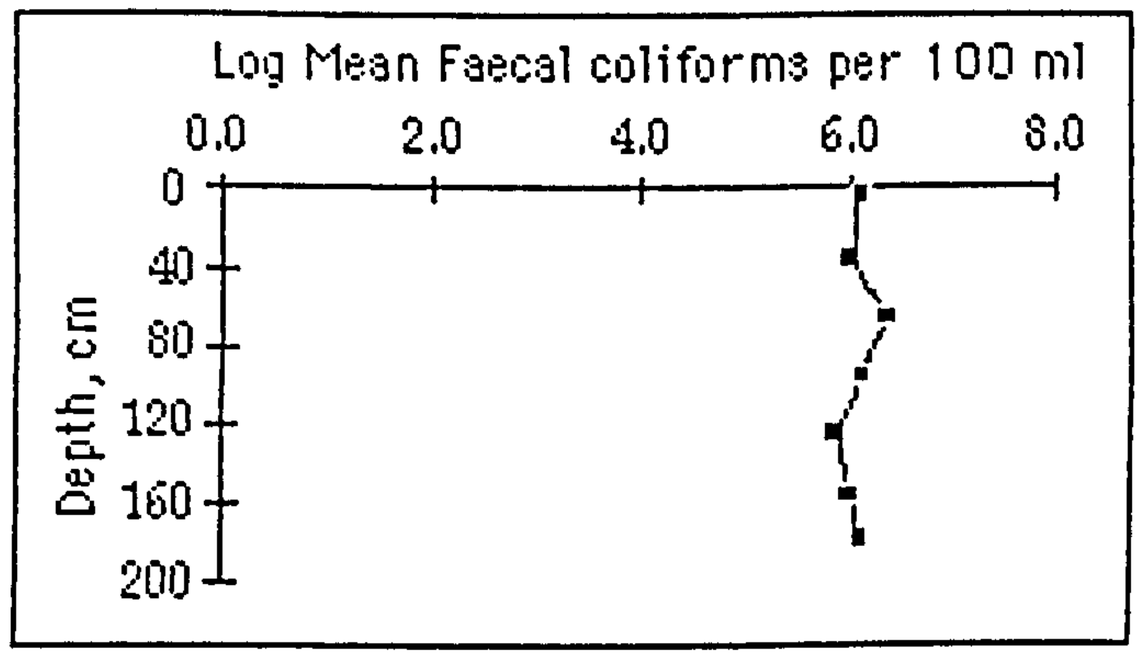
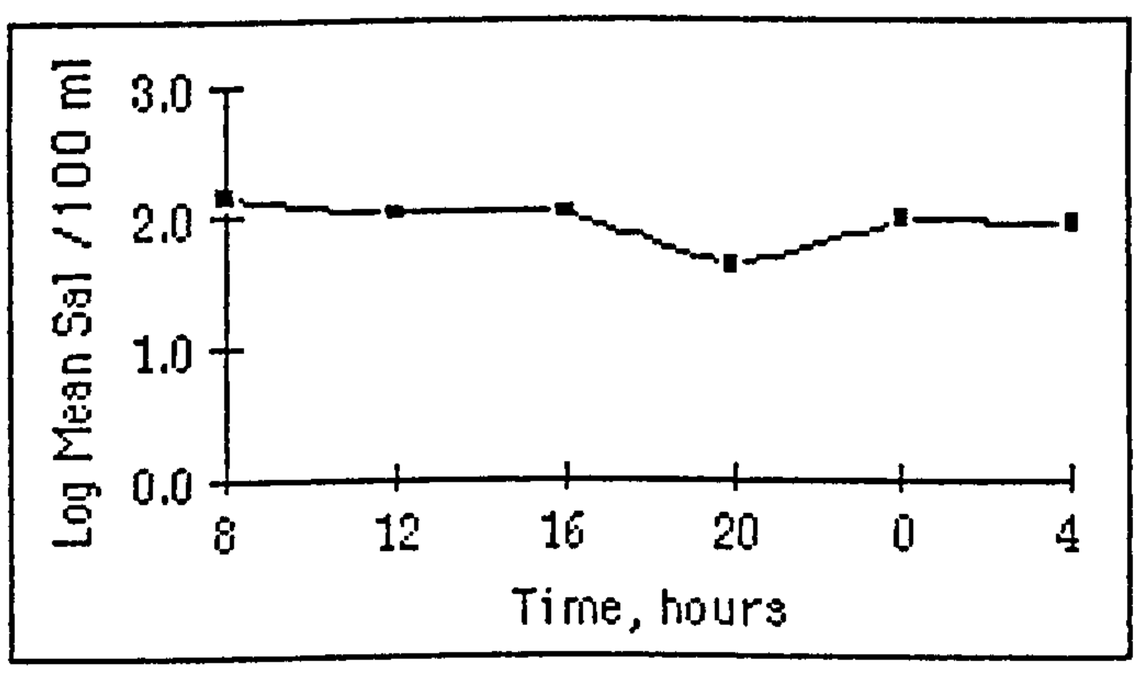
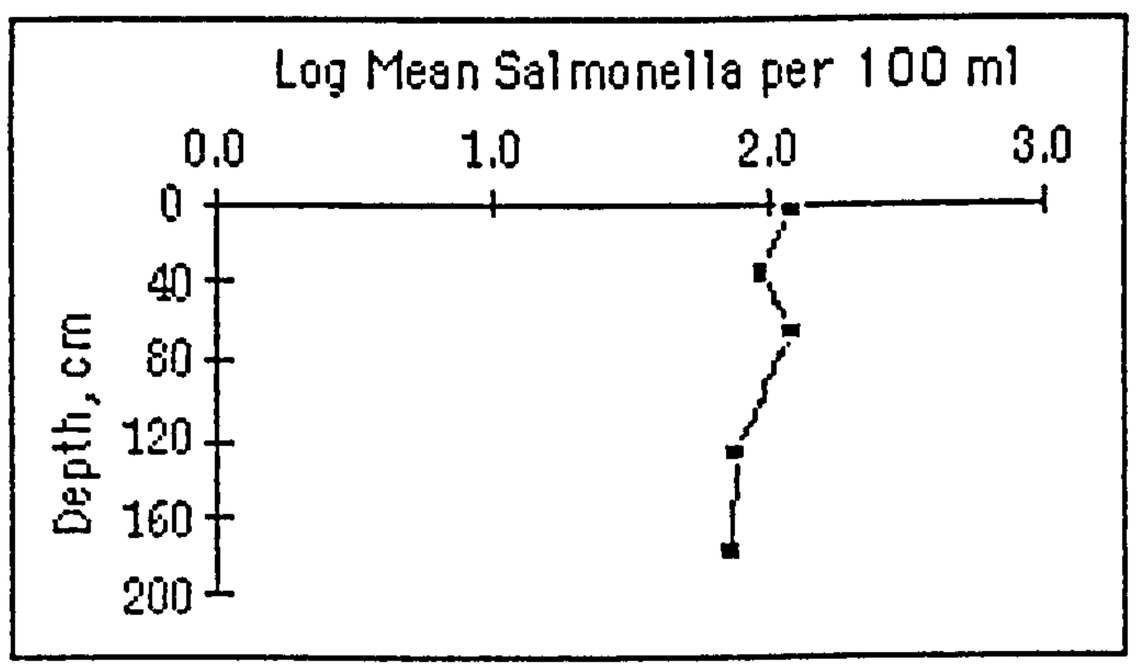


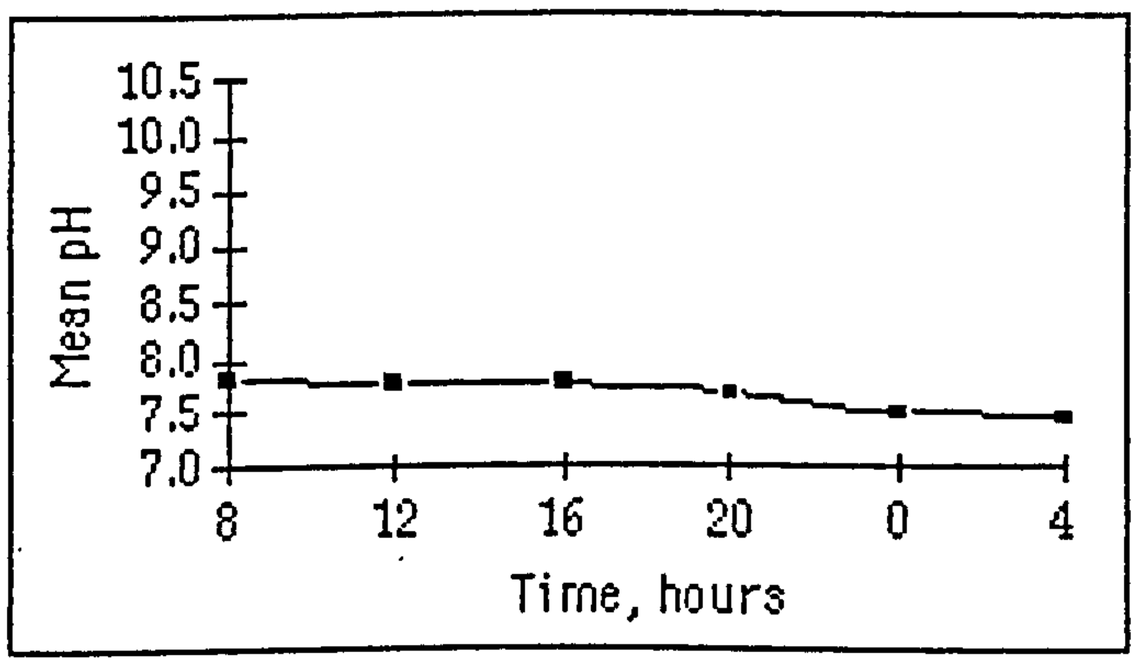
Figure A3/1b **Mean Results of the Profile carried out on F24 on 29.11.92.**

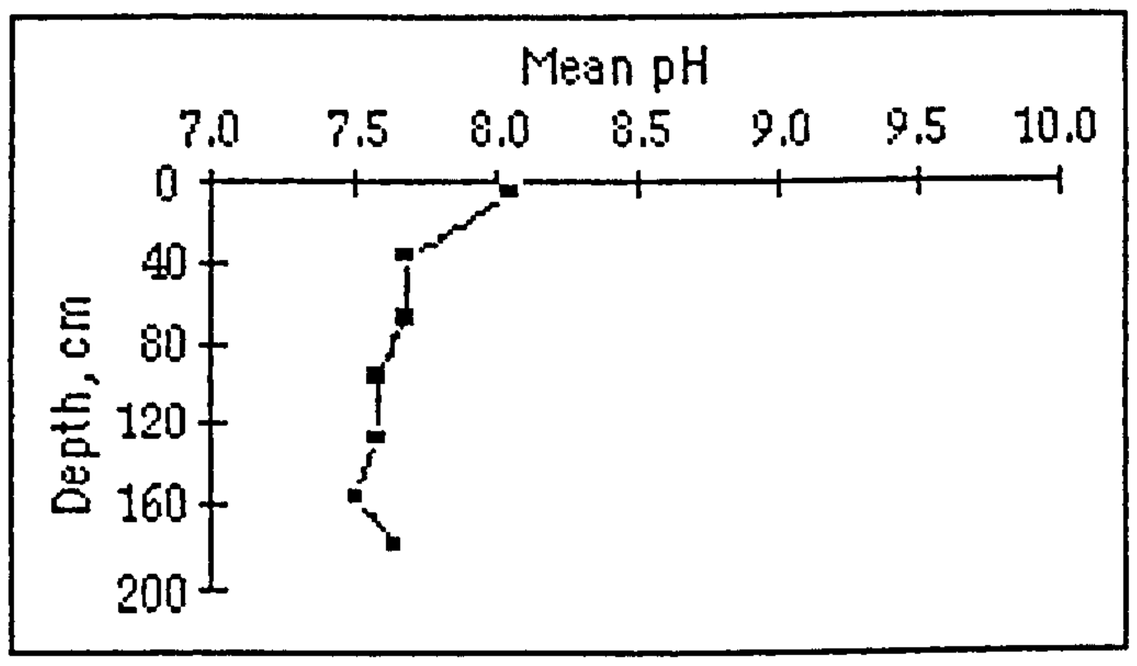


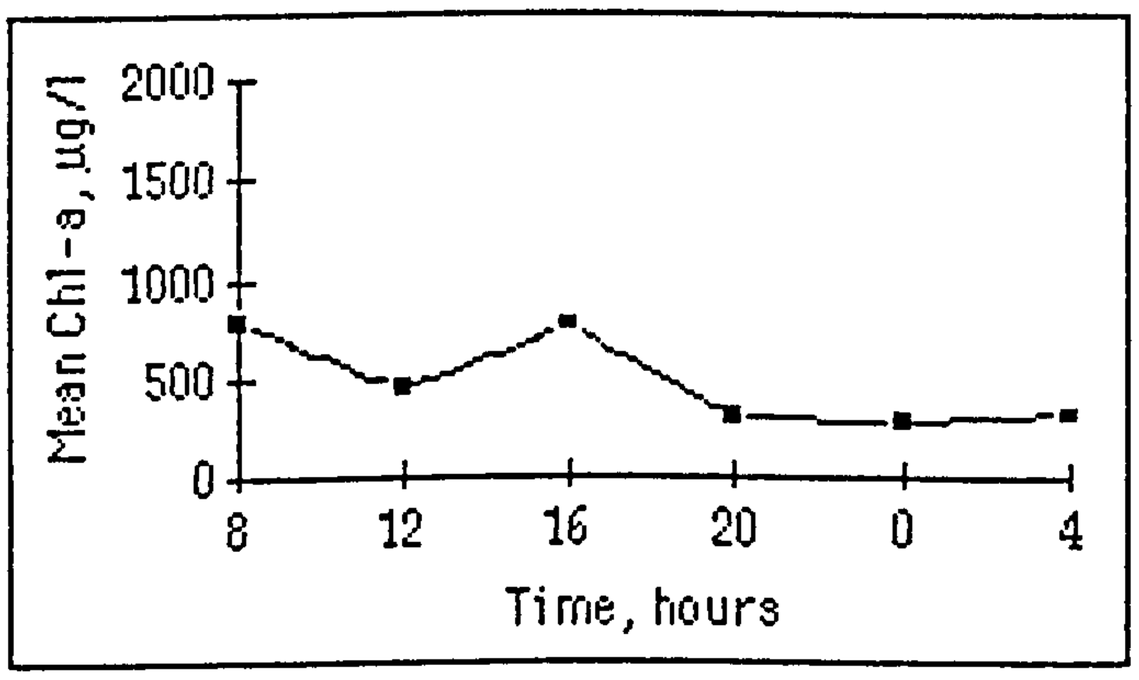












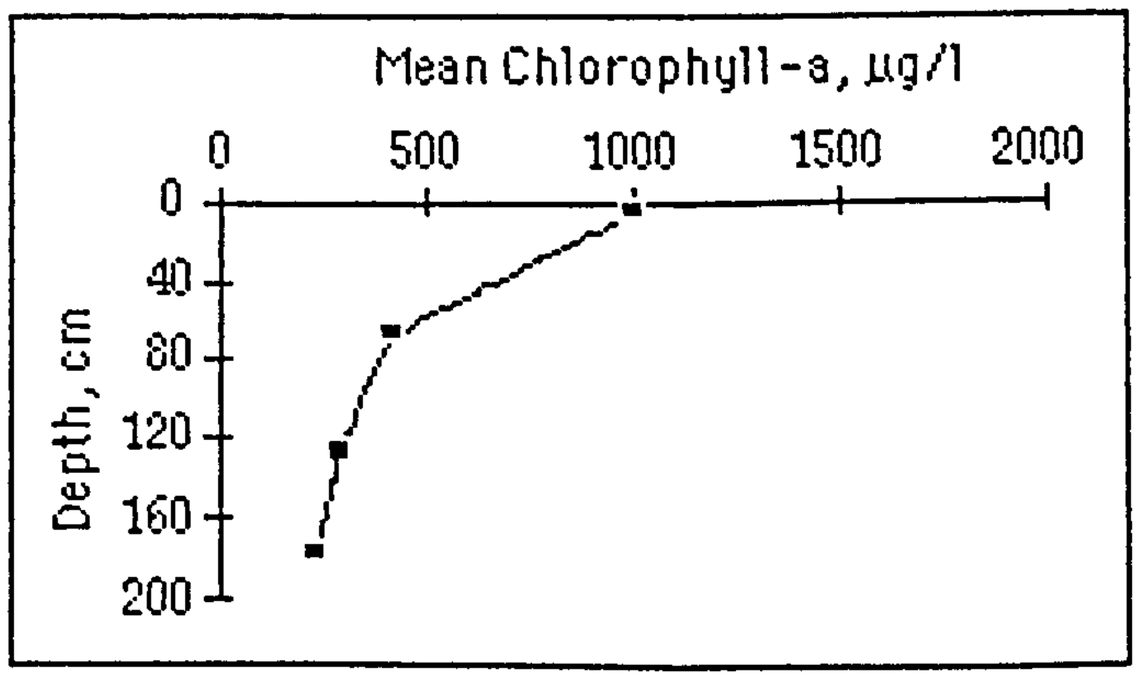
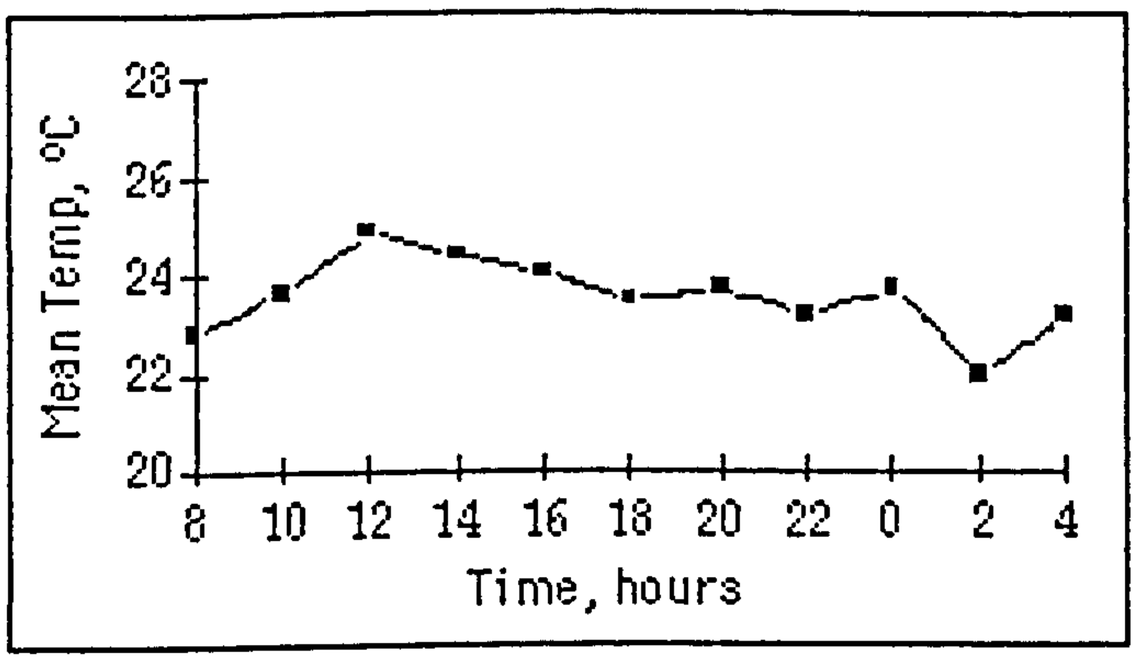
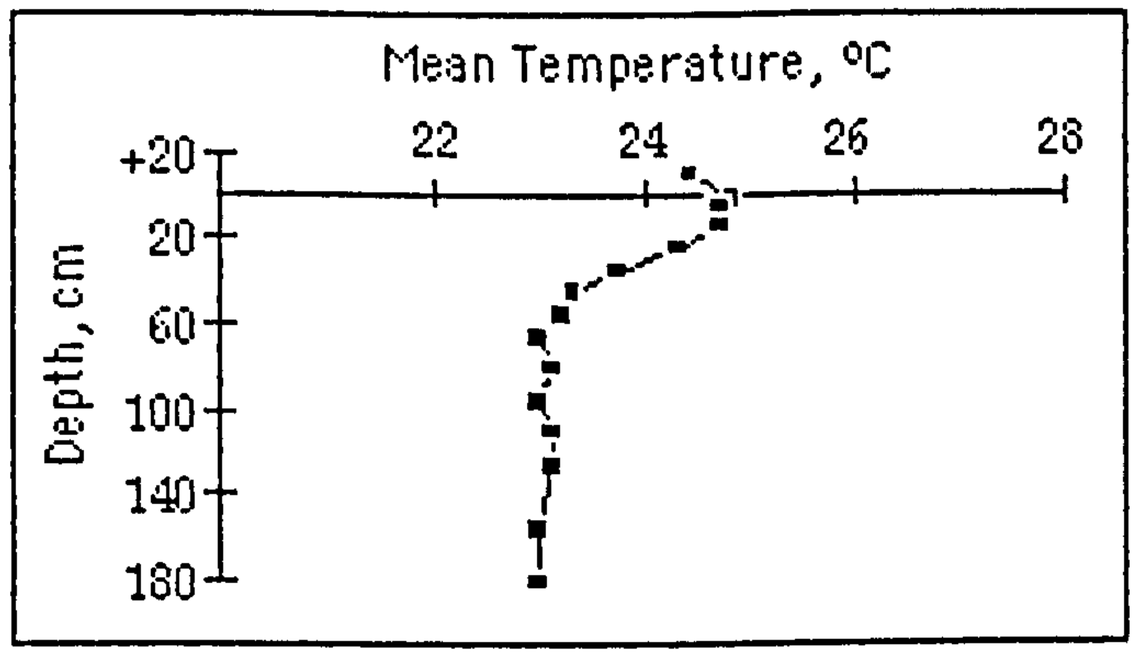
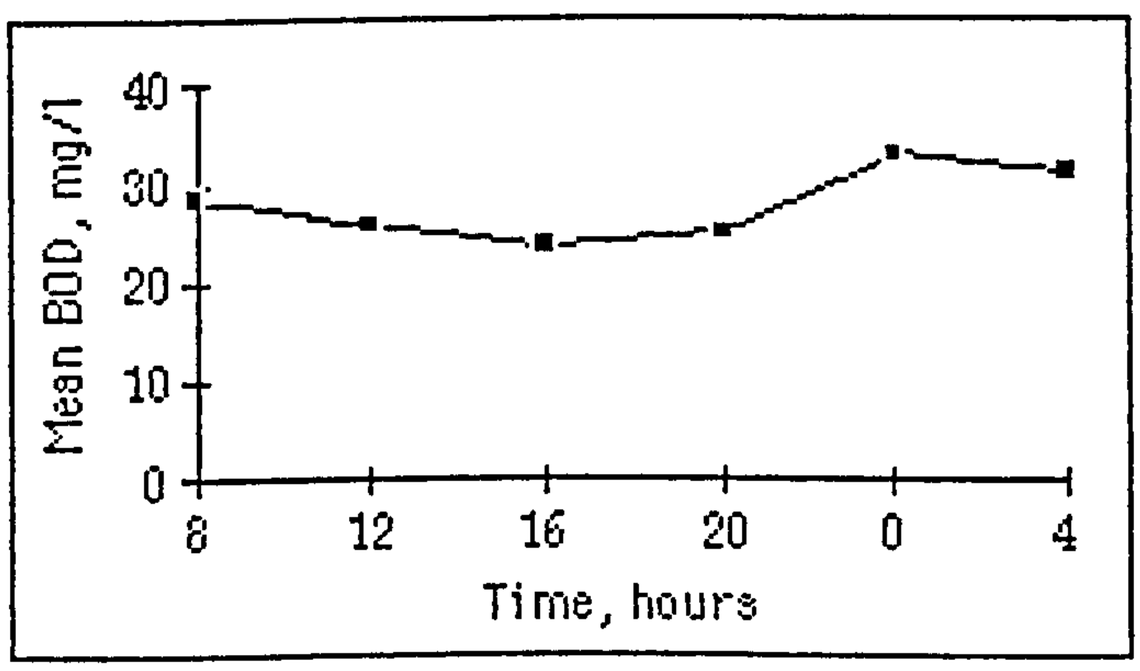
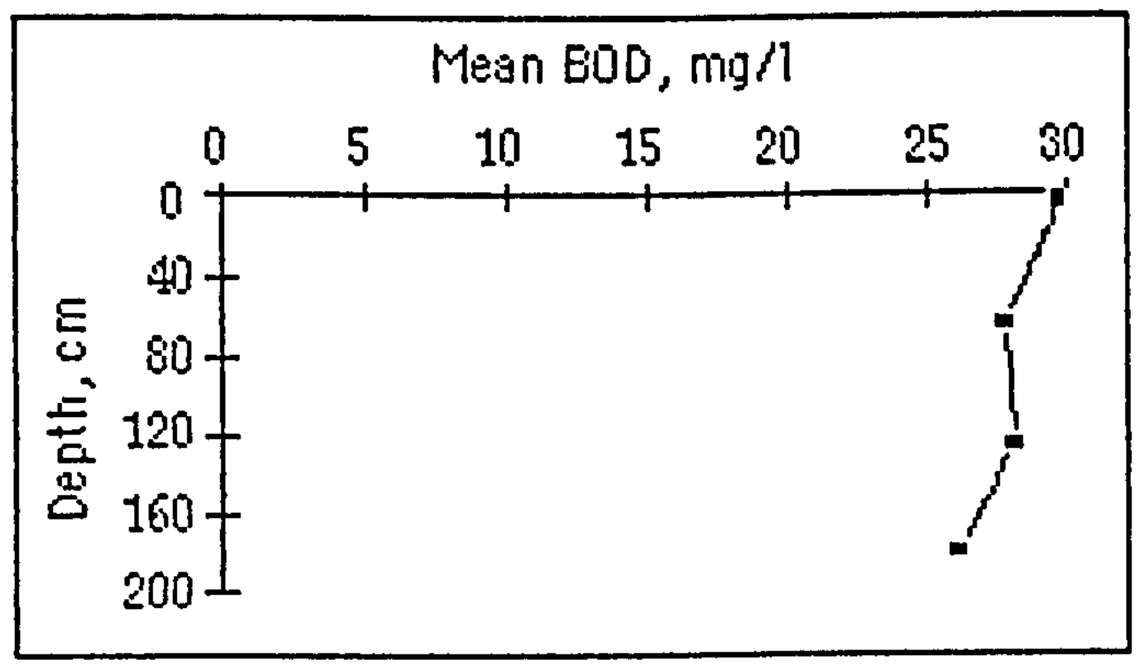


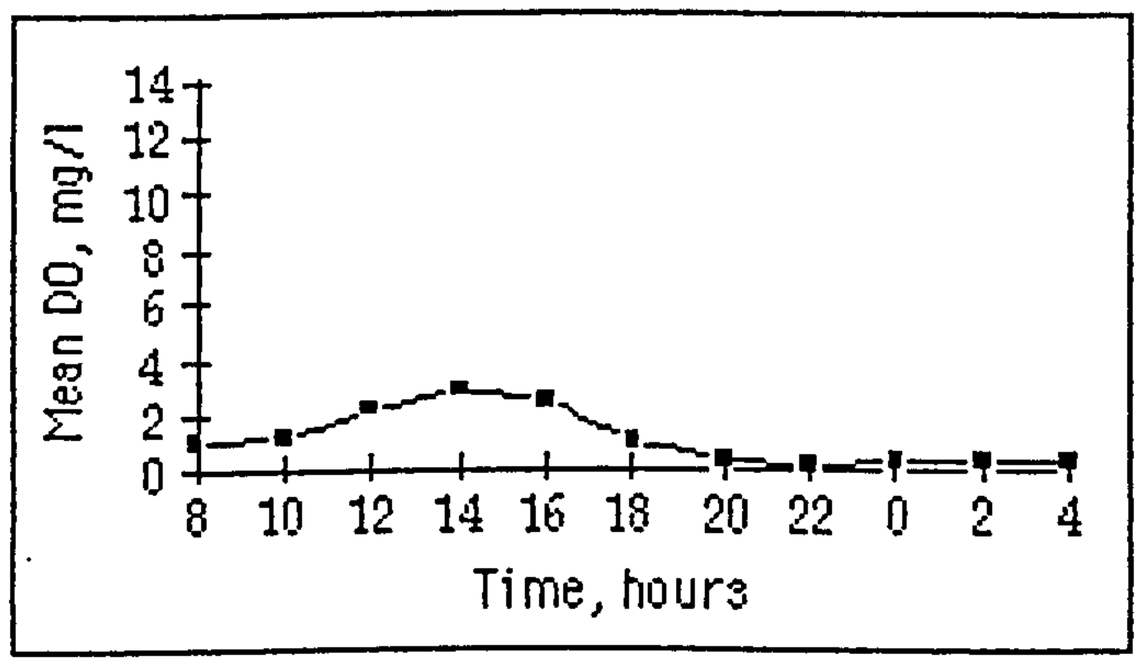
Figure A3/2 **Mean Results of the Profile carried out on F25 on 2.12.92.**

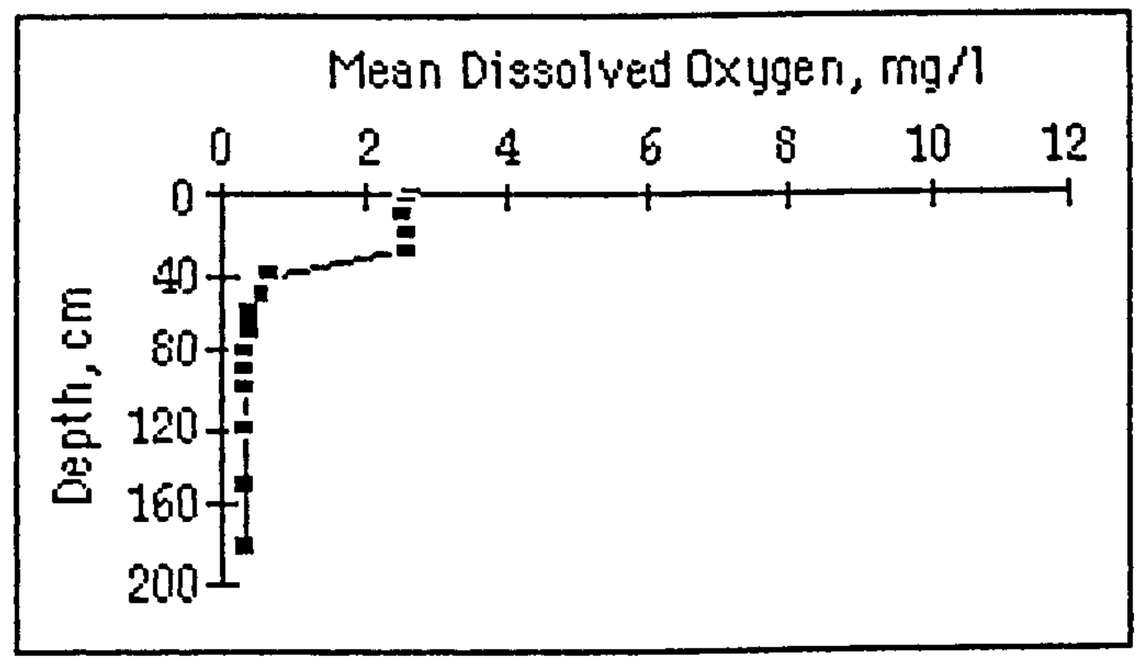


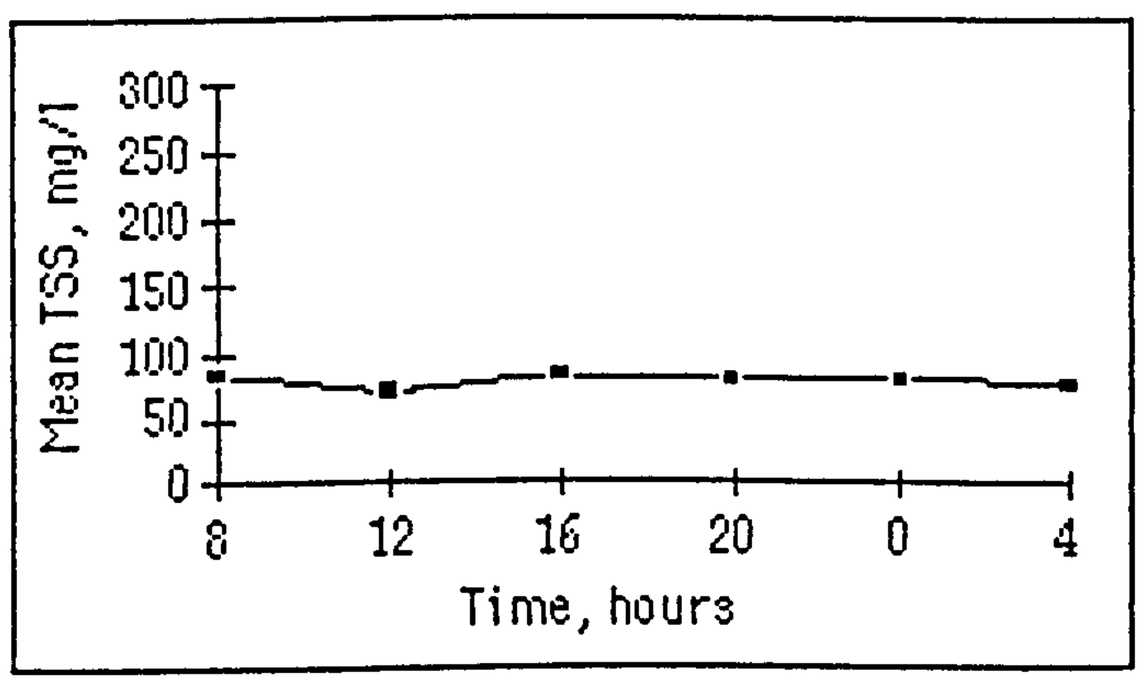












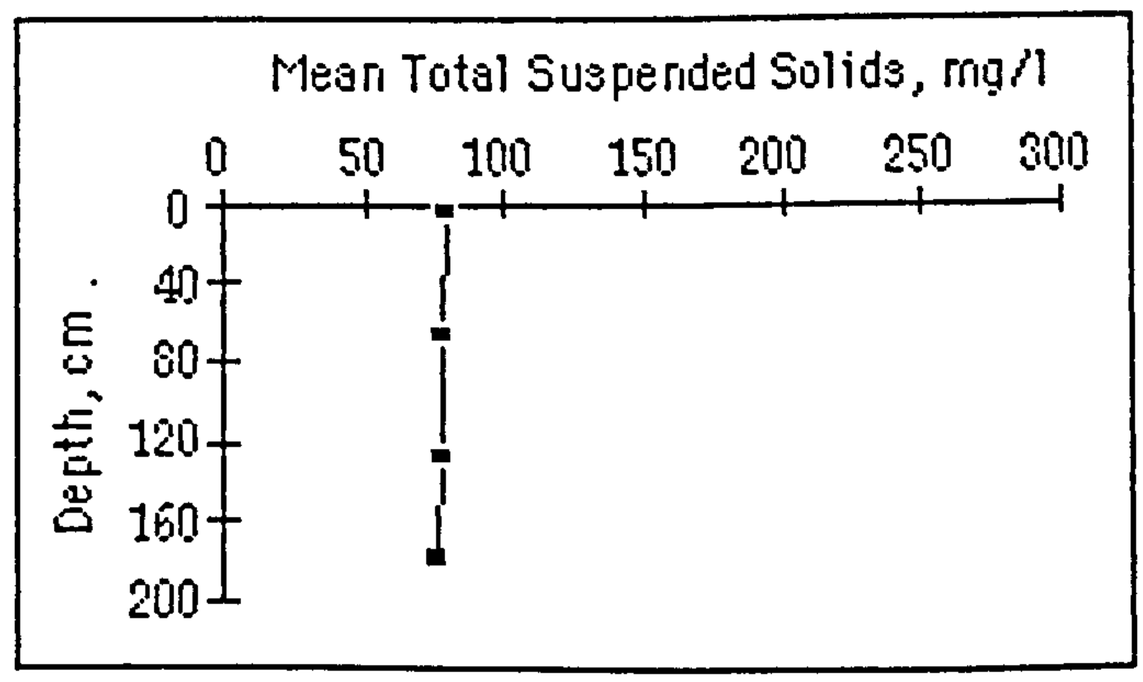
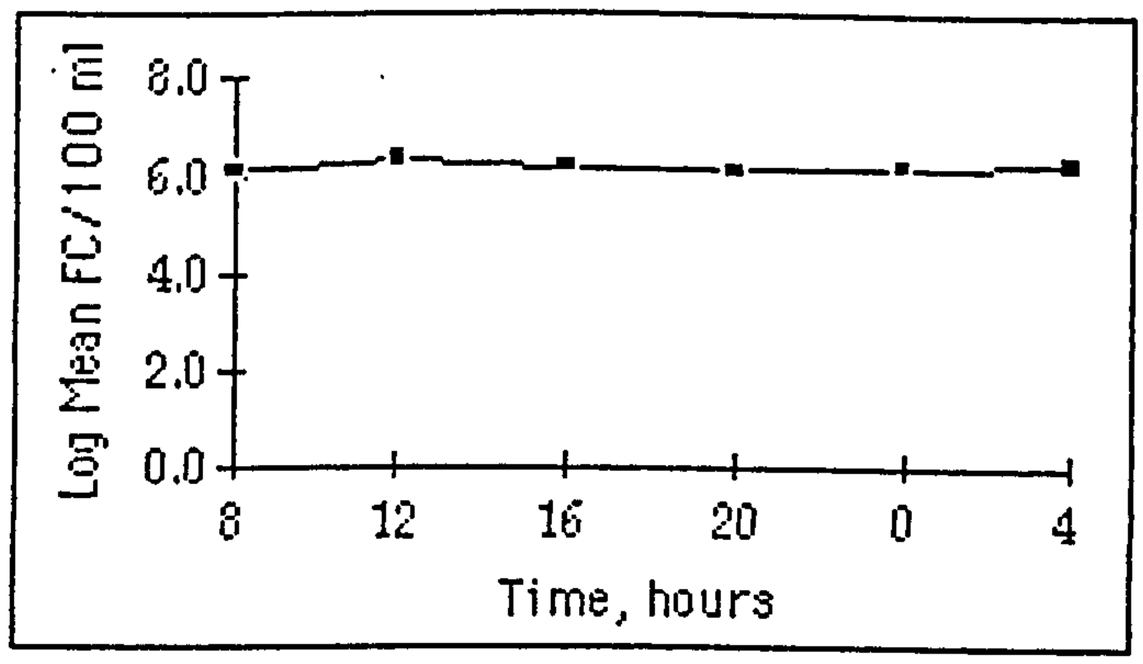
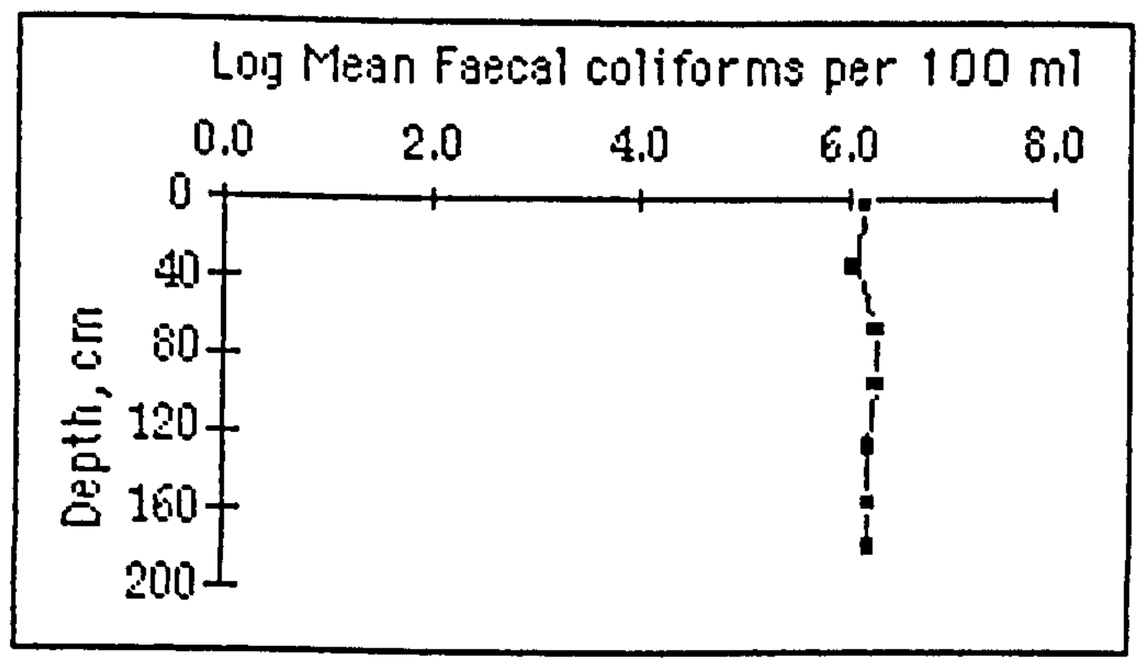
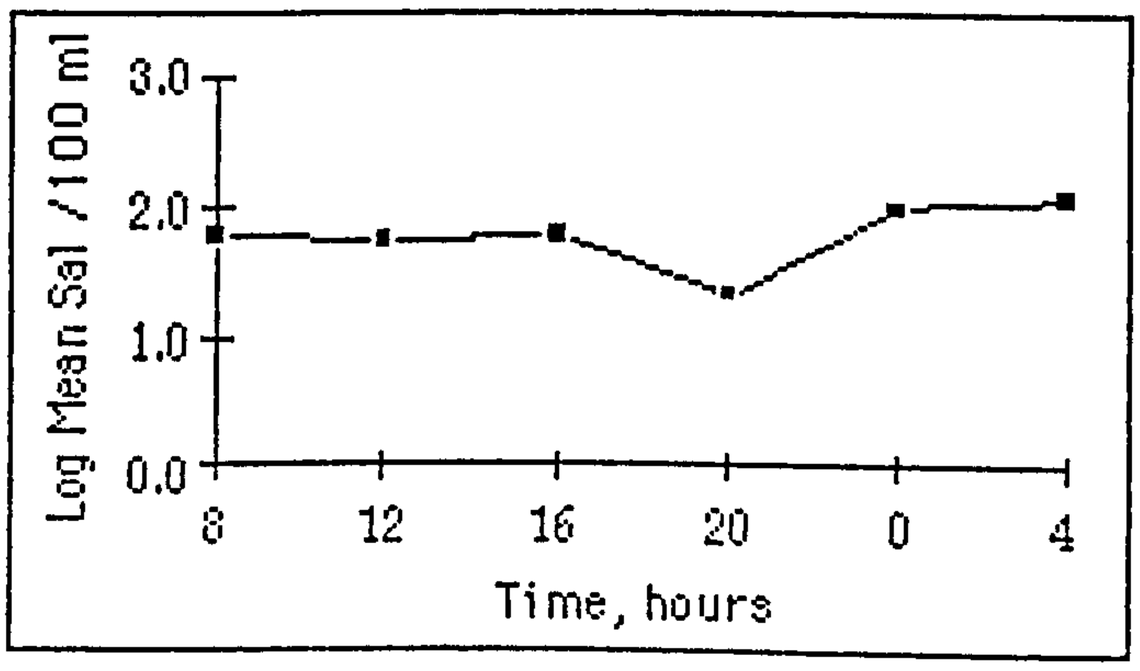
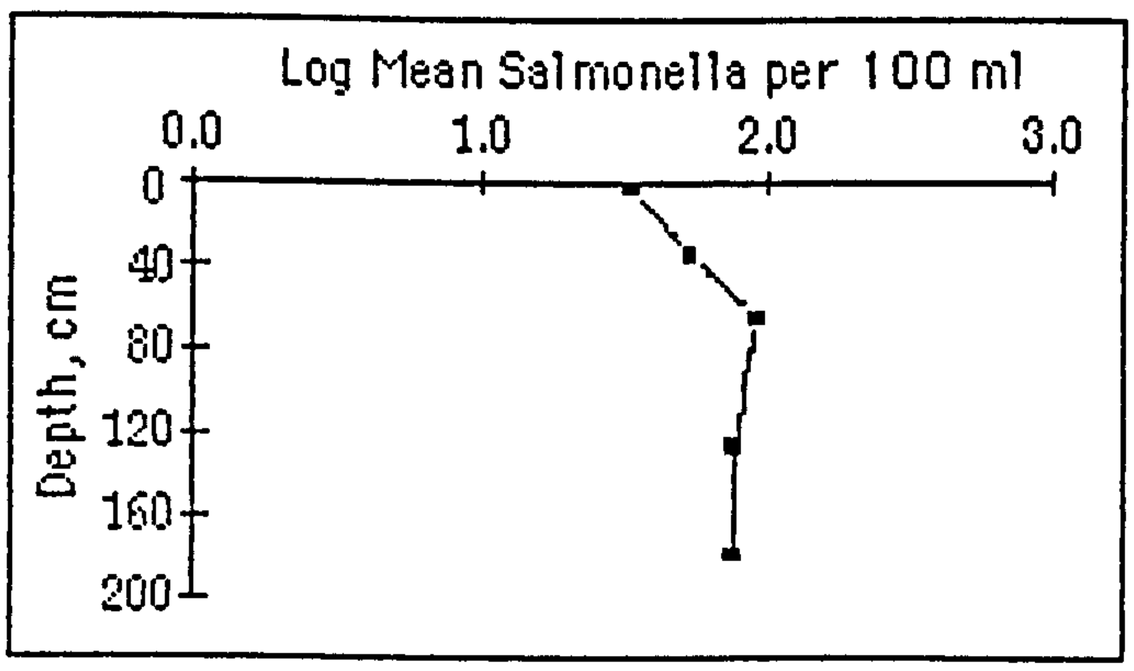


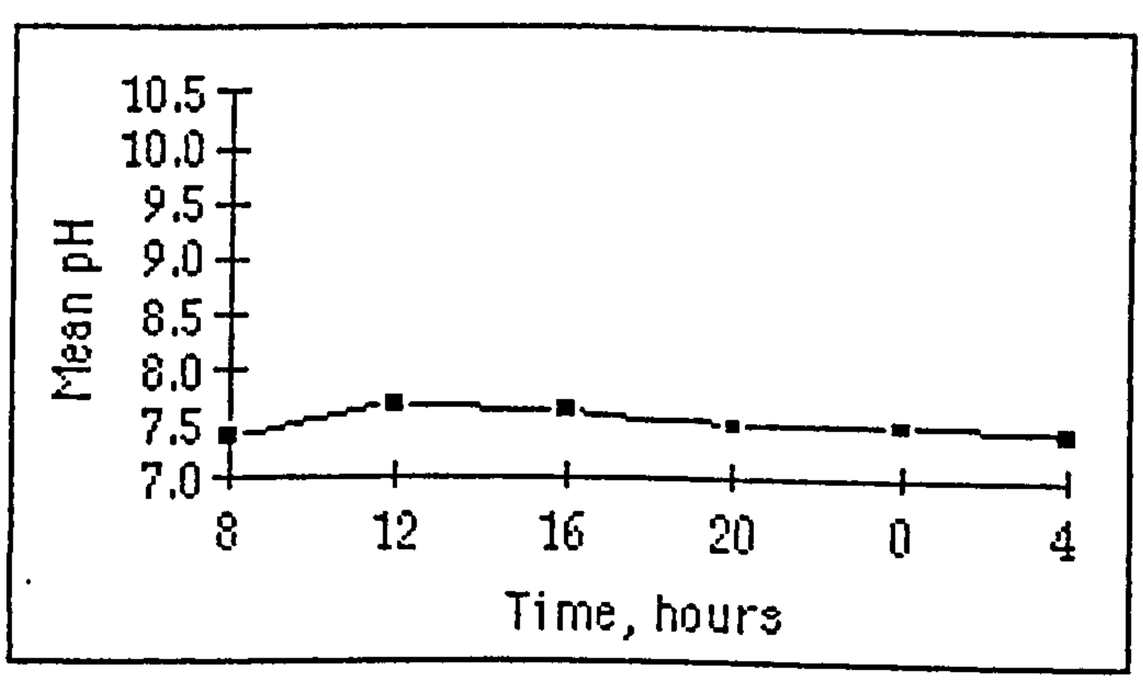
Figure A3/2b **Mean Results of the Profile carried out on F25 on 2.12.92.**

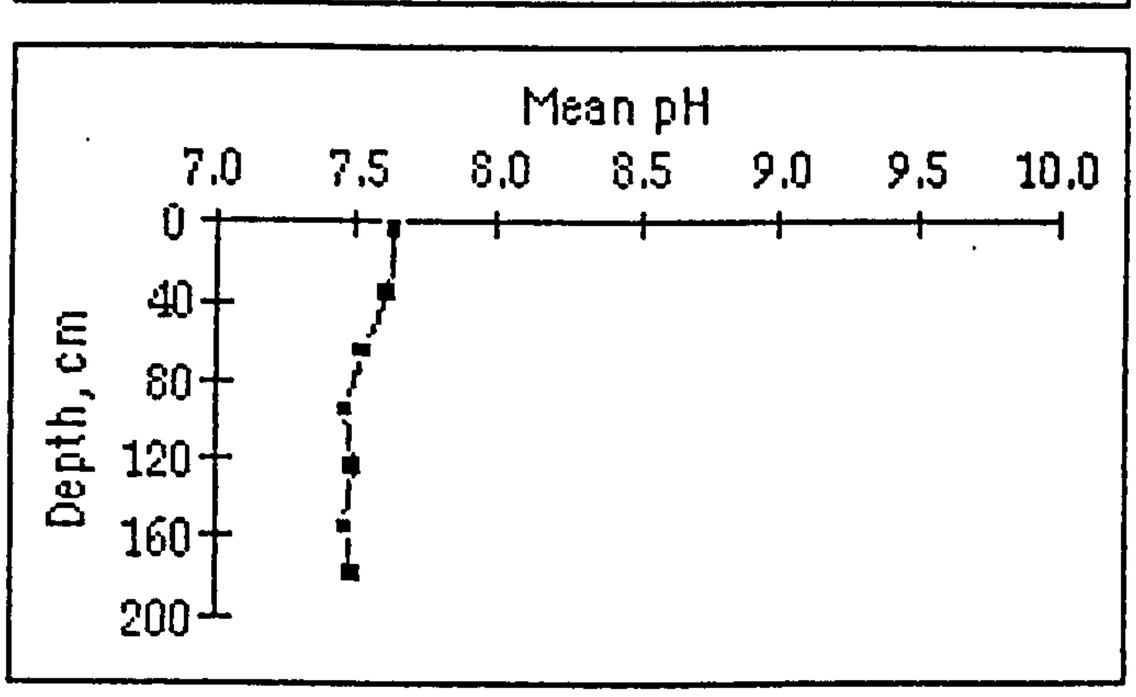


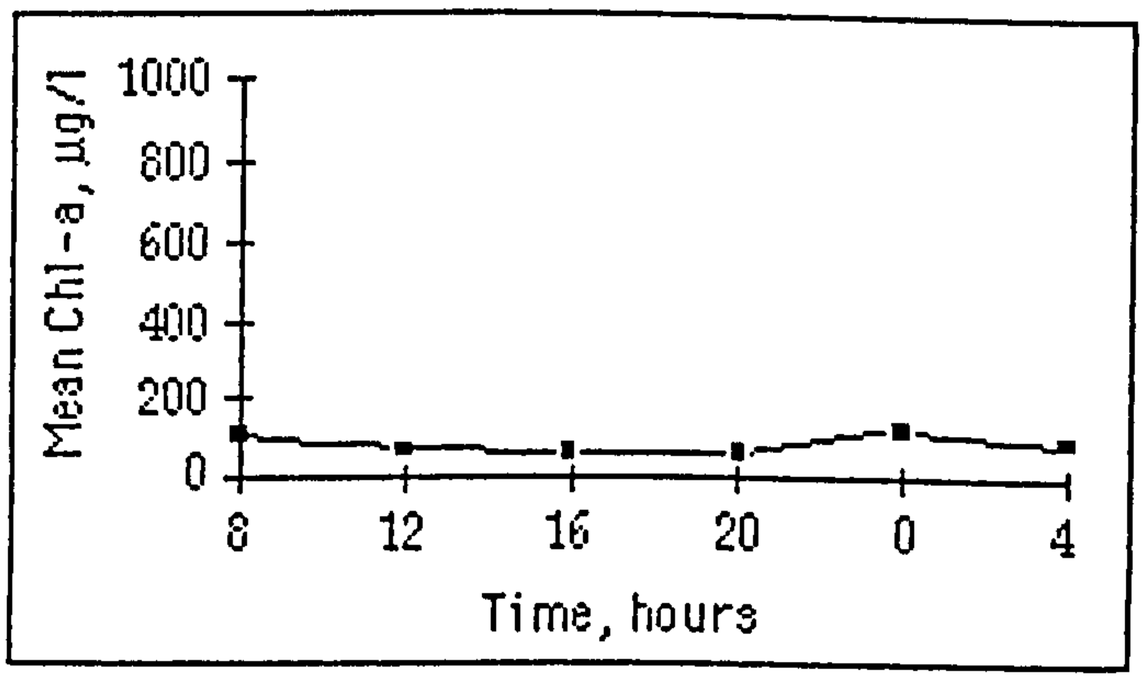












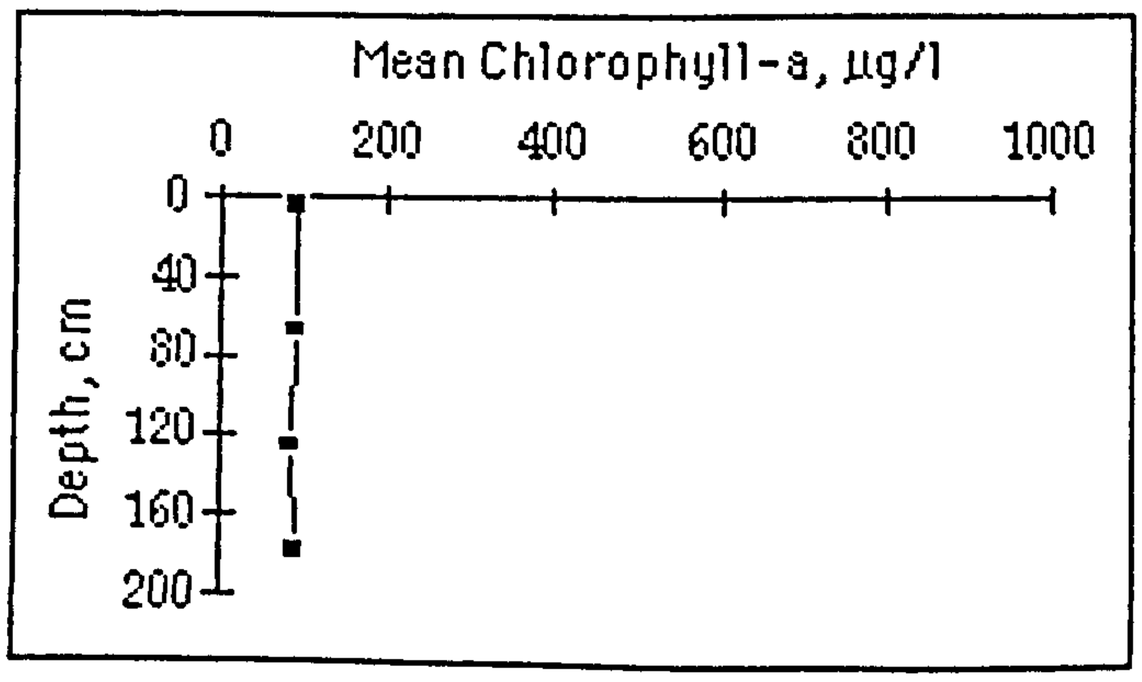
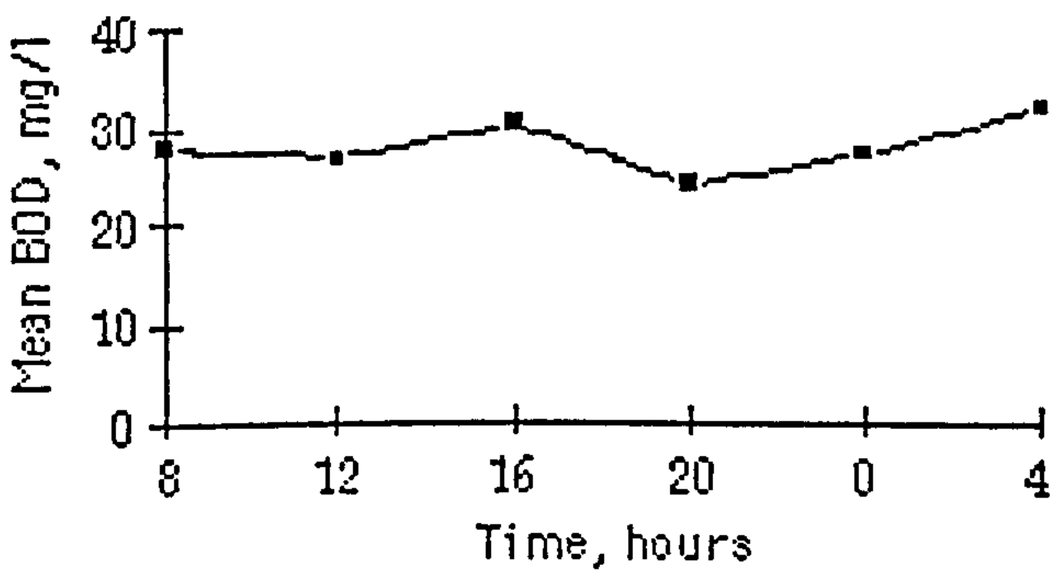
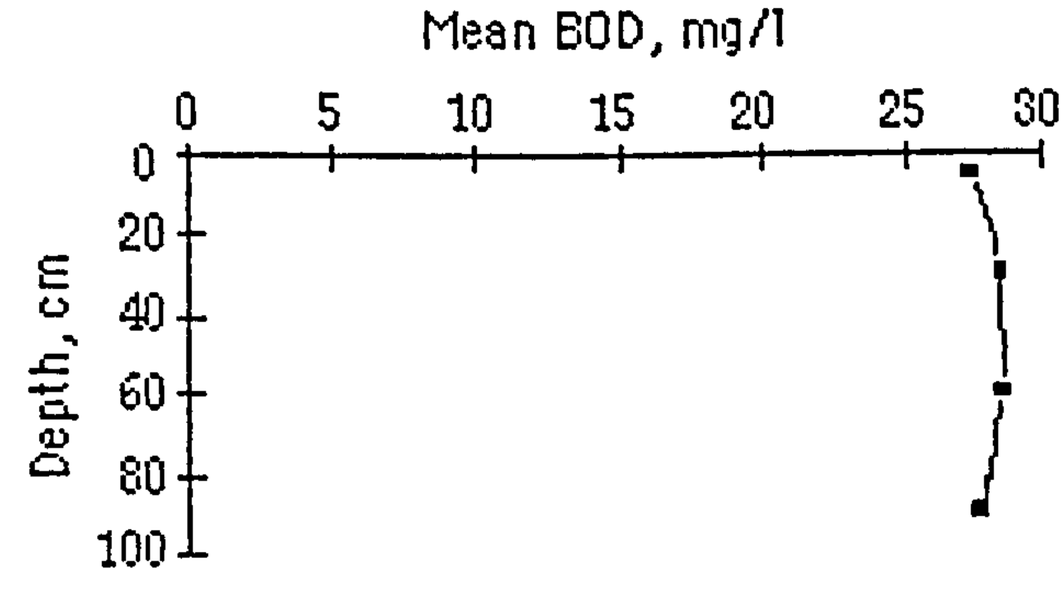


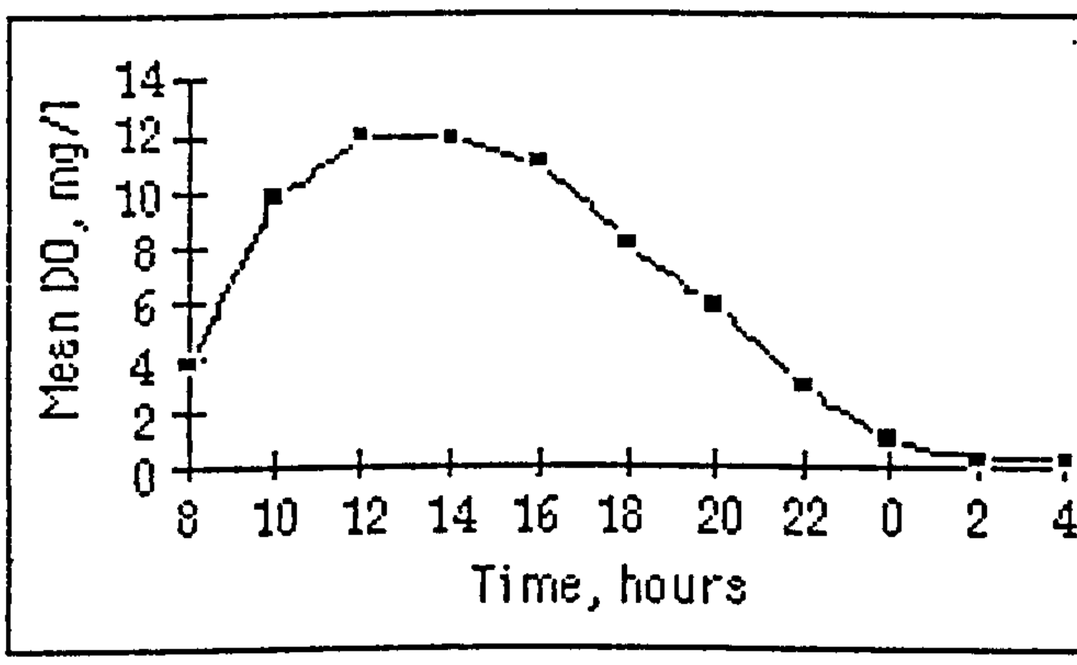
Figure A3/3 **Mean Results of the Profile carried out on M15 on 6.12.92.**

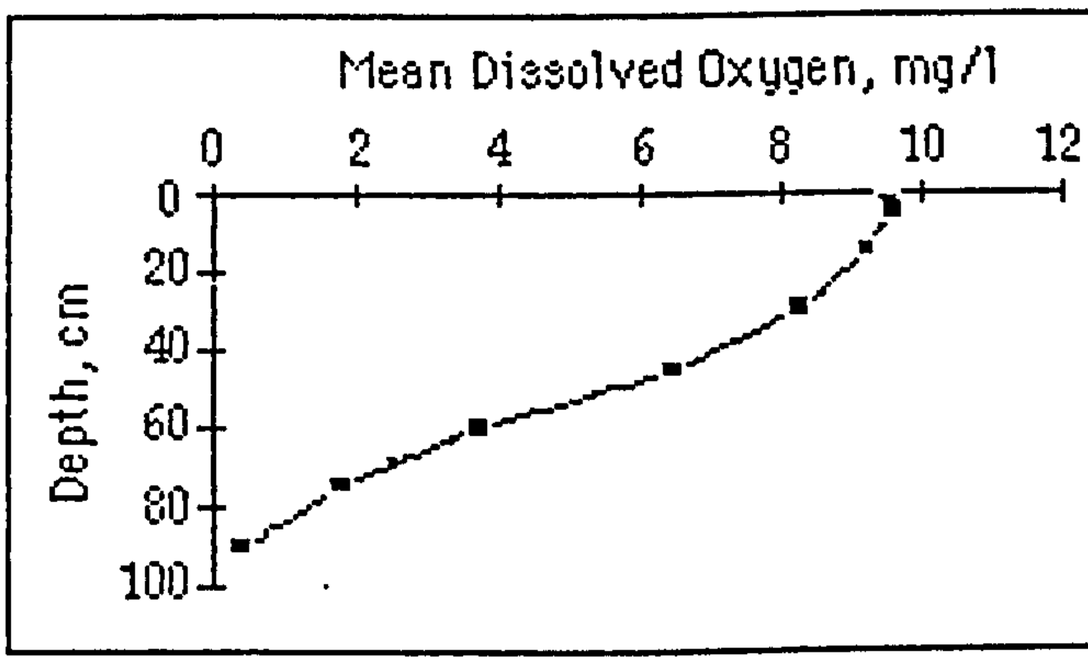
No Temperature Results

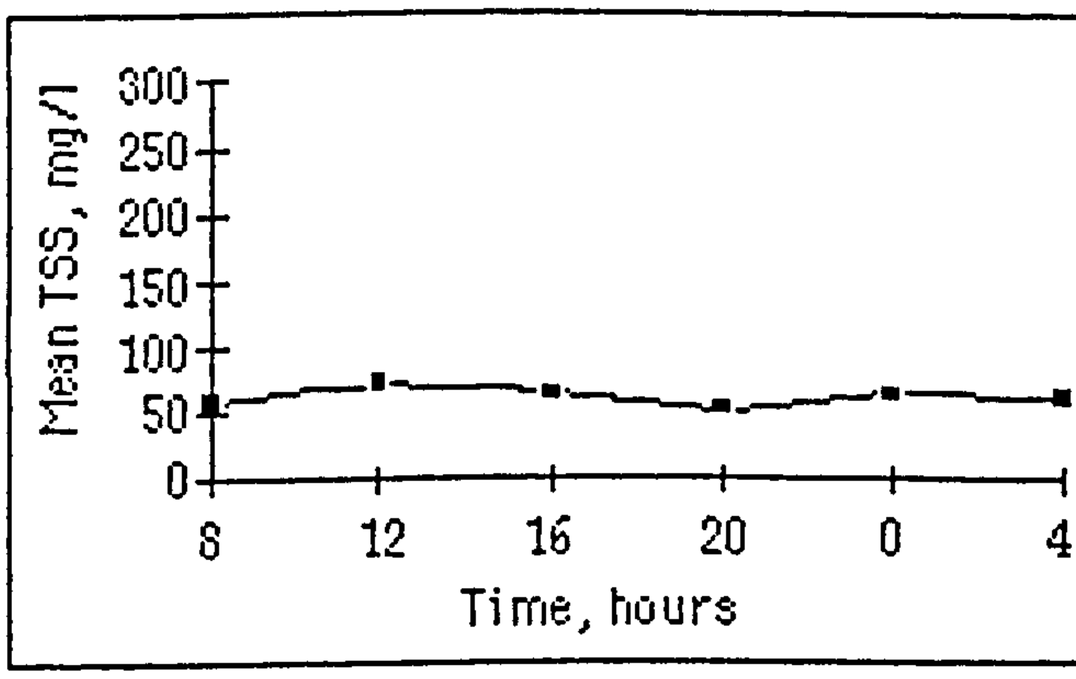
No Temperature Results











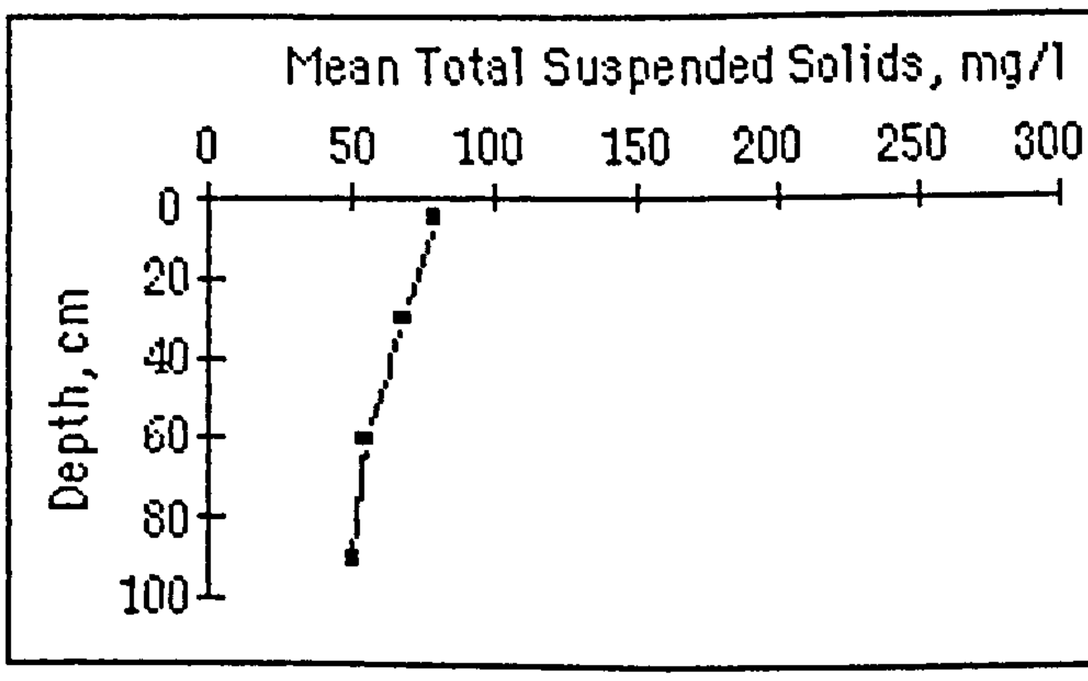
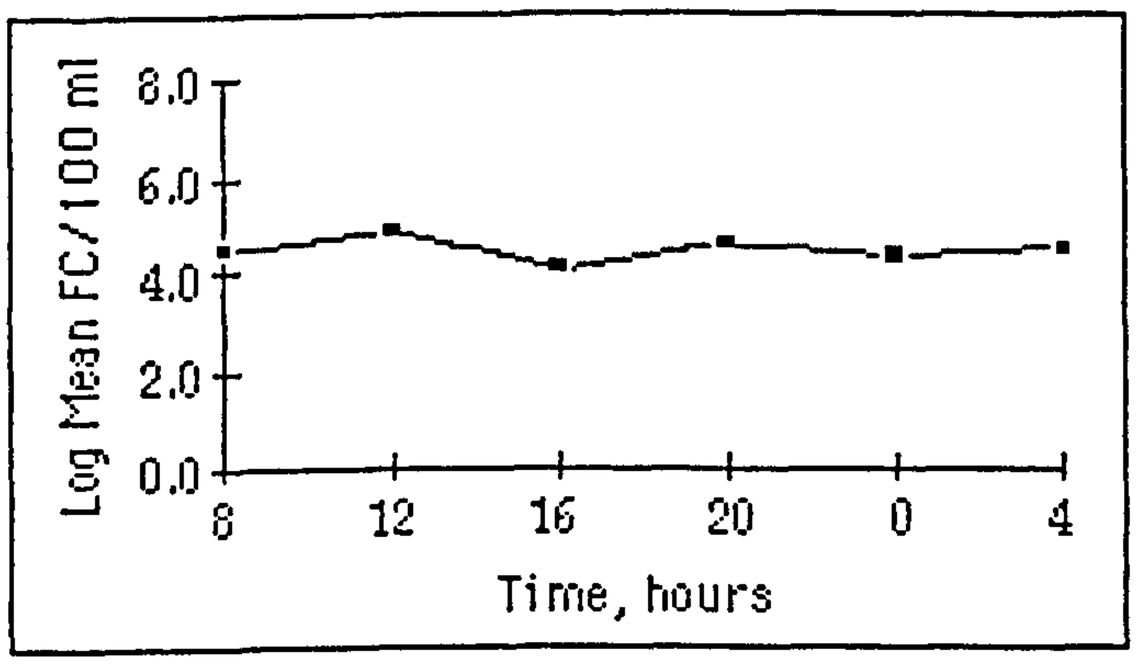
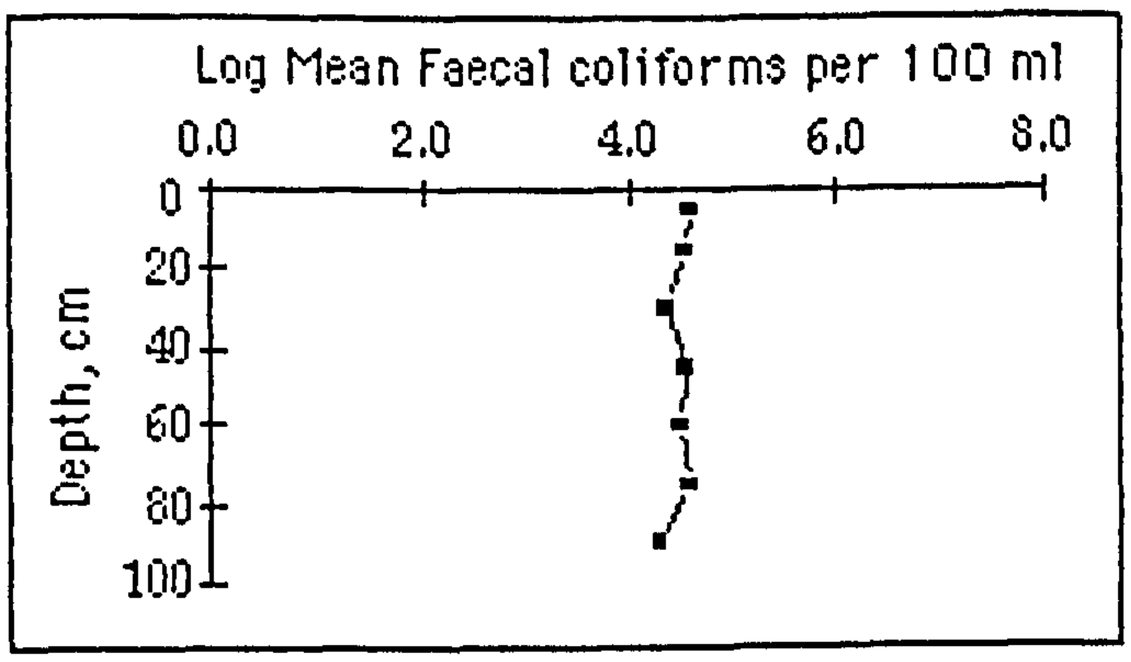
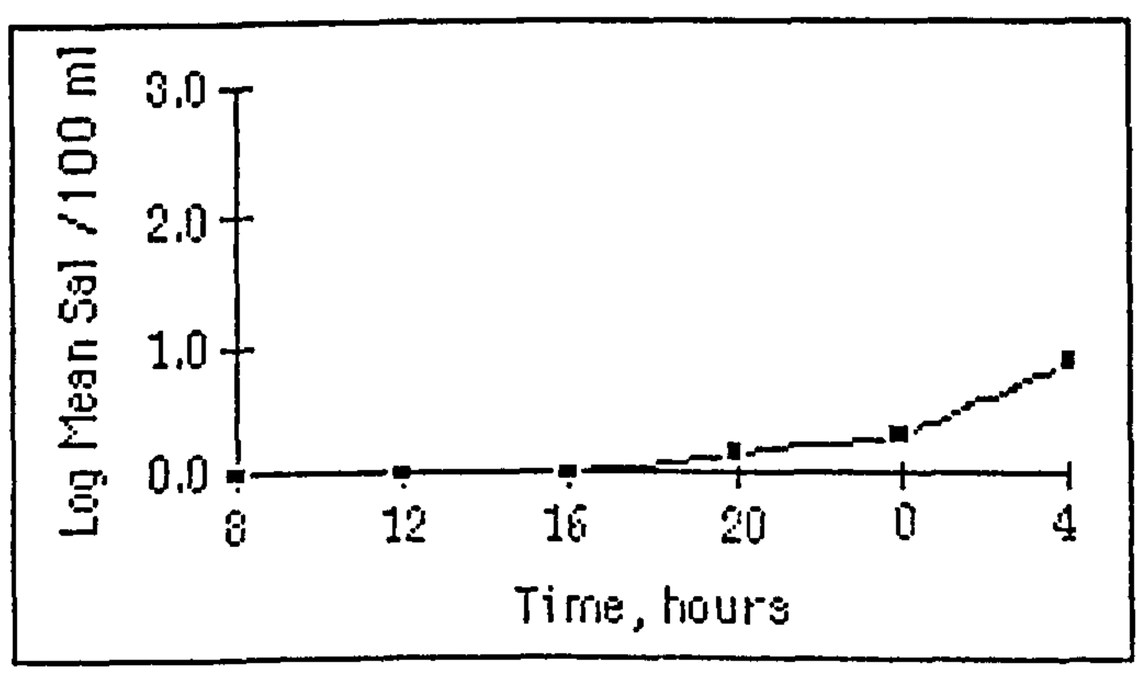
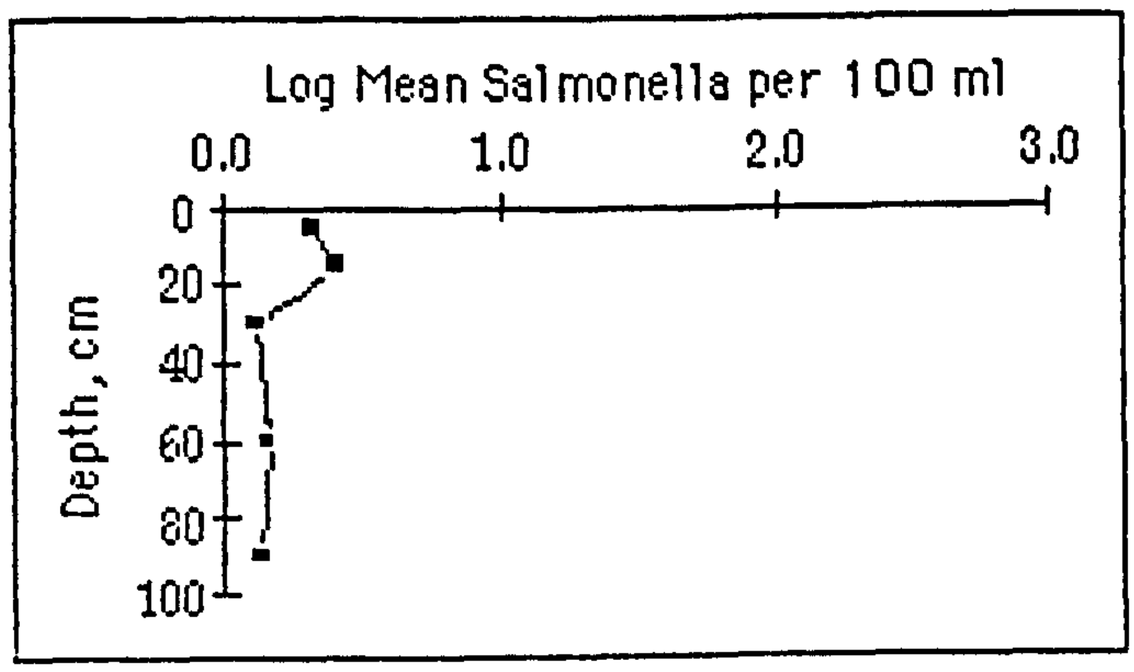


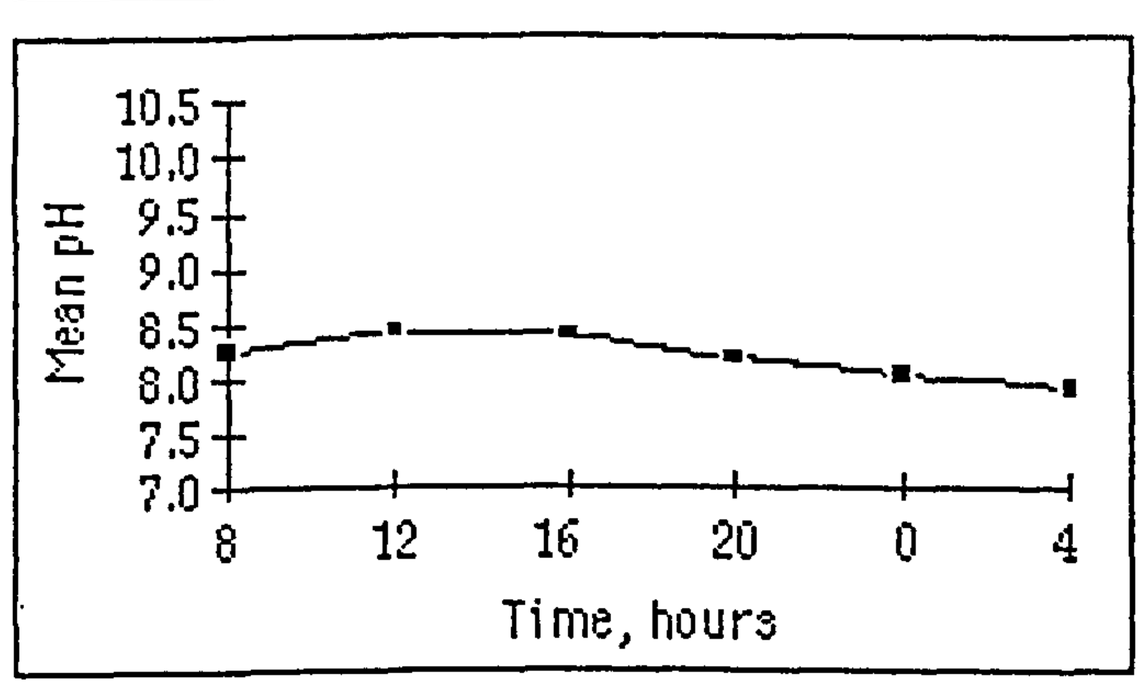
Figure A3/3b **Mean Results of the Profile carried out on M15 on 6.12.92.**

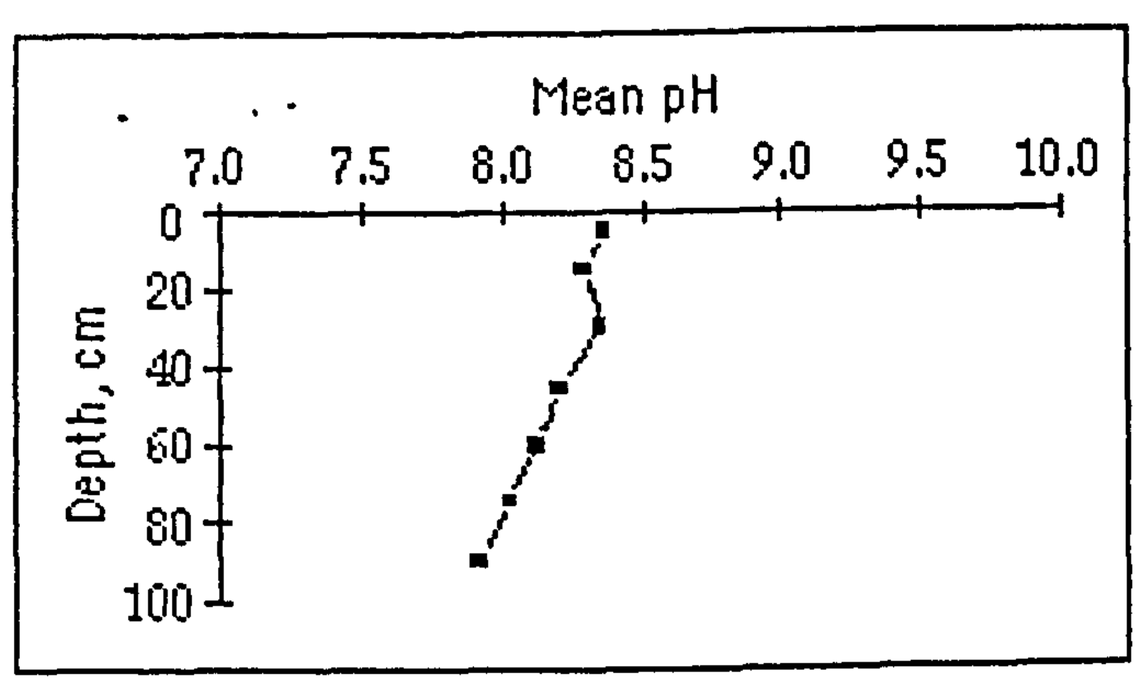


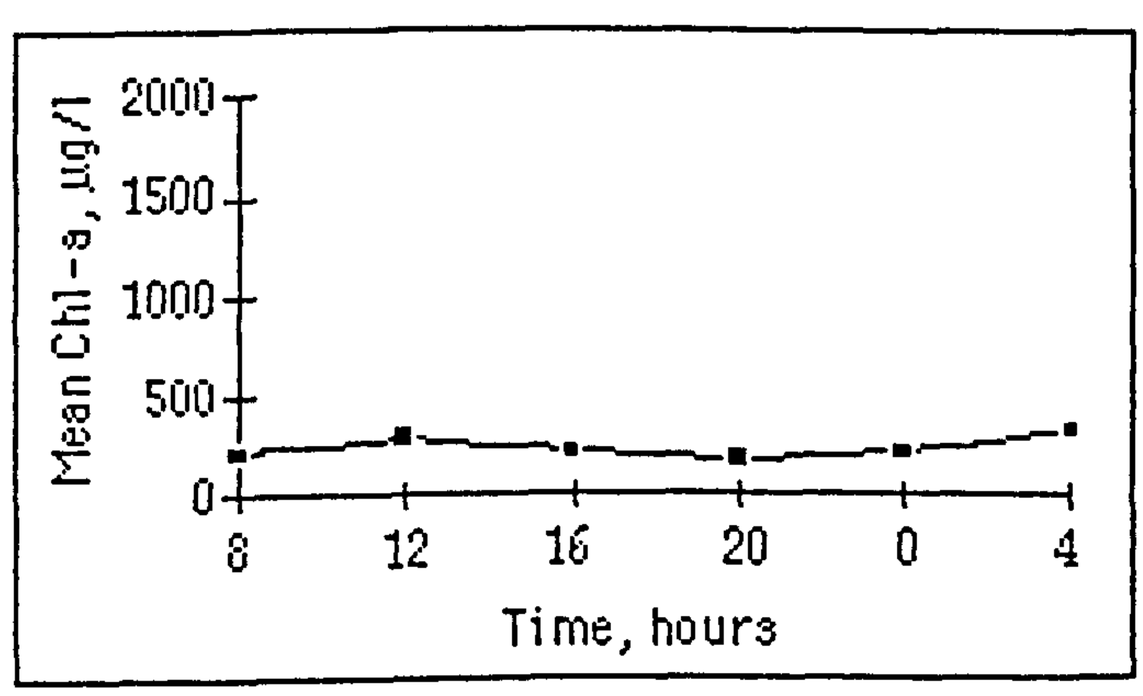












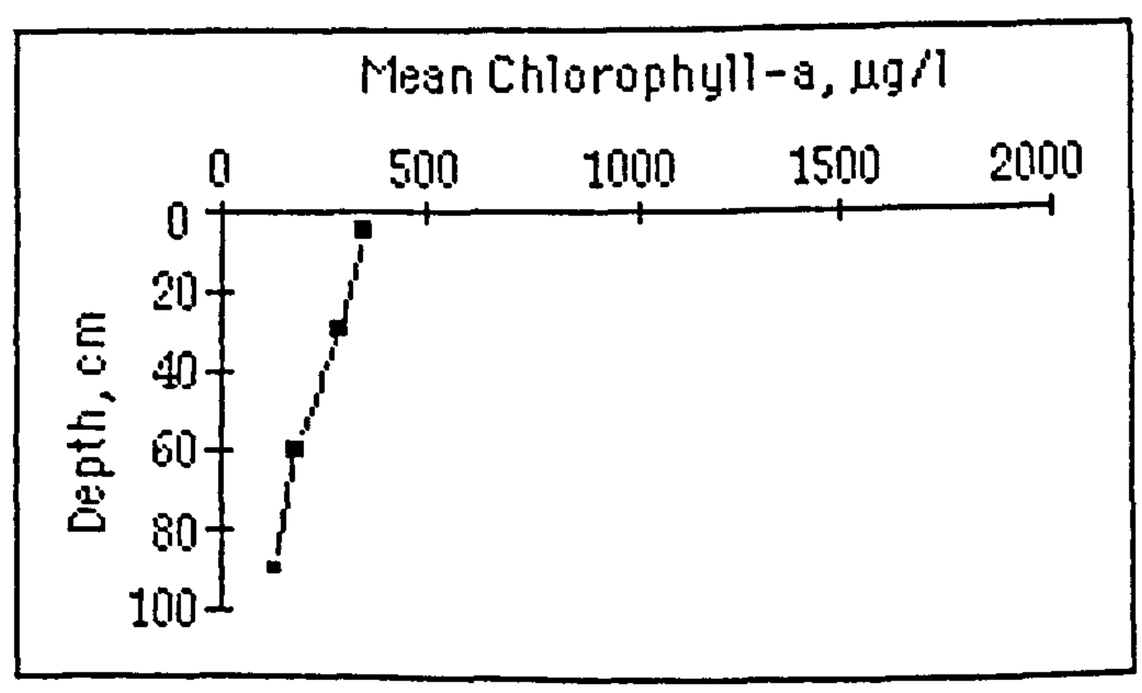
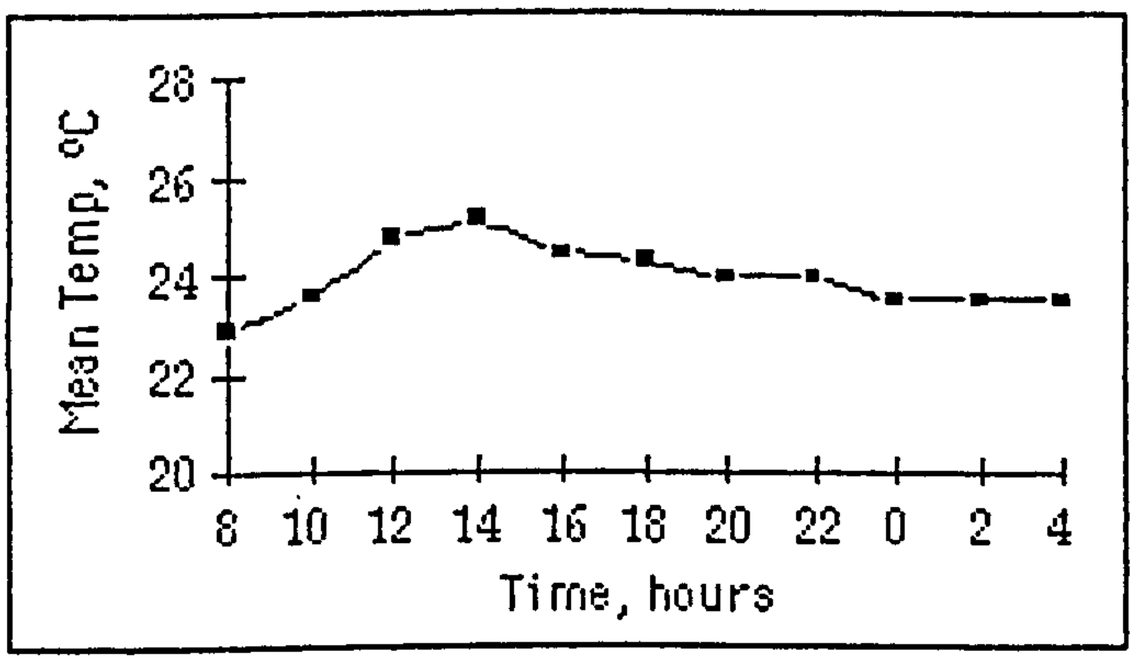
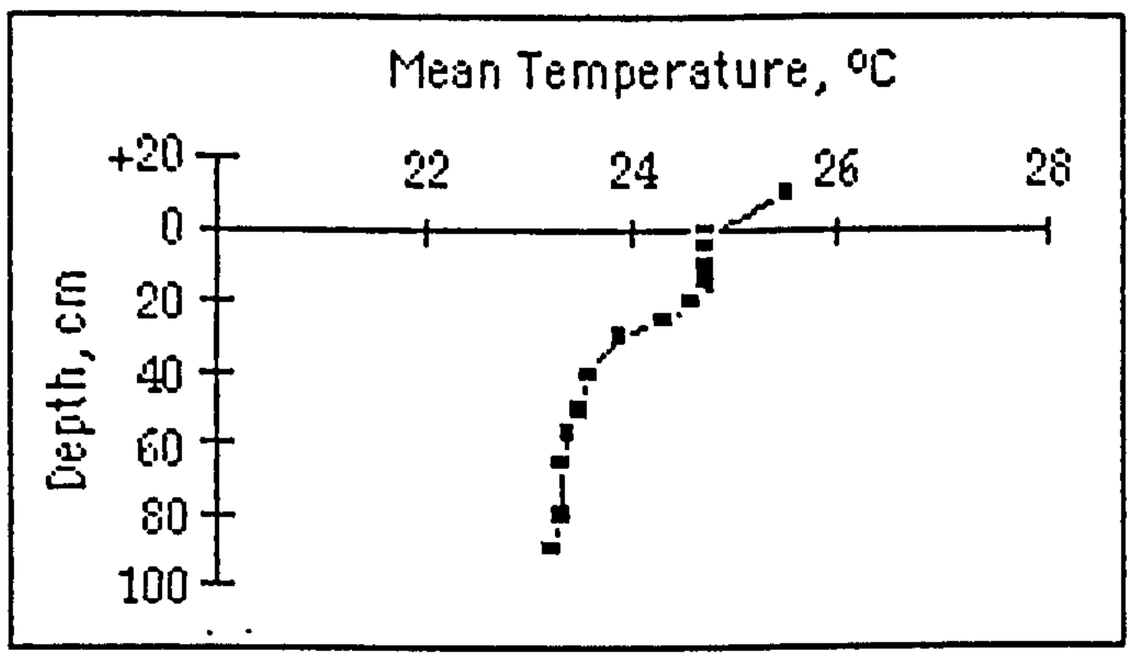
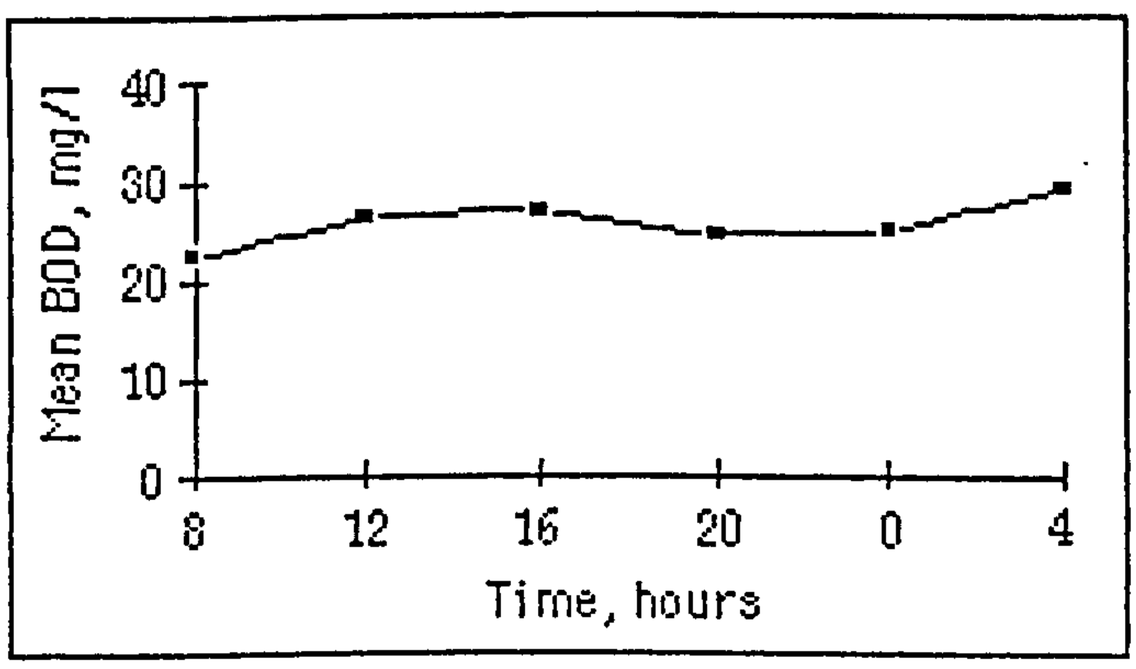
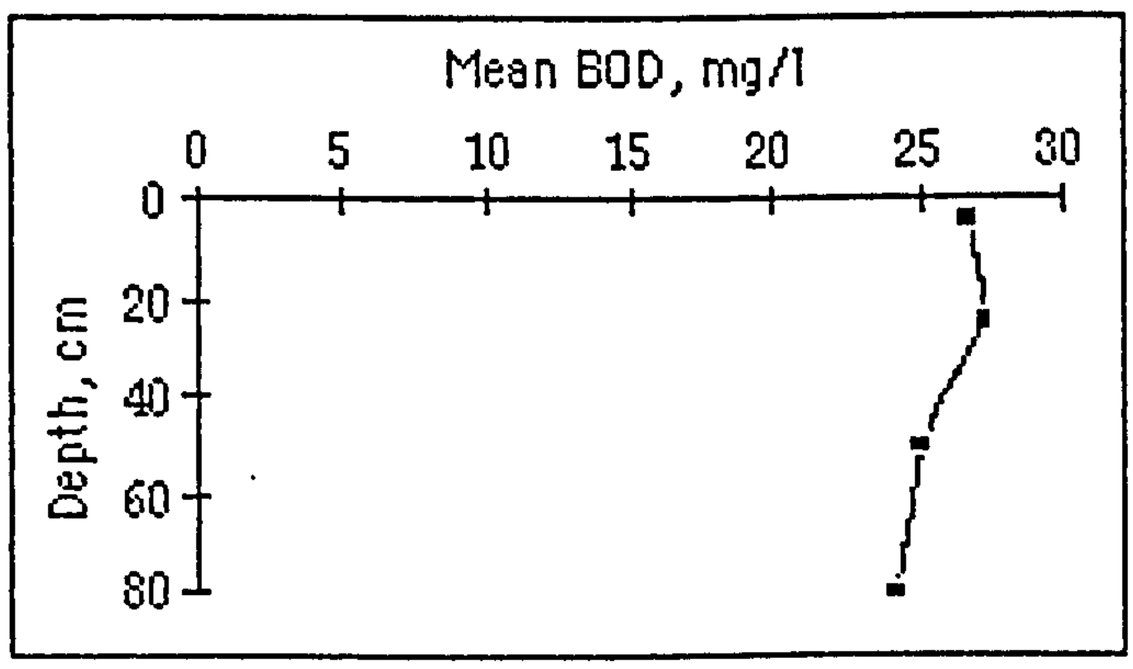


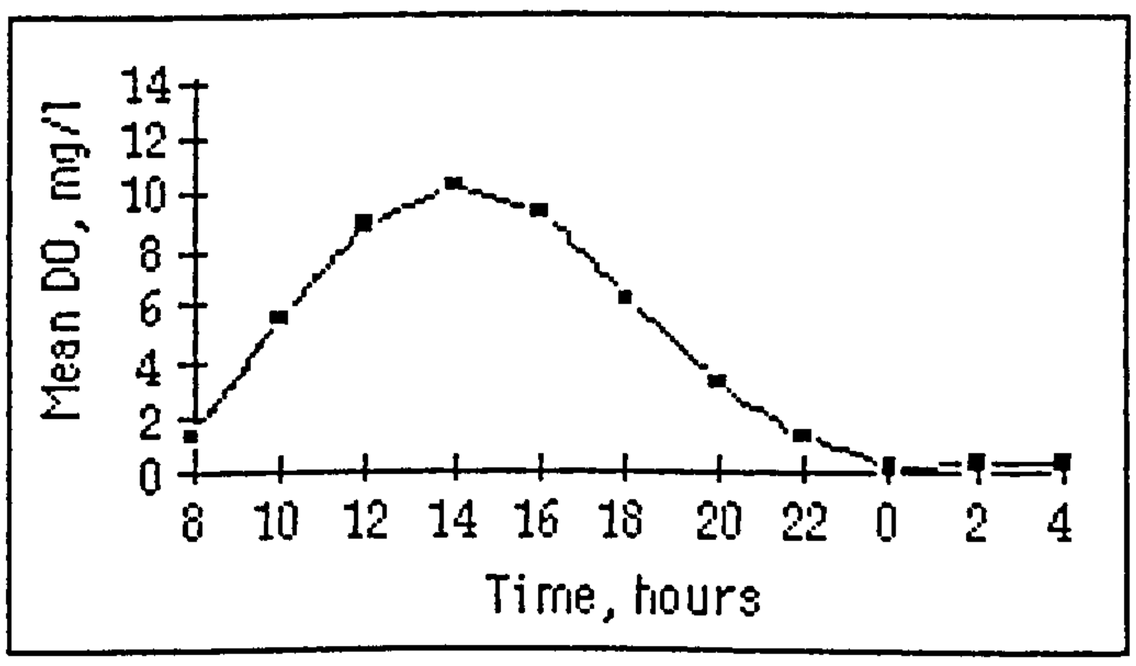
Figure A3/4 **Mean Results of the Profile carried out on M16 on 9.12.92.**

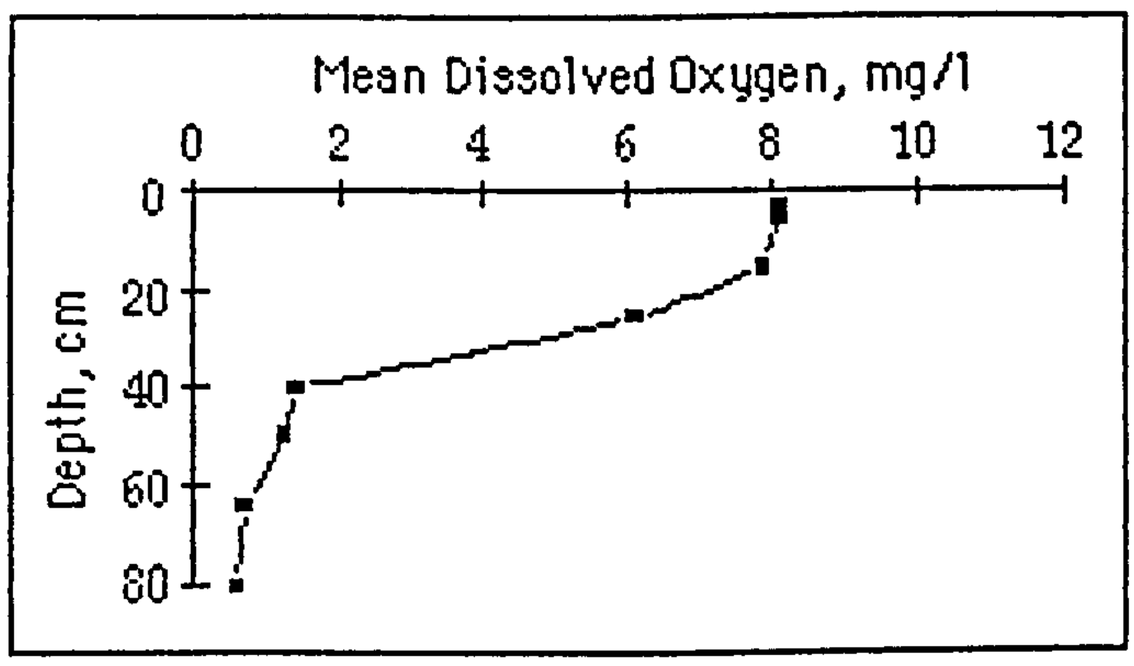


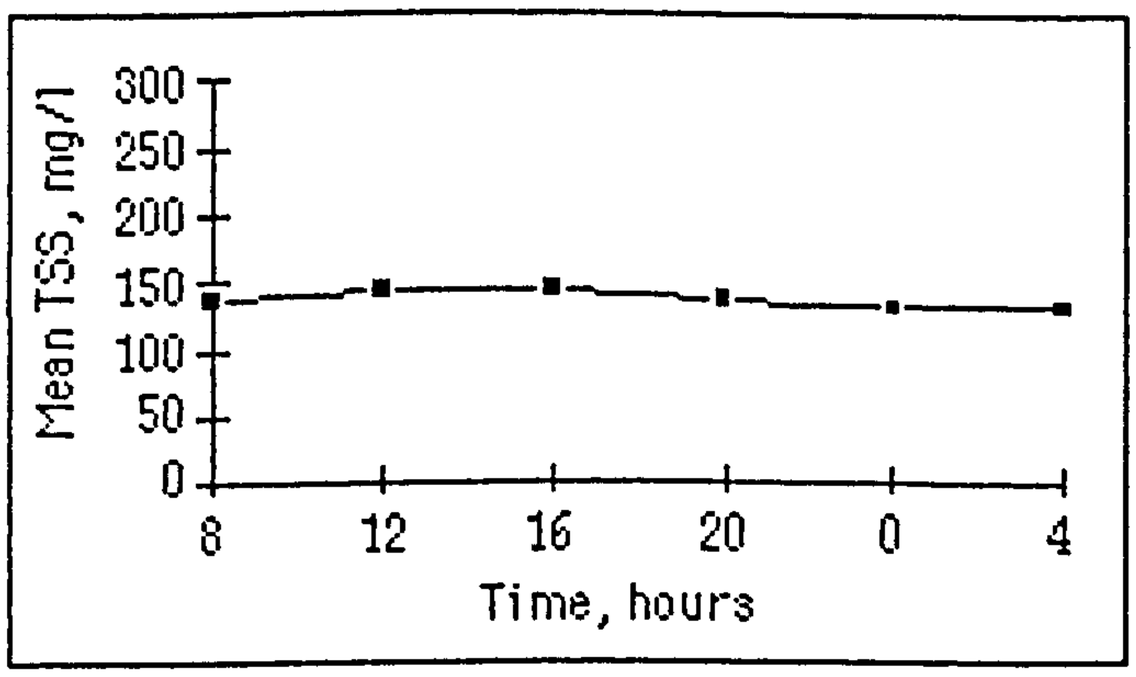












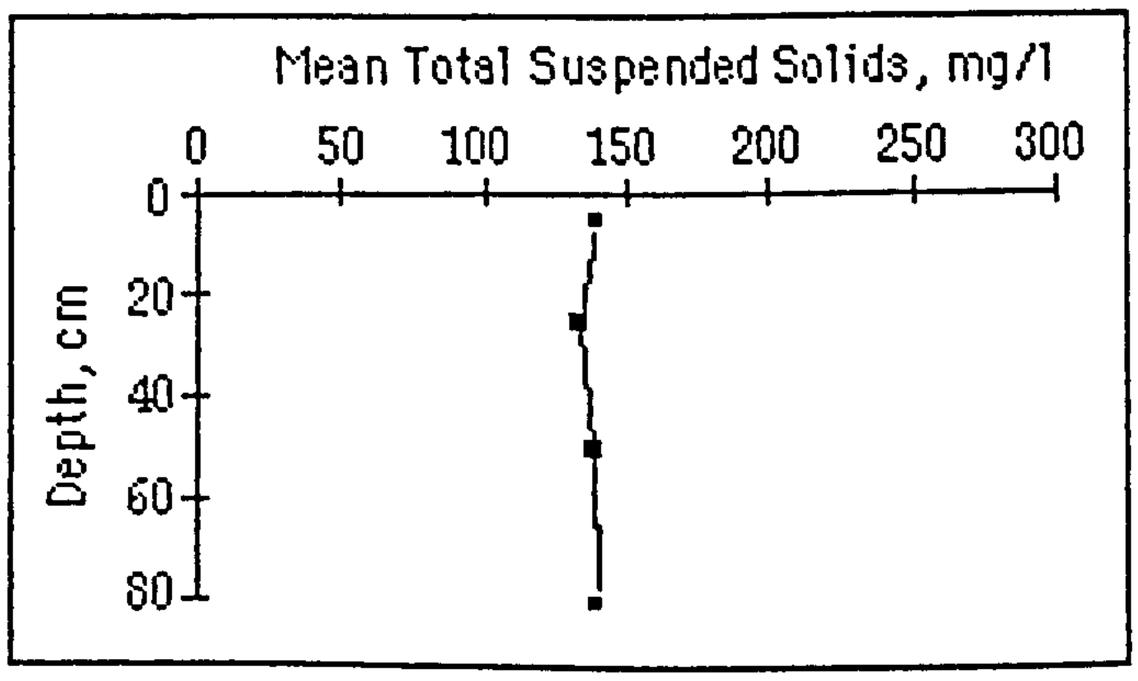
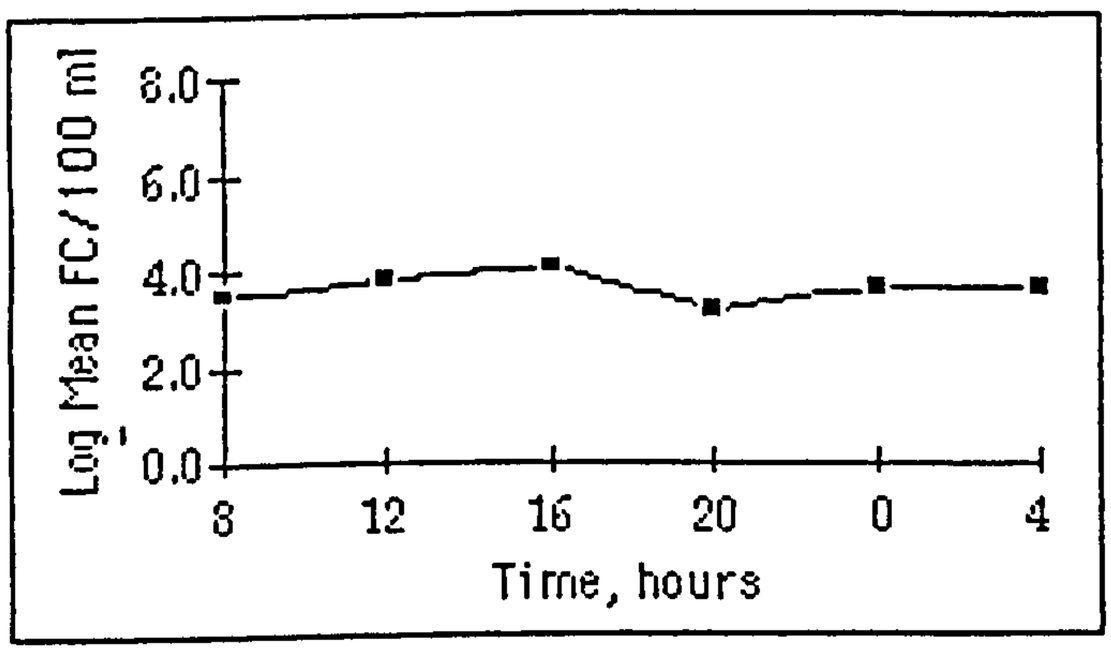
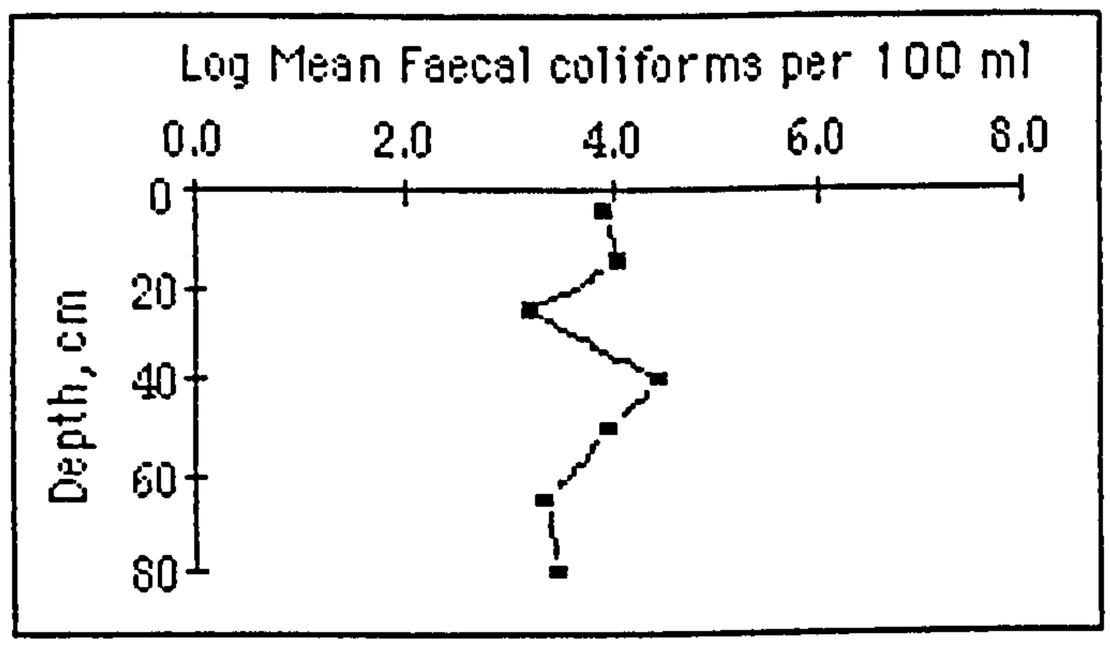


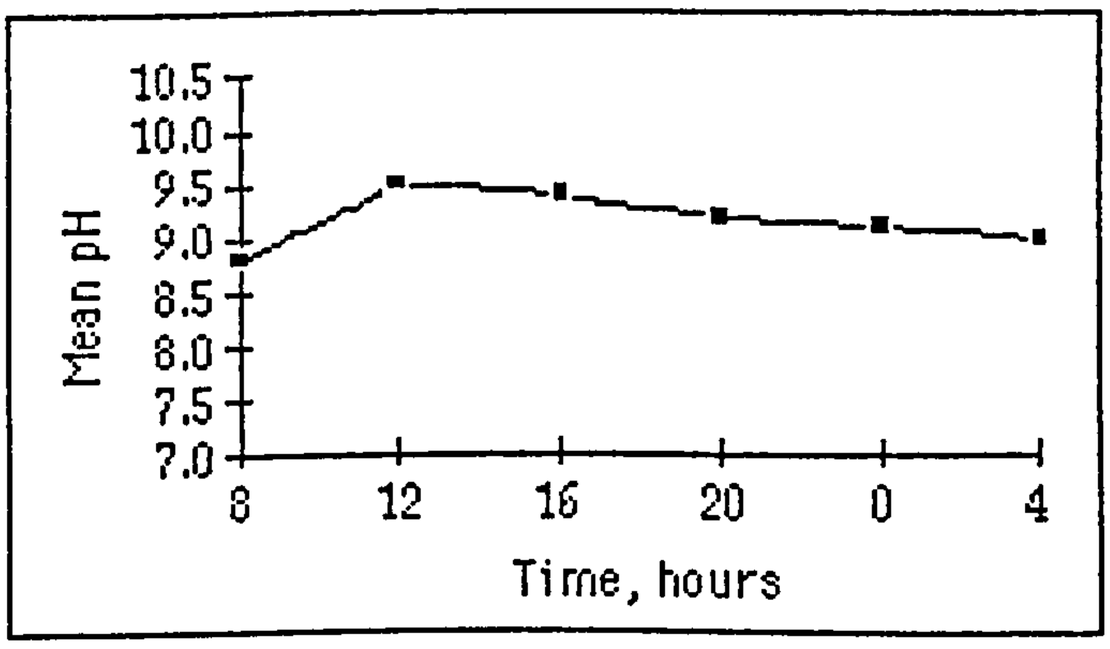
Figure A3/4b **Mean Results of the Profile carried out on M16 on 9.12.92.**

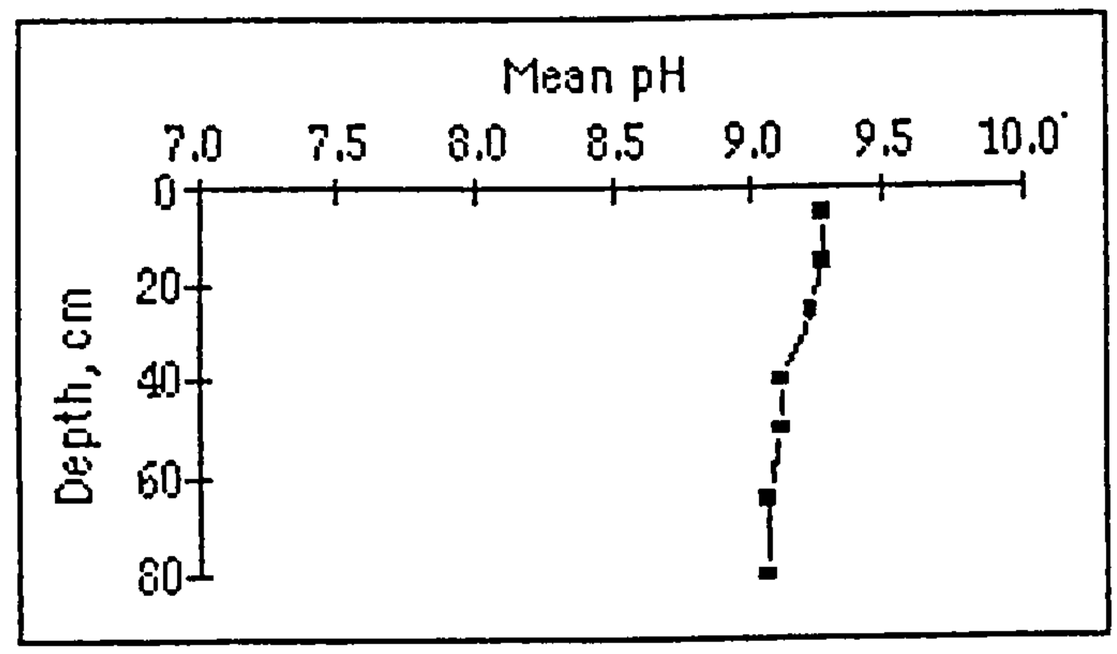


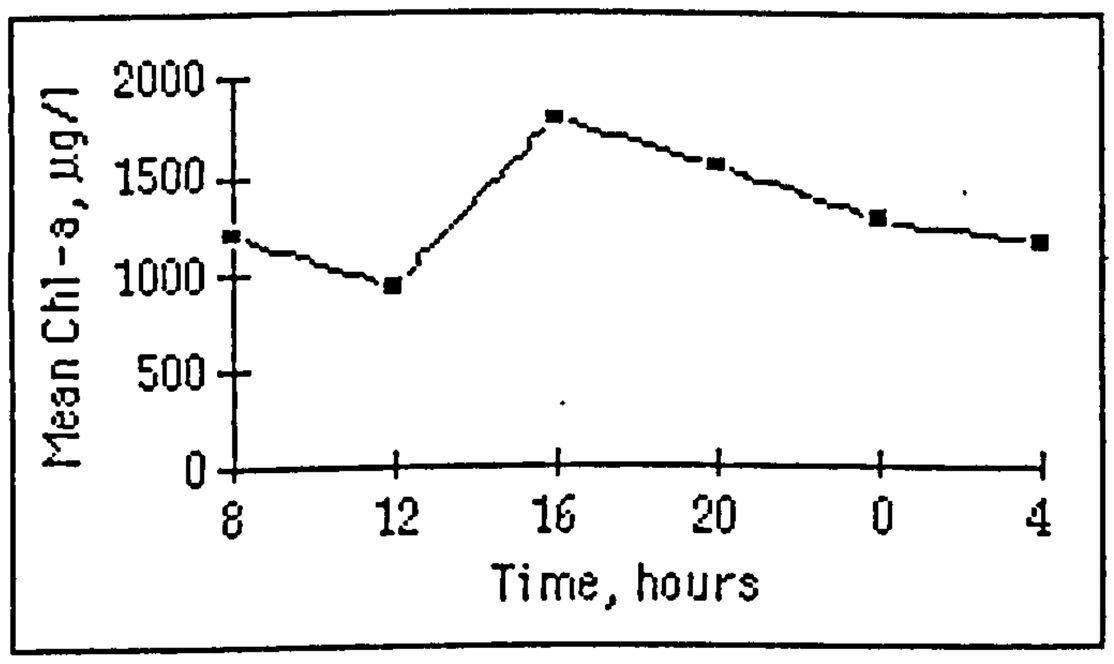


No *Salmonella* Detected

No *Salmonella* Detected







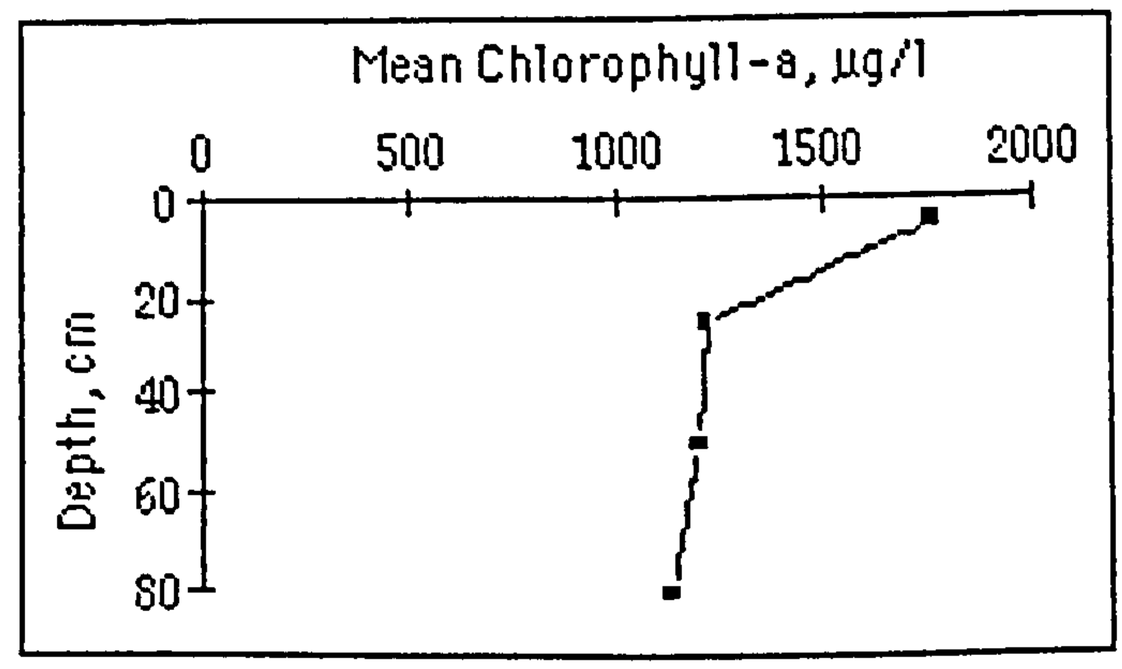
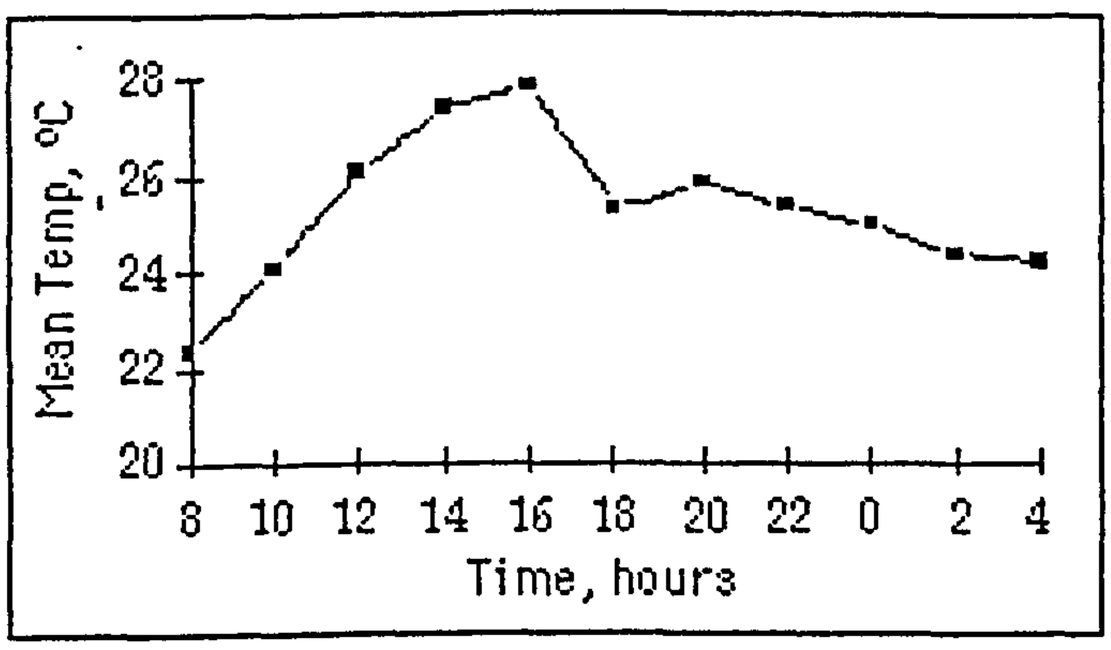
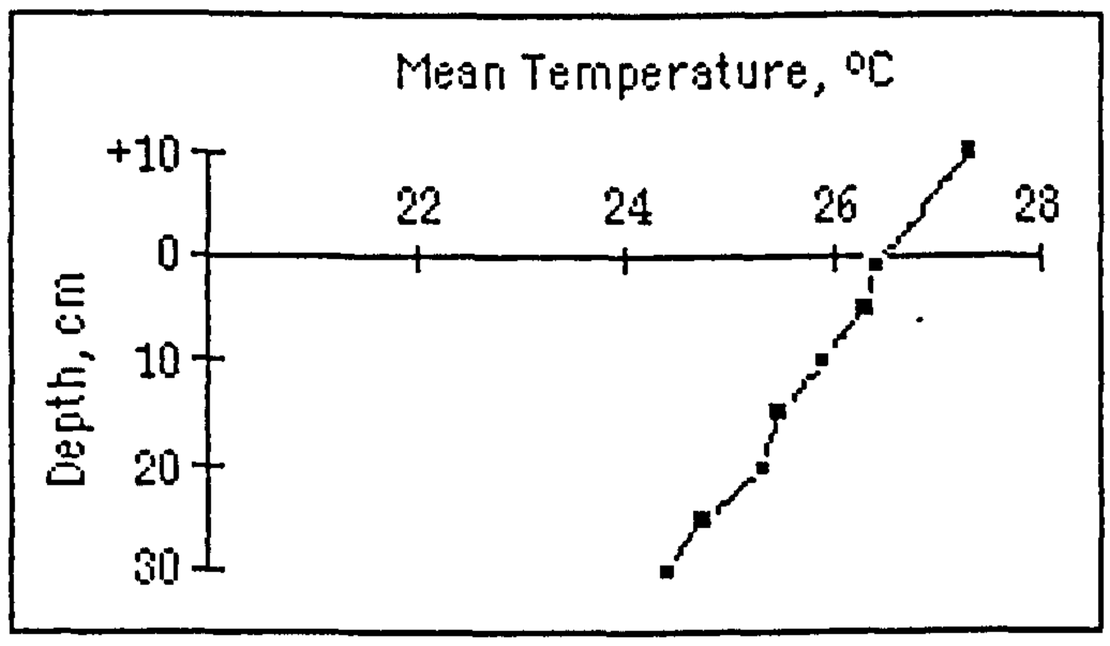
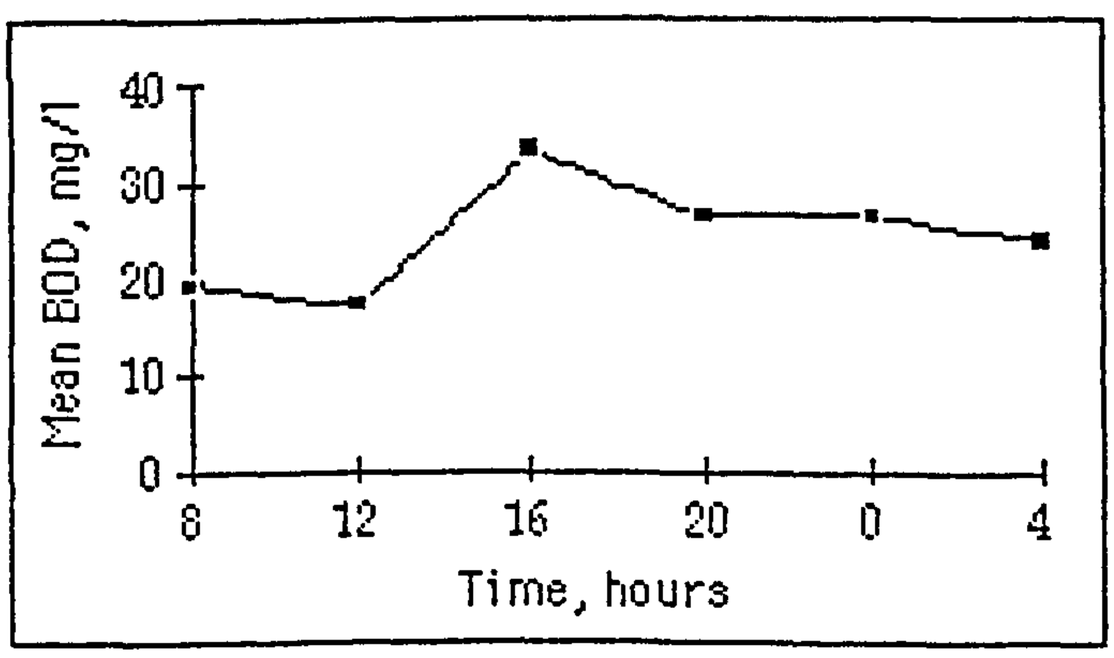
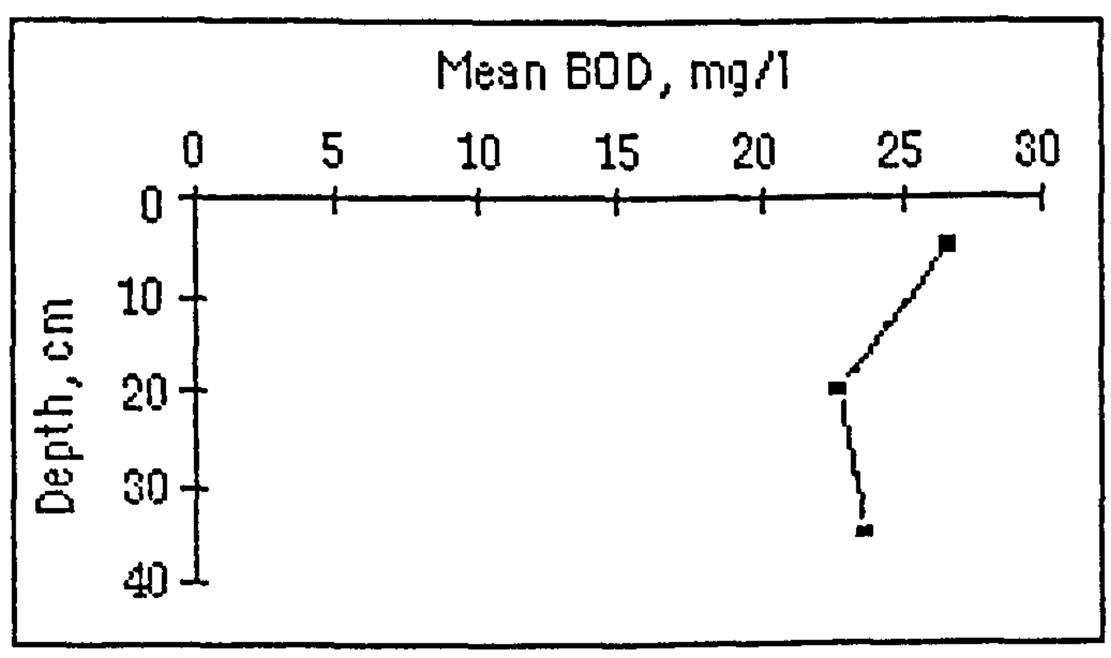


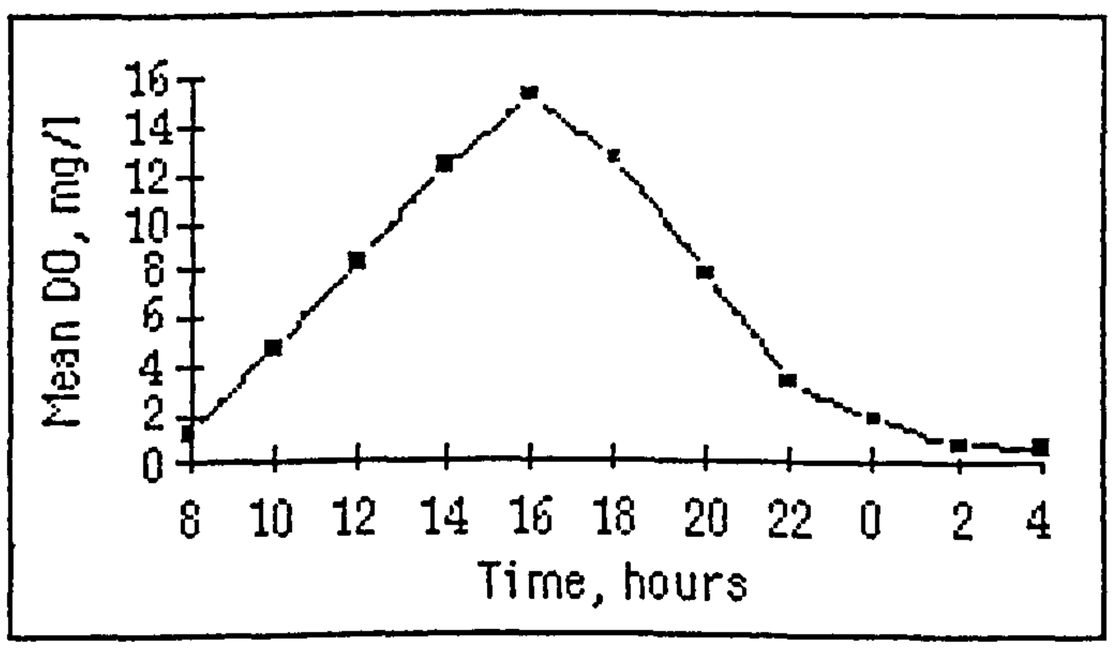
Figure A3/5 **Mean Results of the Profile carried out on M20 on 13.12.92.**

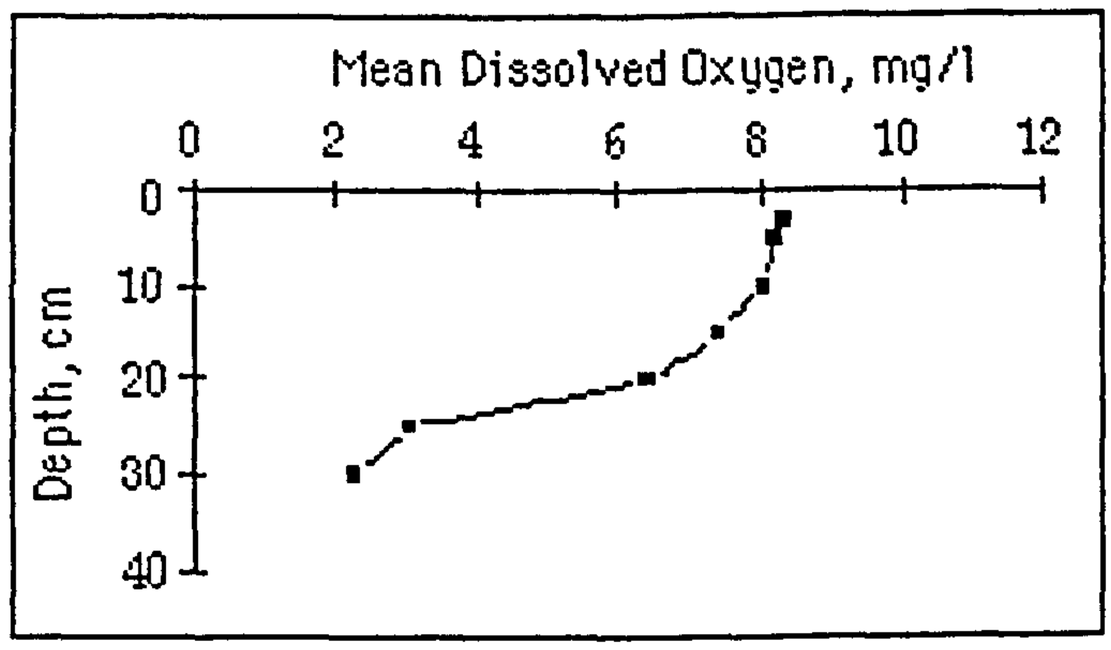


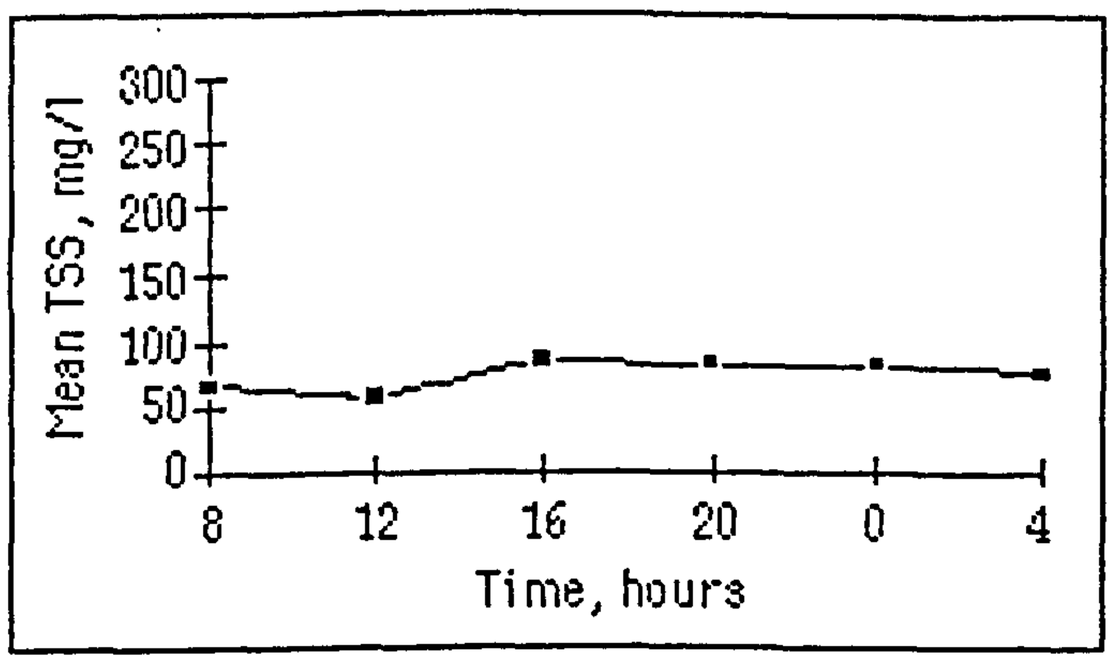












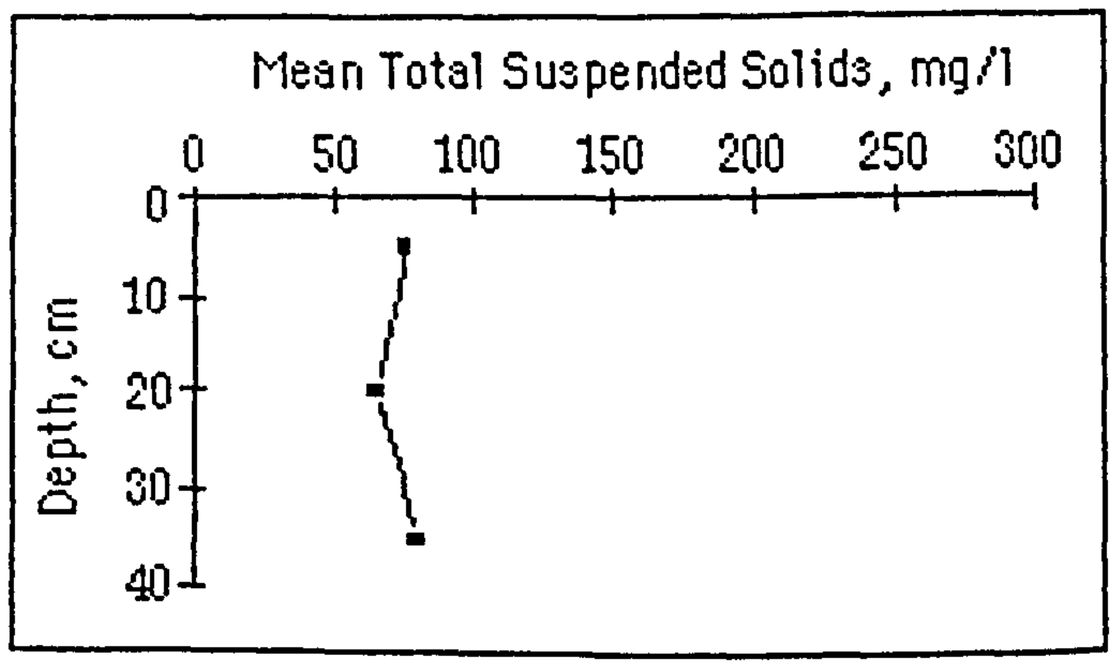
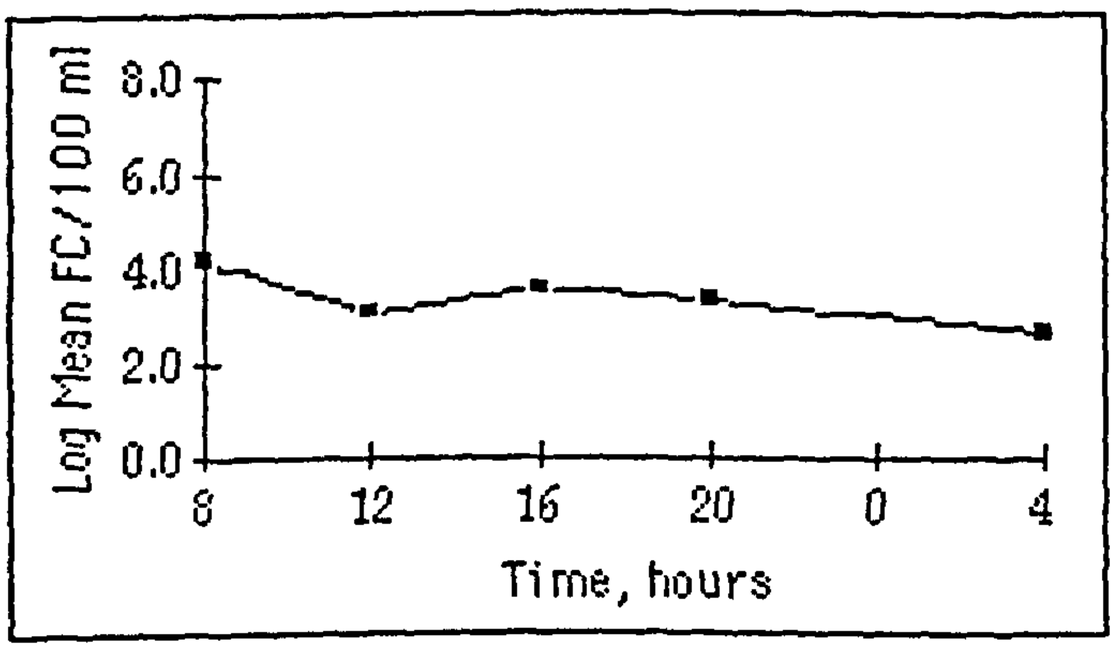
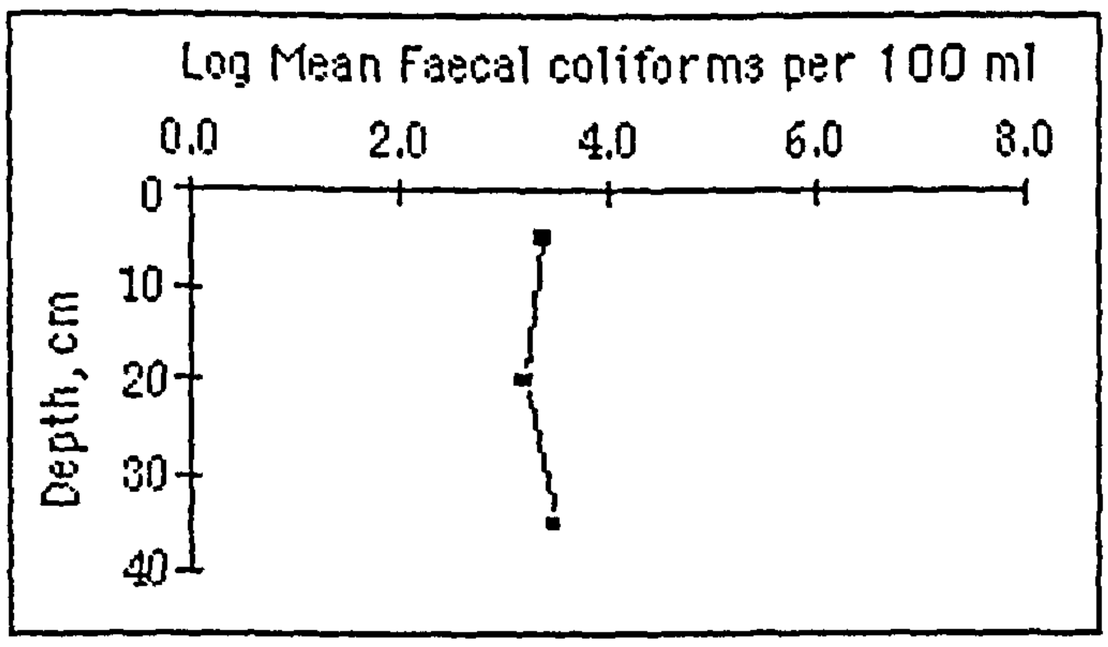


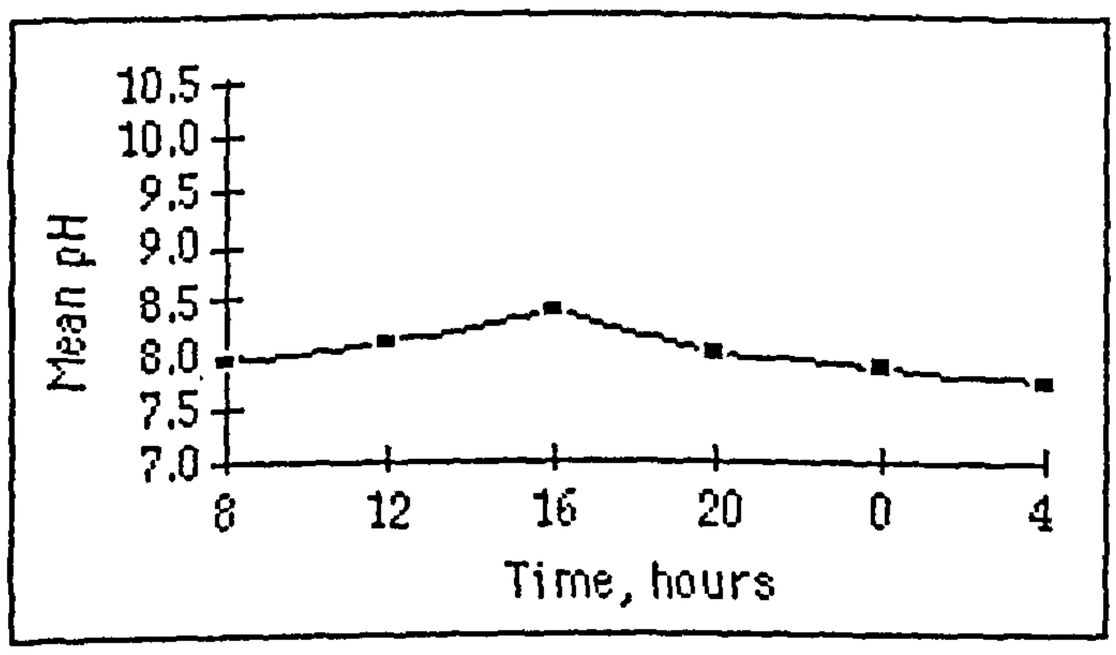
Figure A3/5b <u>Mean Results of the Profile carried out on M20 on 13.12.92.</u>

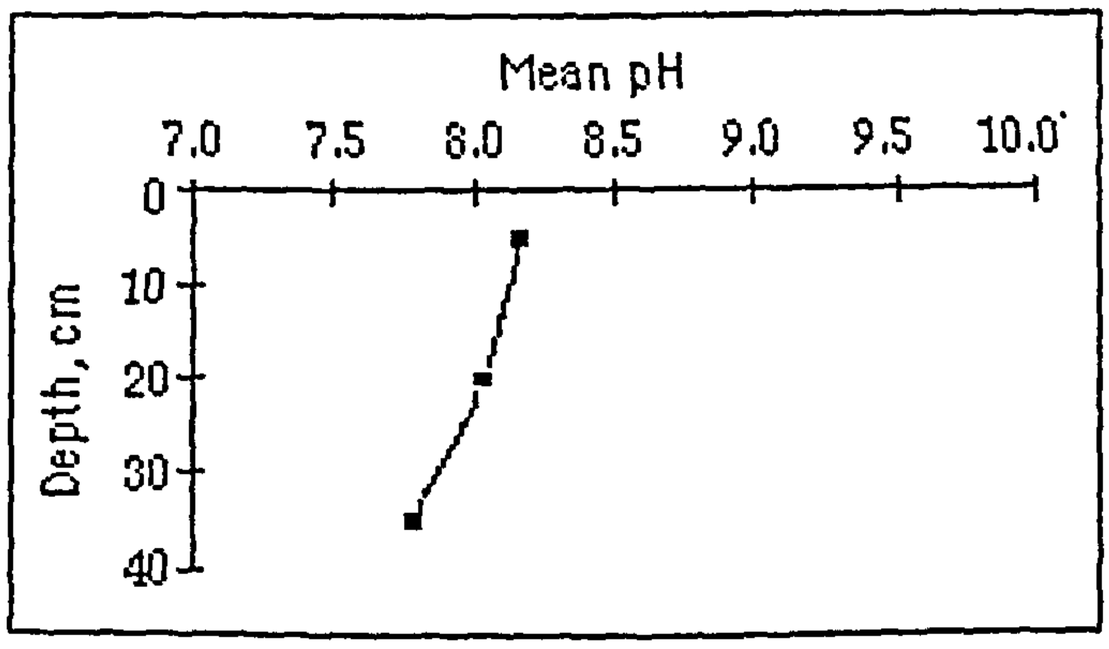


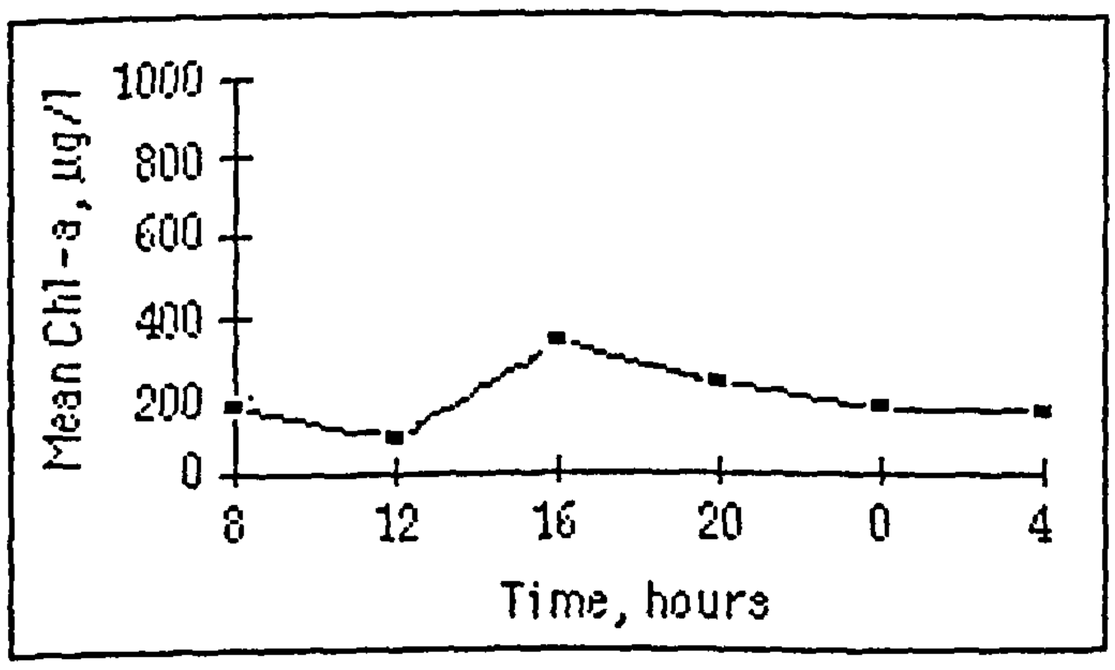


:No *Salmonella* Detected

:No *Salmonella* Detected







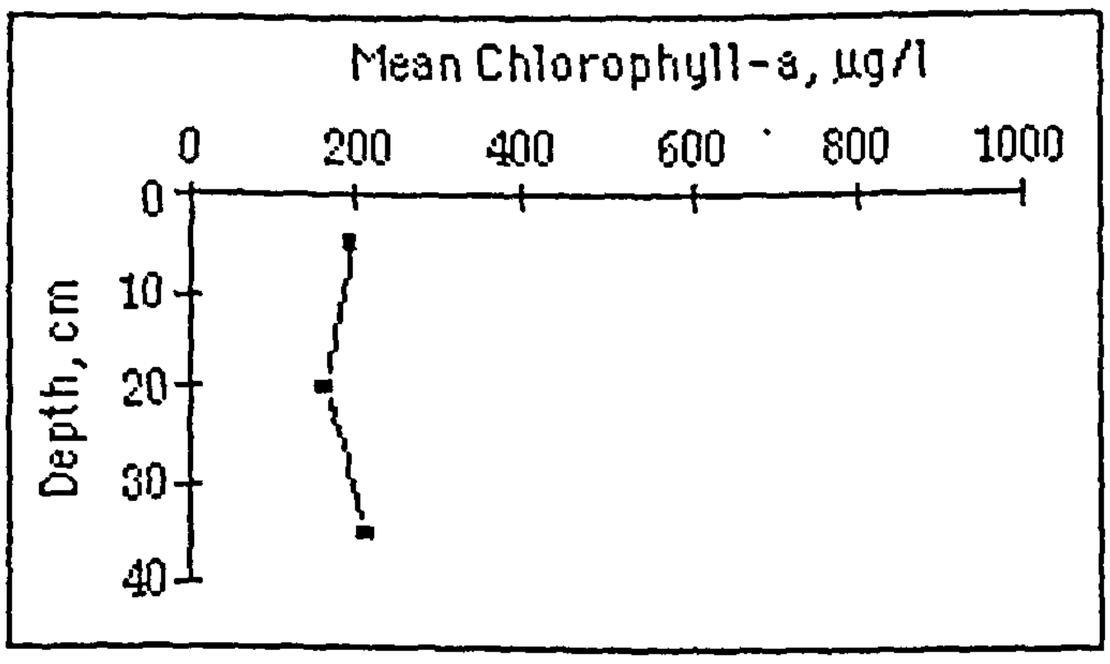
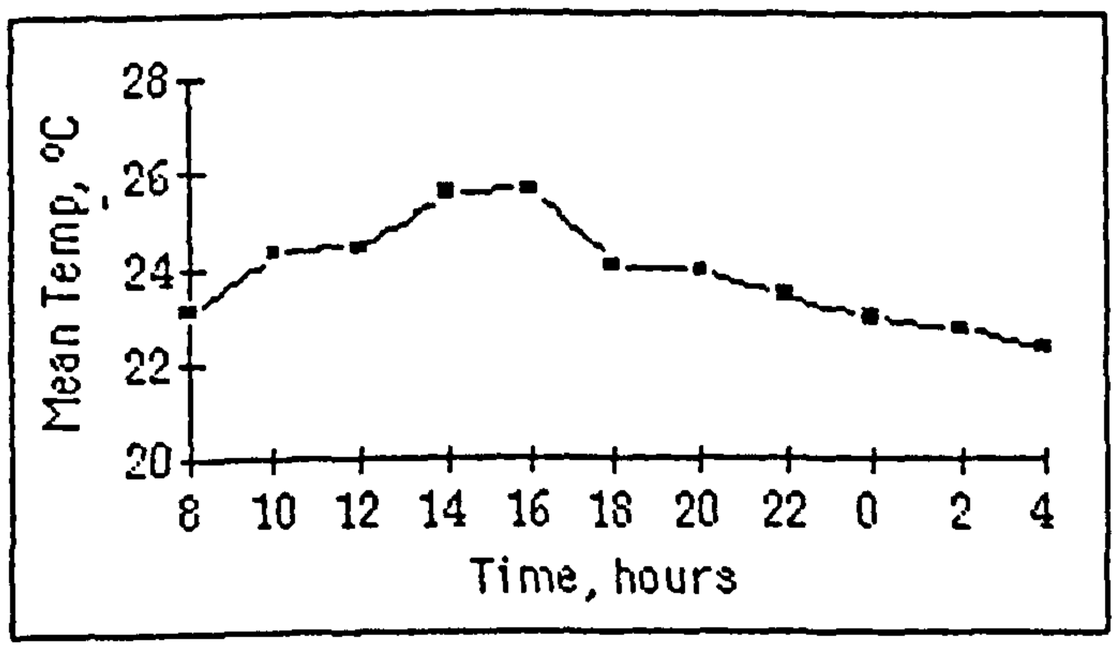
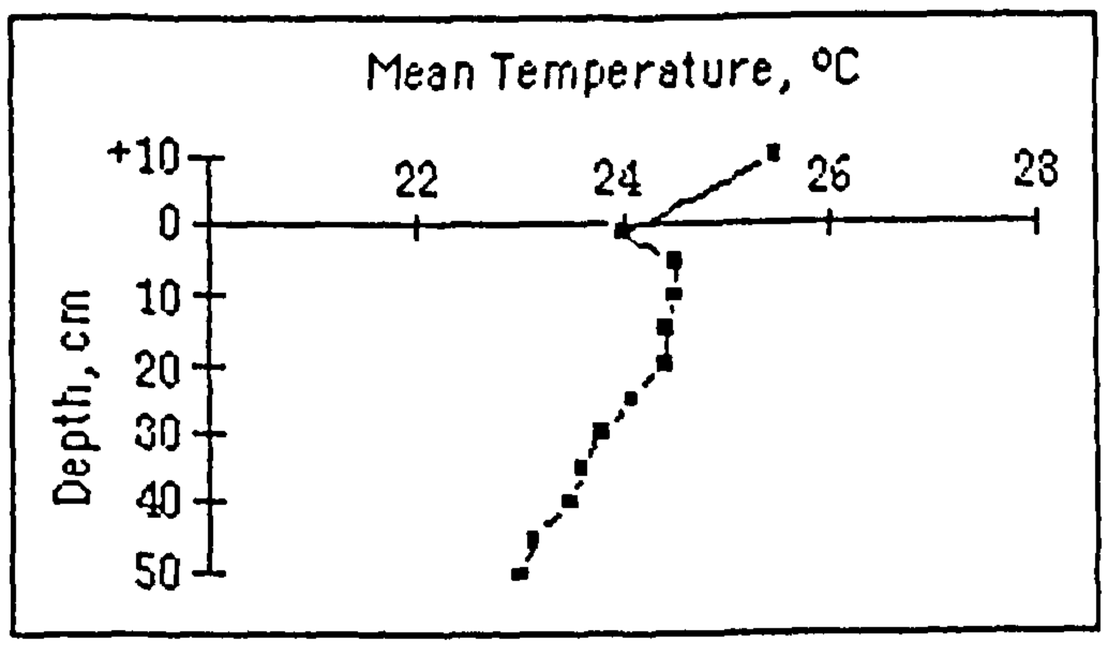
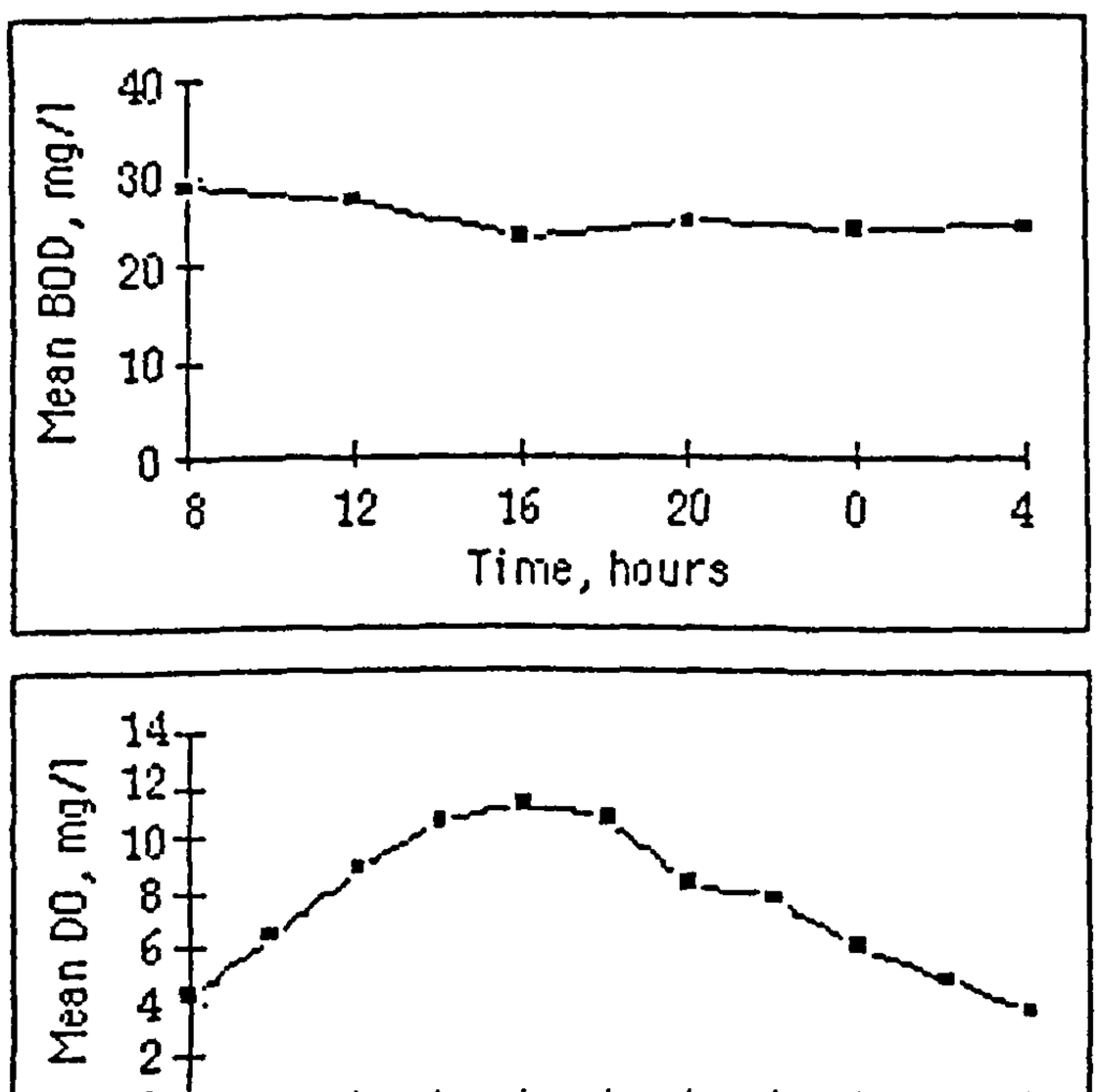
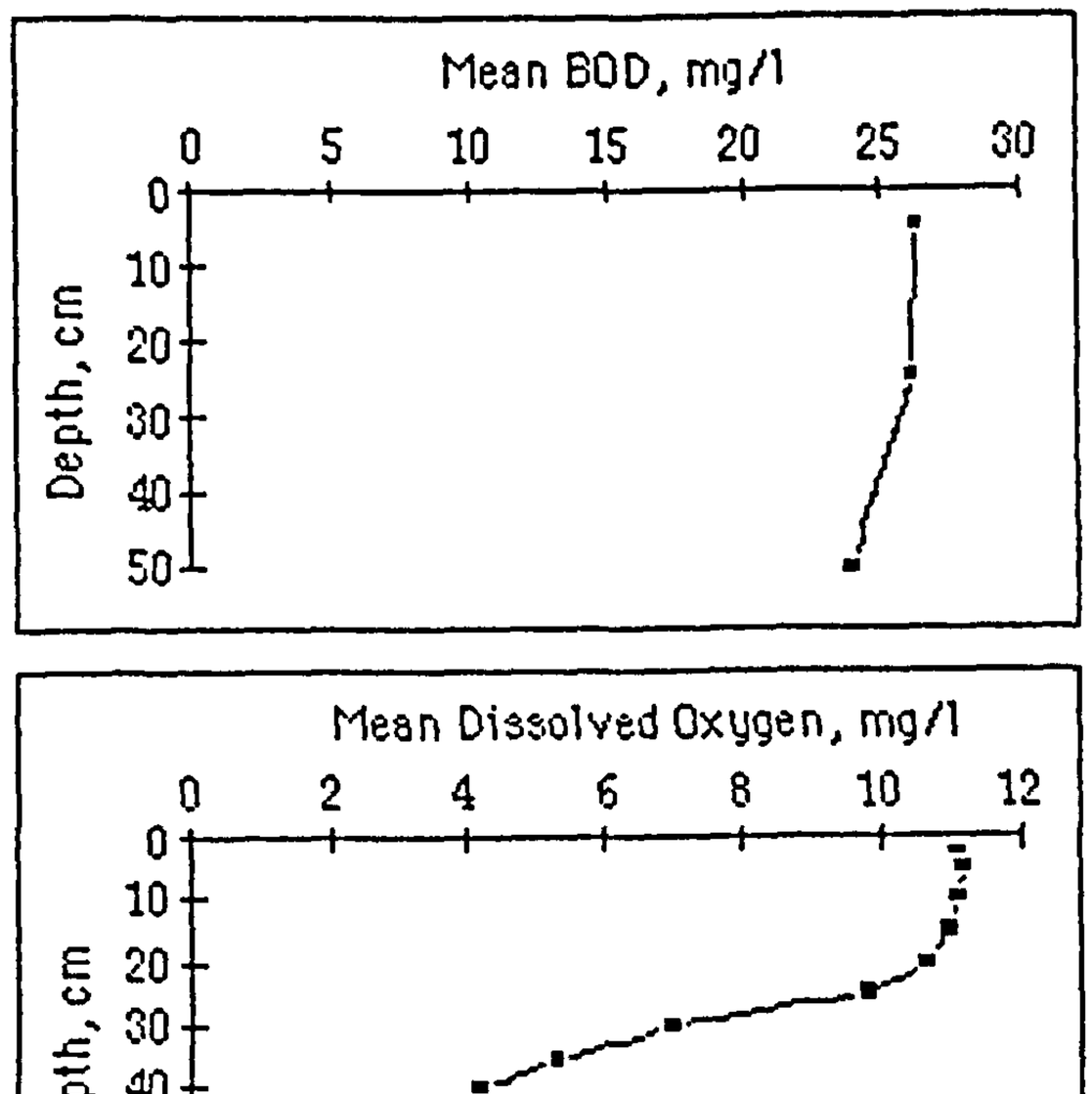


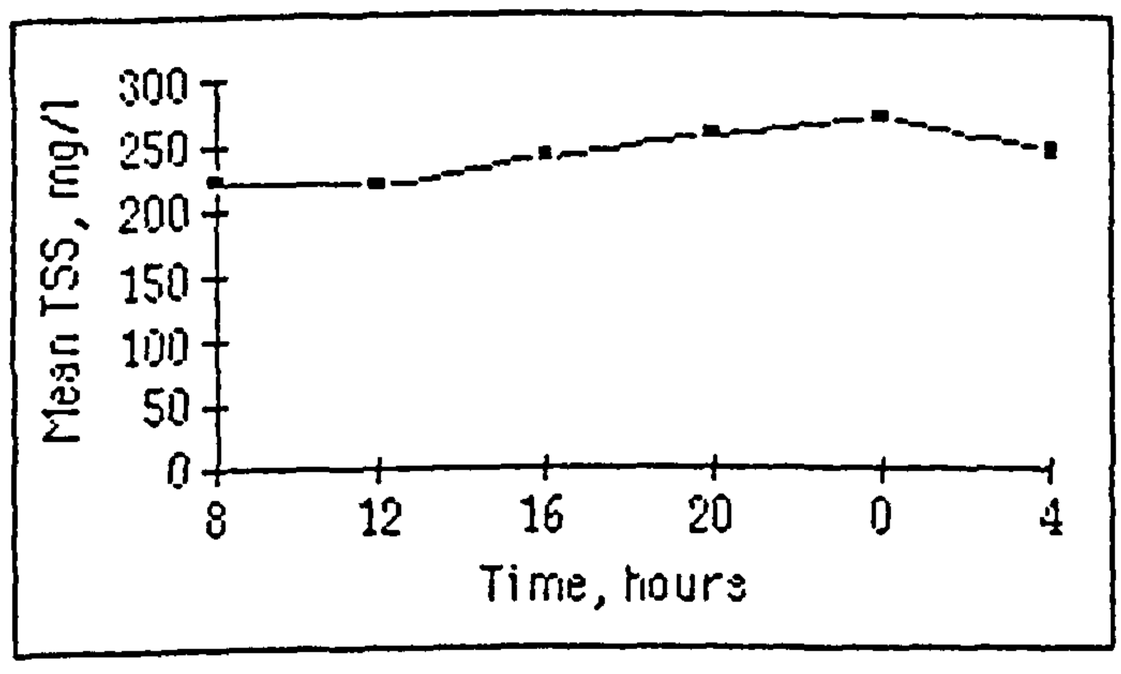
Figure A3/6 <u>Mean Results of the Profile carried out on M23 on 16.12.92.</u>











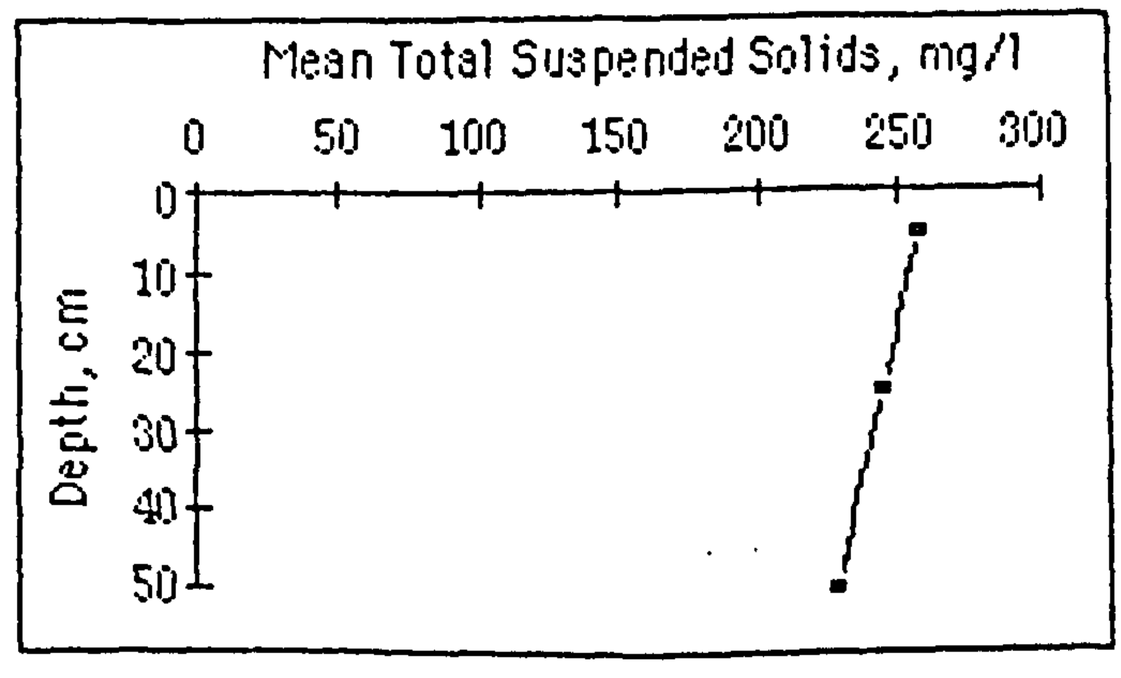
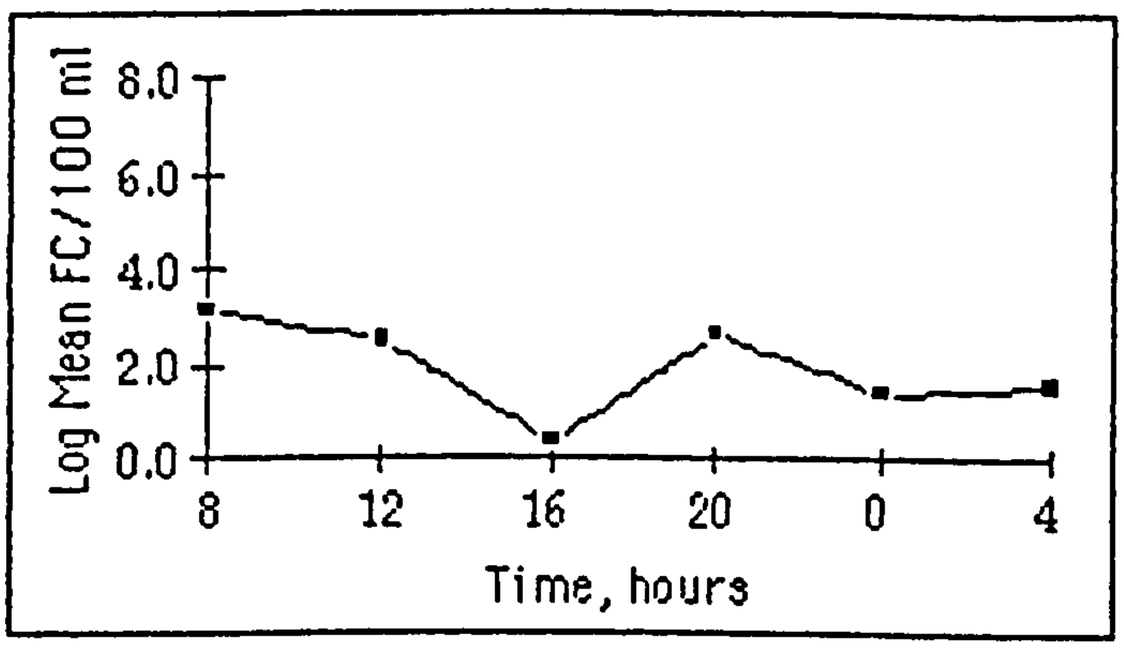
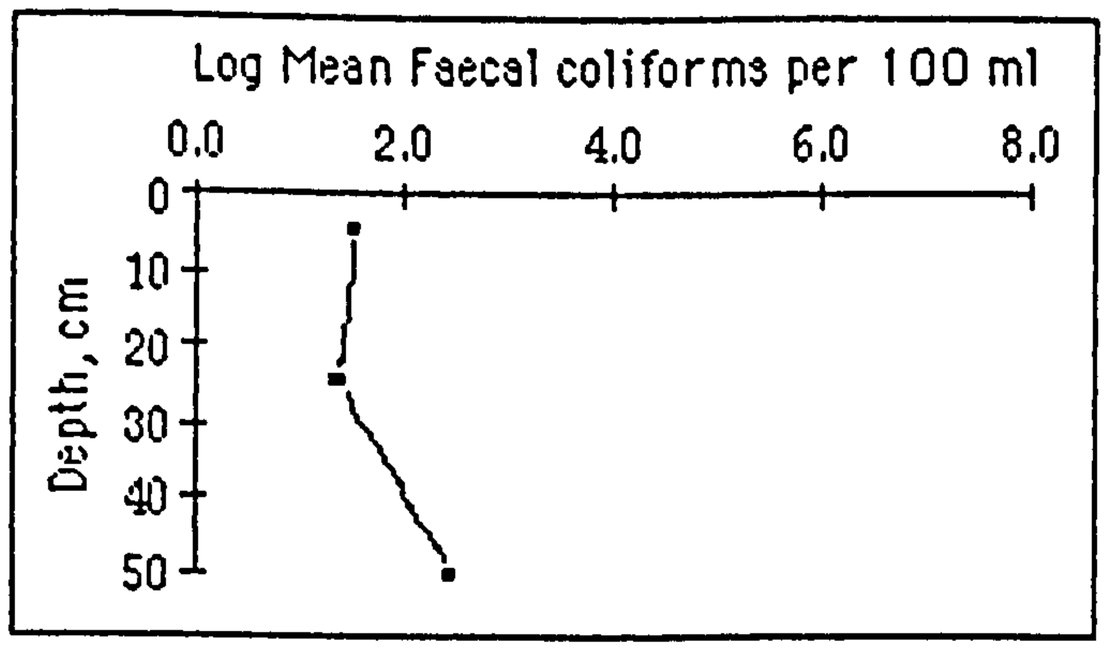


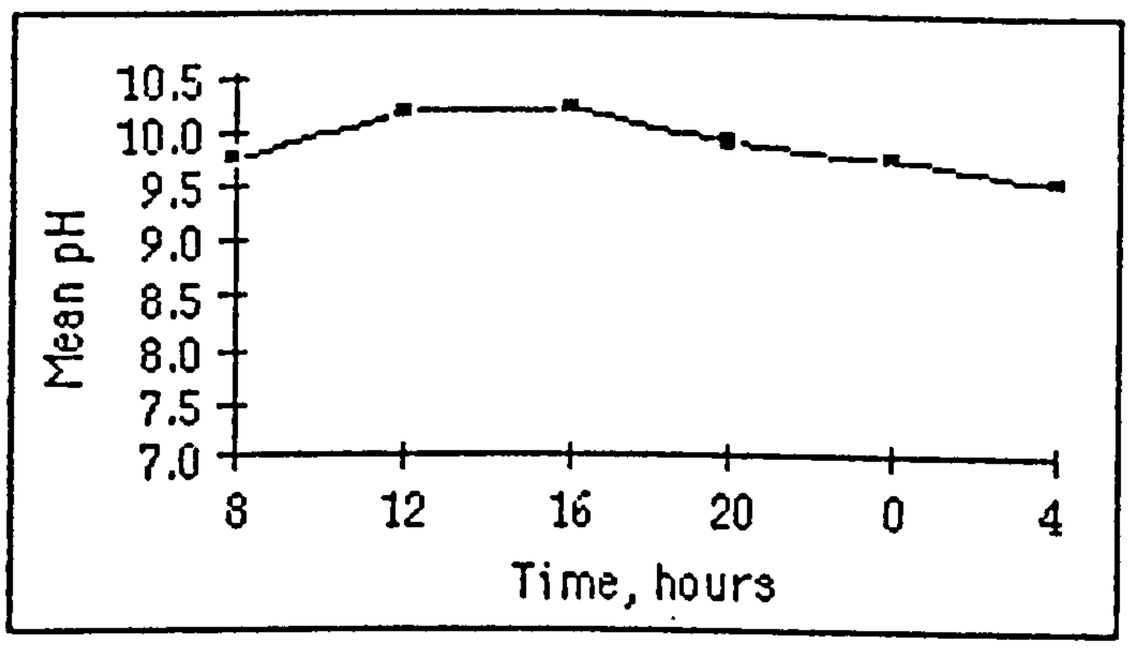
Figure A3/6b Mean Results of the Profile carried out on M23 on 16.12.92.

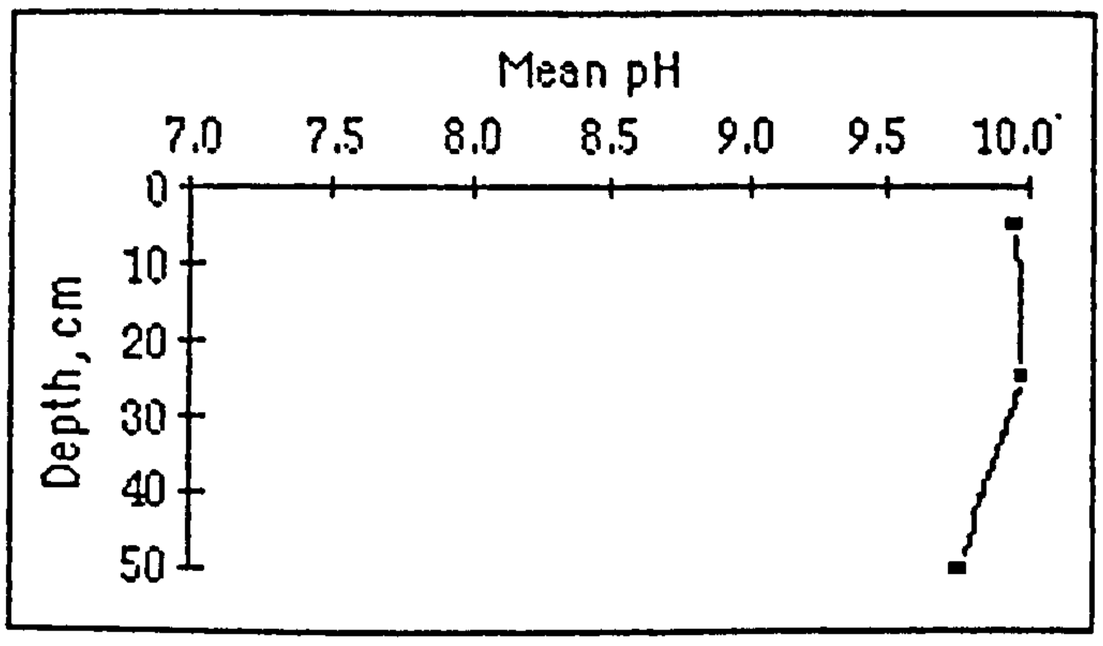


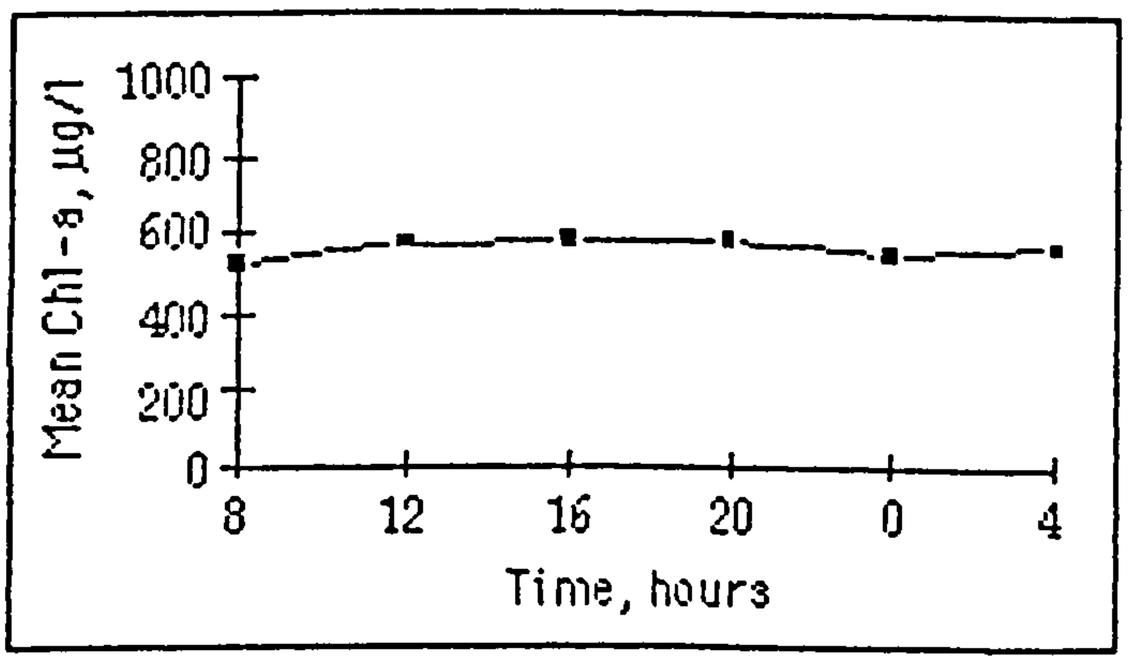


No *Salmonella* Detected

No *Salmonella* Detected







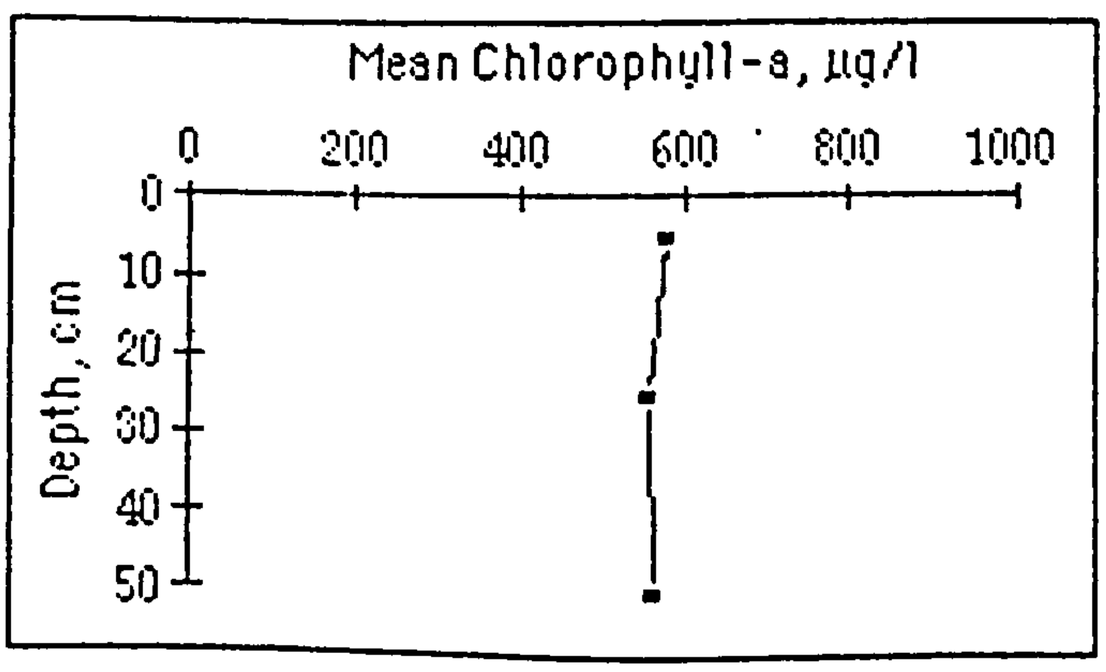
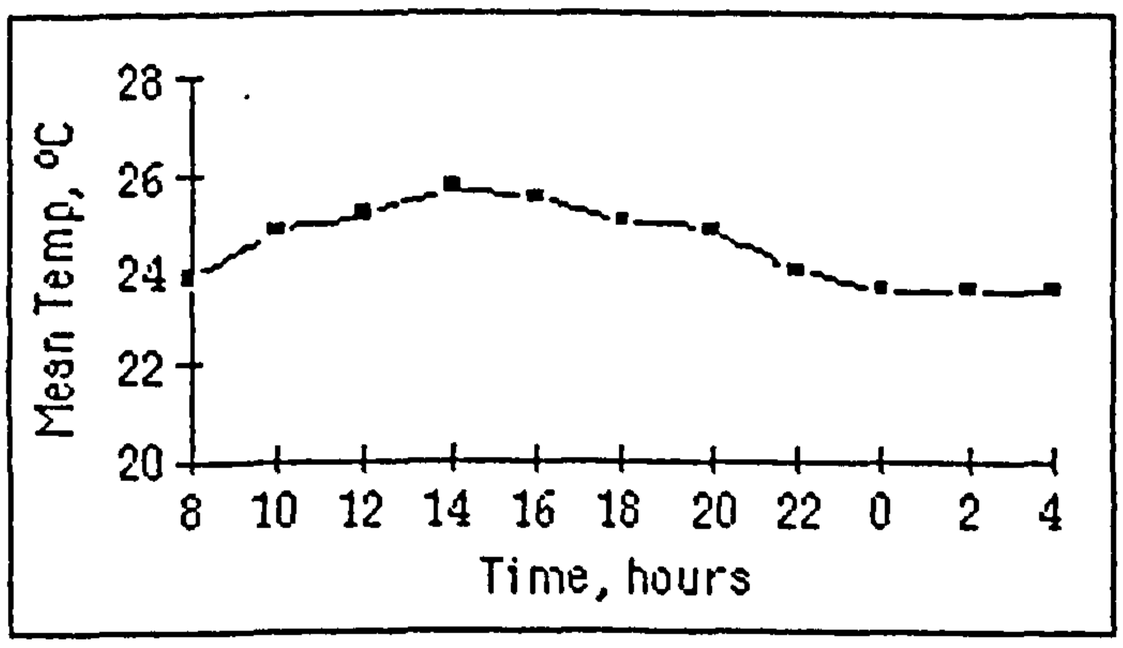
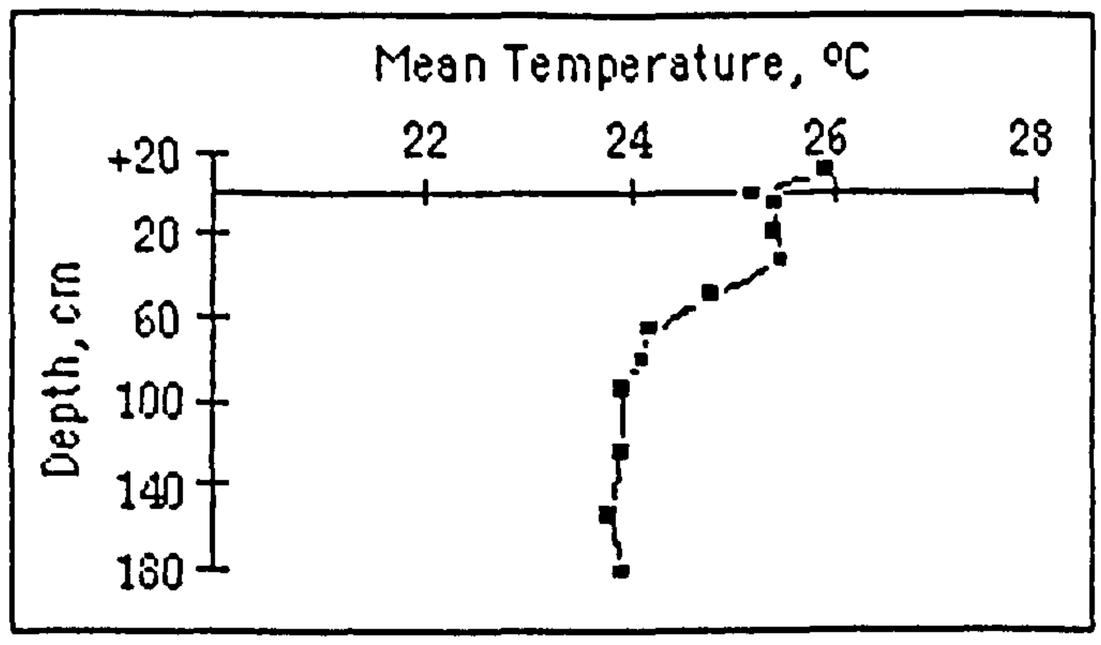
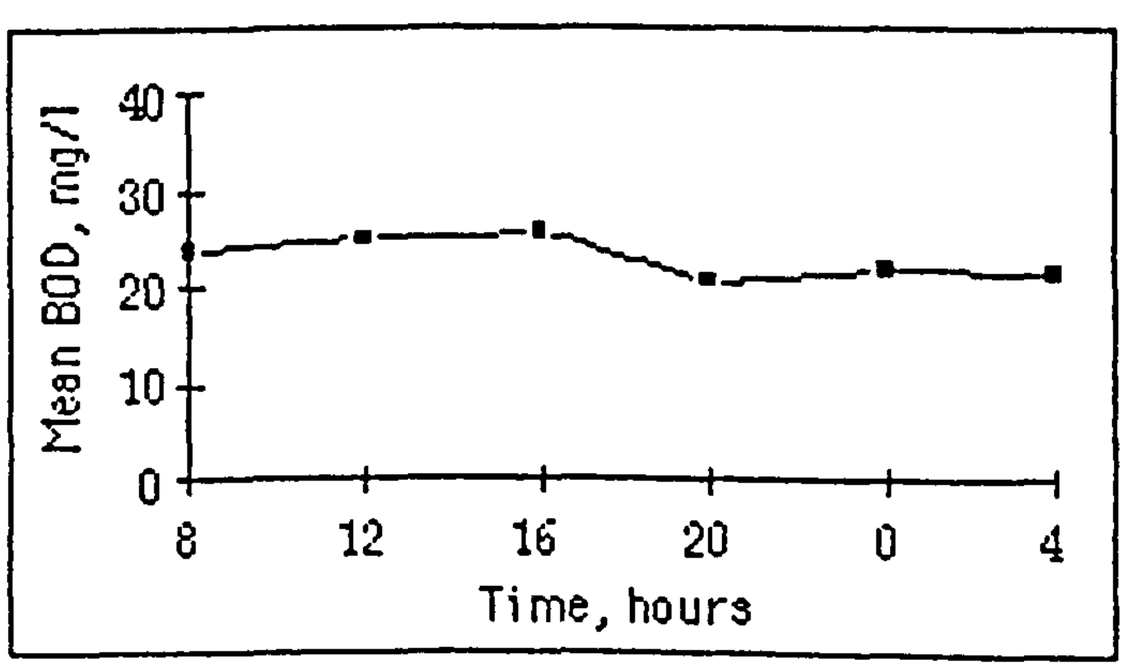
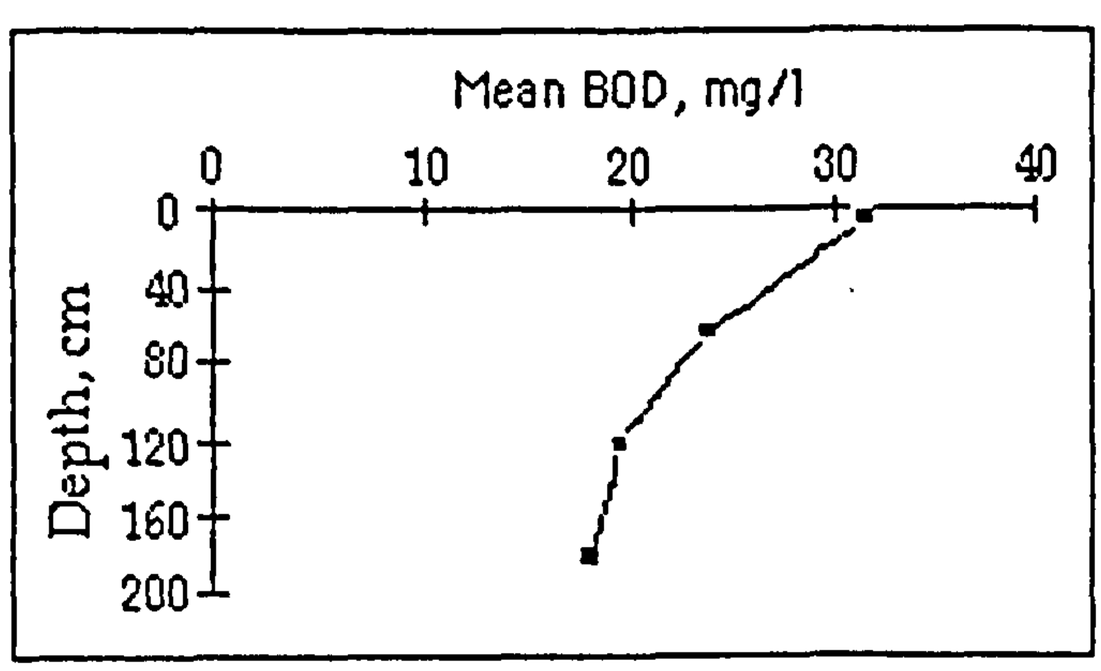


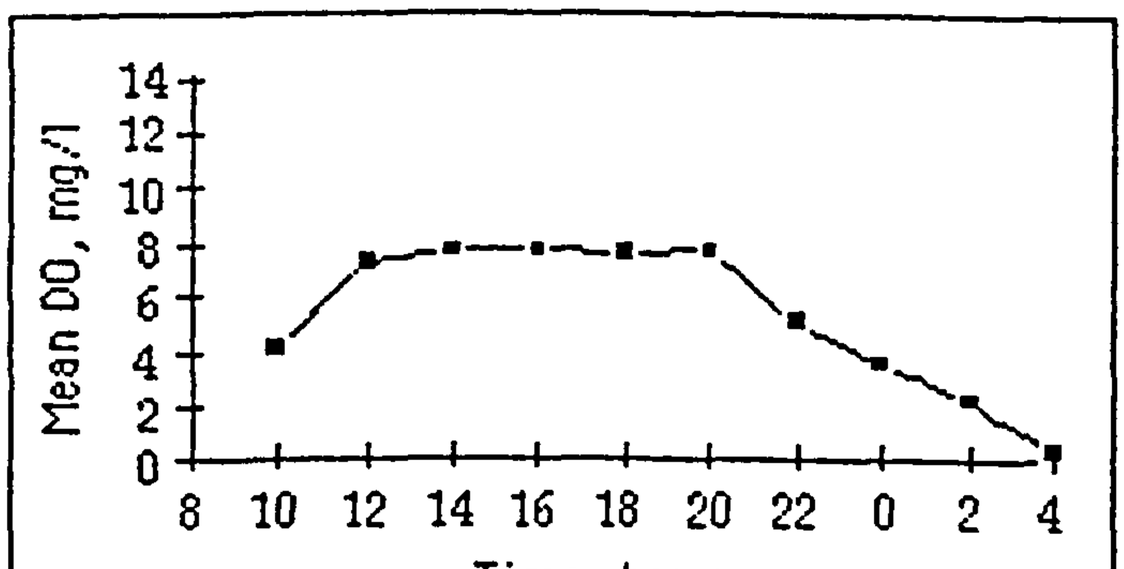
Figure A3/7 **Mean Results of the Profile carried out on F24 on 10. 2.93.**

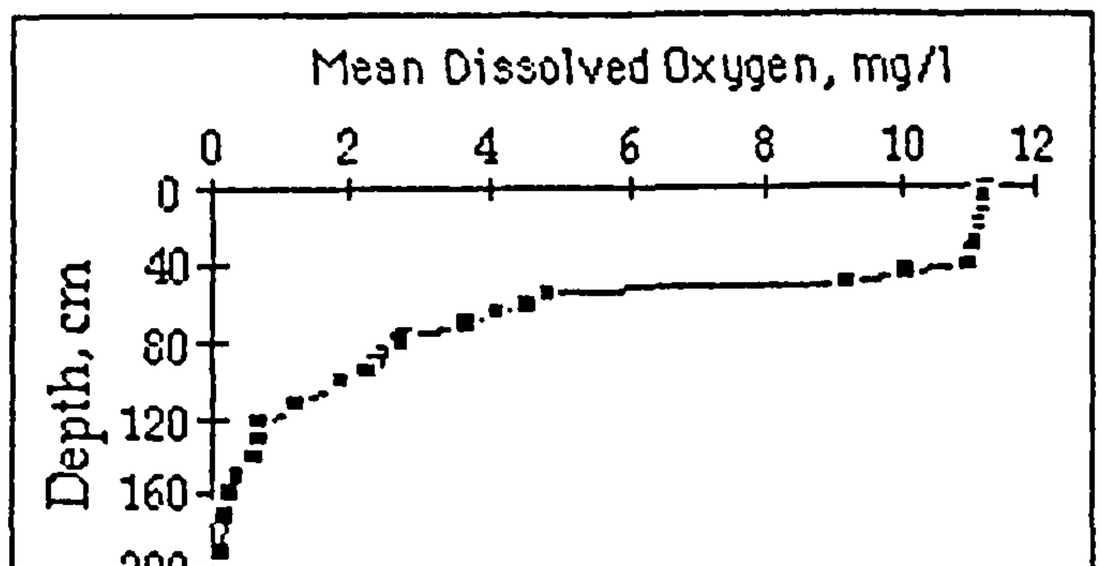


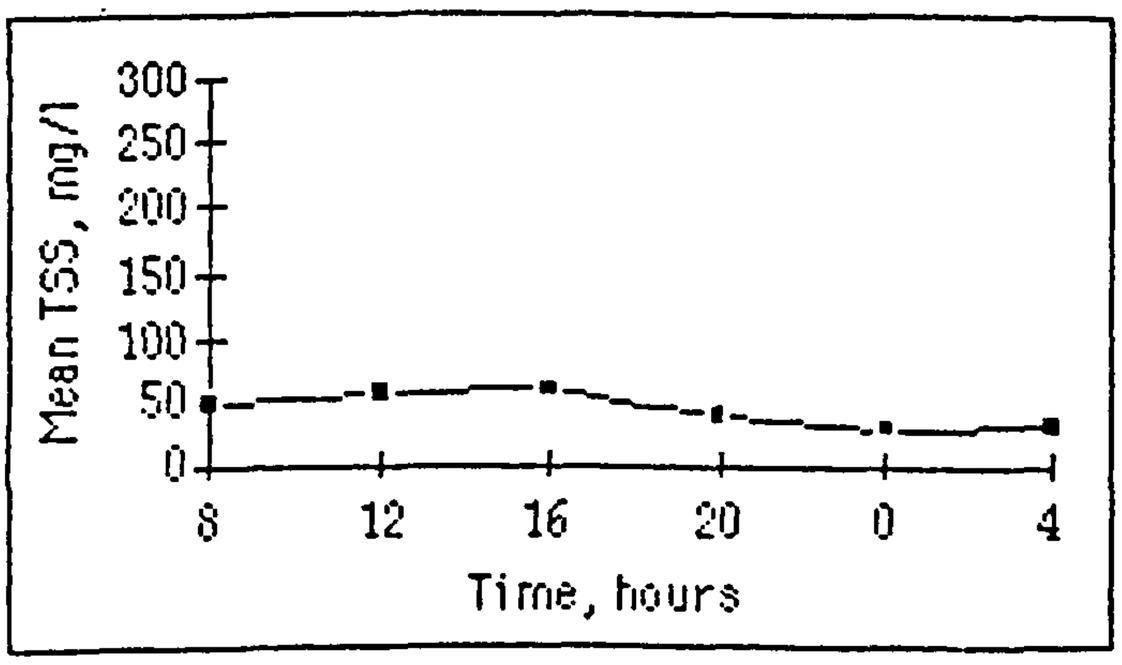












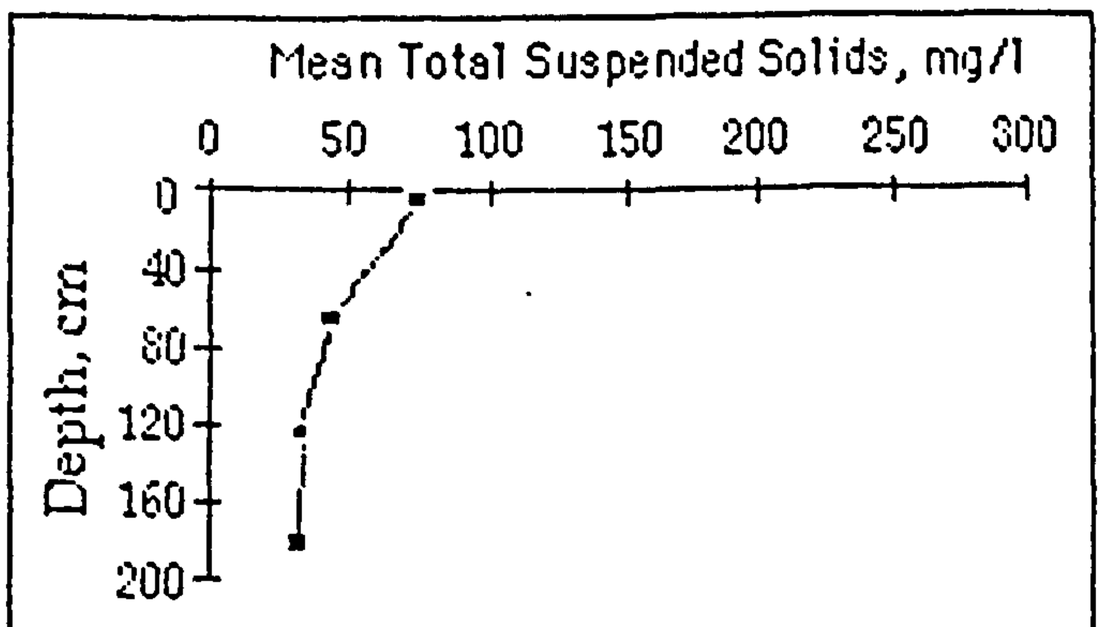
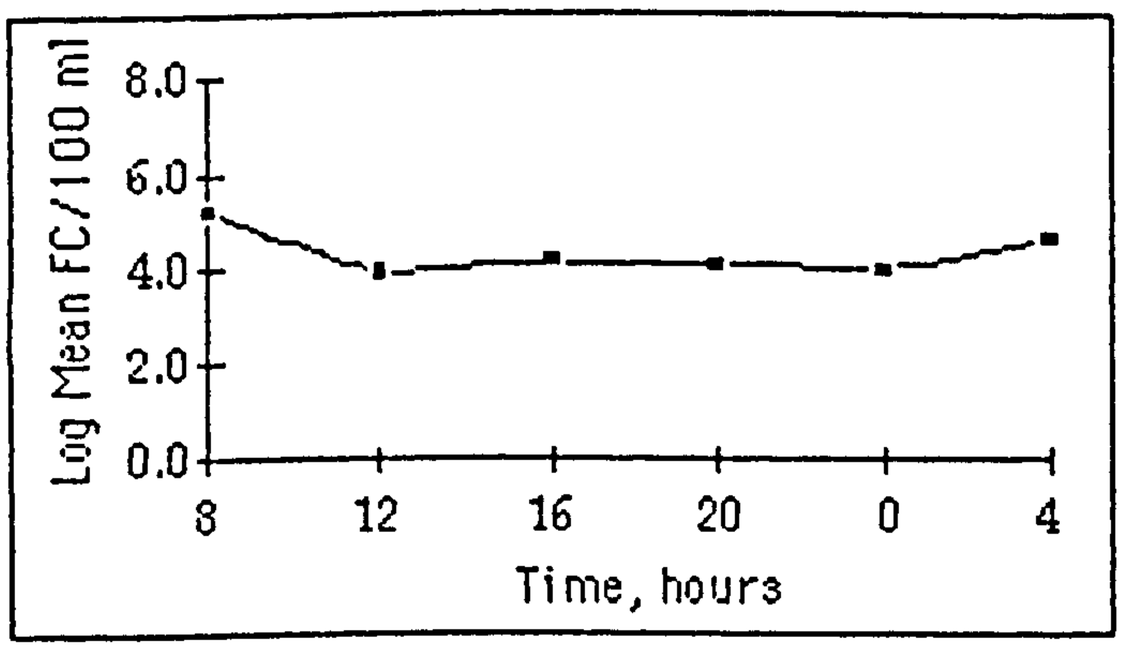
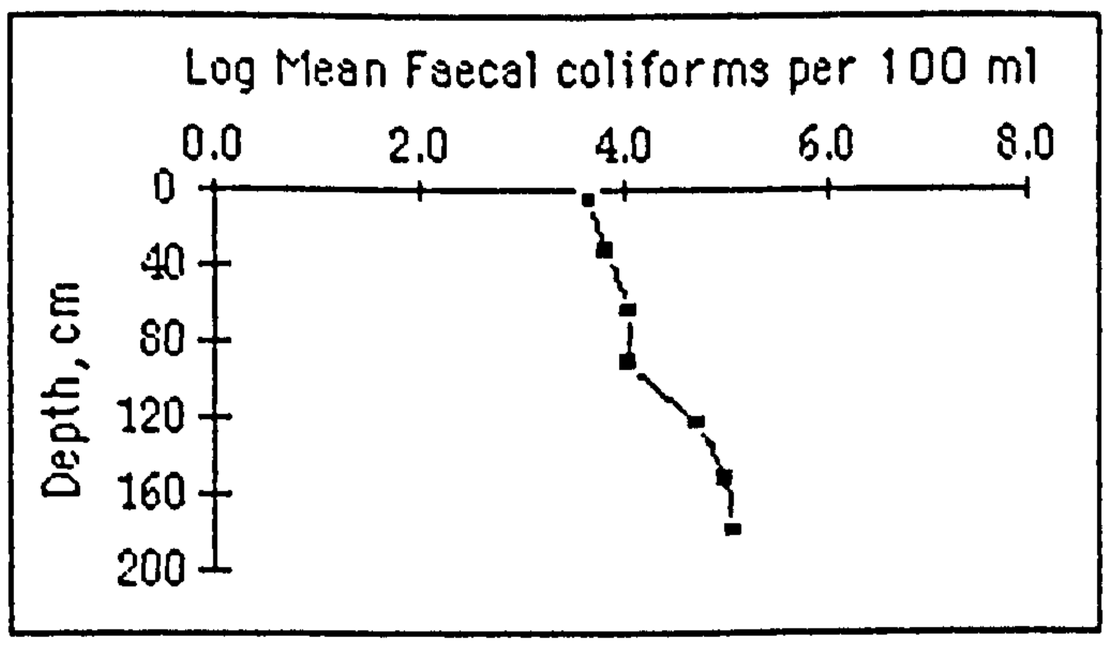
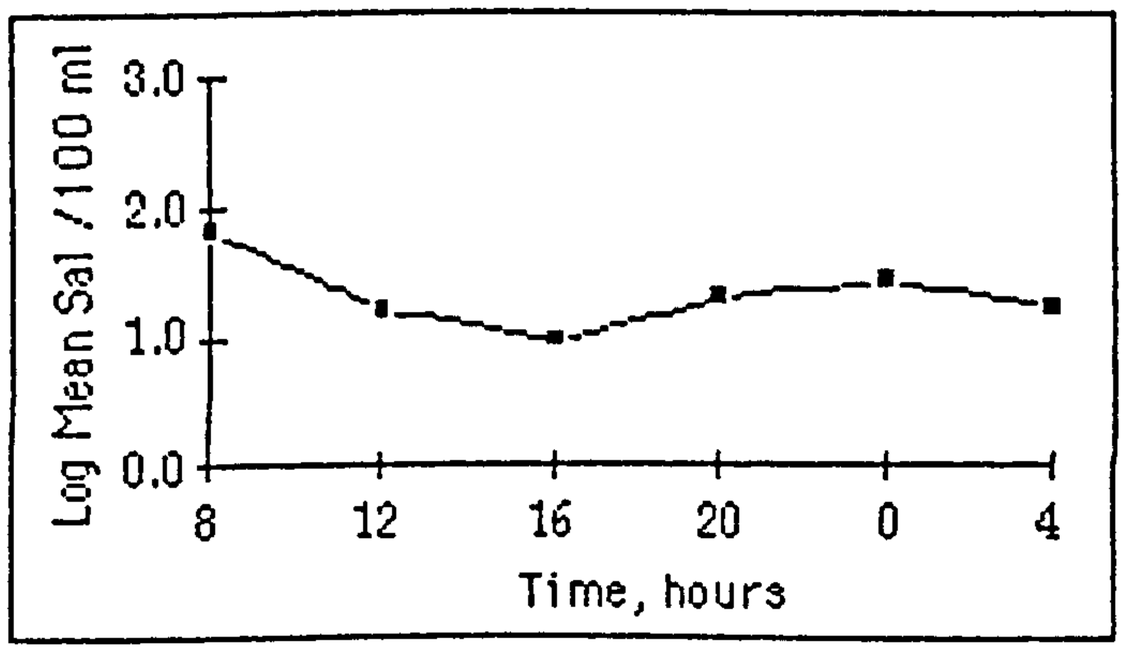
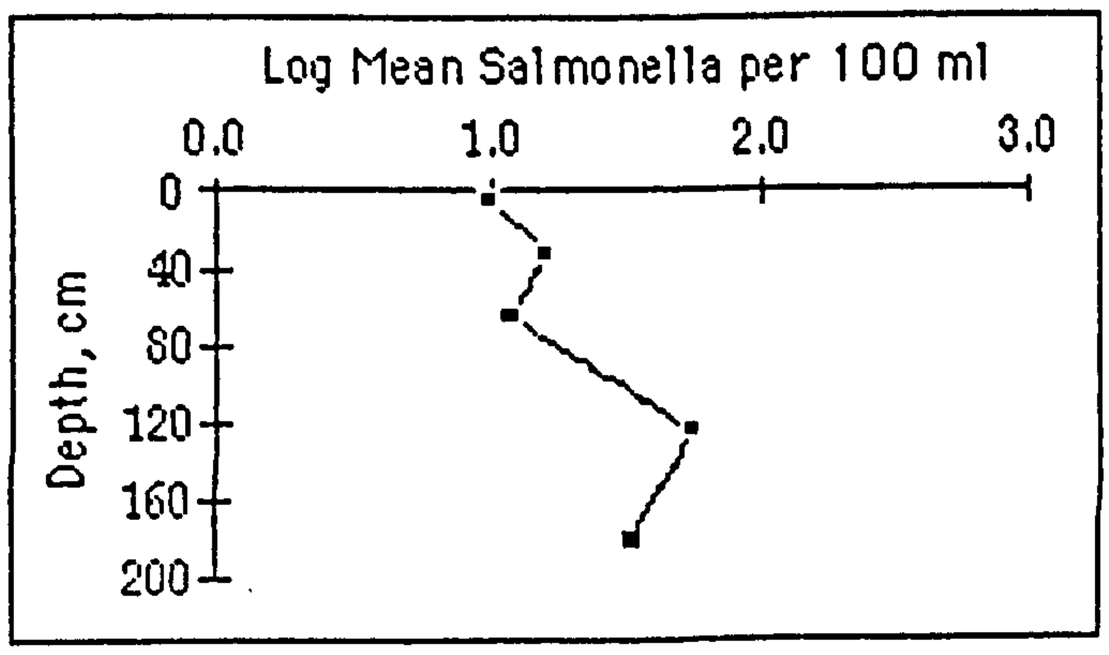


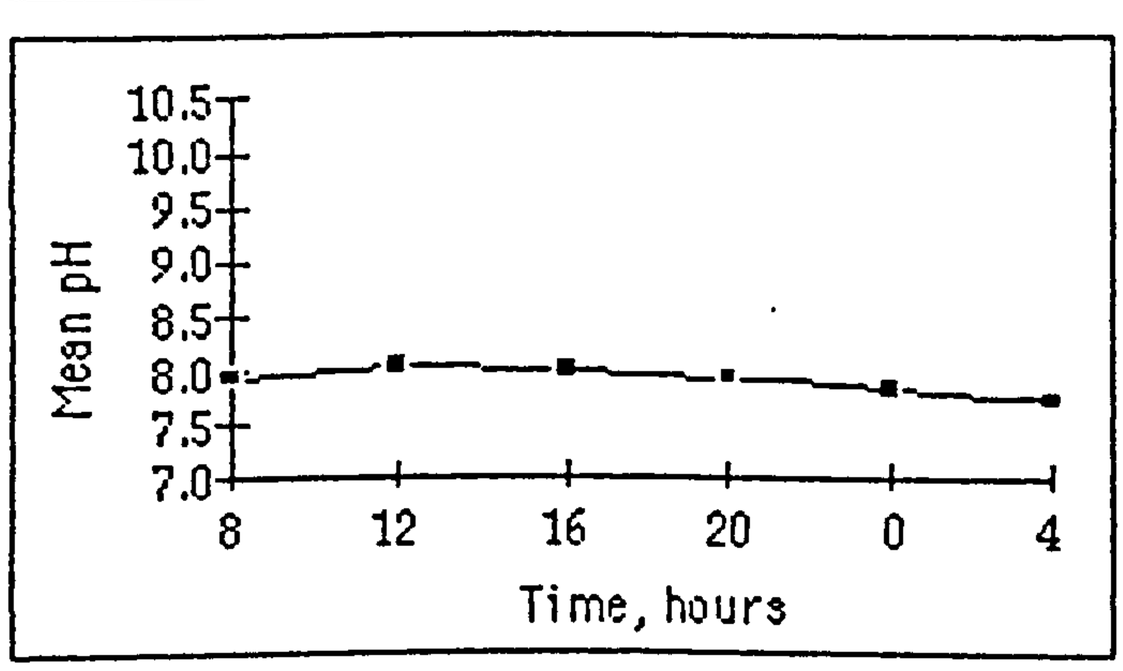
Figure A3/7b <u>Mean Results of the Profile carried out on F24 on 10. 2.93.</u>











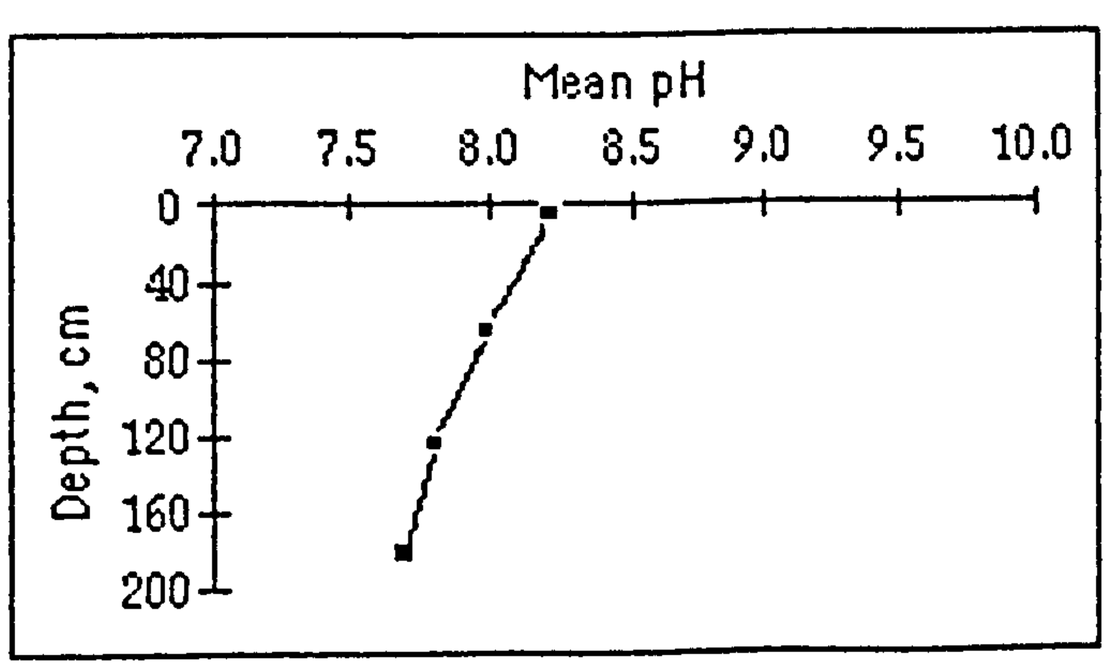
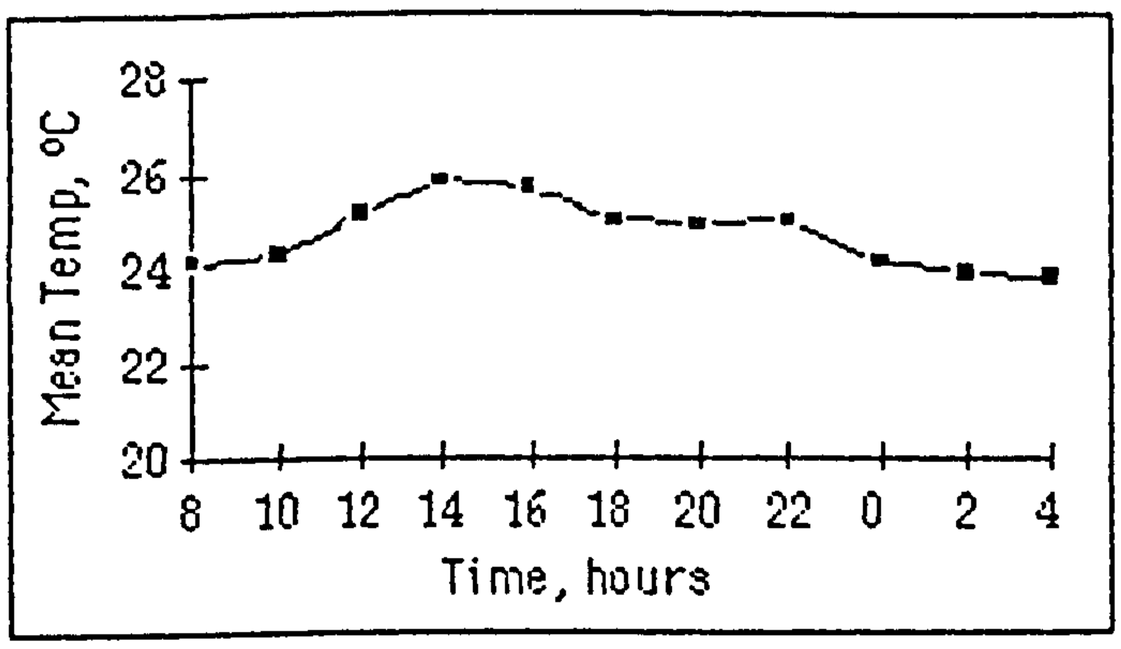
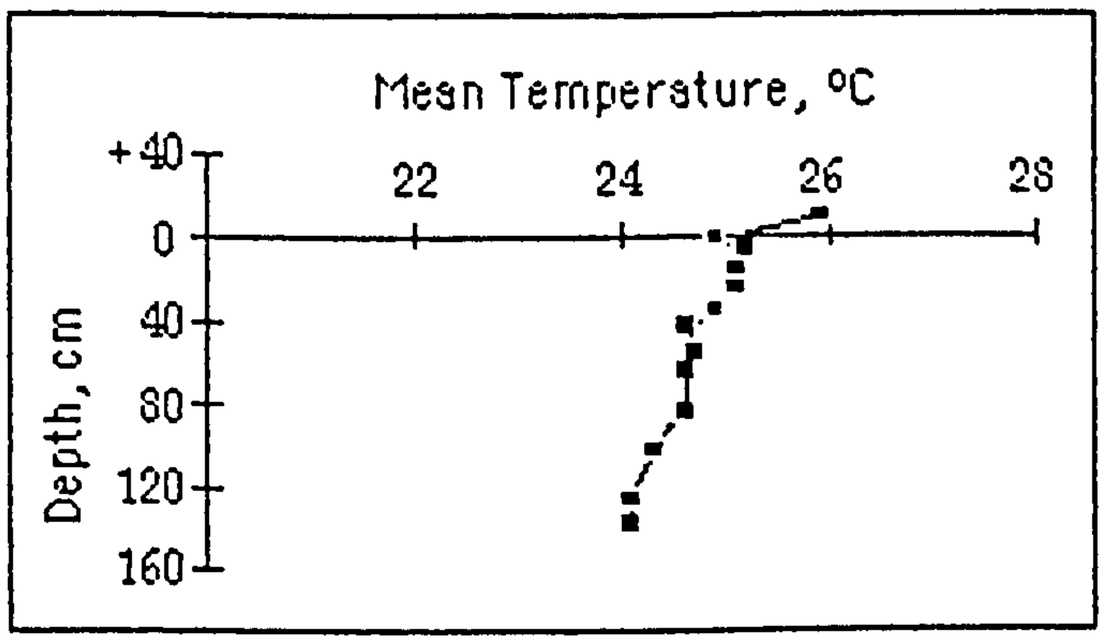
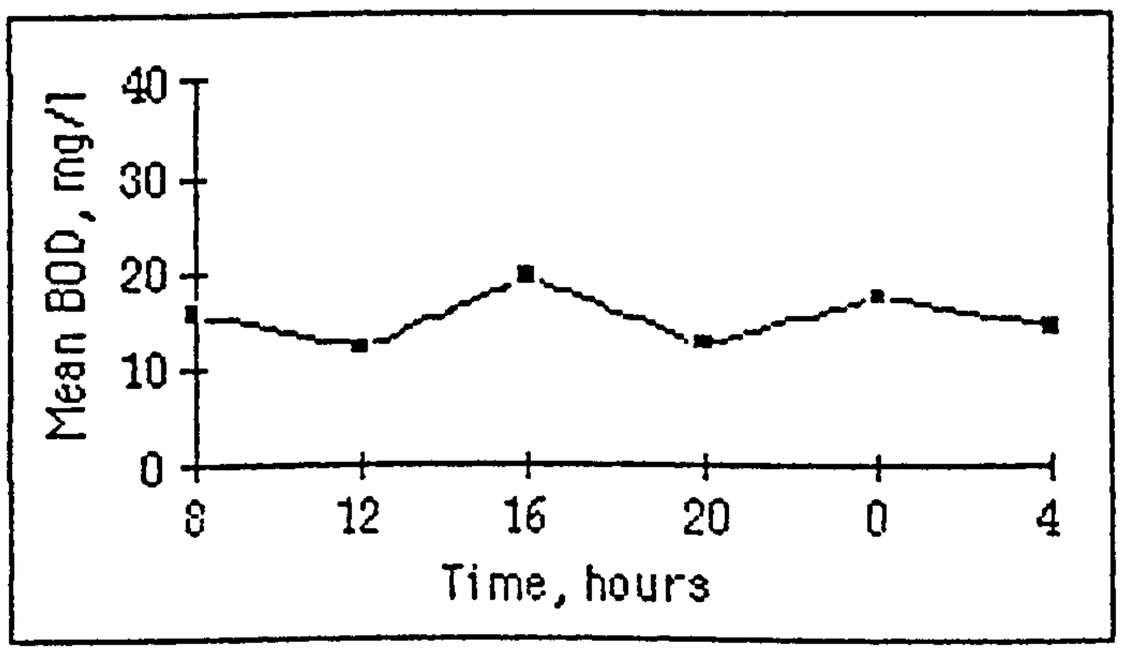
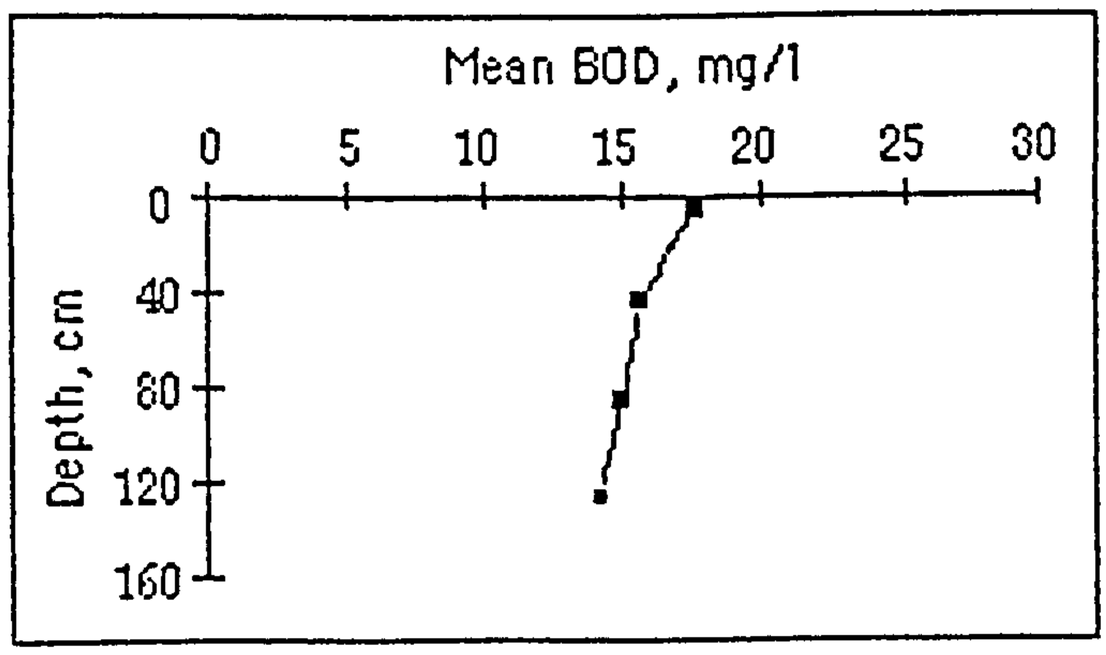


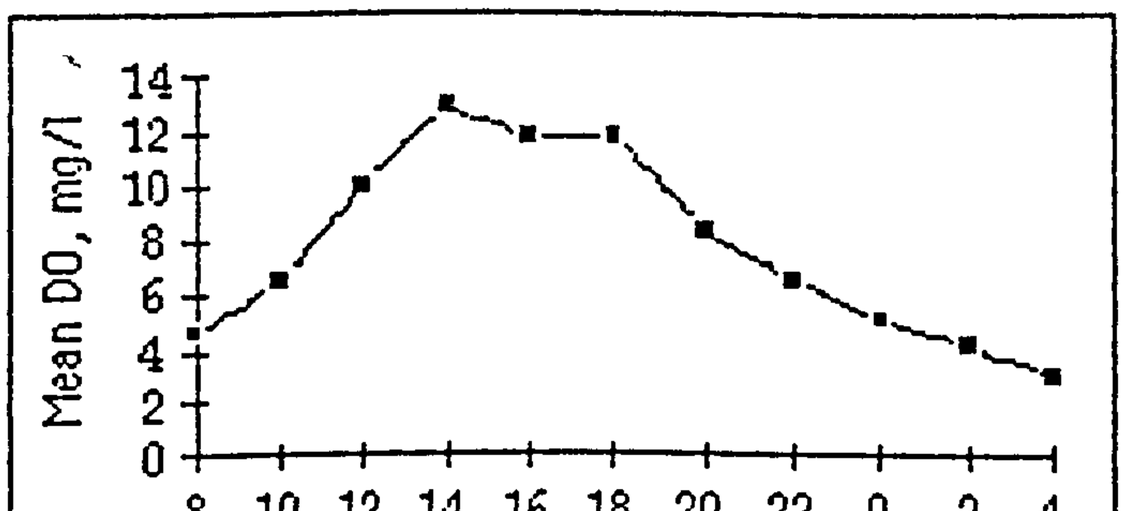
Figure A3/8 **Mean Results of the Profile carried out on M31 on 17.2.93.**

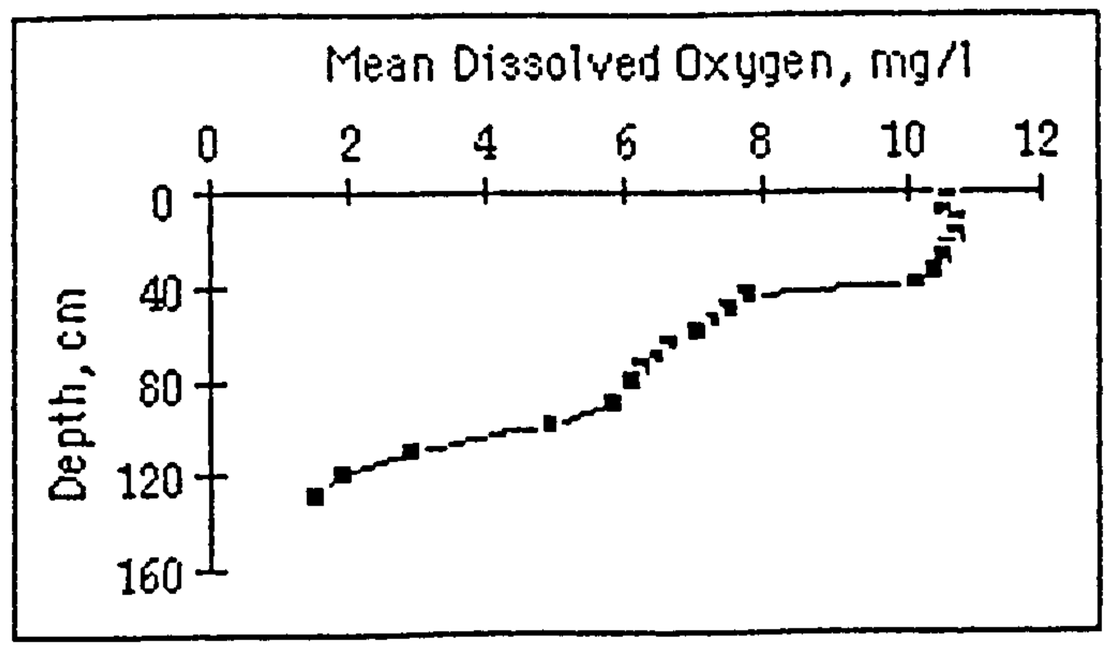


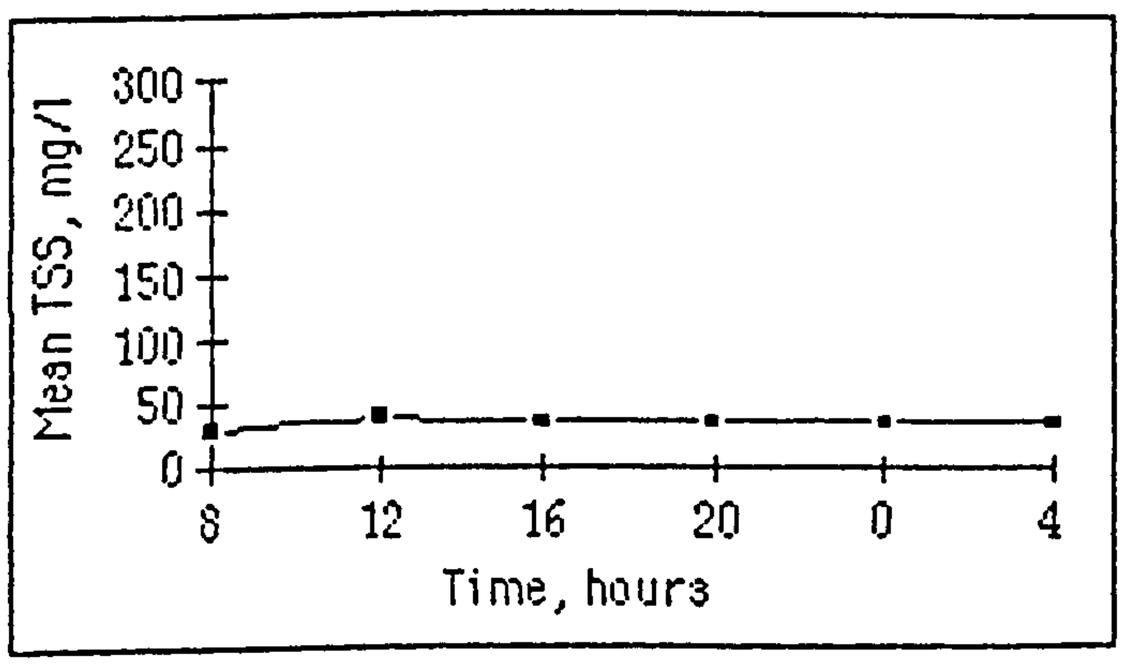












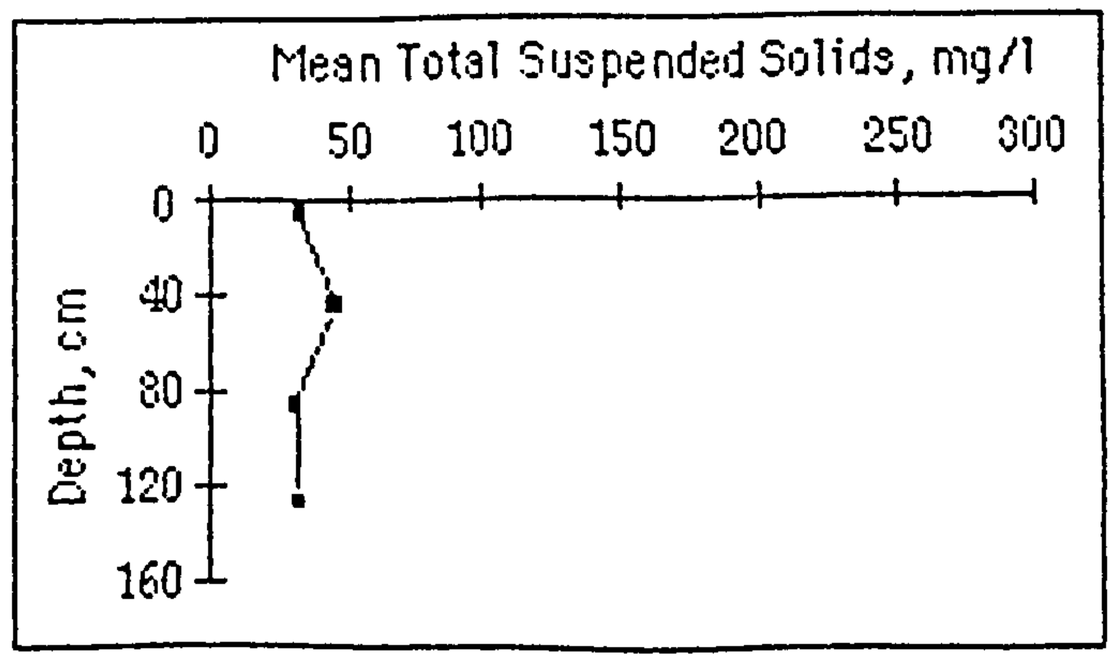
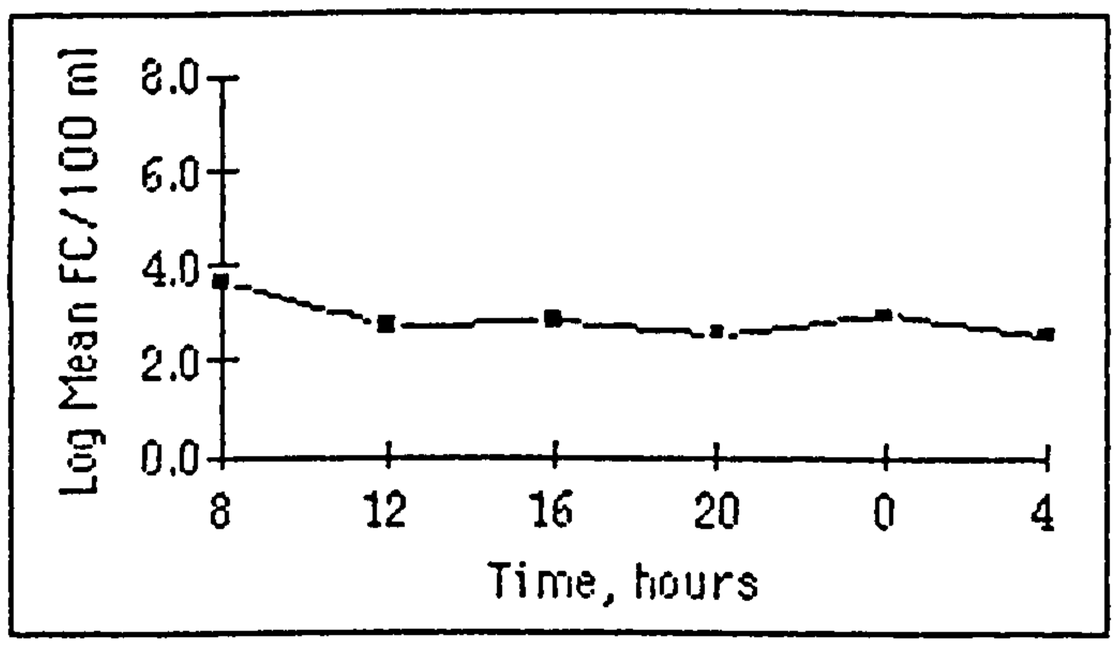
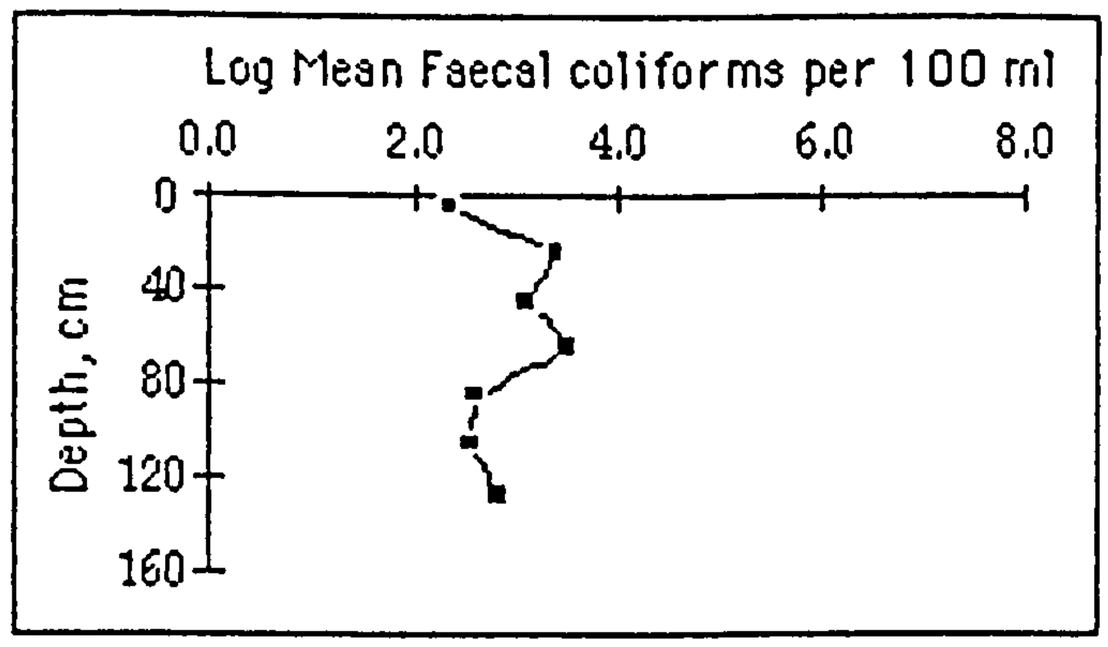


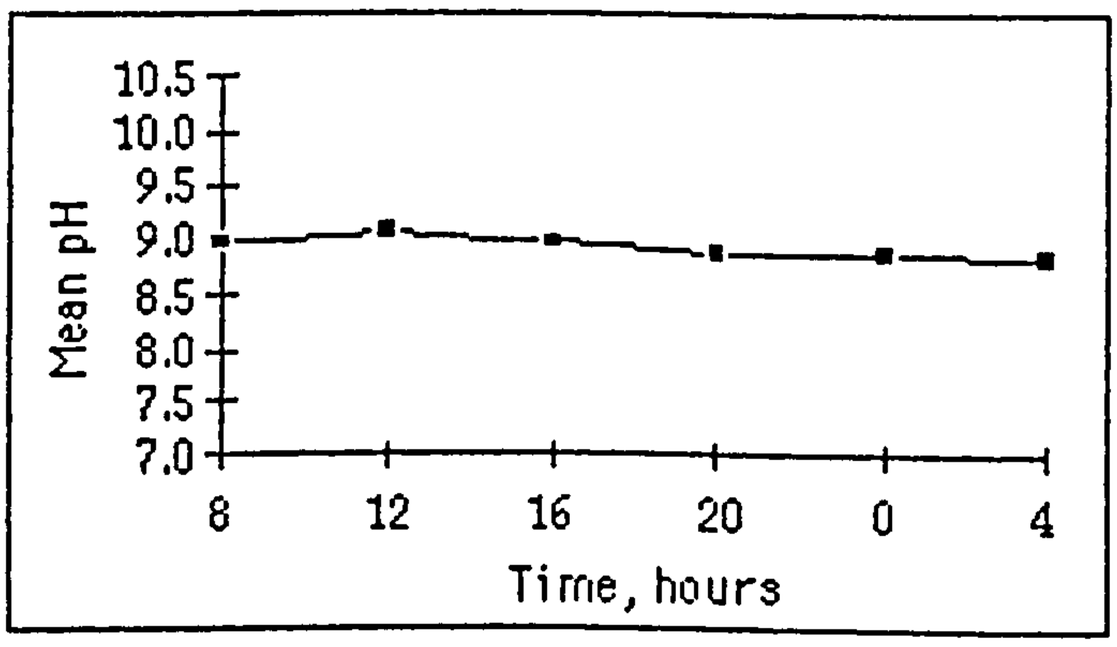
Figure A3/8b <u>Mean Results of the Profile carried out on M31 on 17. 2.93.</u>

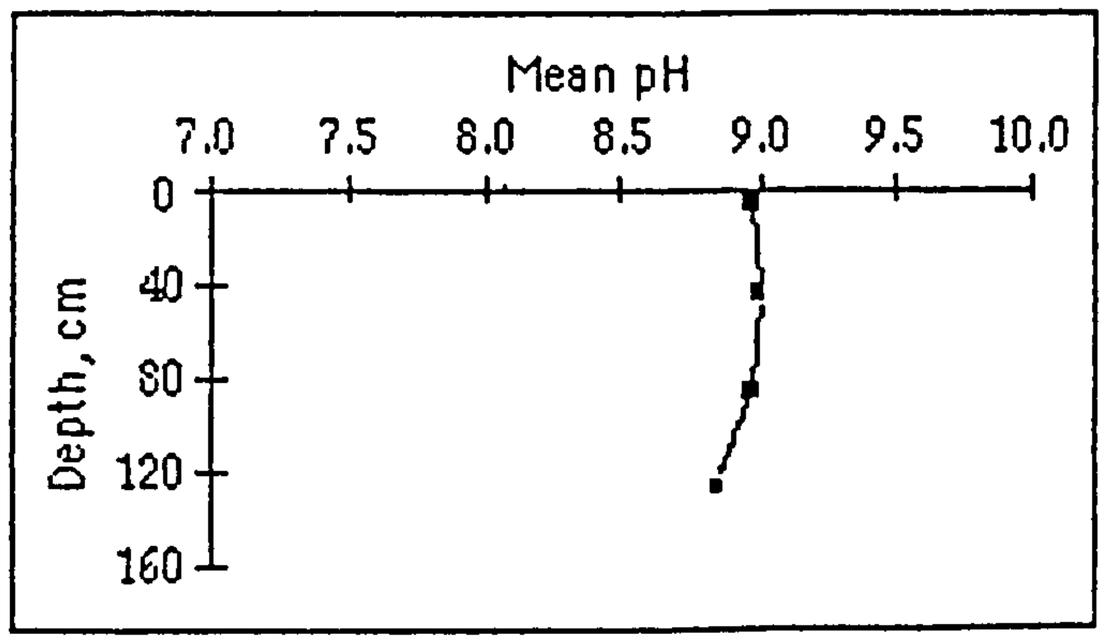


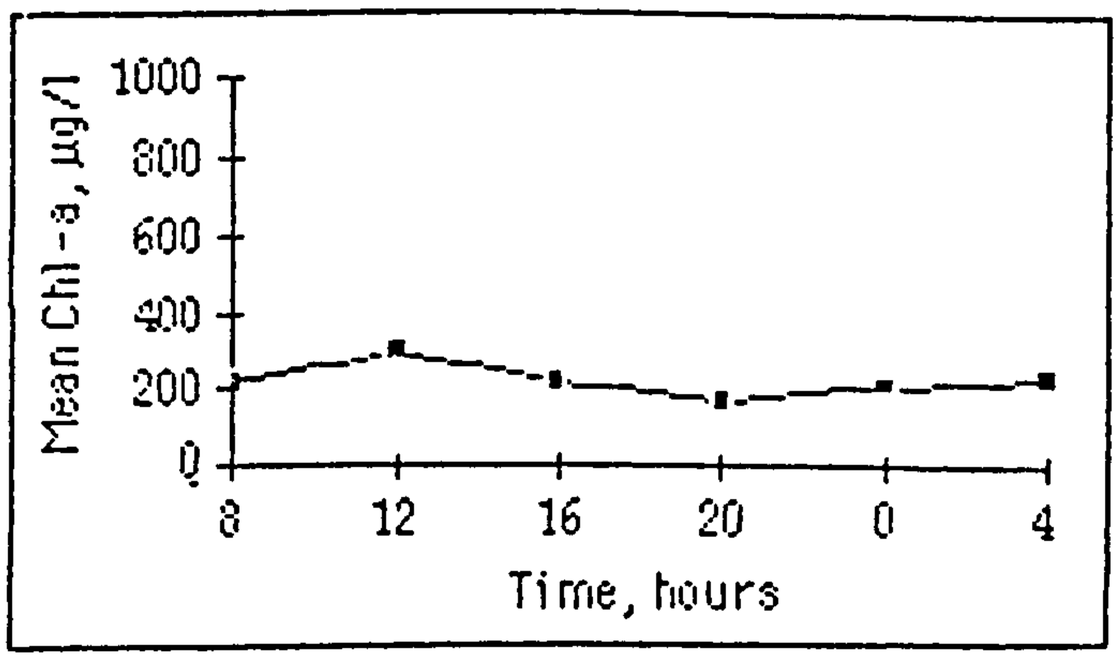


No *Salmonella* Detected

No *Salmonella* Detected







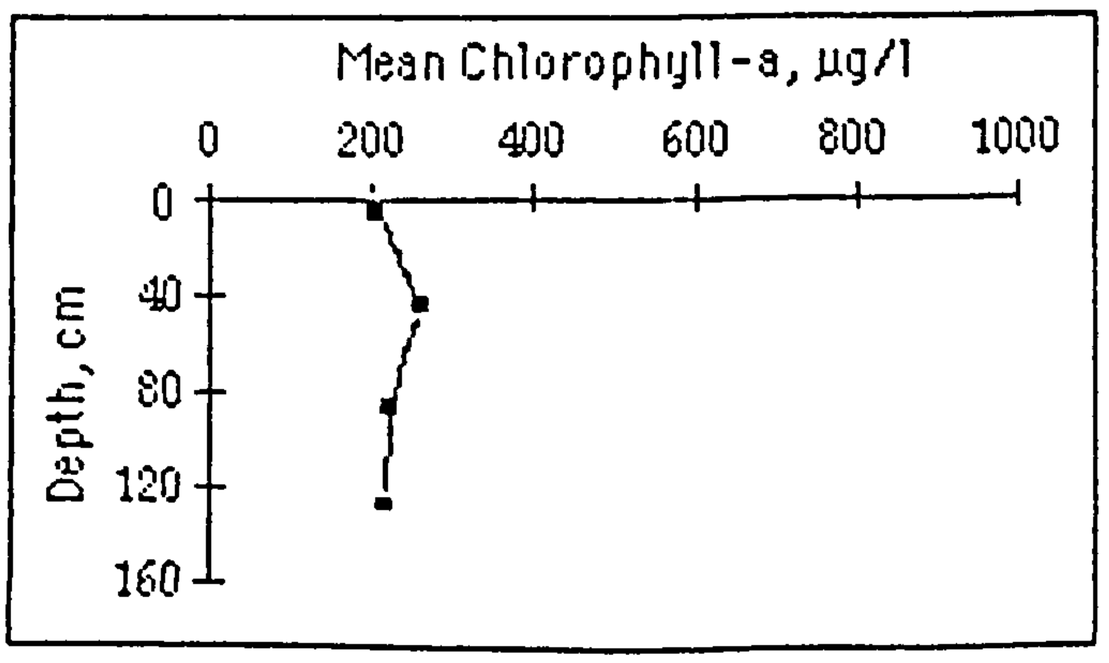
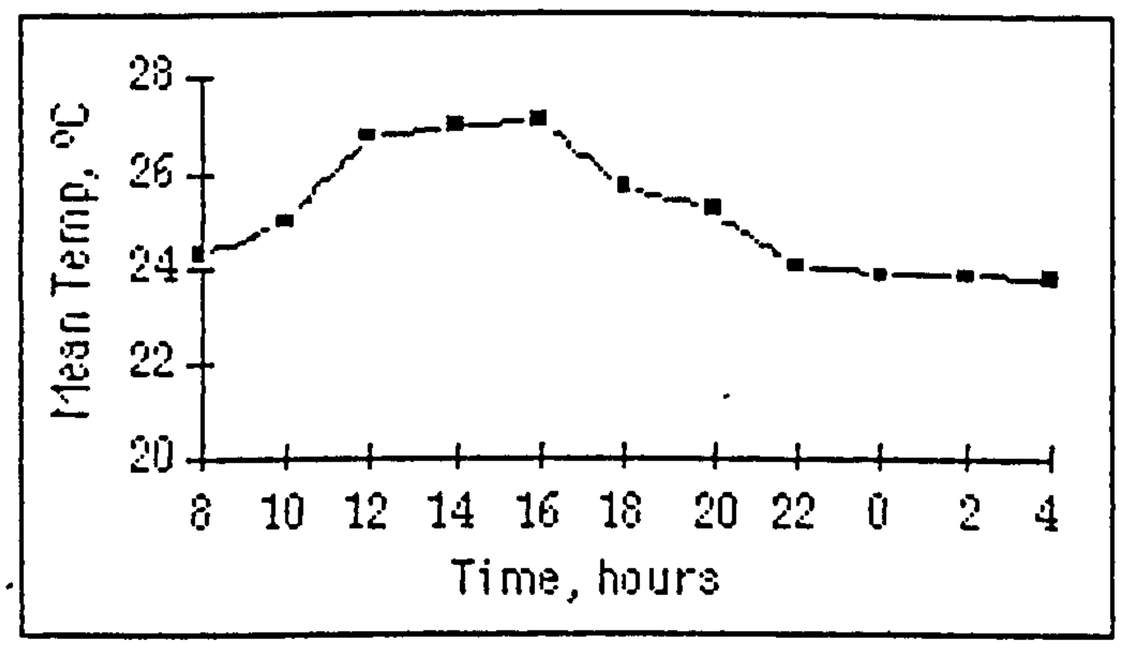
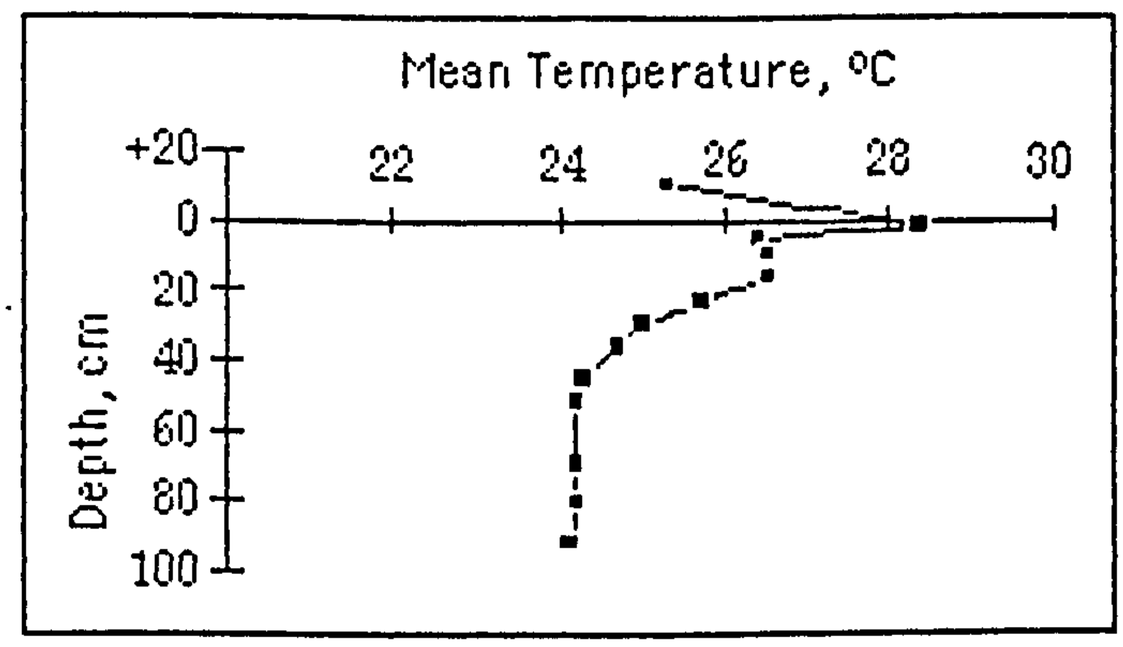


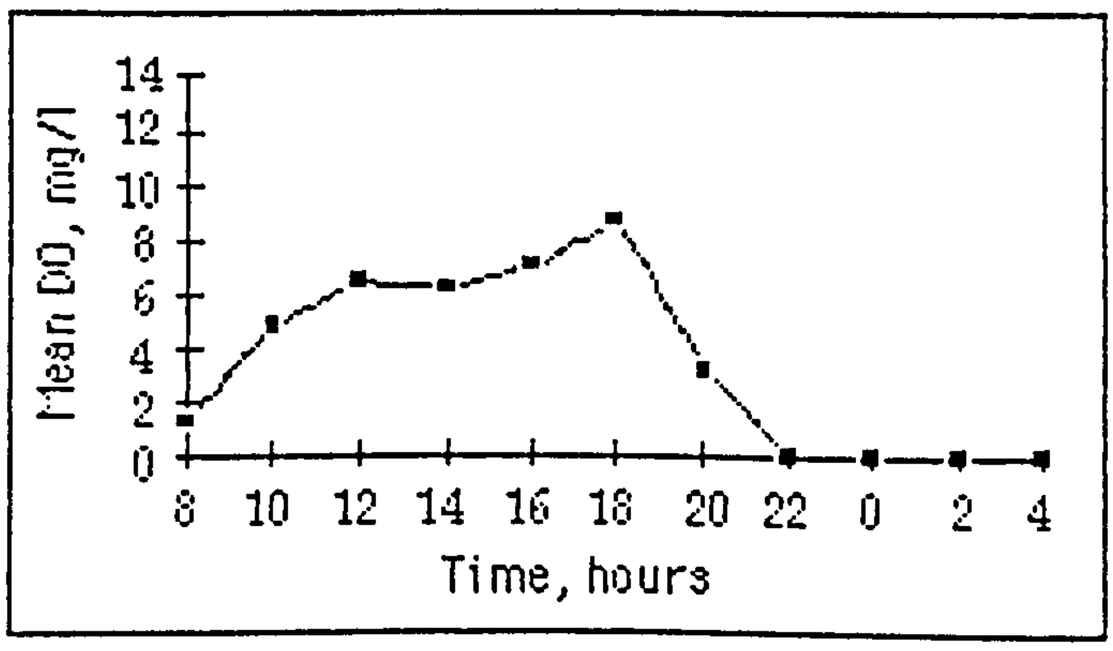
Figure A3/9 **Mean Results of the Profile carried out on F21 on 10. 3.93.**

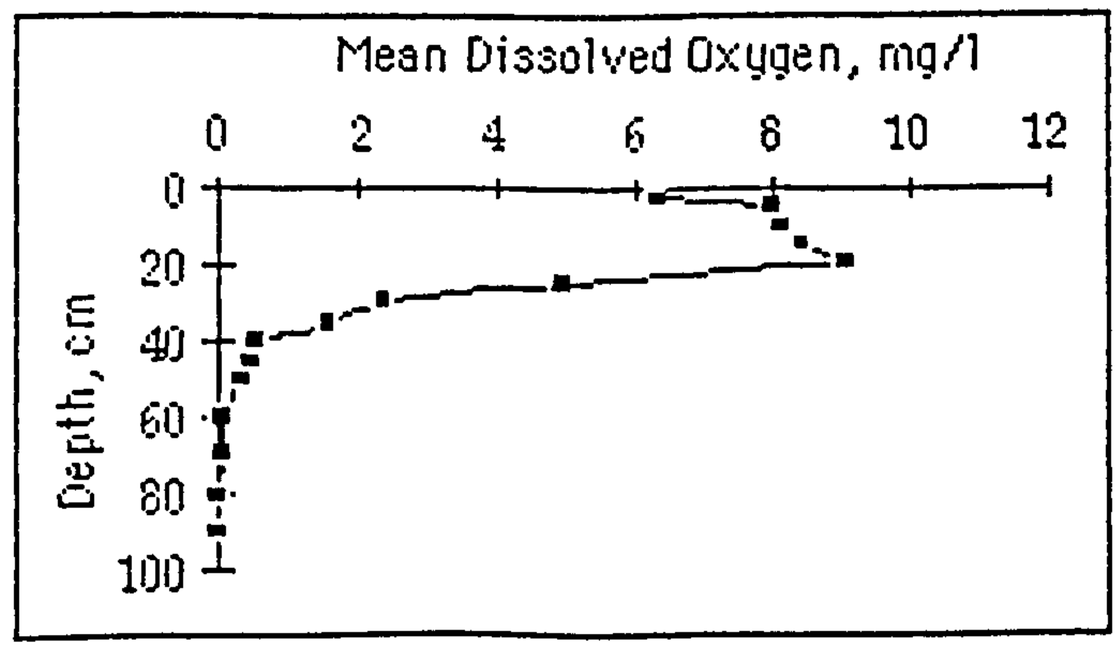


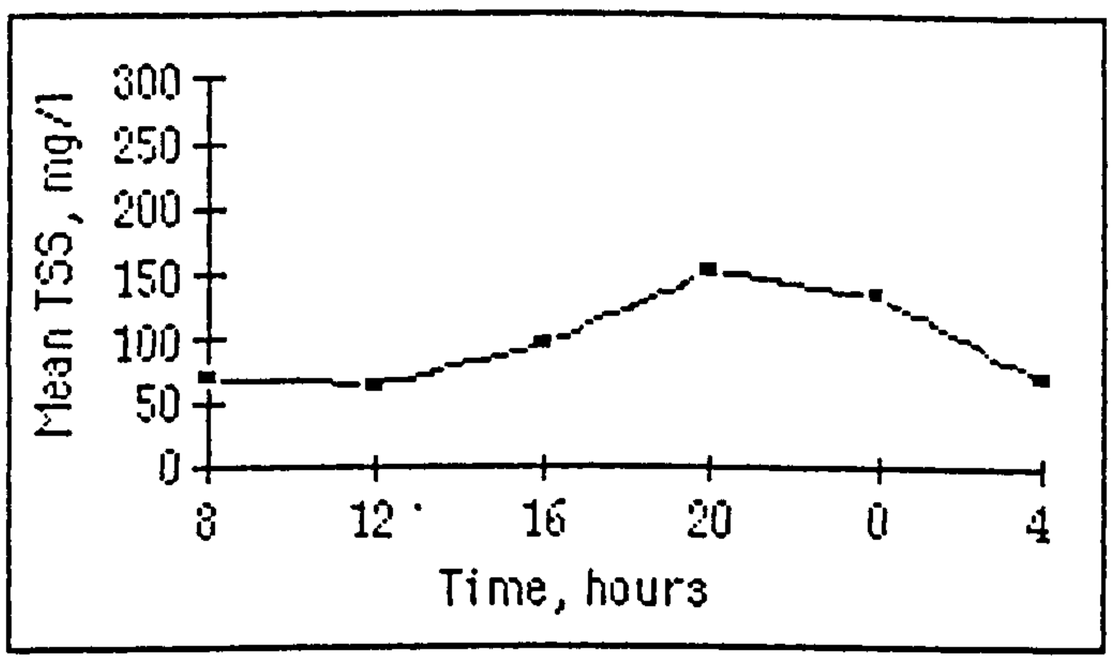


No BOD Results

No BOD Results







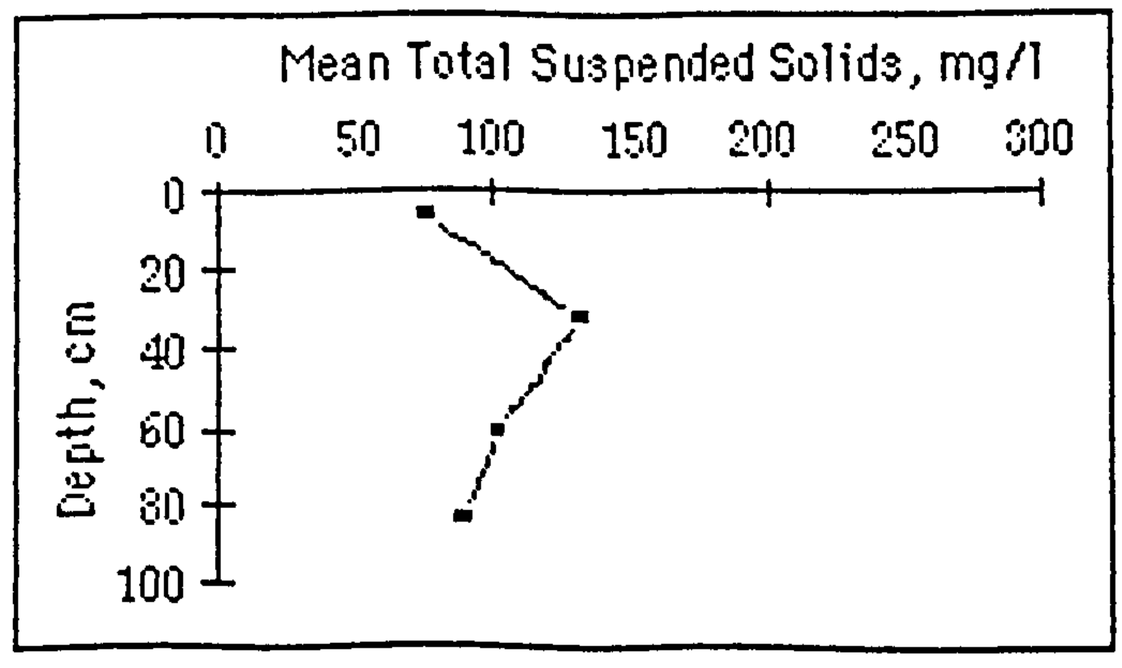
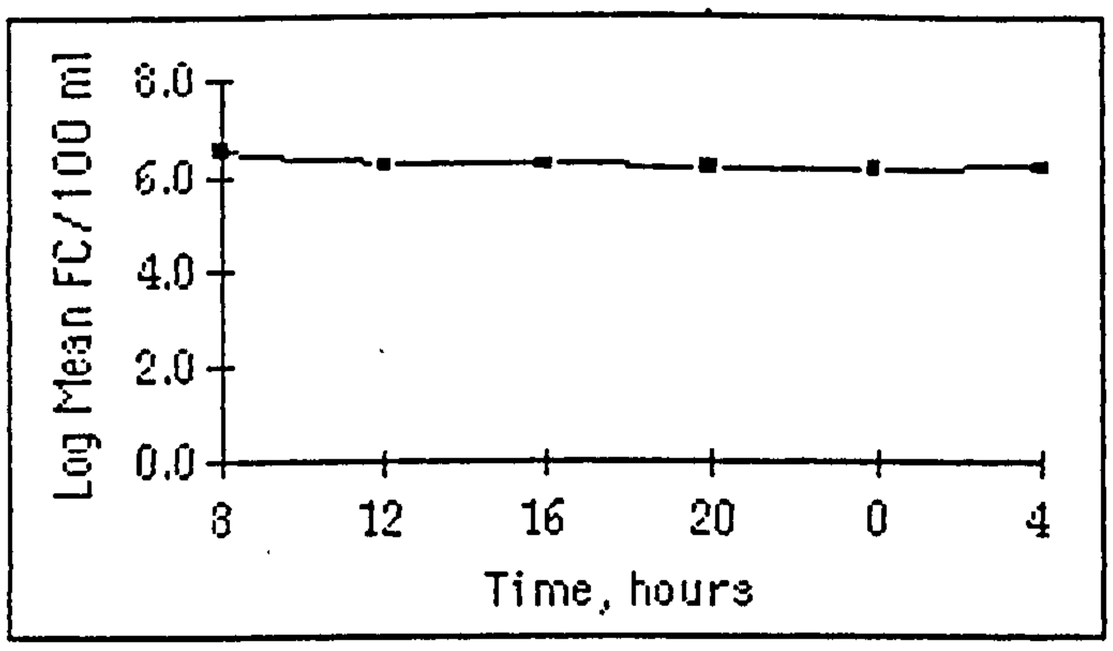
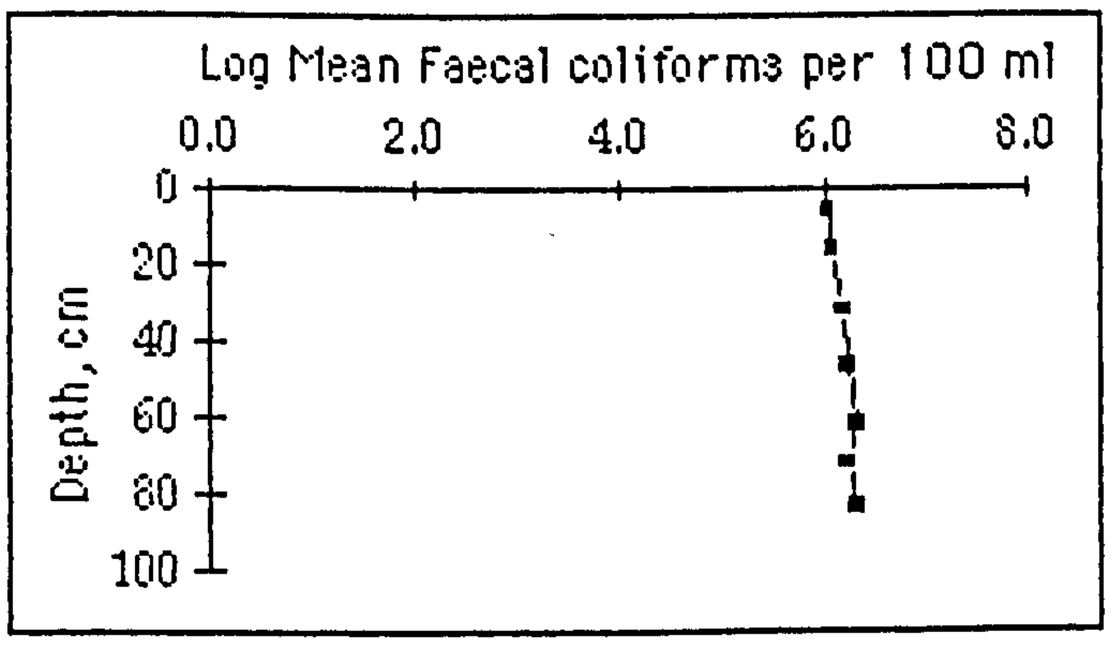
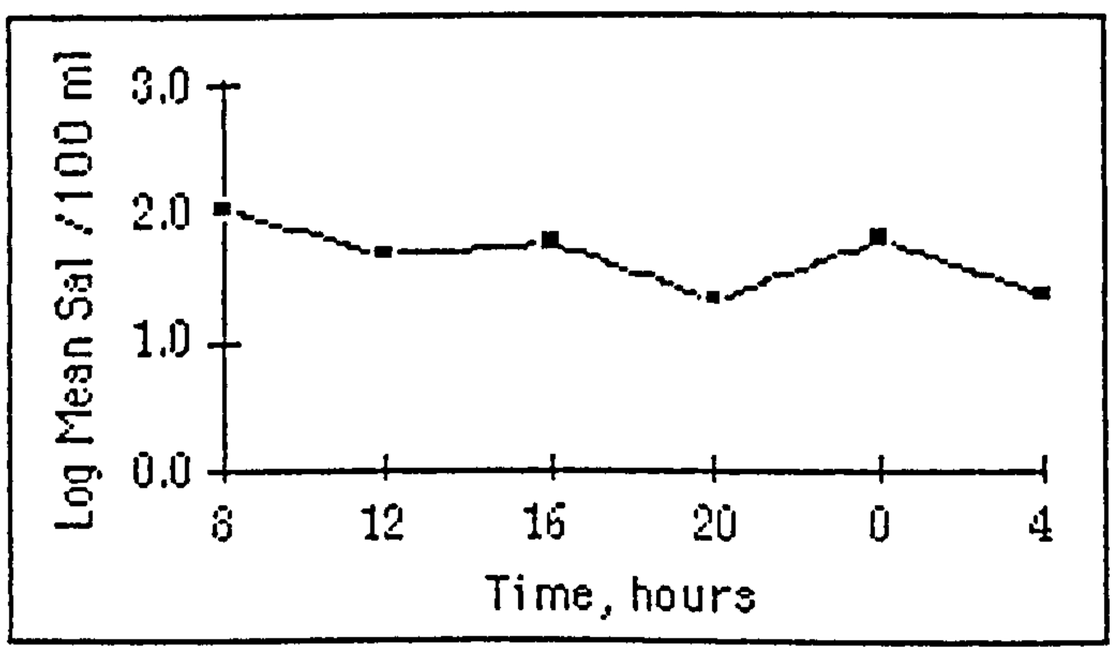
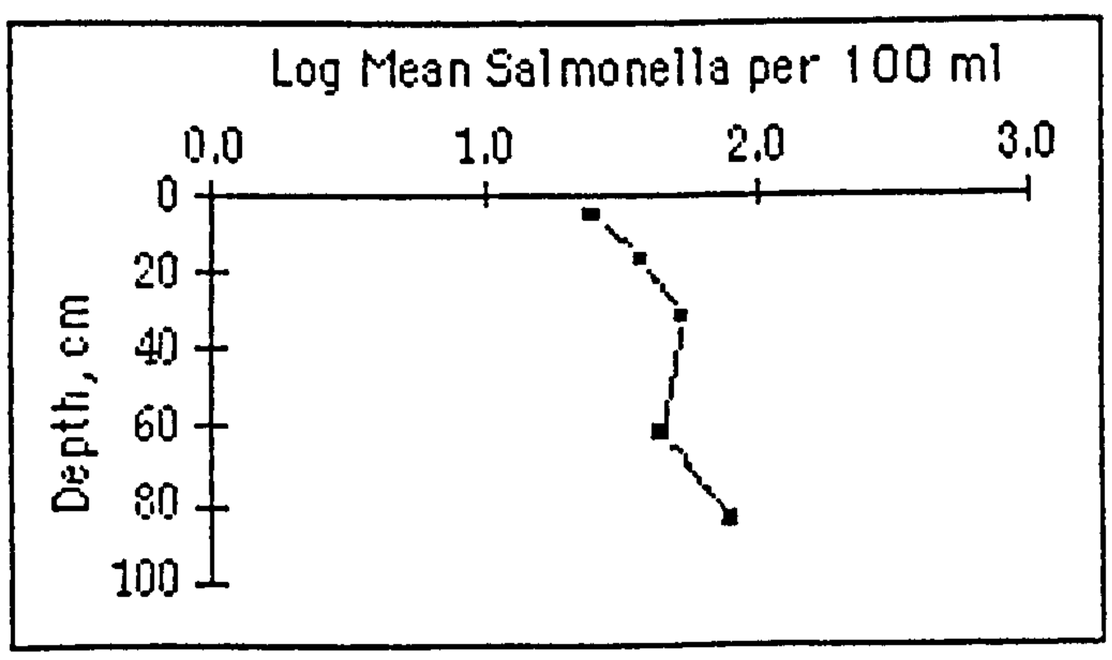


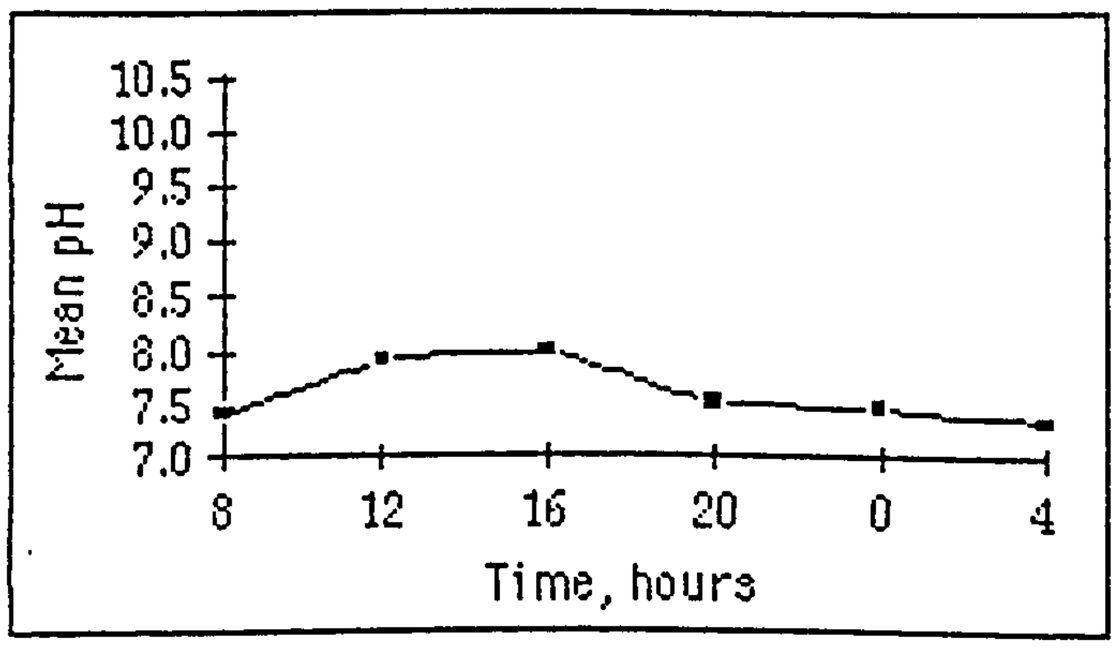
Figure A3/9b **Mean Results of the Profile carried out on F21 on 10. 3.93.**

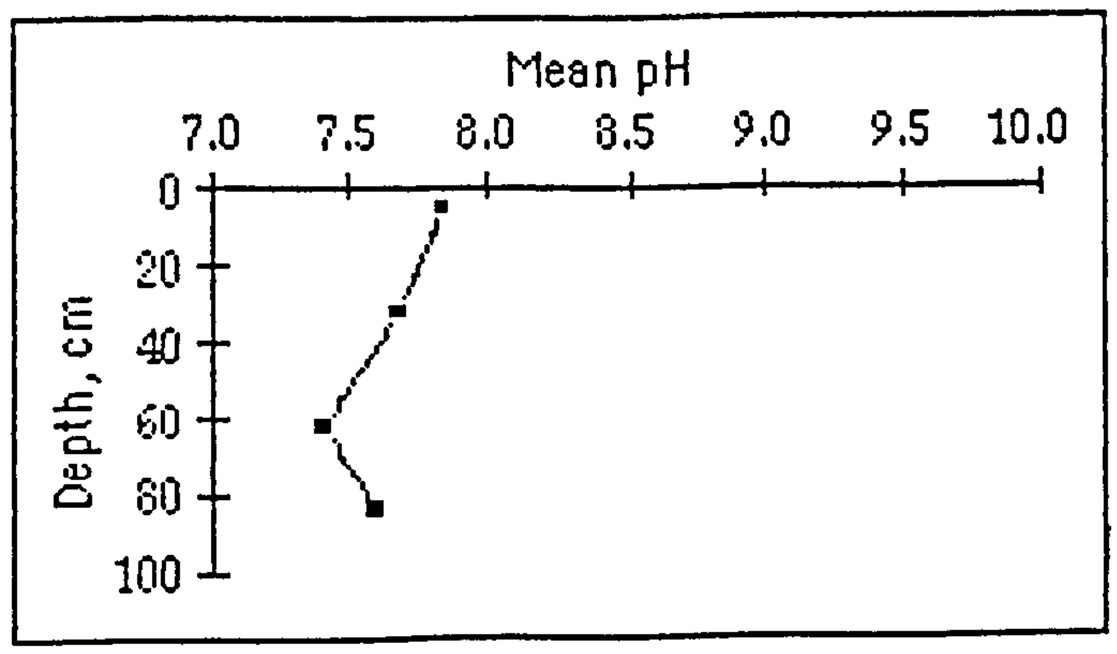


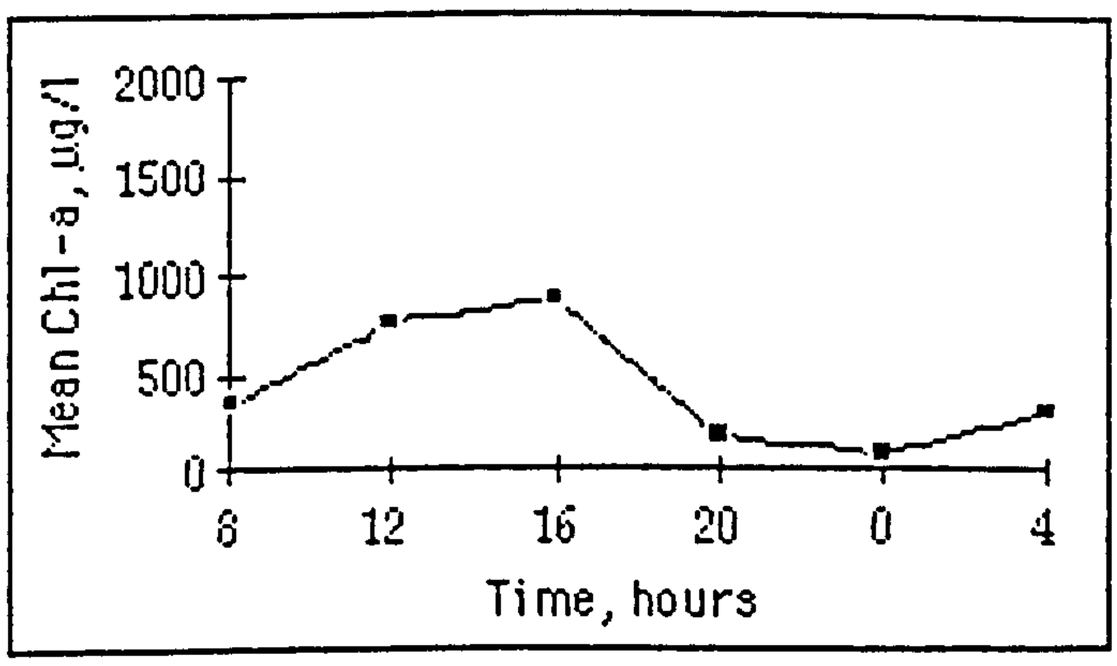












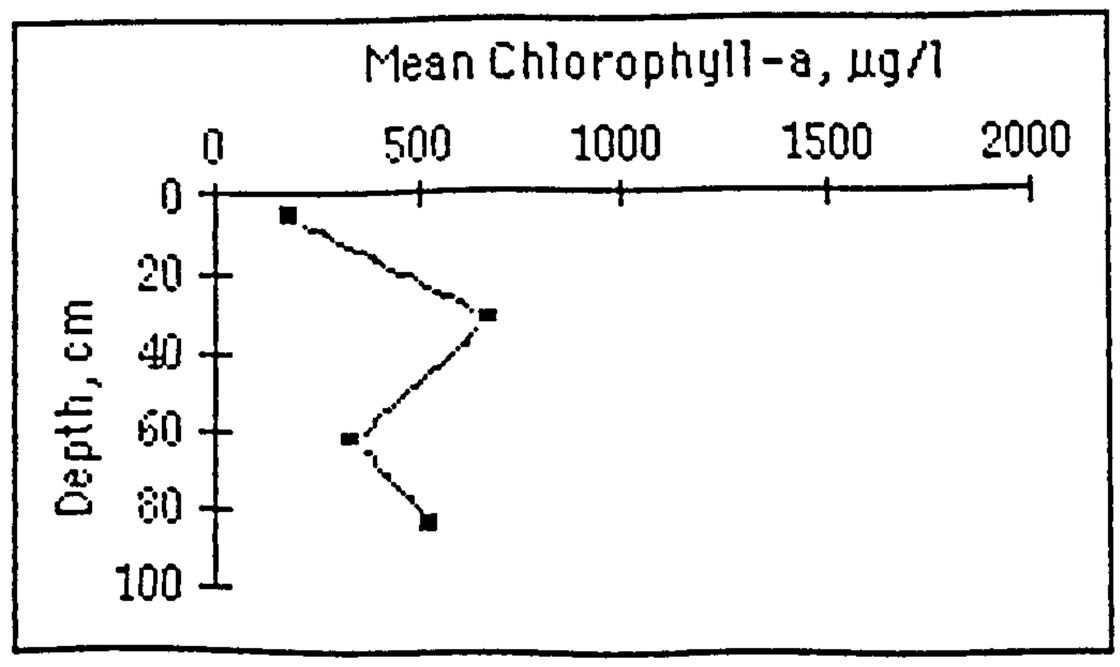
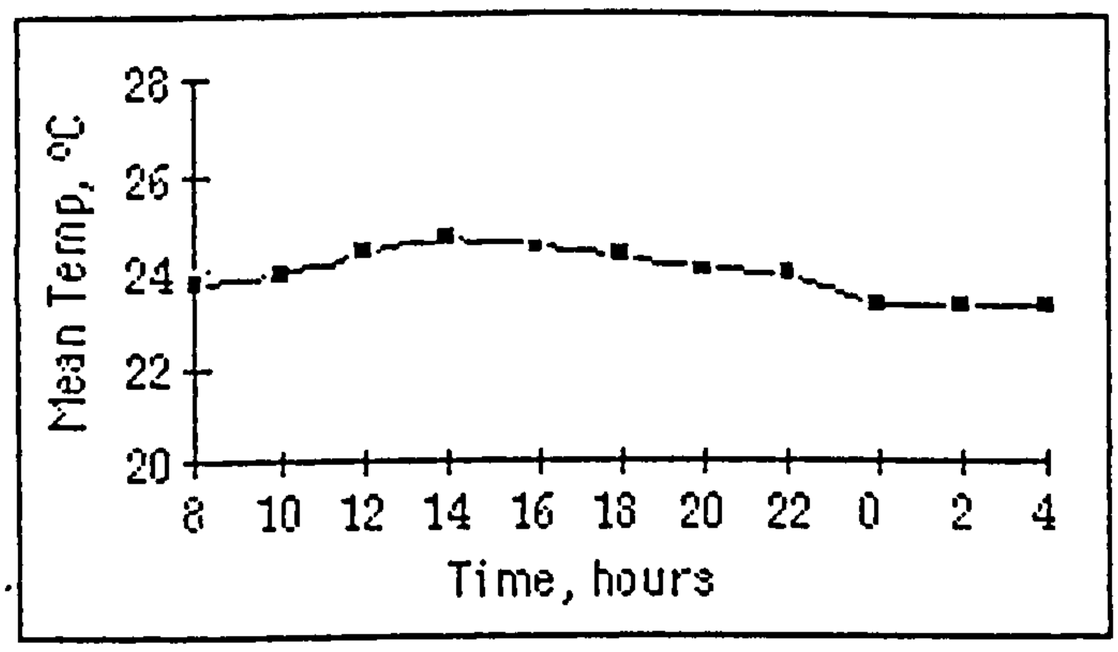
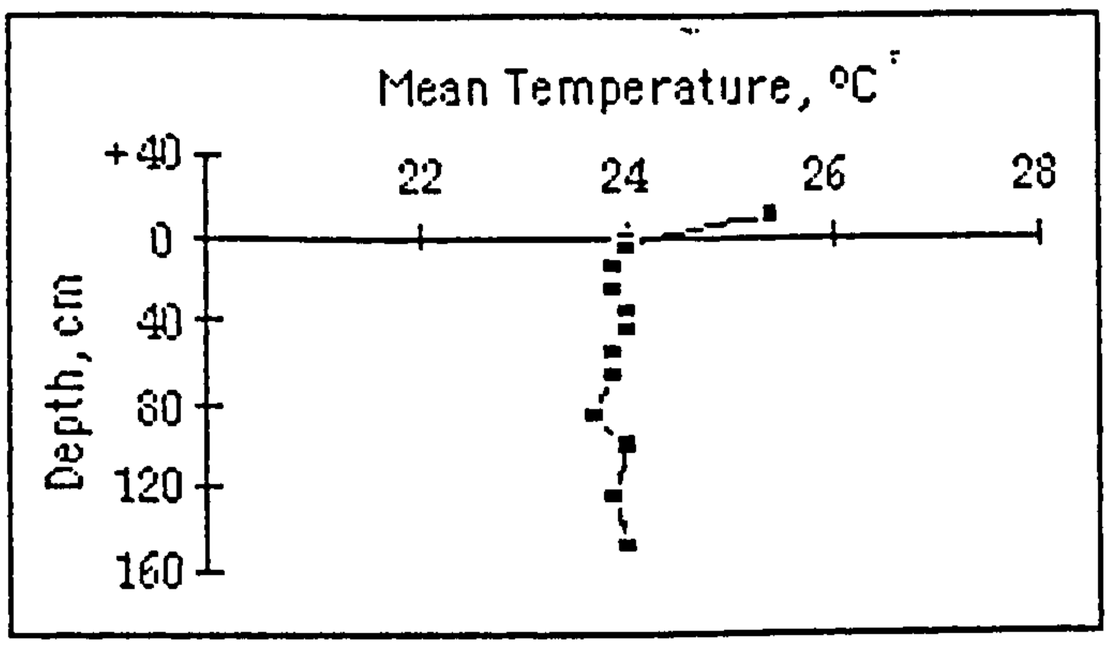


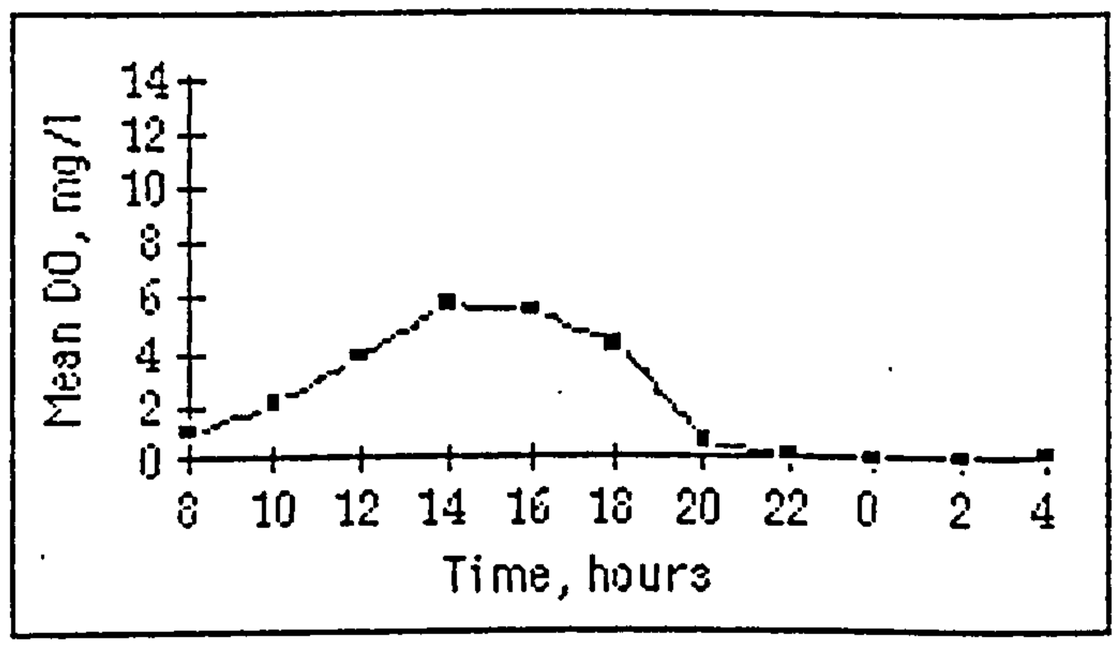
Figure A3/10 **Mean Results of the Profile carried out on M26 on 17.3.93.**

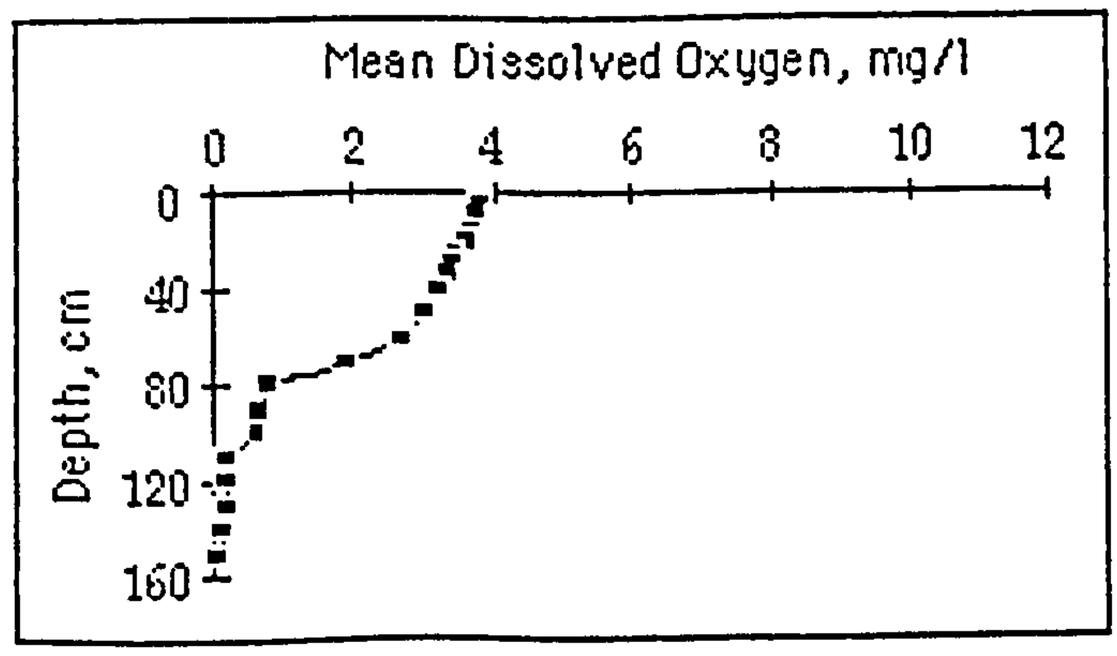


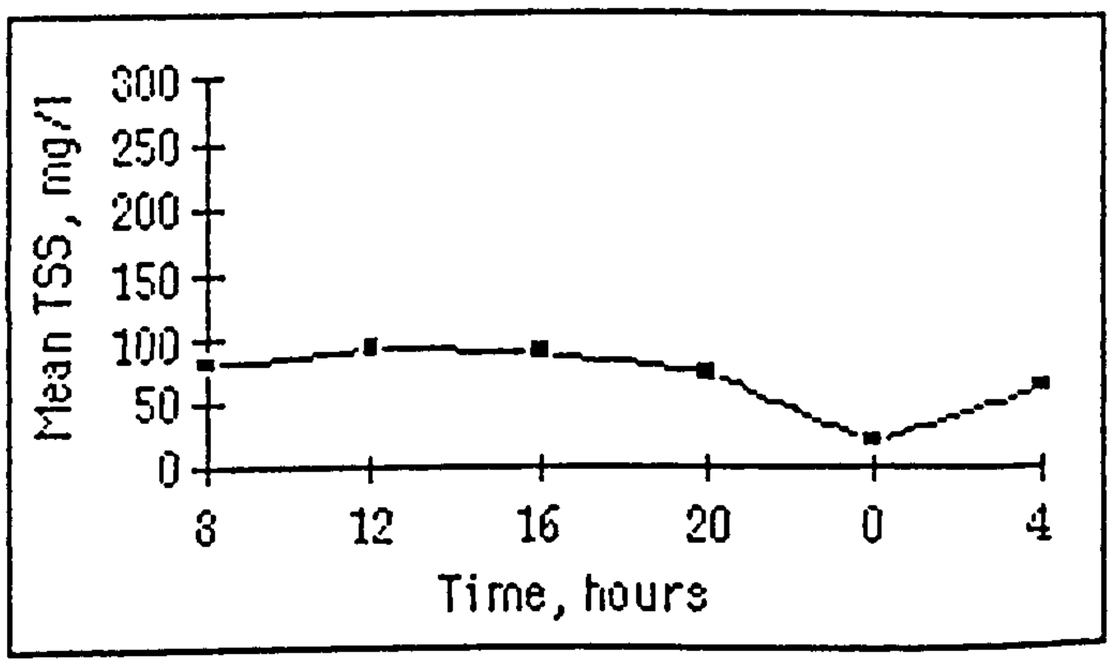


No BOD Results

No BOD Results







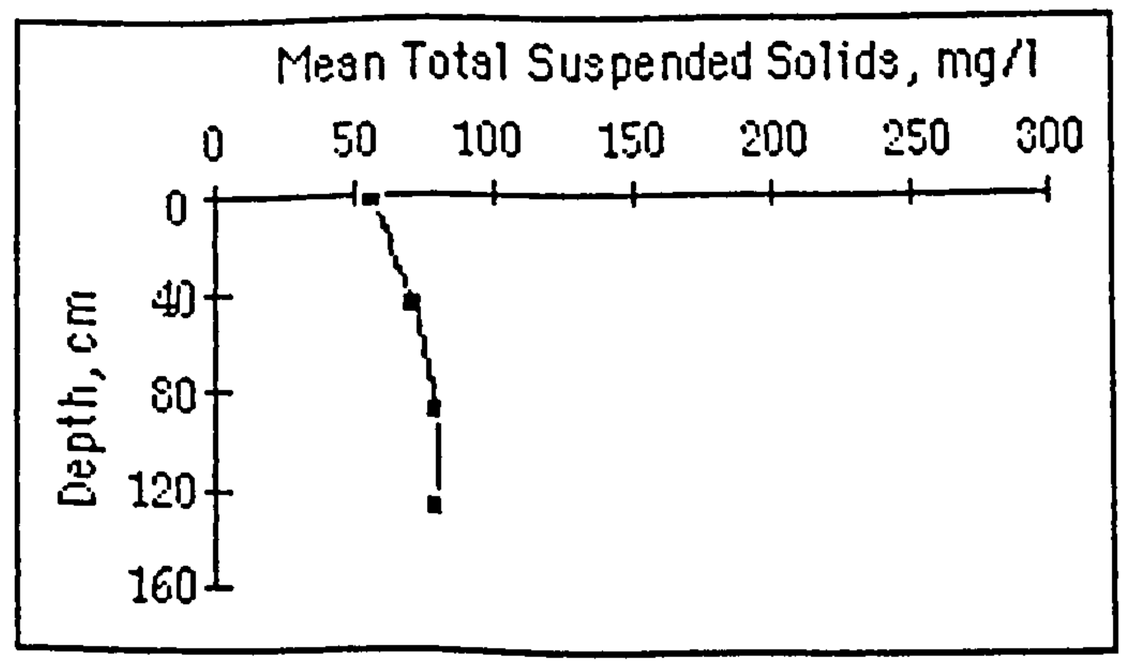
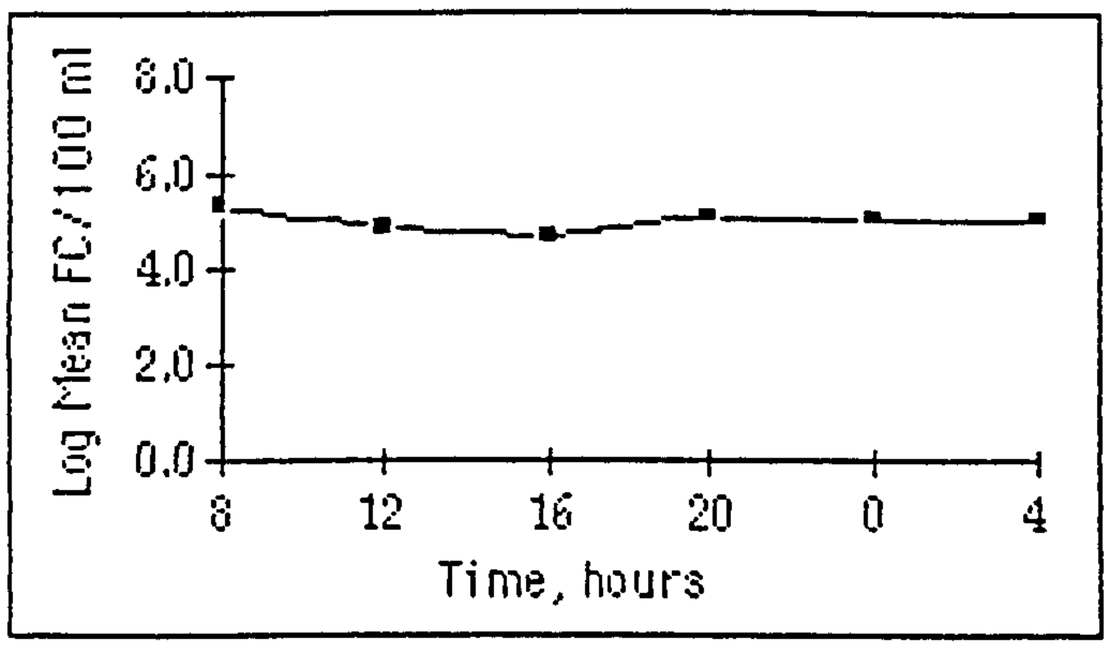
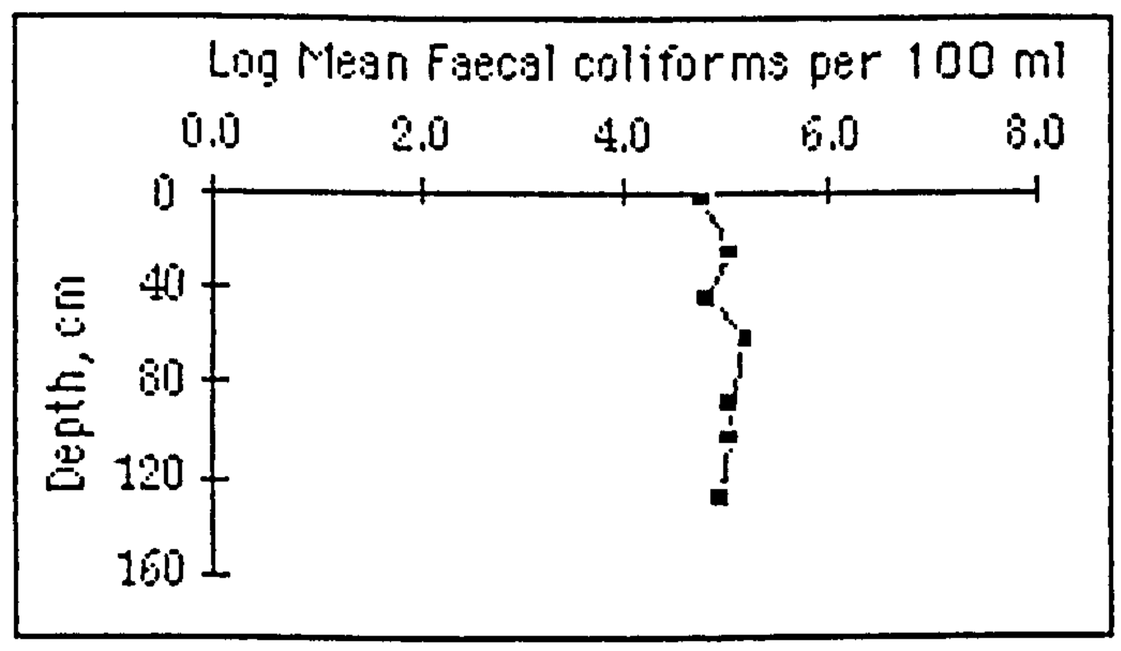
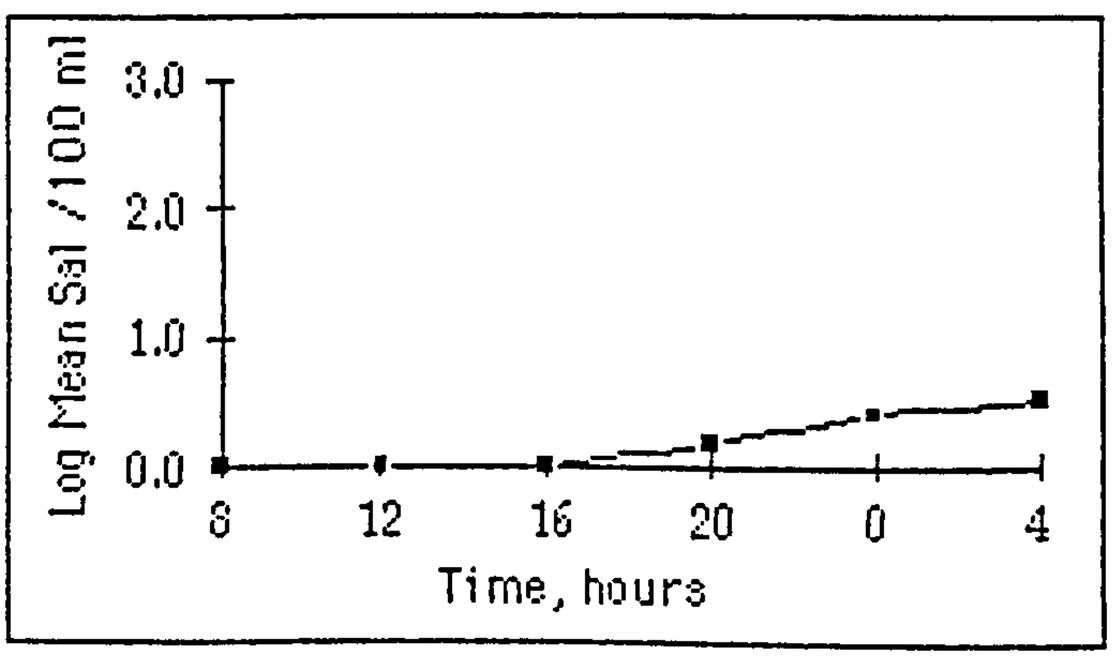
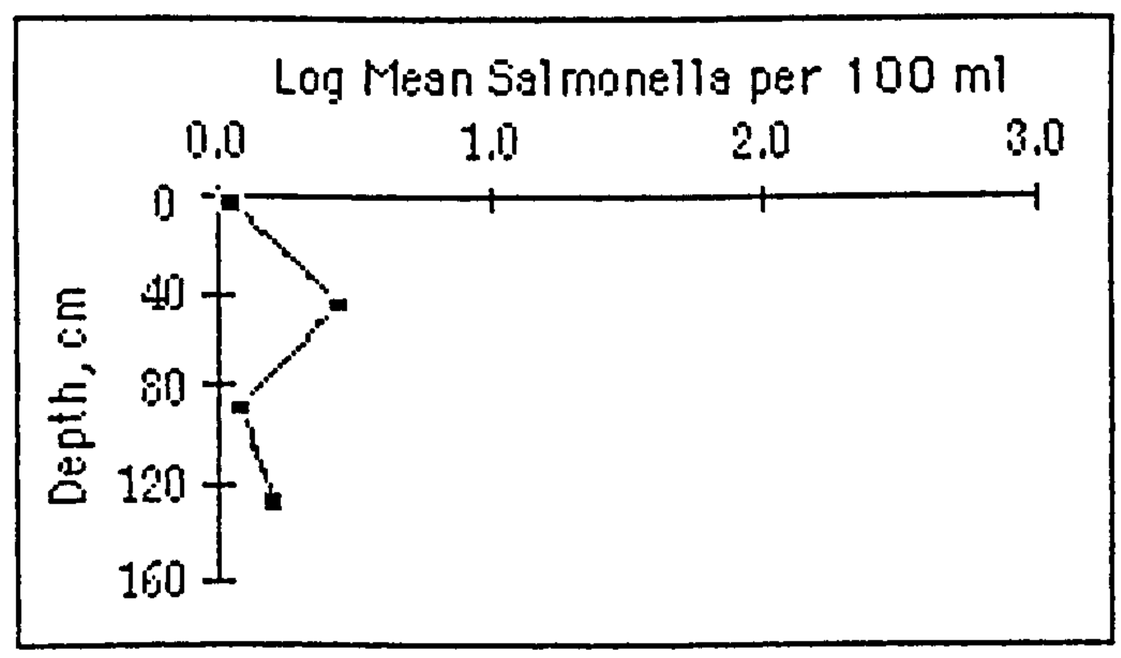


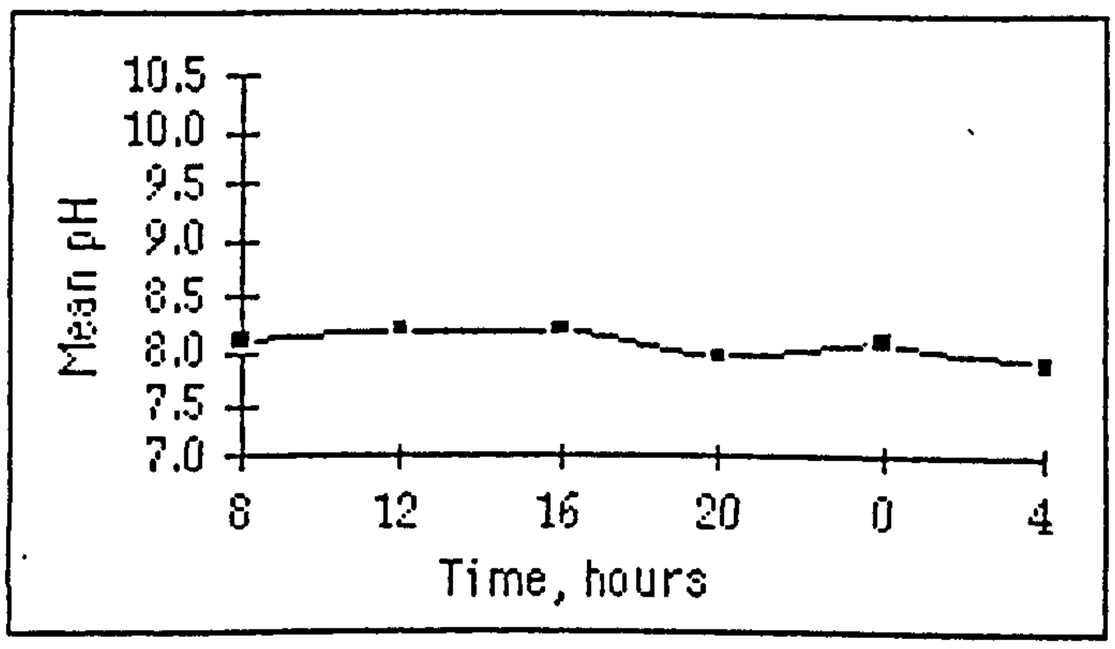
Figure A3/10b <u>Mean Results of the Profile carried out on M26 on 17. 3.93.</u>

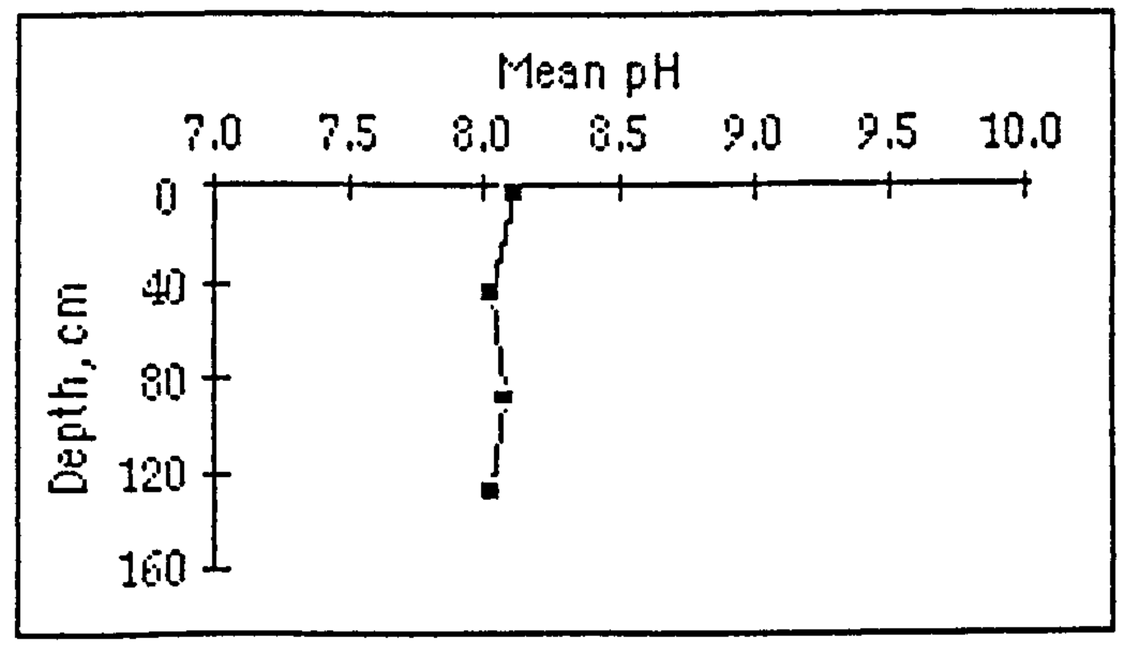


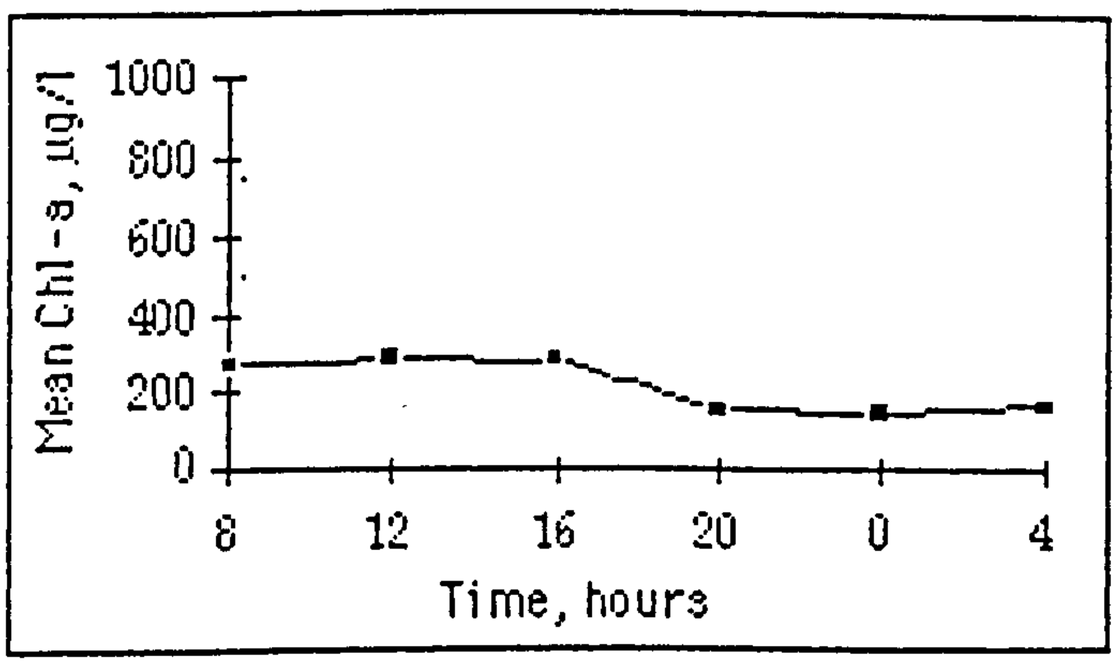












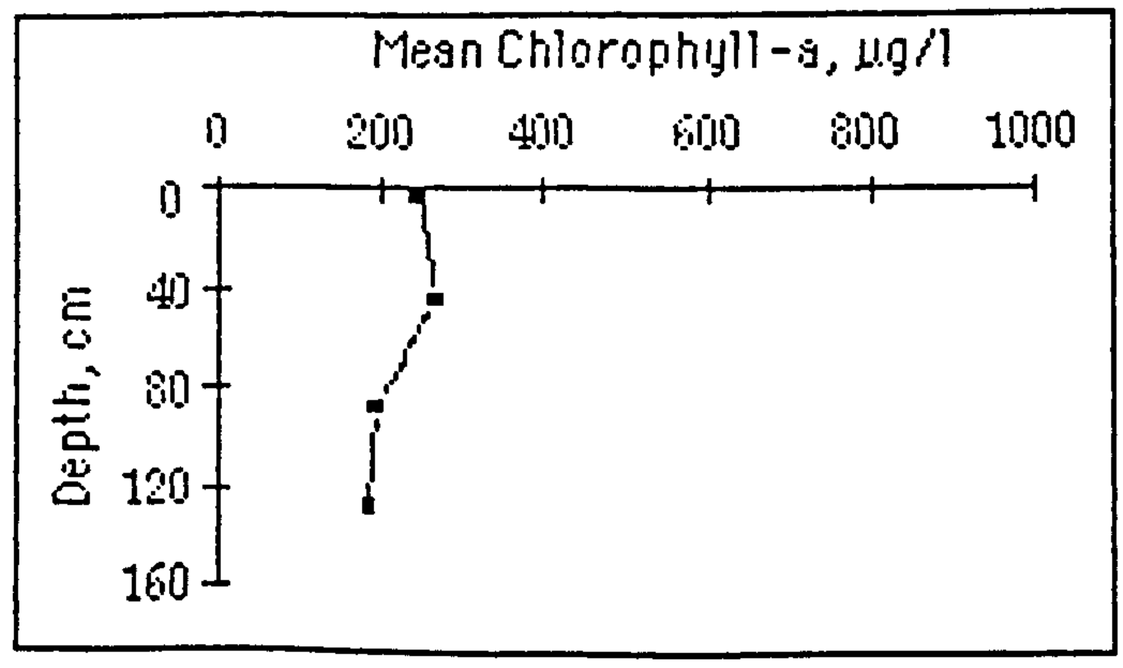
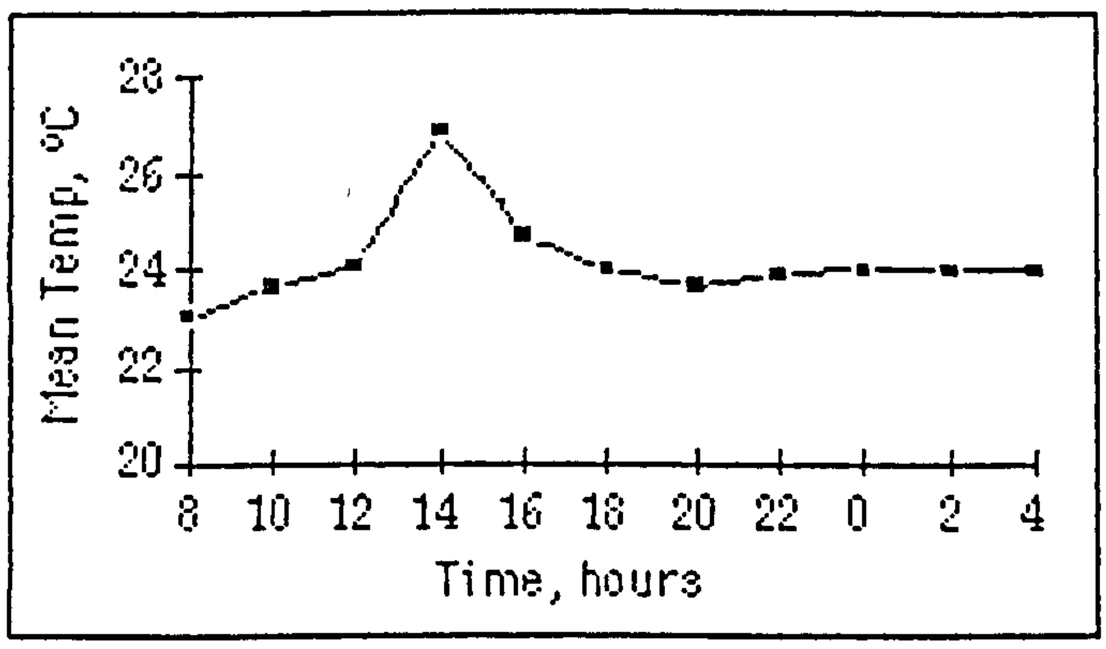
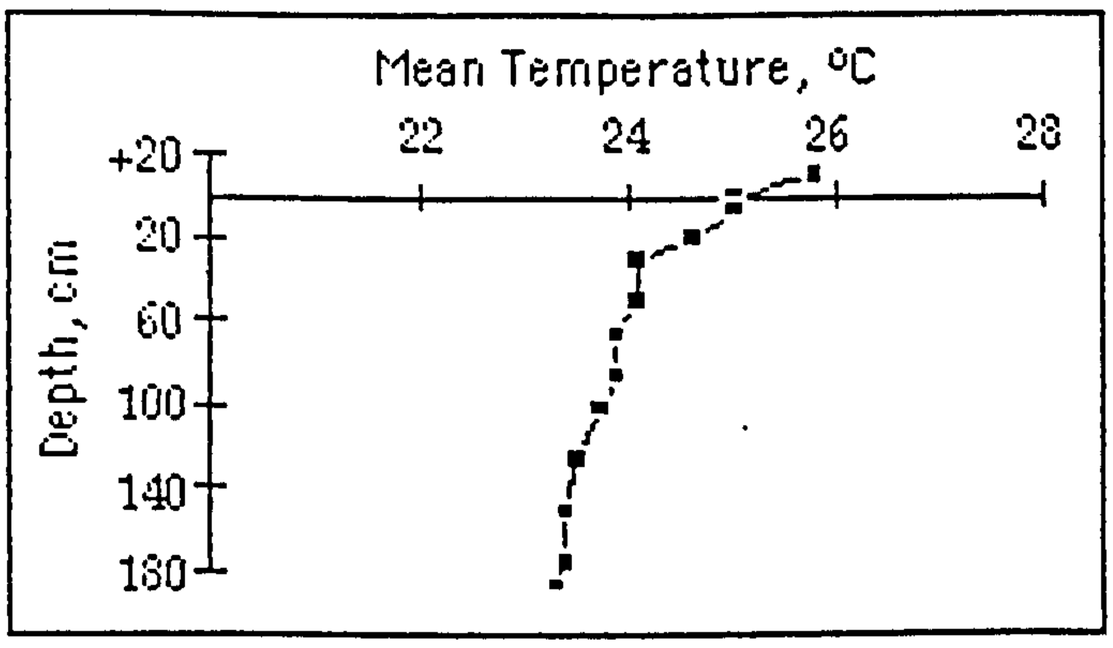


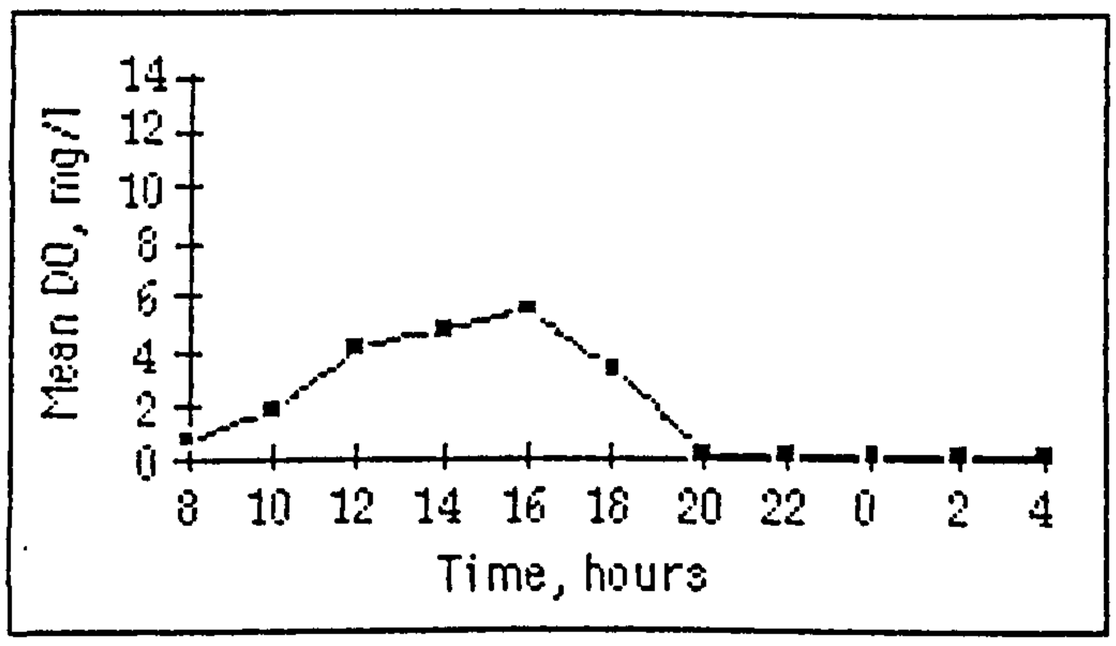
Figure A3/11 Mean Results of the Profile carried out on F25 on 24.3.93.

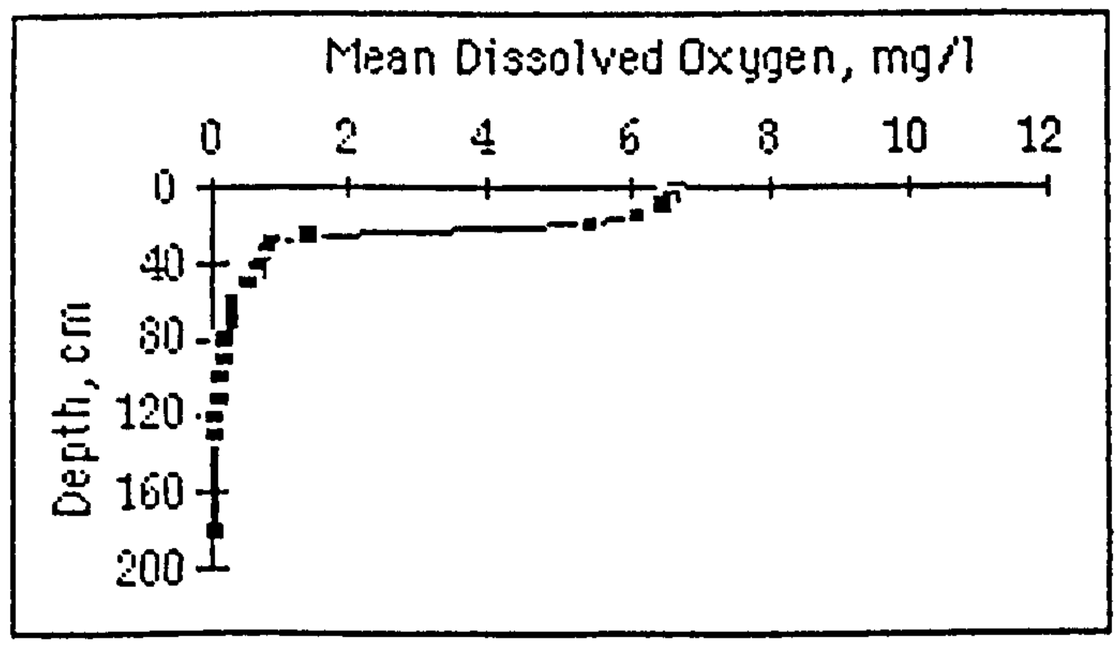


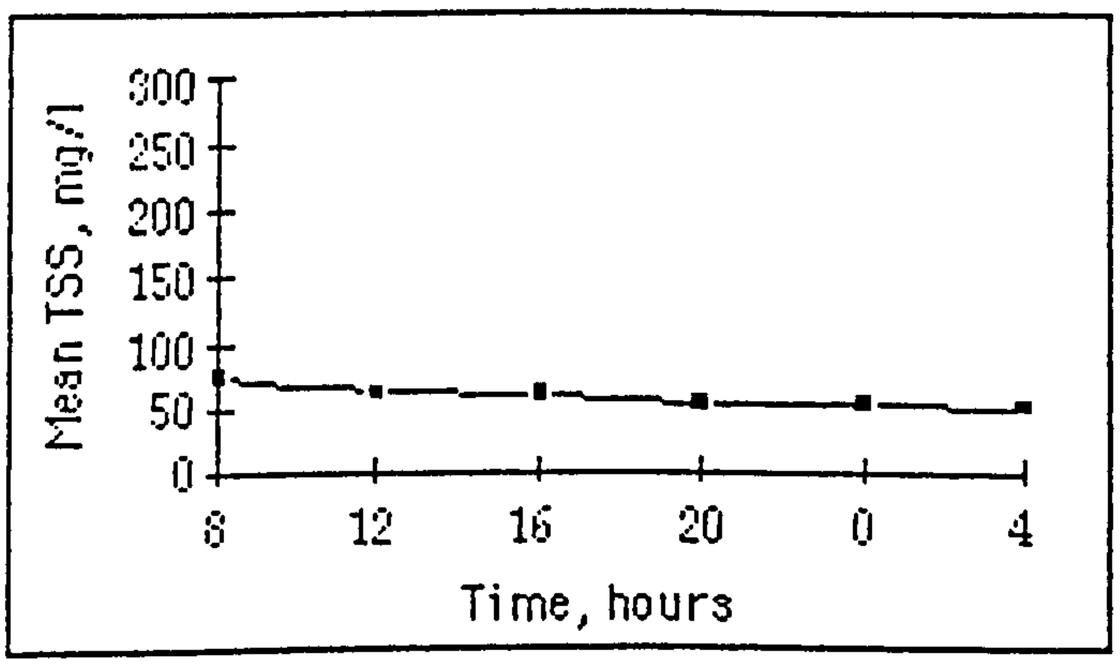


No BOD Results

No BOD Results







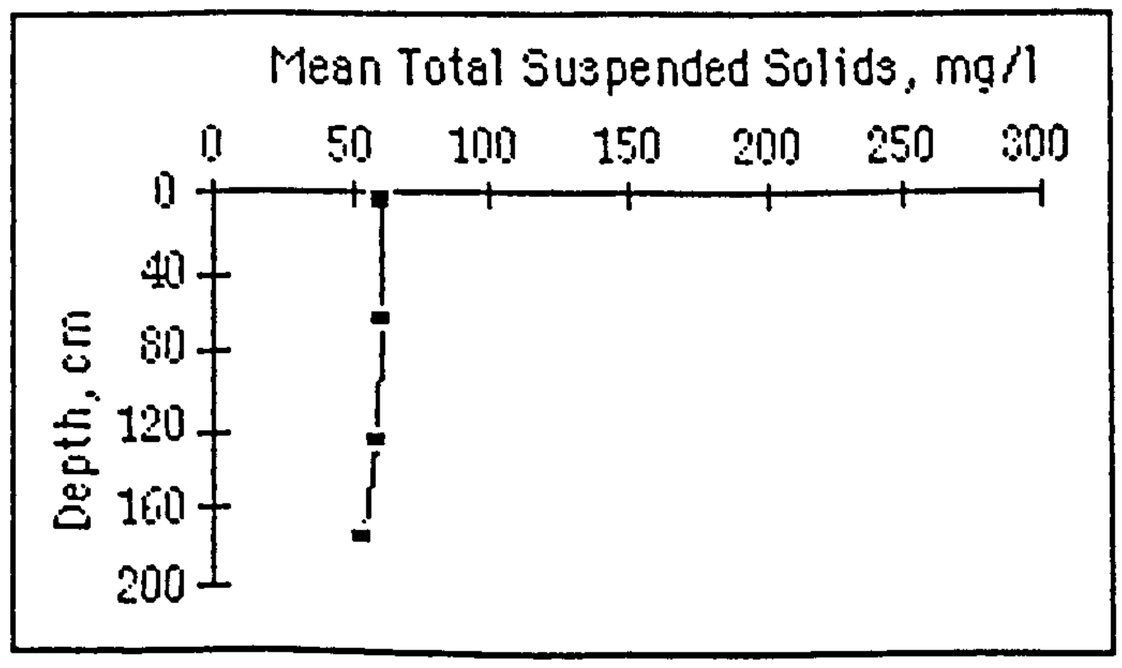
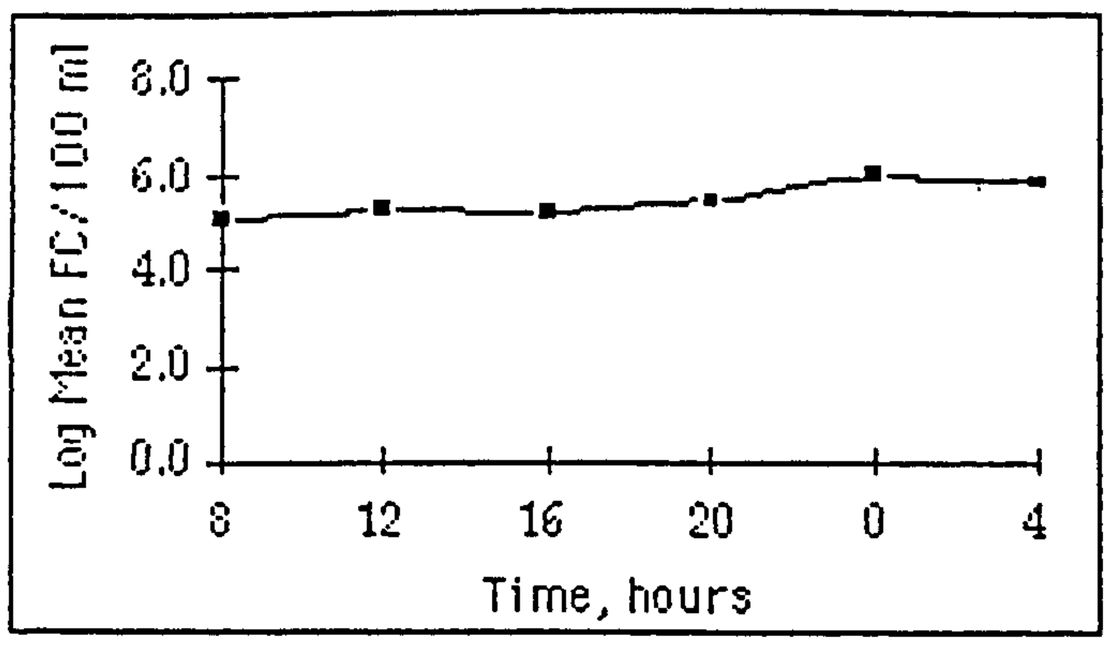
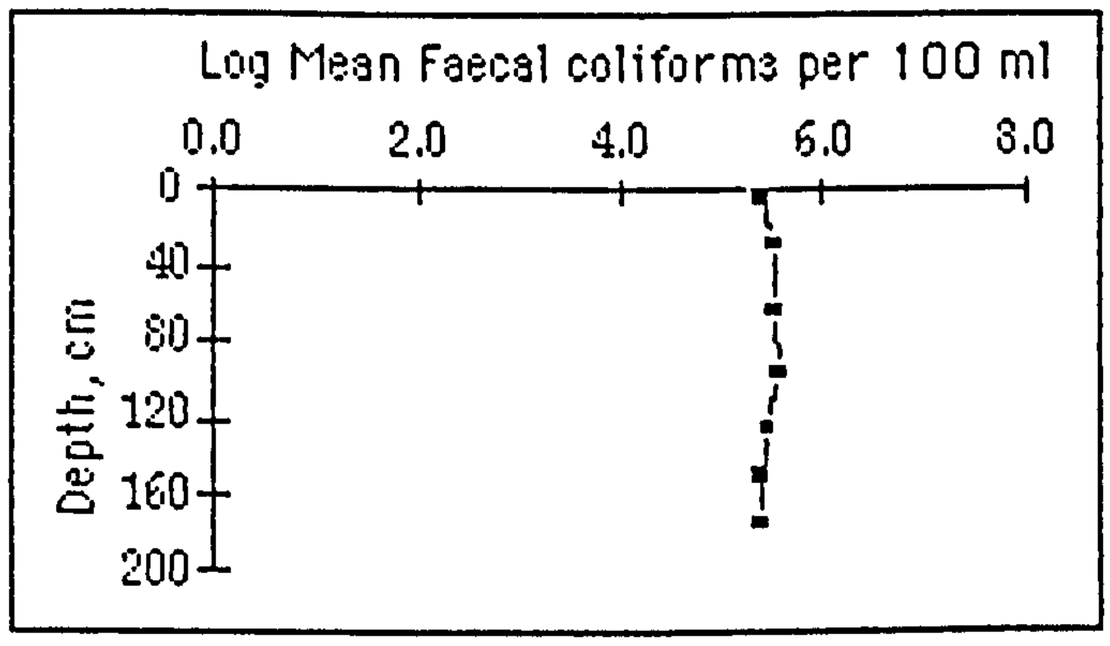
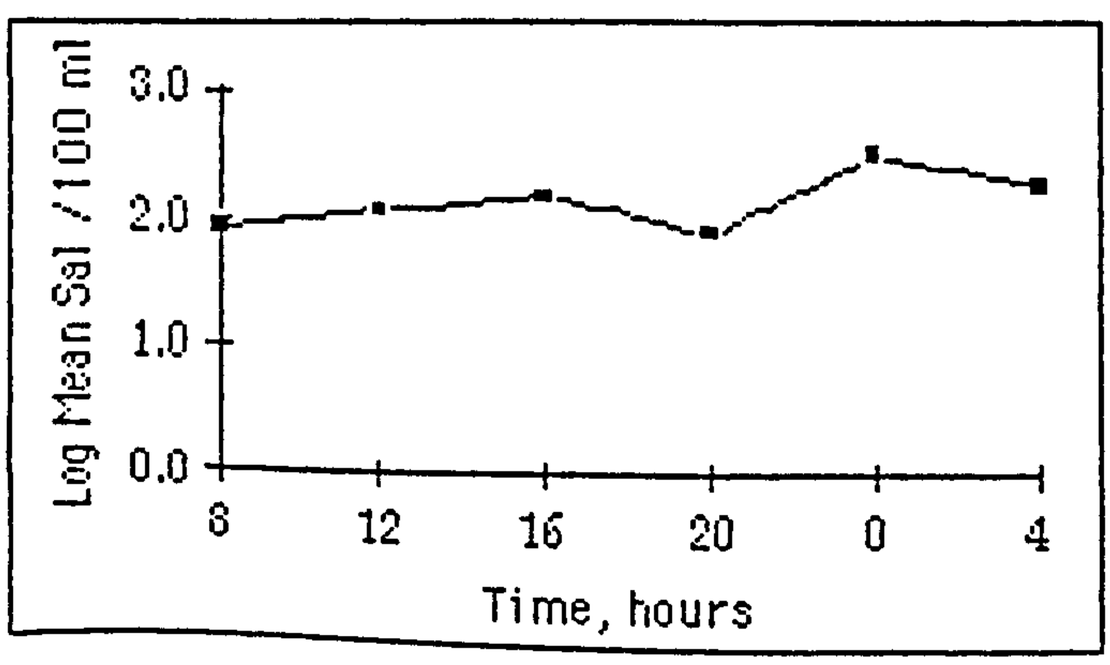
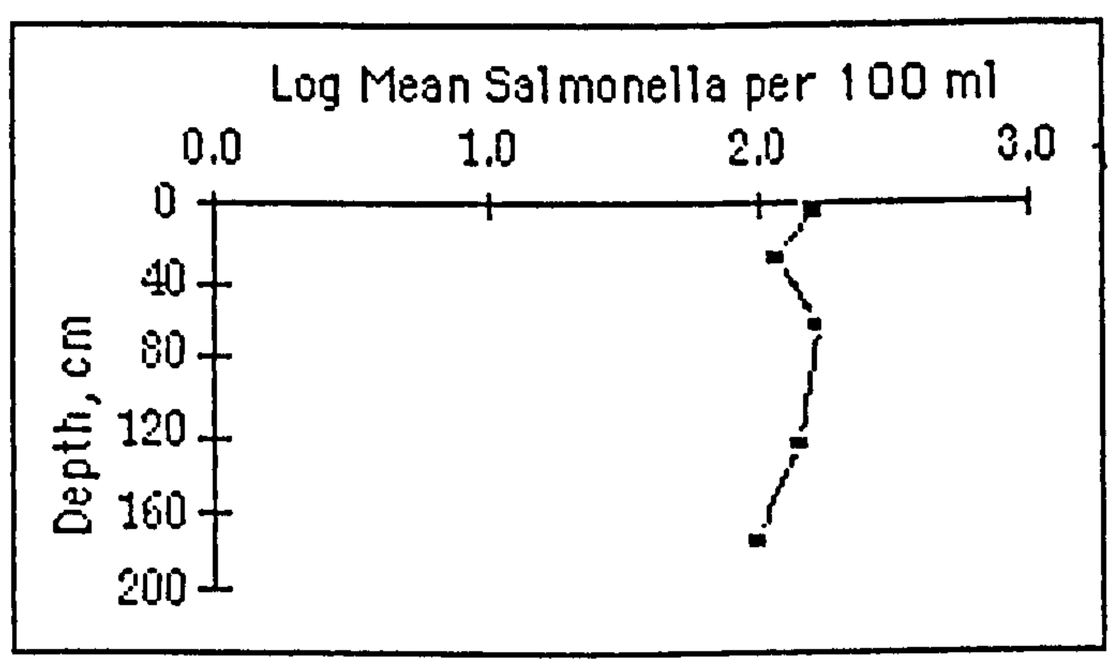


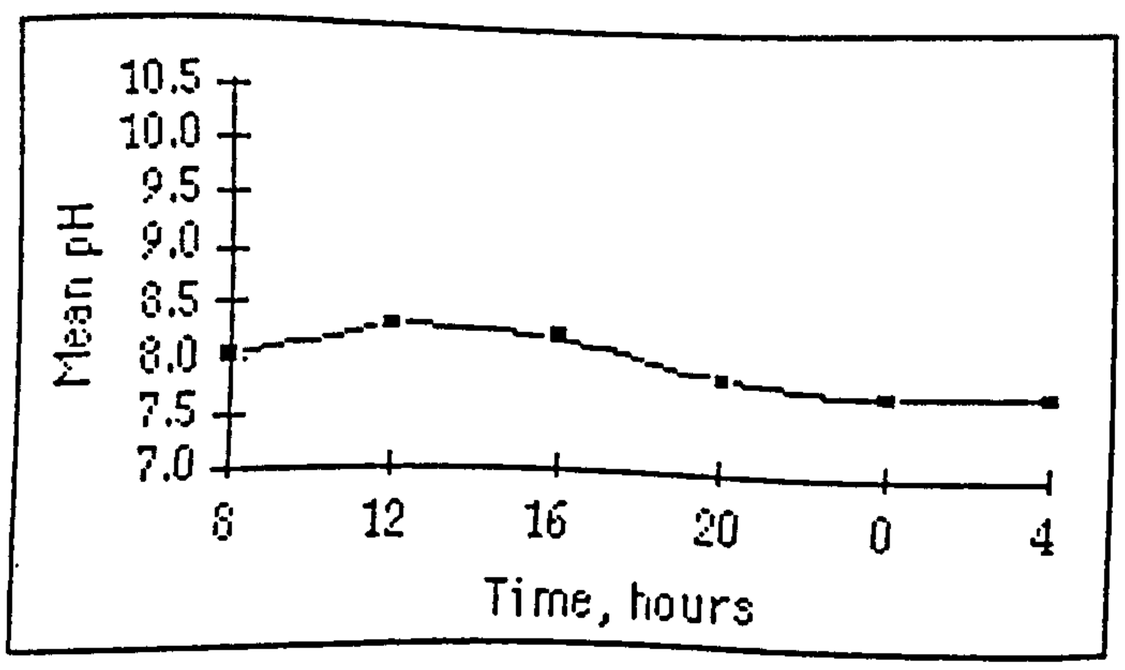
Figure A3/11b Mean Results of the Profile carried out on F25 on 24. 3.93.

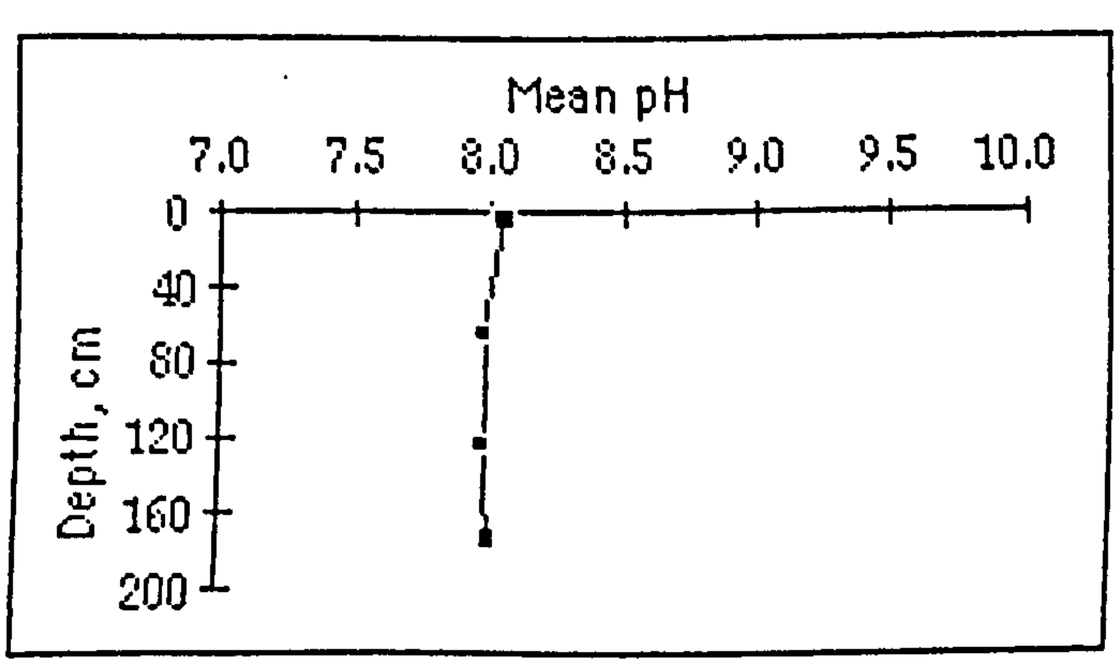


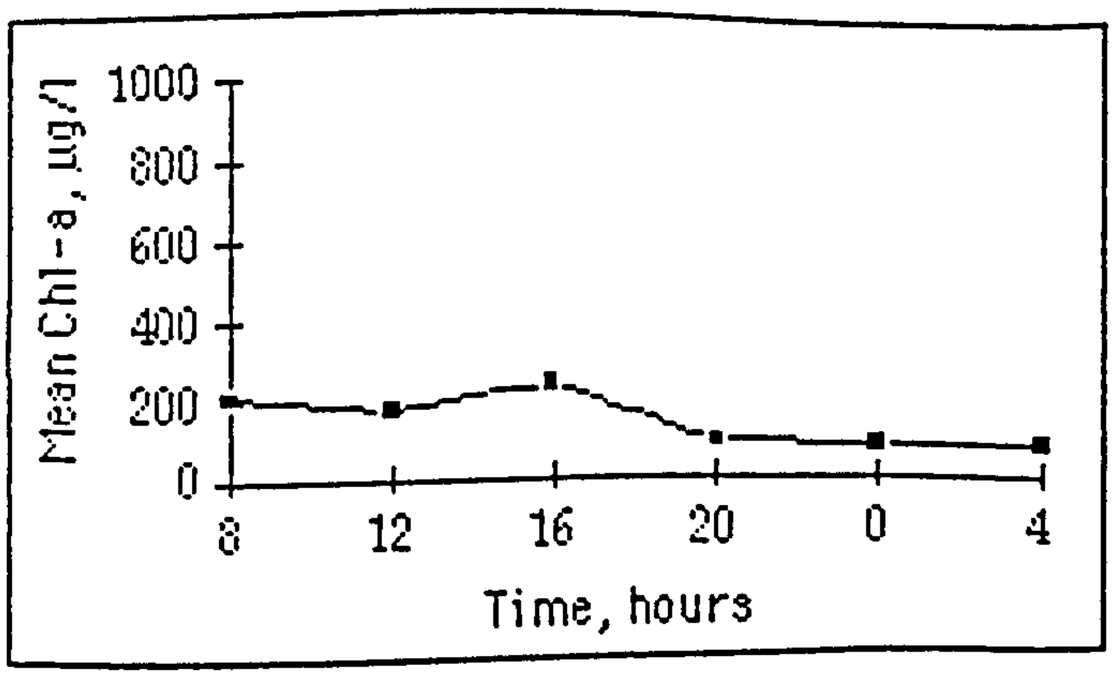












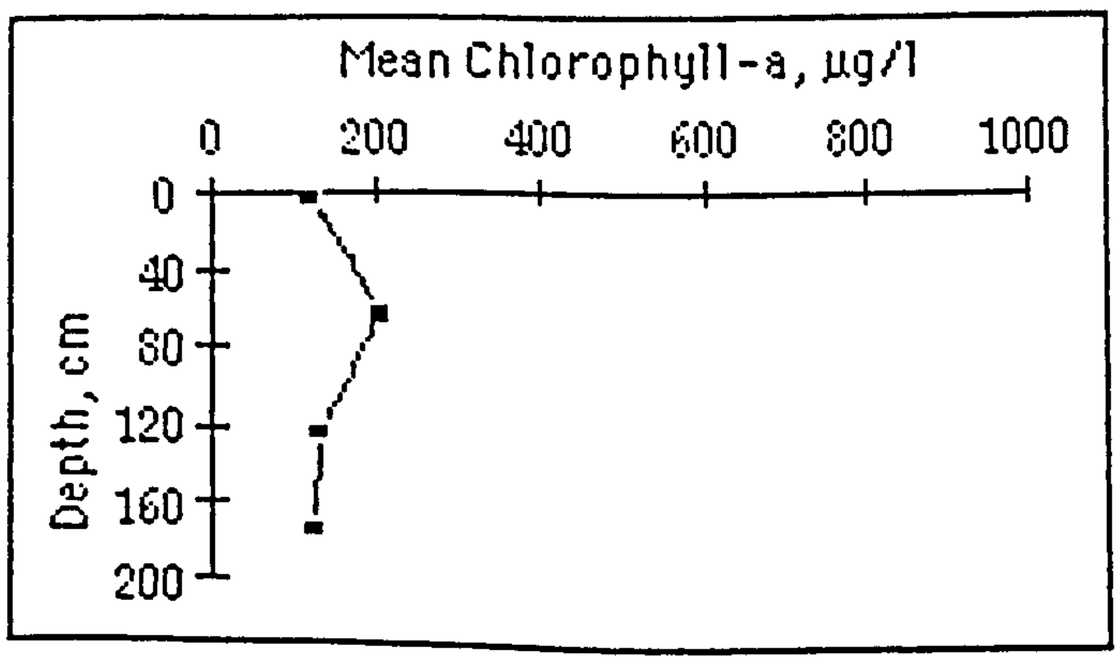
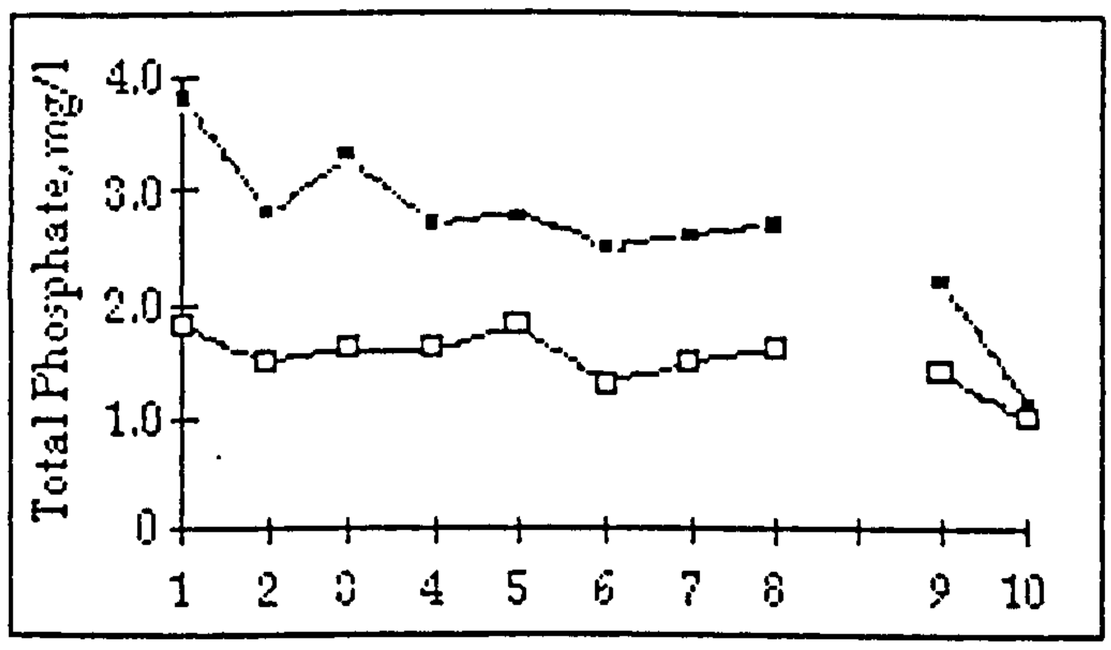
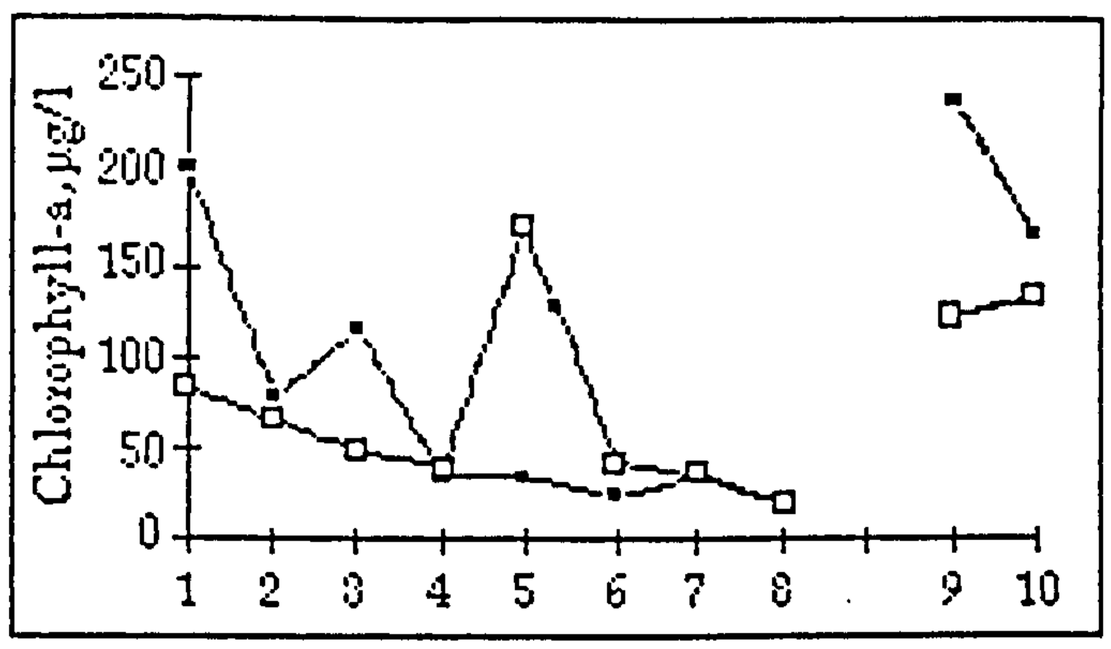
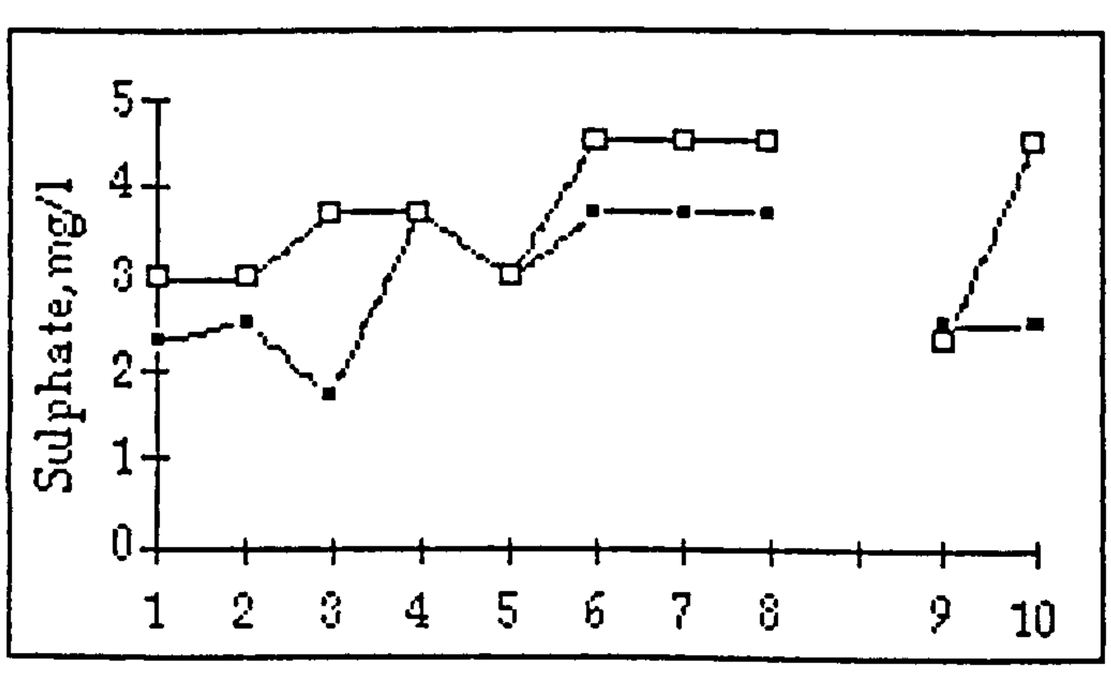
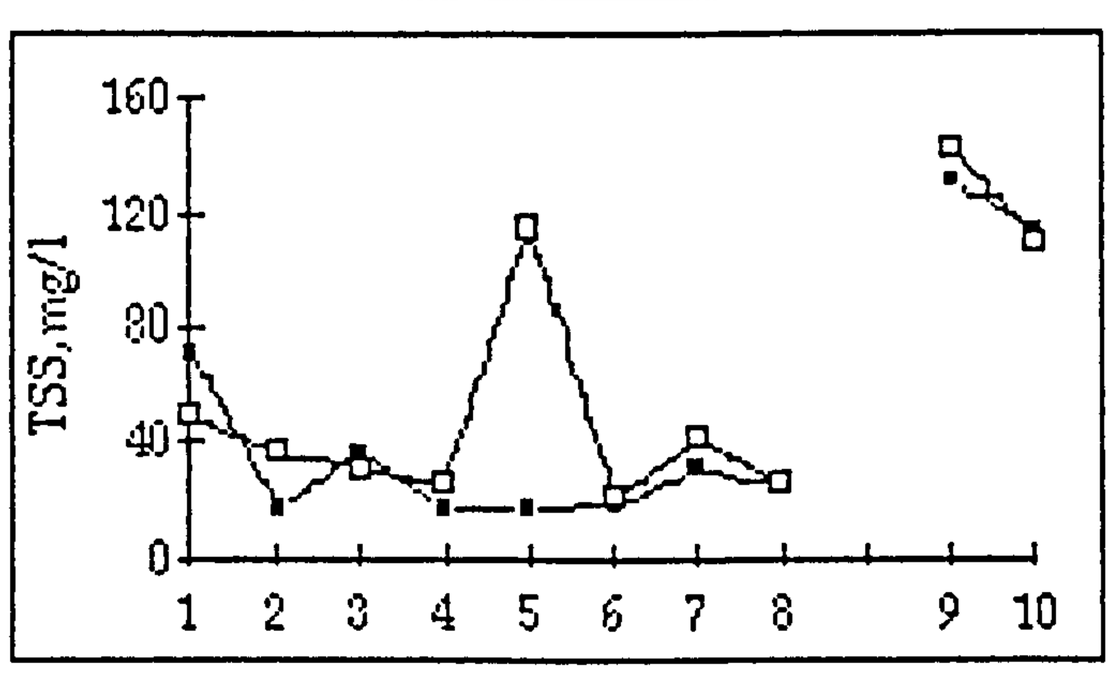


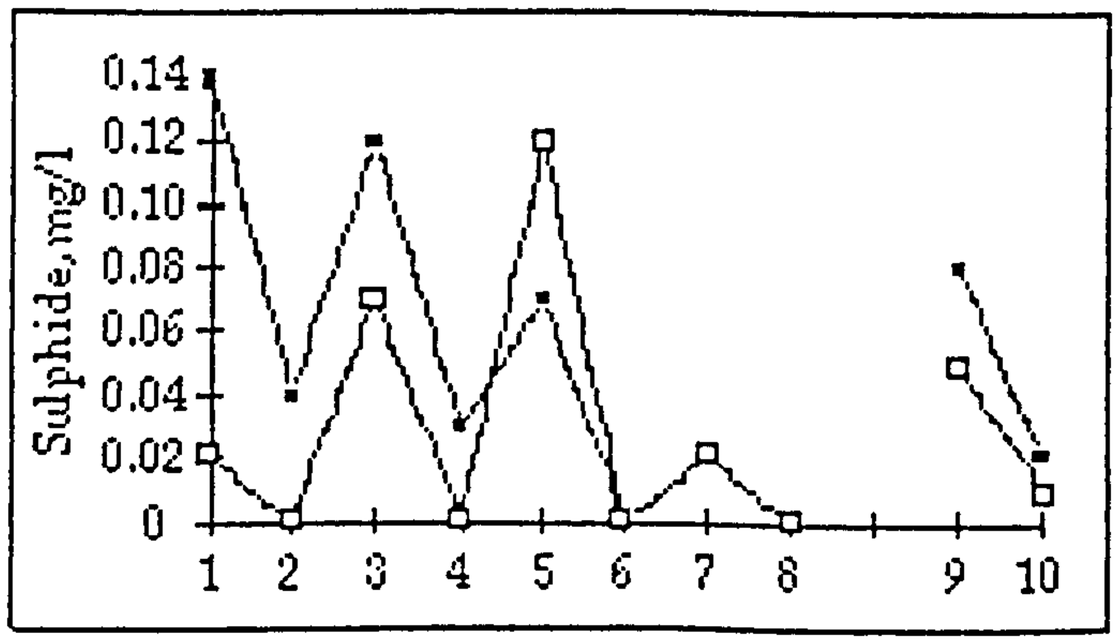
Figure A3/12 Results of the Linear Profile carried out on M24 on 3.2.93.
Samples taken at 8.00 hours —■— and 15.00 hours —□—.

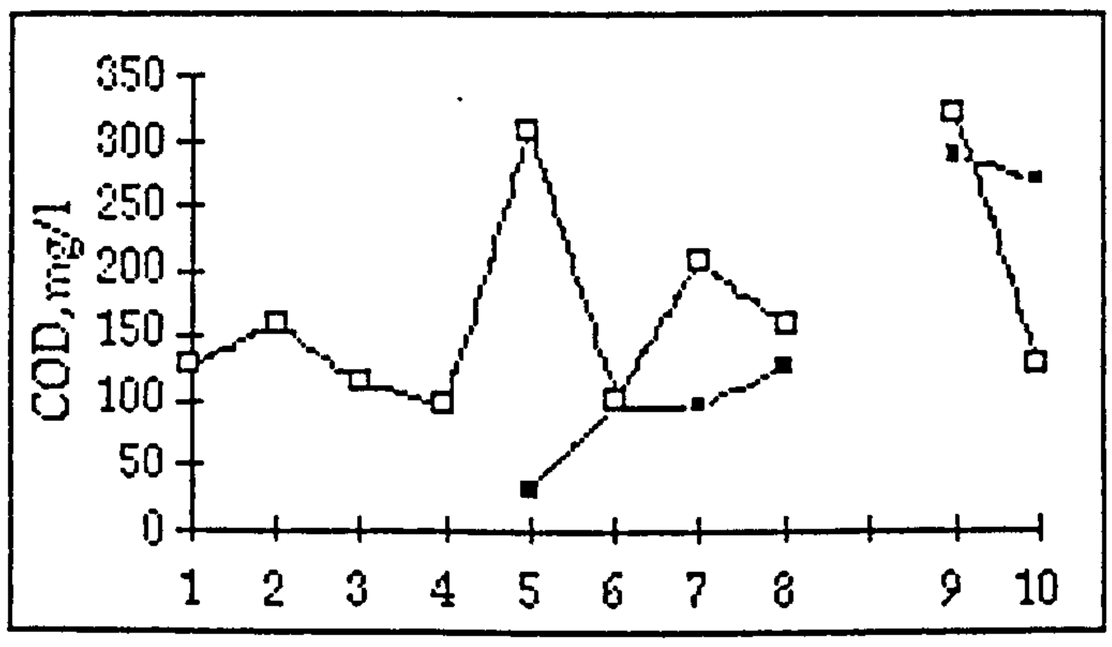


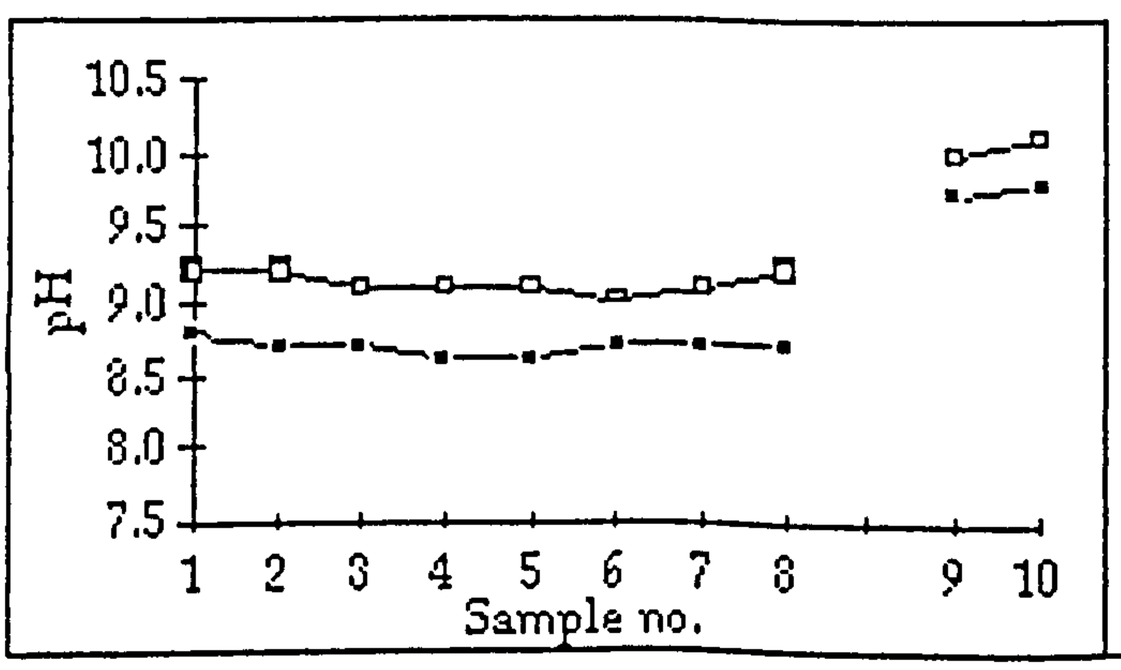












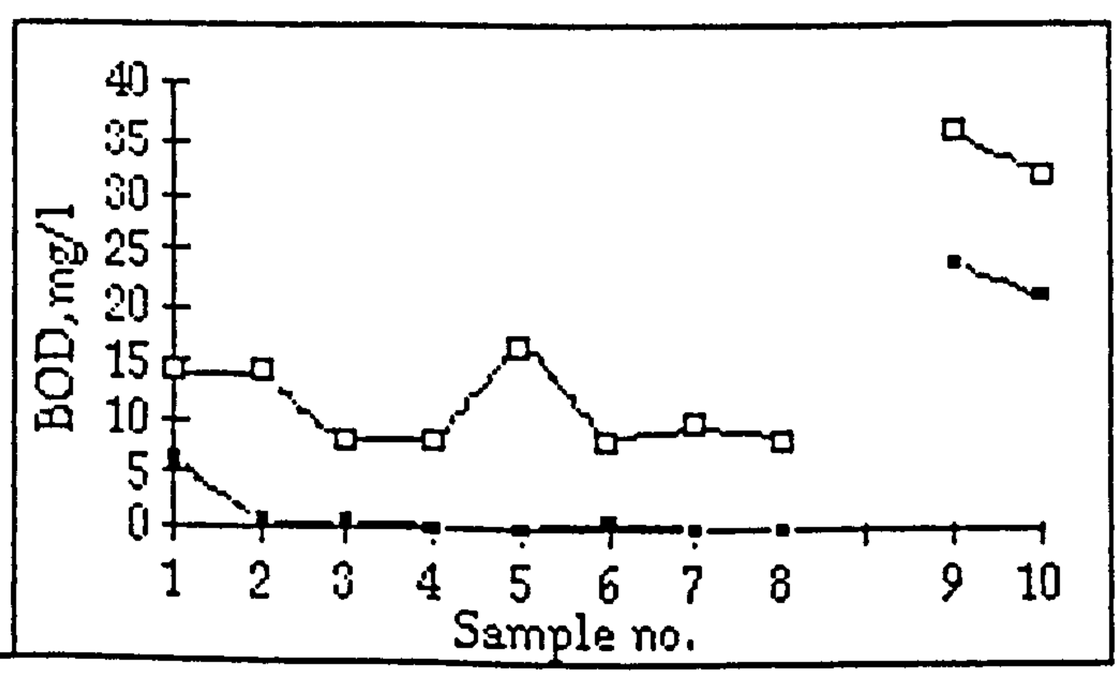
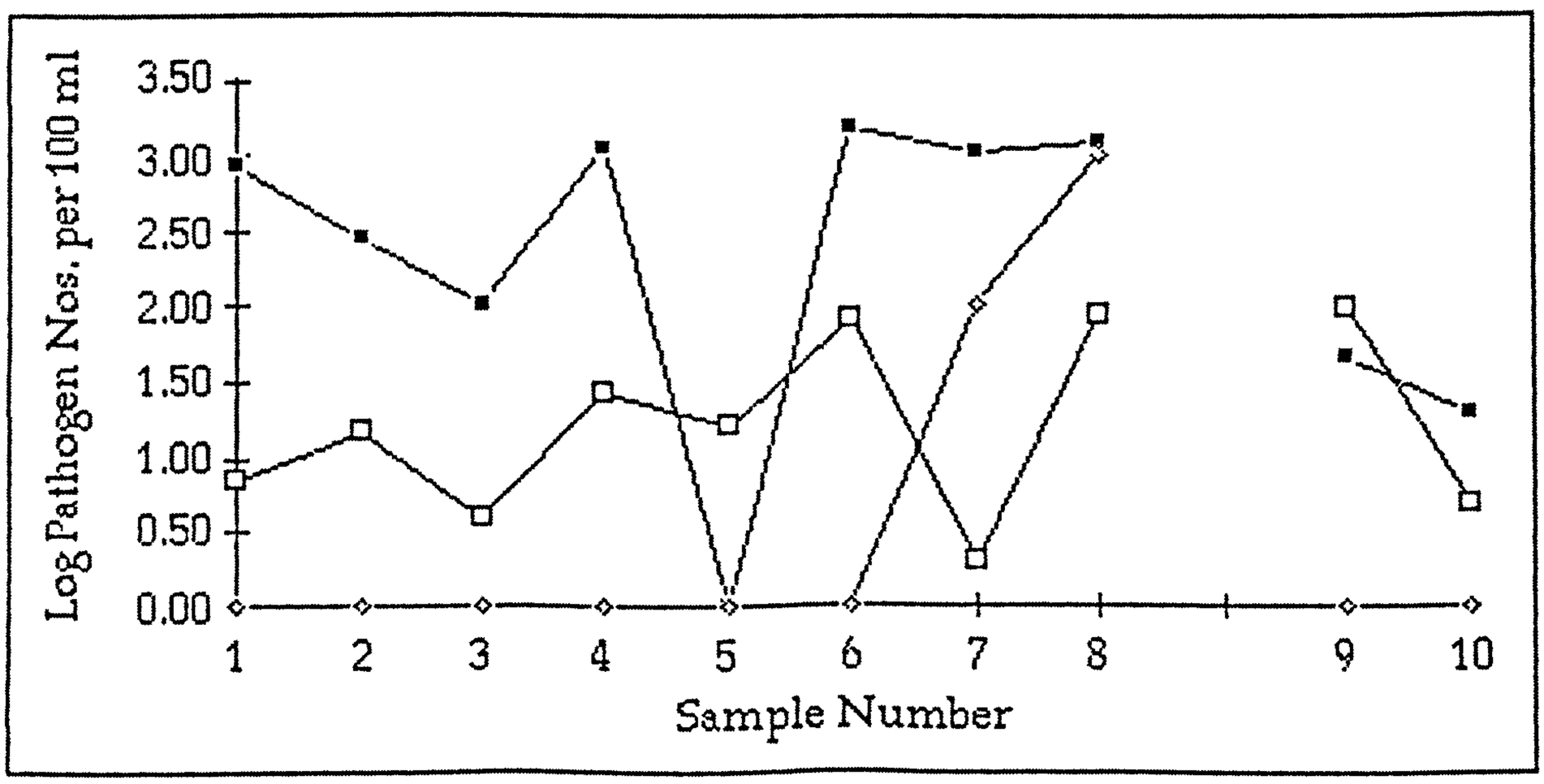
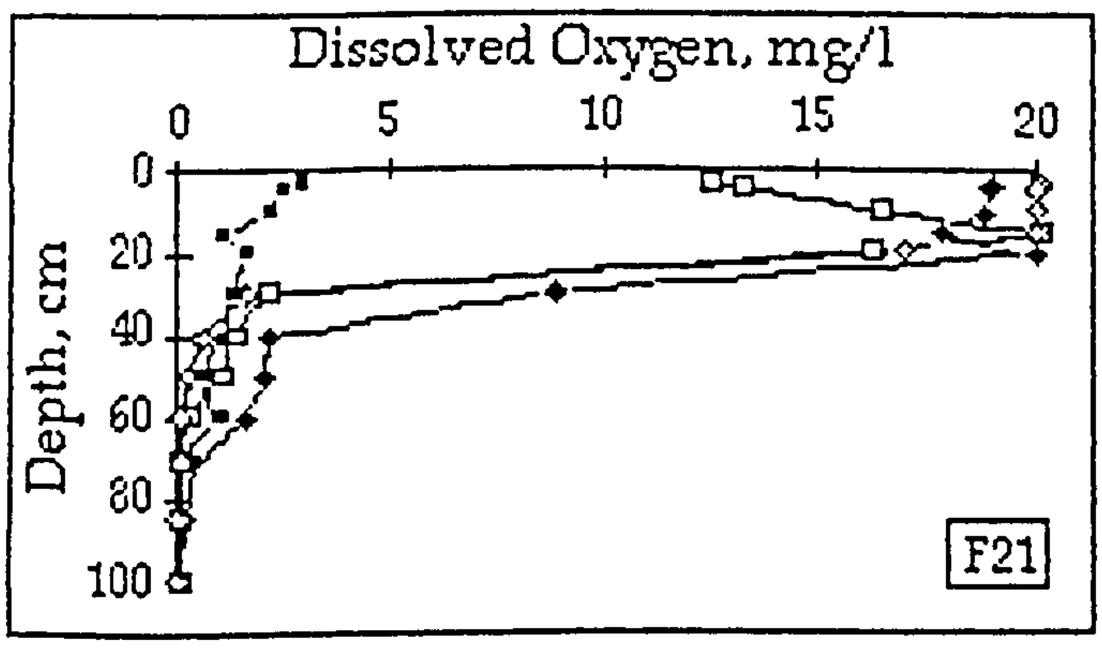


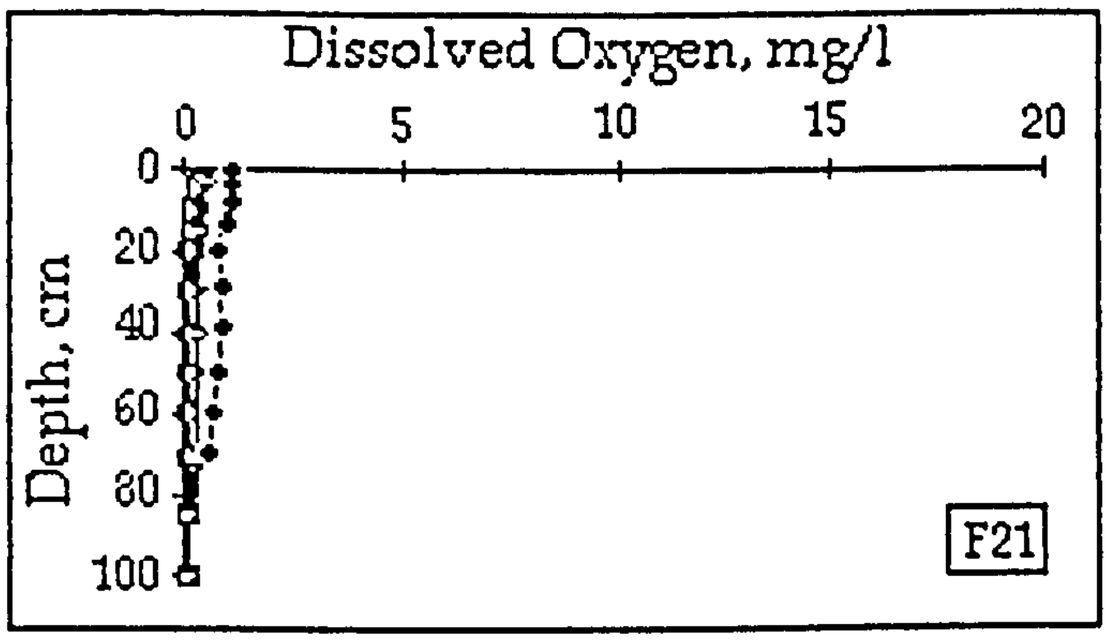
Figure A3/12b Results of the Linear Profile carried out on M24 on 3.2.93. Samples of Faecal coliforms taken at 8.00 hours —▪— and 15.00 hours —□— and *Salmonella** at 8.00 hours —◇— .

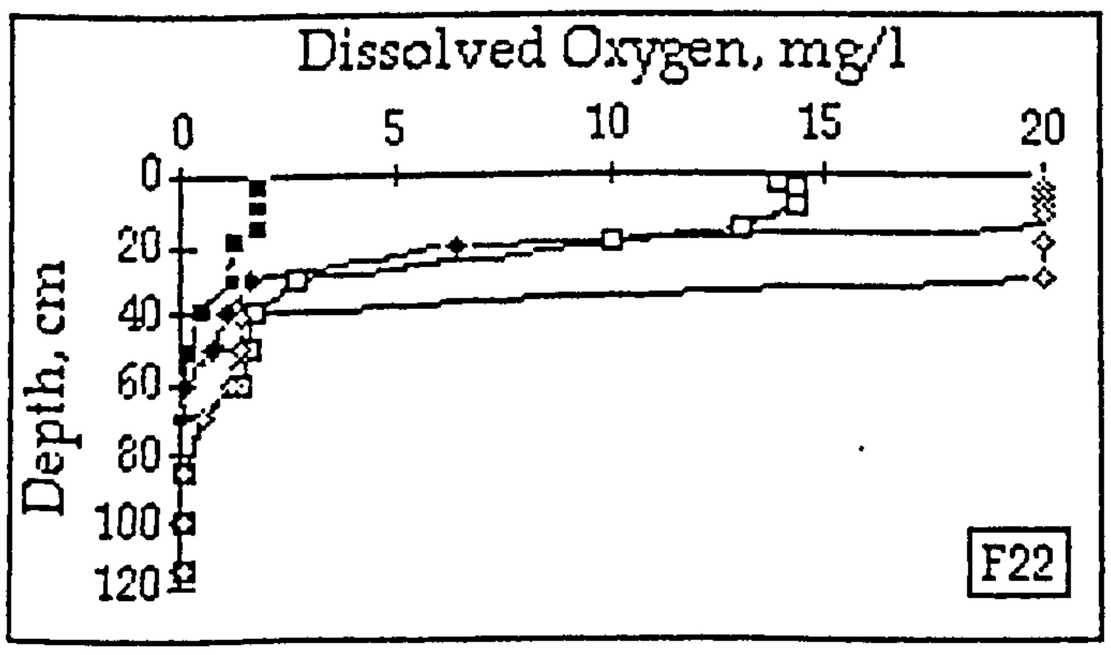


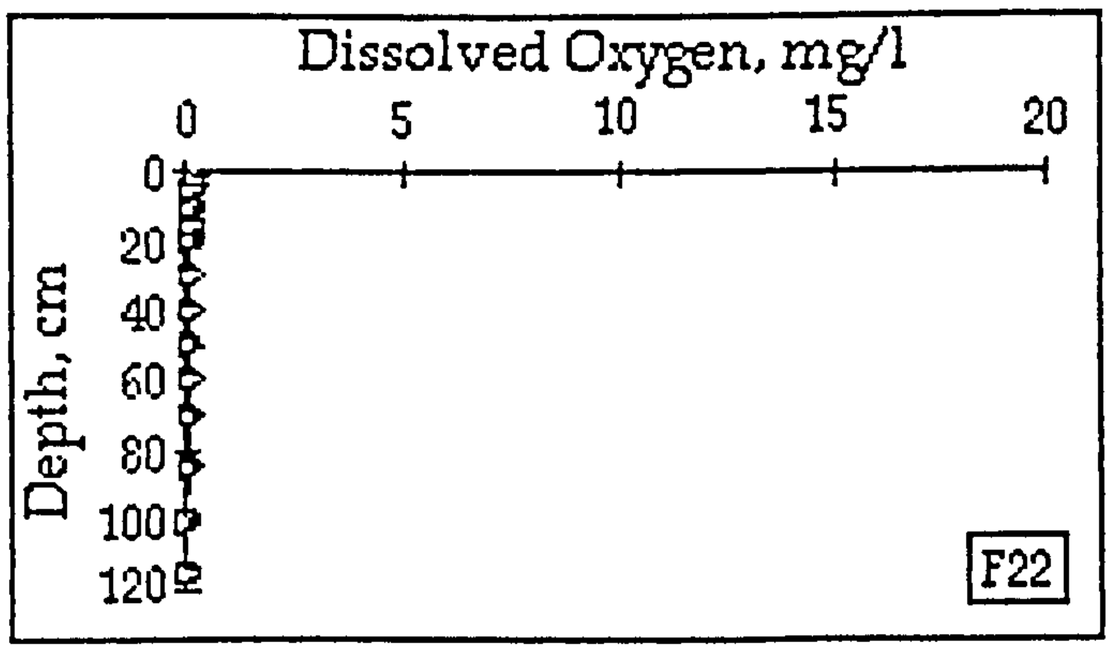
* No *Salmonella* detected at 15.00 hours

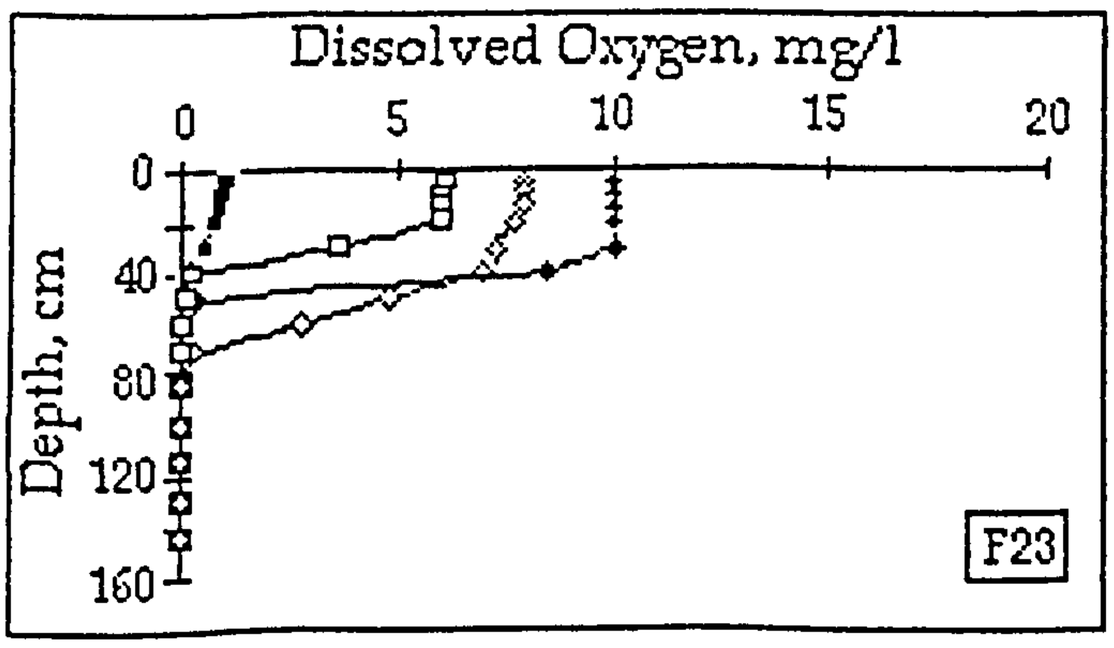
Figure A3/13 Dissolved Oxygen Profiles of the Ponds Investigated on 31.3.93.
Samples taken at 8.00 hours —▪—, 11.00 hours —□—,
14.00 hours —•—, 17.00 hours —◇—, 20.00 hours —▲—,
23.00 hours —△—, 2.00 hours —•— and 5.00 hours —○—.

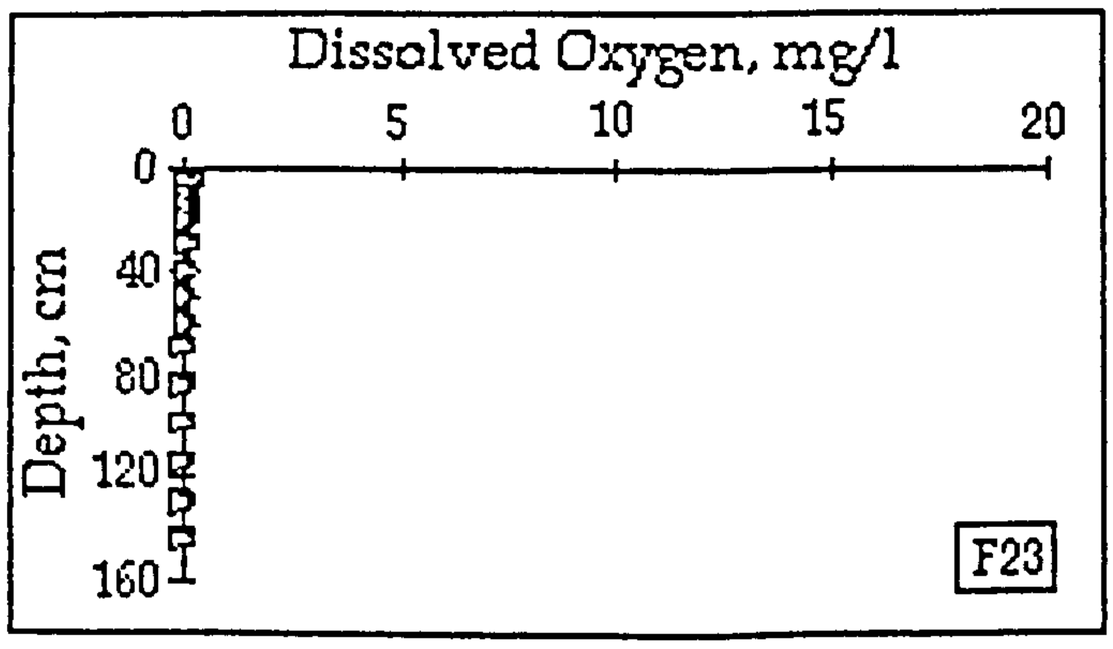


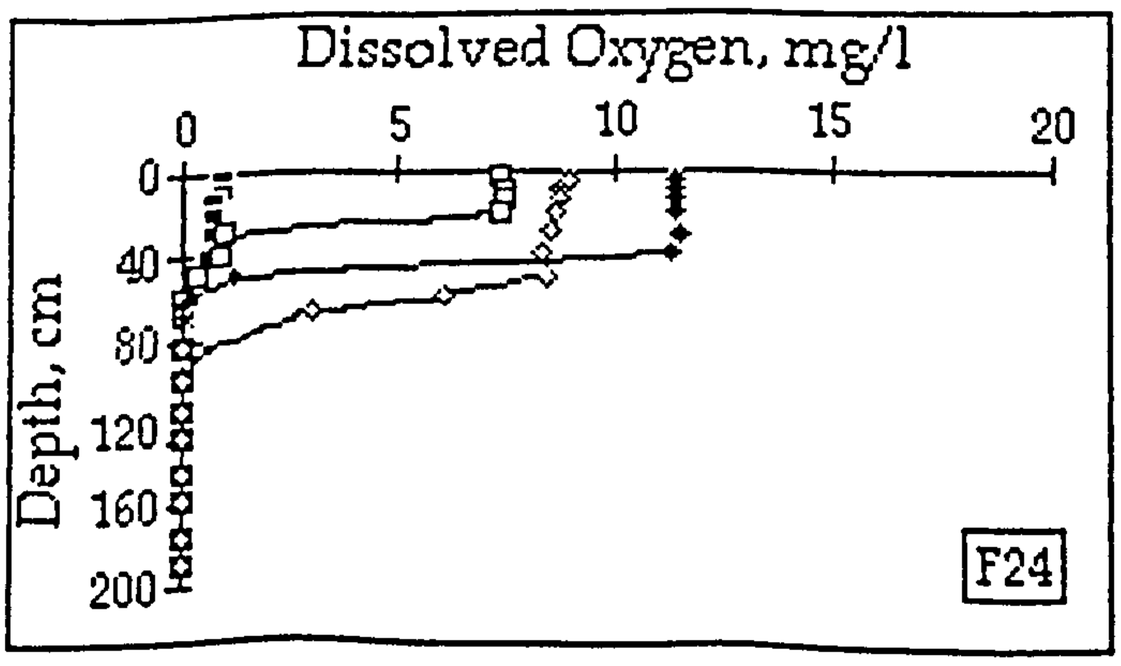












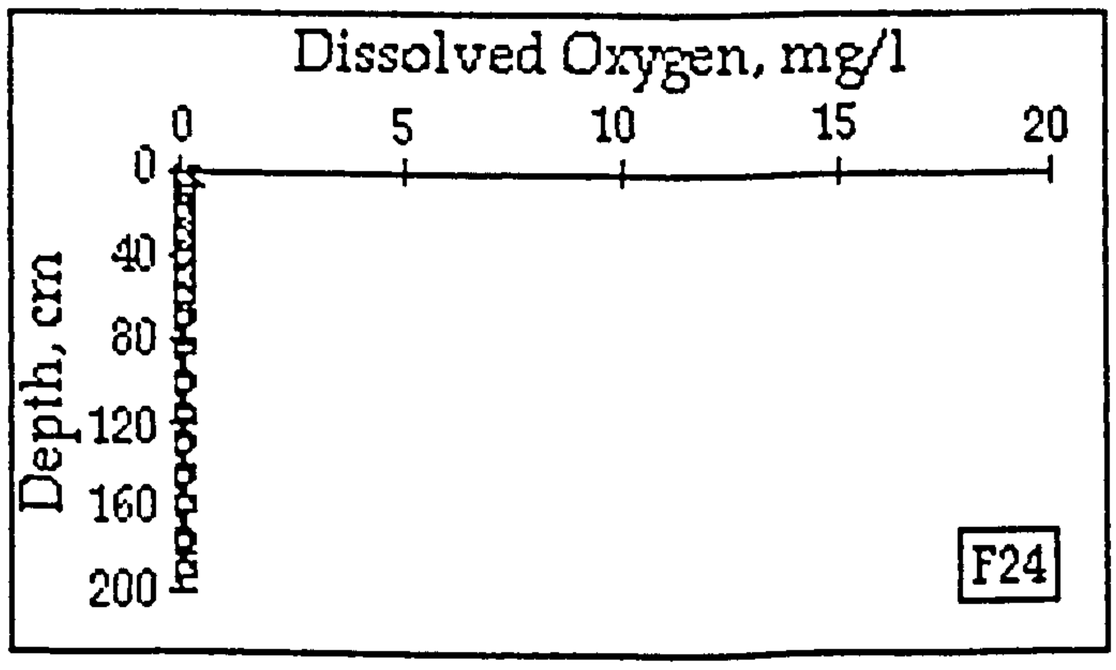
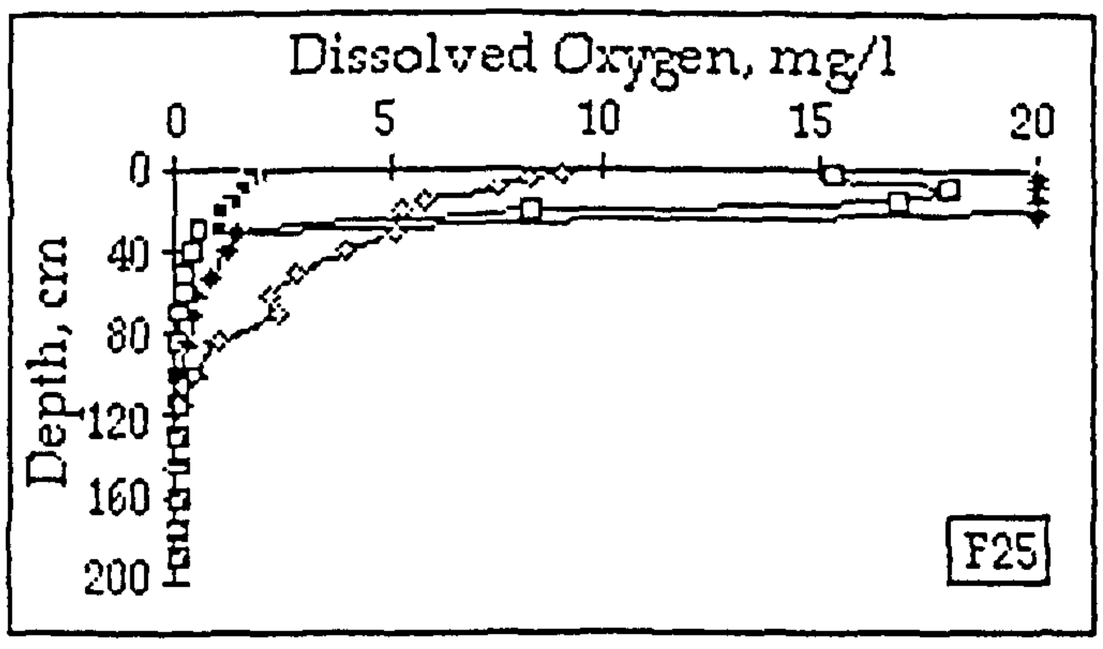
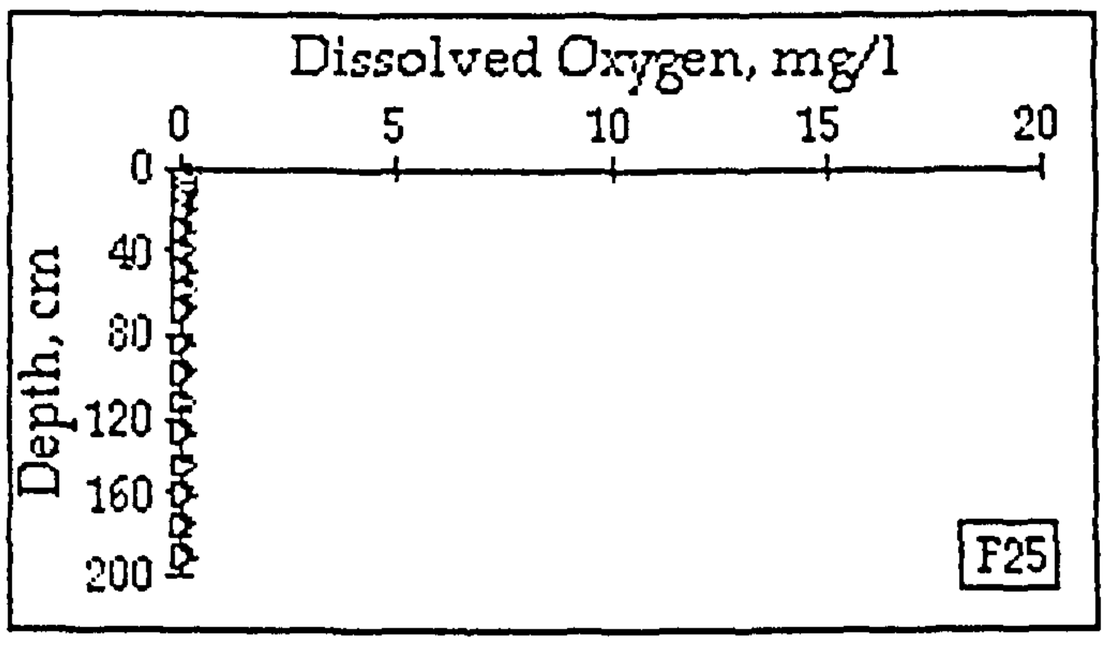
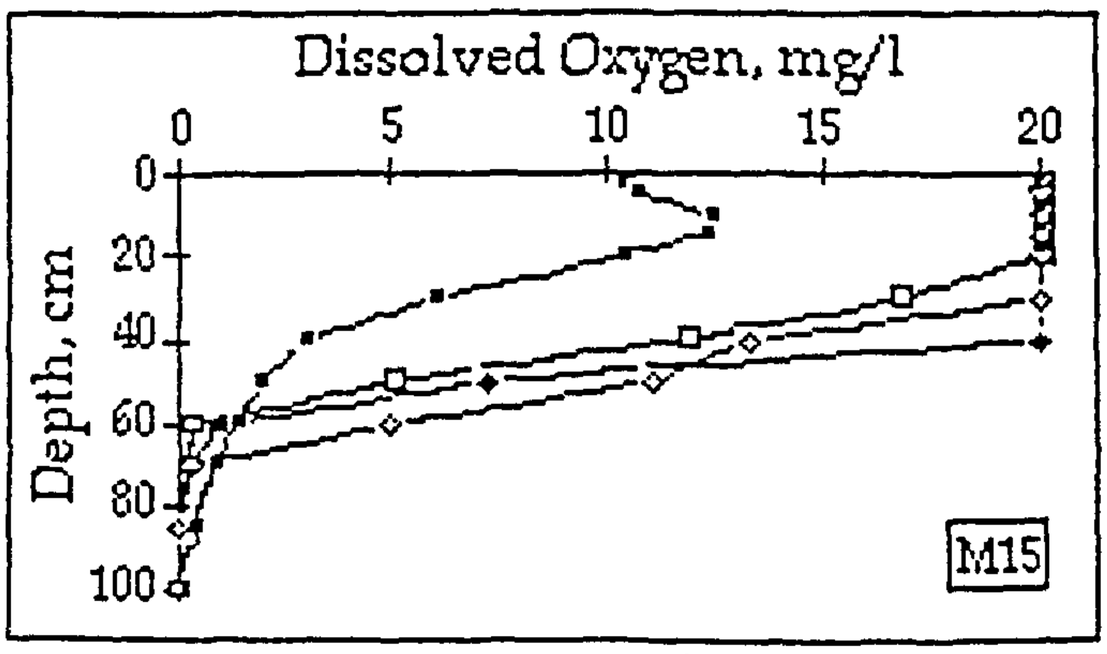


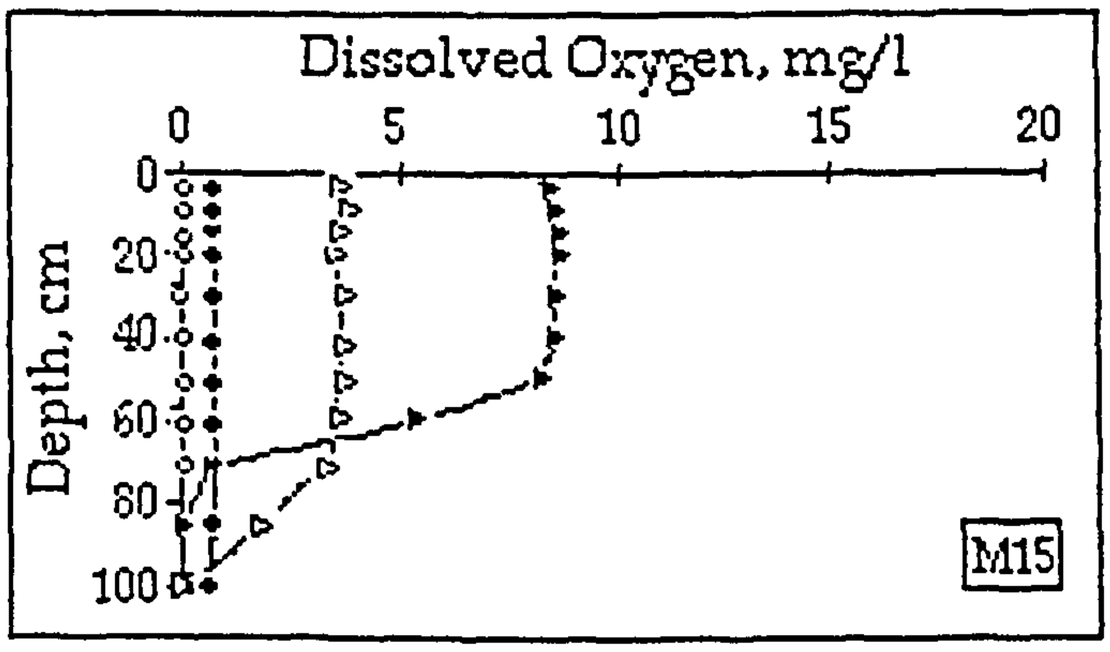
Figure A3/13b Dissolved Oxygen Profiles of the Ponds Investigated on 31.3.93.

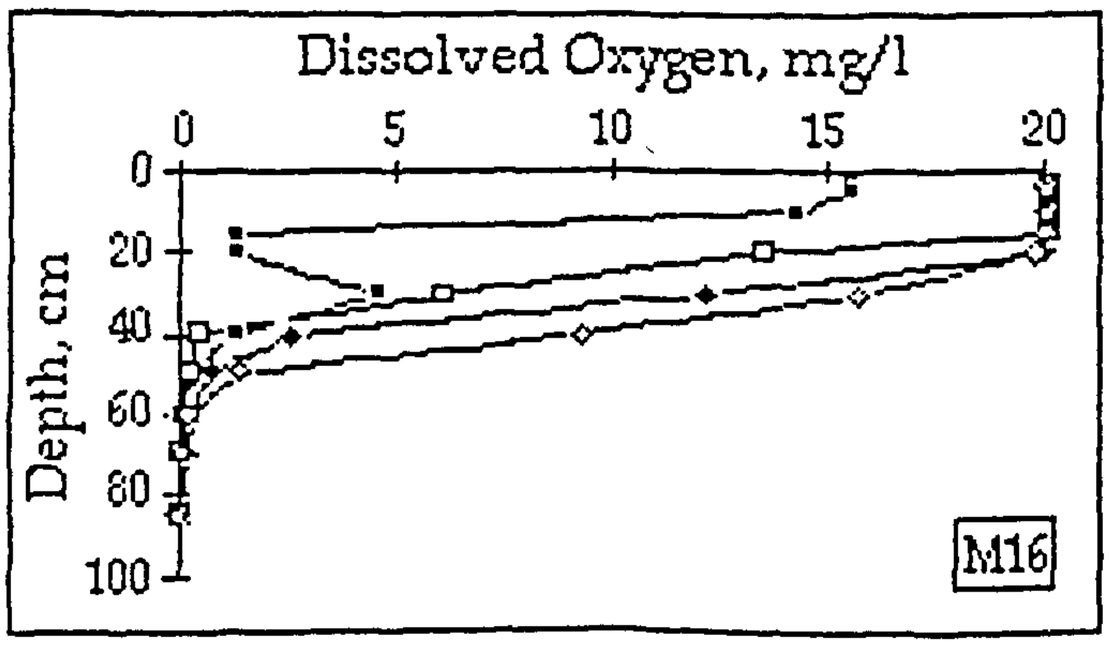
Samples taken at 8.00 hours —▪—, 11.00 hours —□—,
14.00 hours —•—, 17.00 hours —◇—, 20.00 hours —▲—,
23.00 hours —△—, 2.00 hours —•— and 5.00 hours —○—.

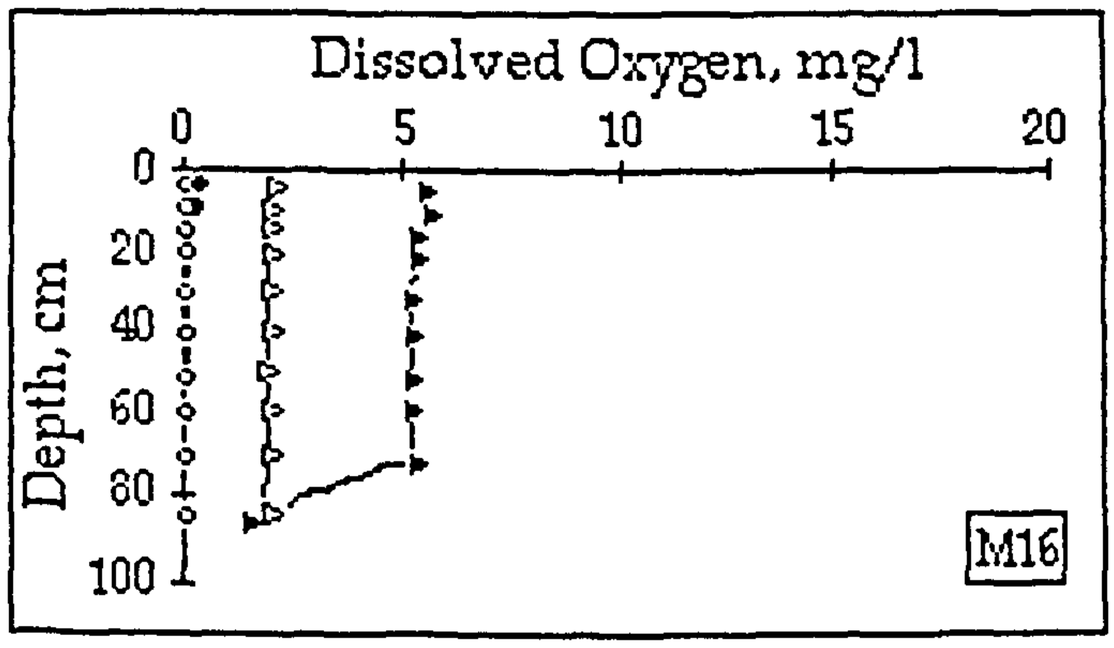


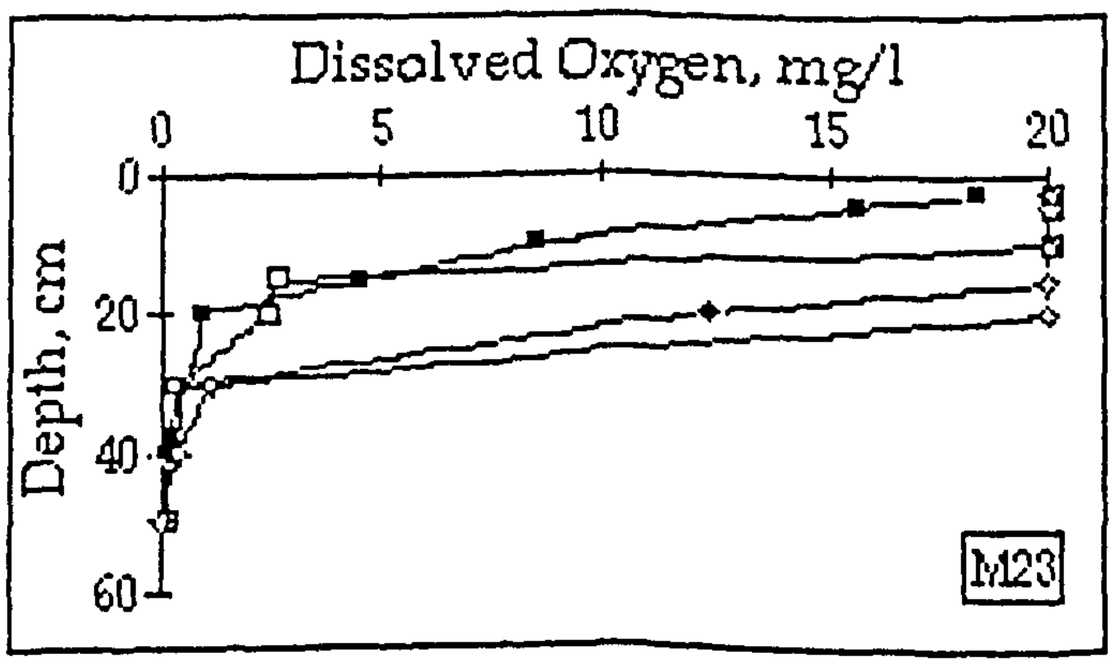












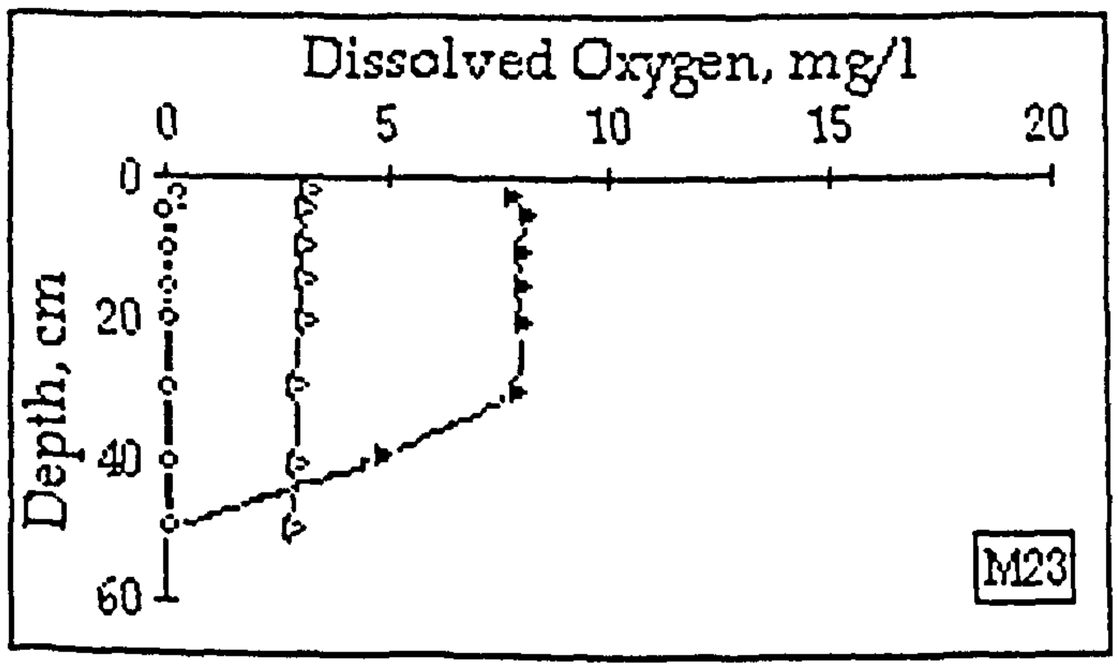
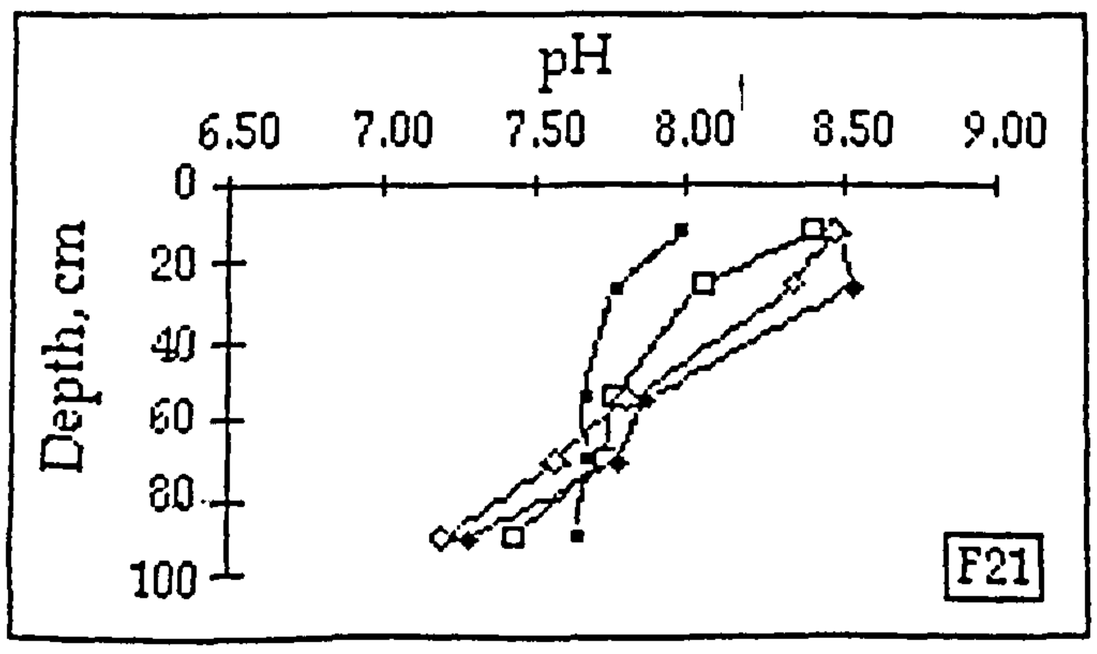
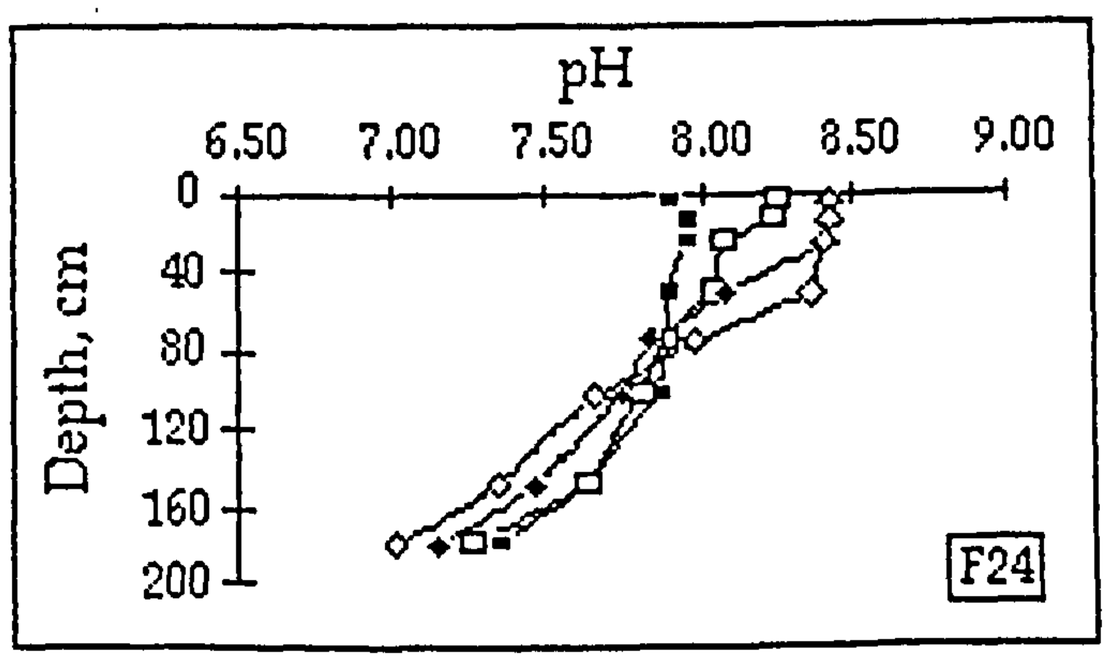
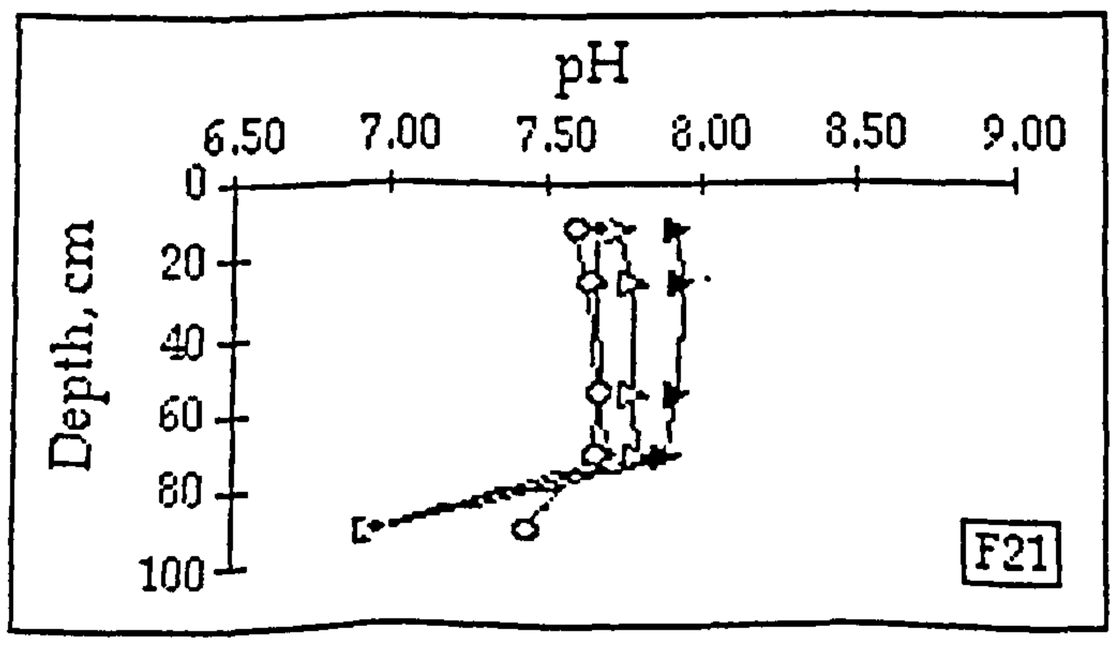


Figure A3/14 pH Profiles of F21 and F24 on 31.3.93.

Samples taken at 8.00 hours —▪—, 11.00 hours —□—,
14.00 hours —⬩—, 17.00 hours —◇—, 20.00 hours —▲—,
23.00 hours —△—, 2.00 hours —•— and 5.00 hours —○—.







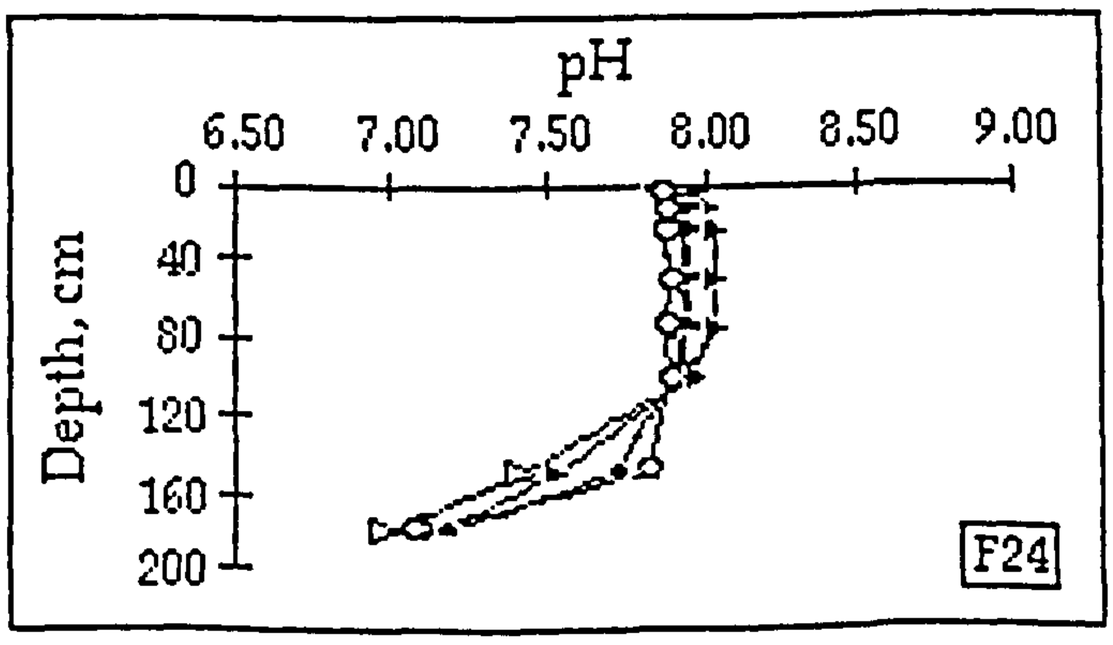
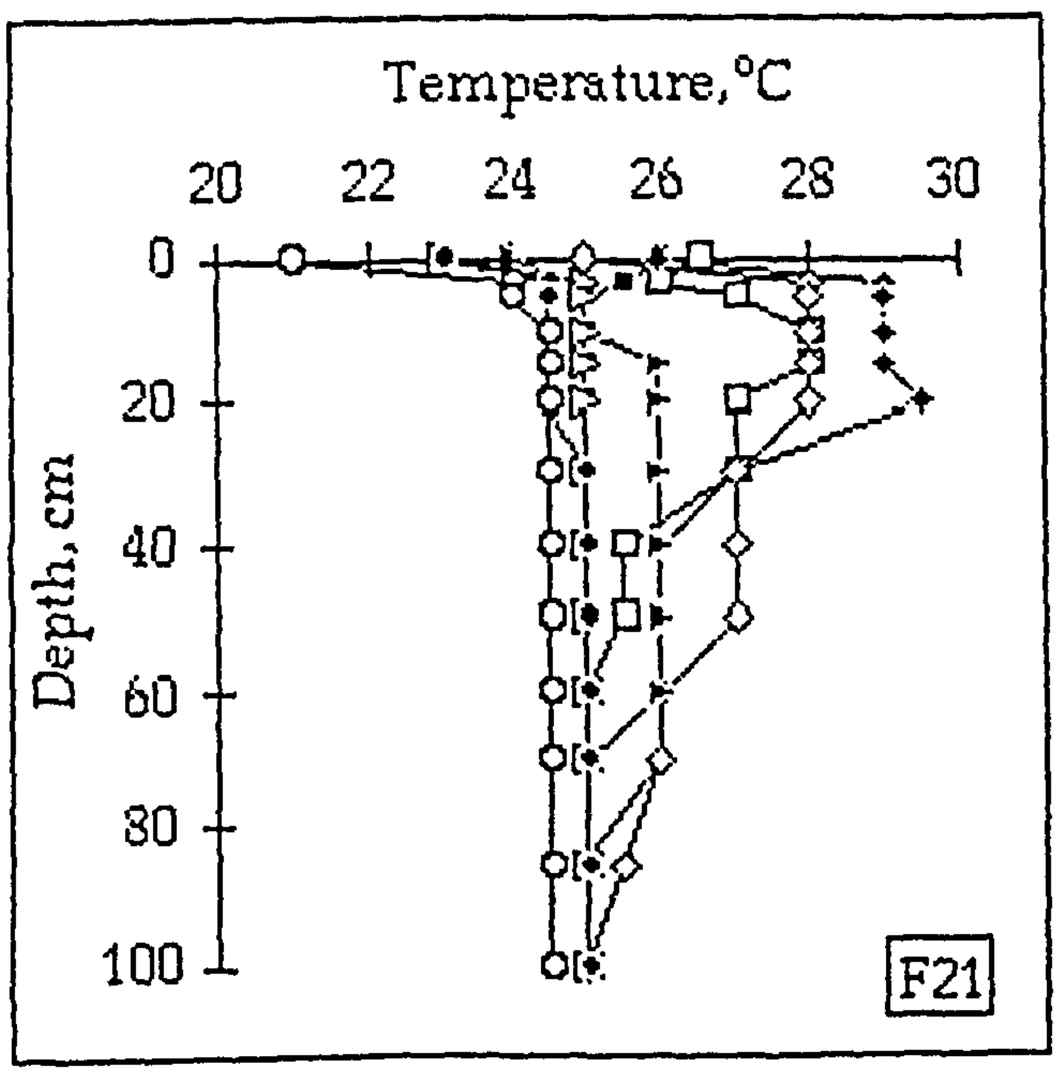
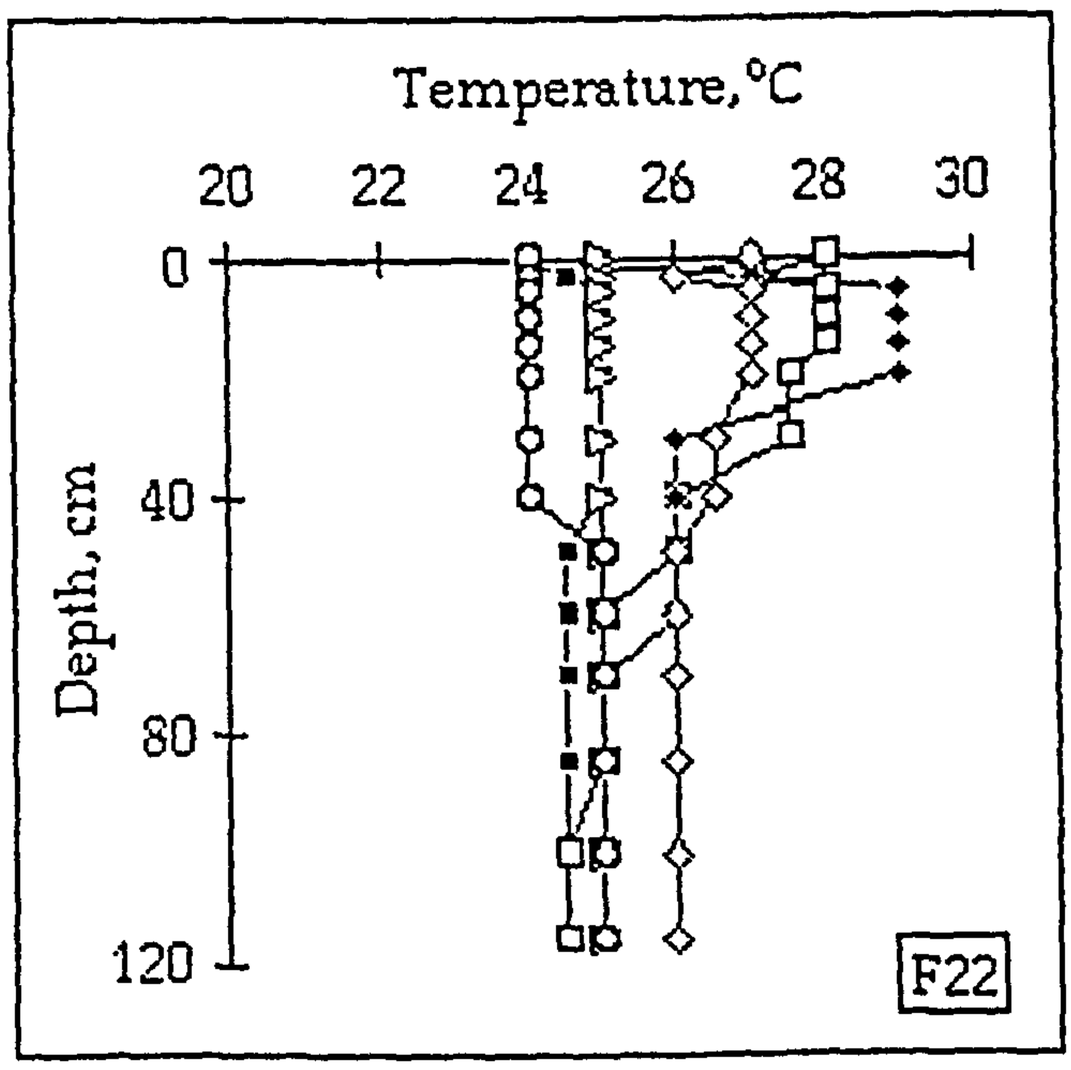
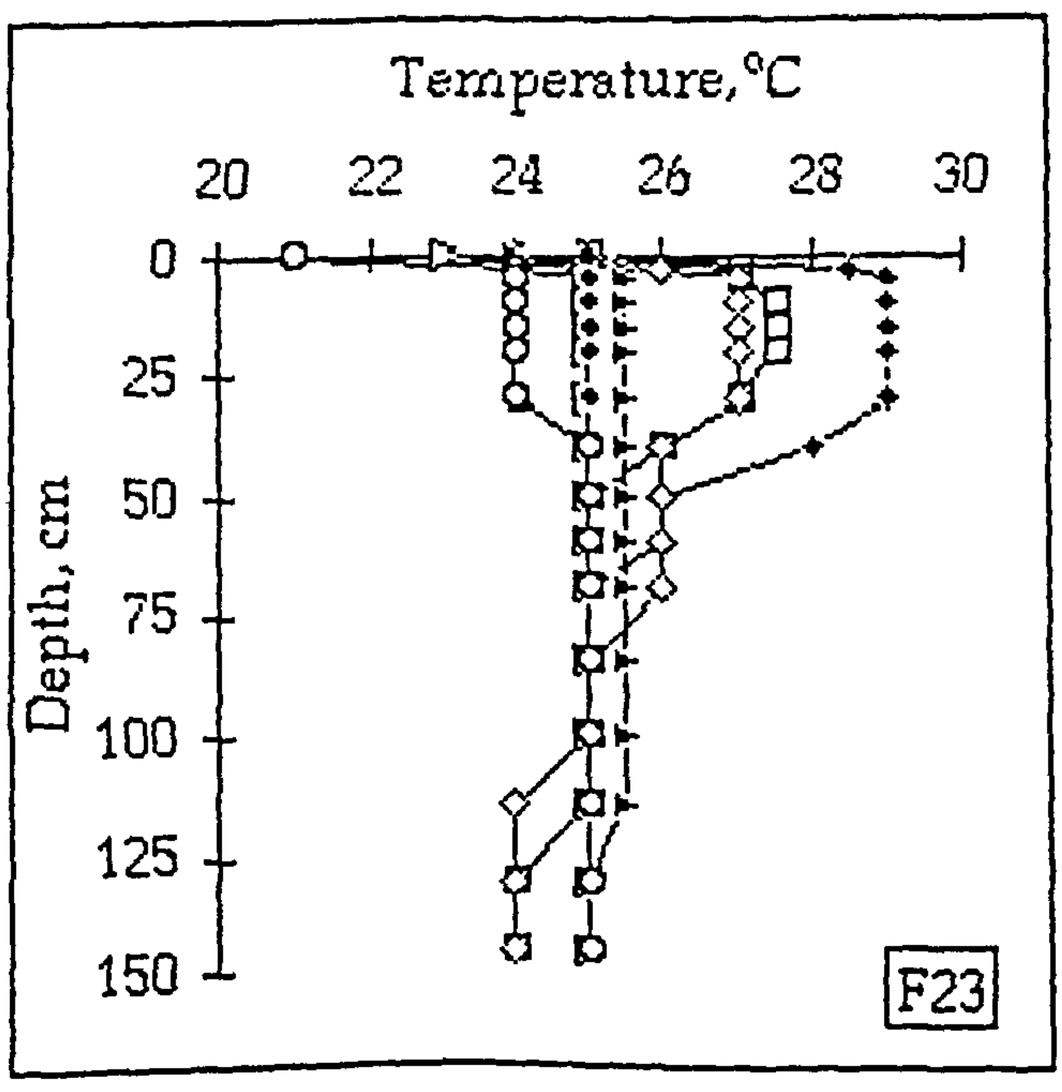


Figure A3/15 Temperature Profiles of the Ponds Investigated on 31.3.93.
Samples taken at 8.00 hours —▪—, 11.00 hours —□—,
14.00 hours —♦—, 17.00 hours —◇—, 20.00 hours —▲—,
23.00 hours —△—, 2.00 hours —•— and 5.00 hours —○—.







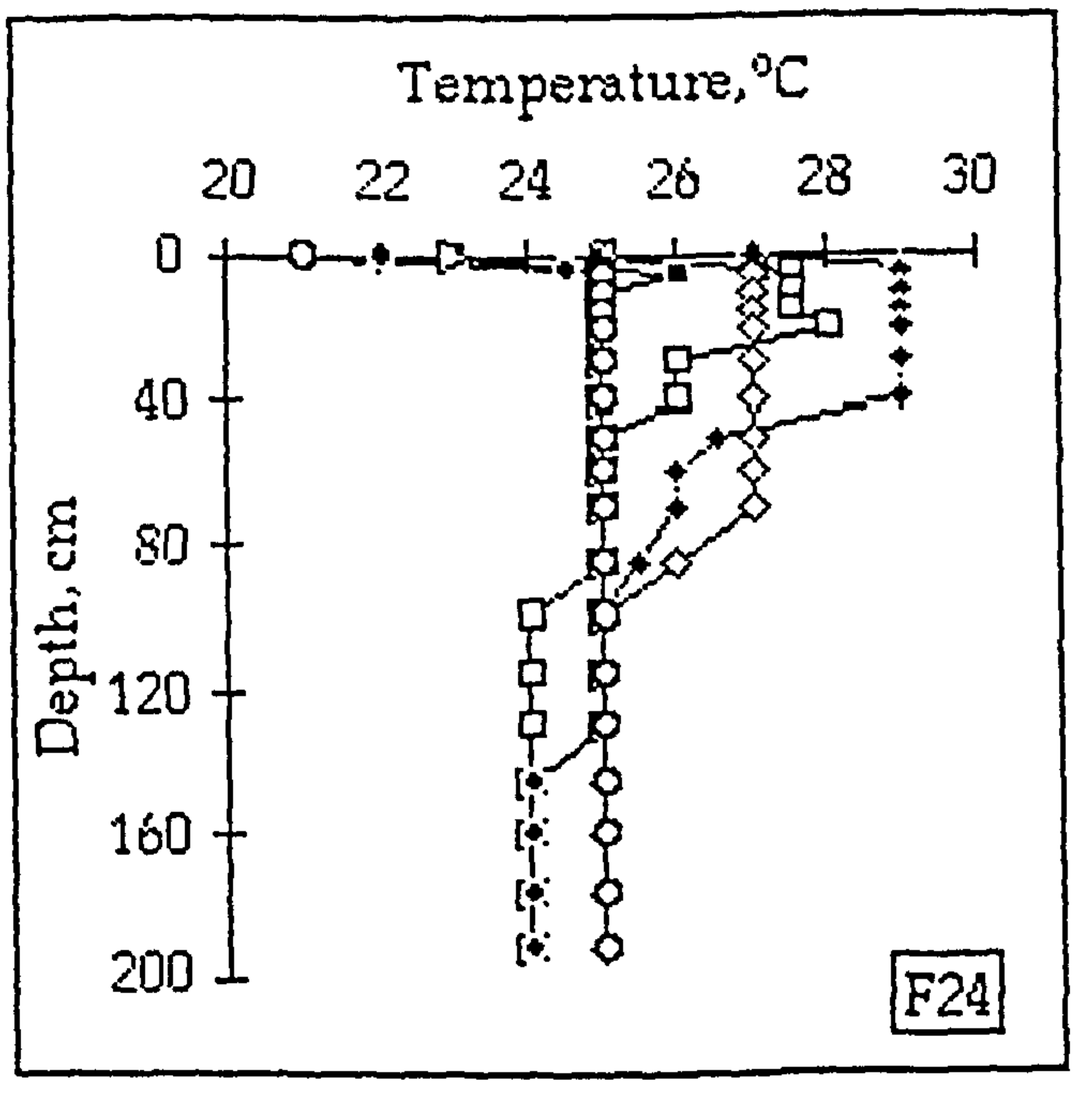


Figure A3/15b Temperature Profiles of the Ponds Investigated on 31.3.93.
Samples taken at 8.00 hours —▪—, 11.00 hours —□—,
14.00 hours —♦—, 17.00 hours —◇—, 20.00 hours —▲—,
23.00 hours —△—, 2.00 hours —•— and 5.00 hours —○—.